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DUCKWEED FORUM

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International Steering Committee on Duckweed Research and Applications

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Lemna minor L., clone 9441

Cover page

Lemna minor L., clone 9441

Lemna minor turned out recently, in the genomics era, to be a very interesting species in as far as a significant variance has been observed in genome sizes and apparent ploidy between natural populations. This is very unusual when compared with the highly conserved genome structure observed in the well characterized genus of Spirodela. At the same time clones of this species are top candidates for large scale cultivation to produce high amounts of biomass because it is a robust species for such practical purposes. Thus, potential use for nutrition and as energy plants is ahead for this species. Clone 9441 is the oldest clone in cultivation and its fascinating story is told inside this issue. This clone is also the preferred duckweed for phytotoxicity assays according to ISO 20079. Photo credit: Dr. Klaus-J. Appenroth, Friedrich Schiller University of Jena, Germany.

In this issue

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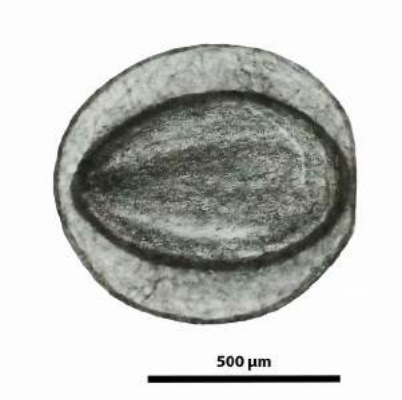
All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: *Wolffia cylindracea* Hegelm.



The genus *Wolffia* contains 11 species. Especially in the section *Wolffia* it is not easy to distinguish the seven different species on a morphological basis. *Wolffia cylindracea* does not have stomata on the upper surface and the ratio between width and length of the fronds is larger than in other species. Edges of the fronds are not translucent. Christoph Friedrich Hegelmaier described this species for the first time in 1868. This species can be found south of the equator, e.g. in Angola. The drawing shows a top view with the ellipsoid dorsal surface of the frond and the widest part of the frond just beneath. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor:

Dear Readers of the DF,

Greetings to you all on behalf of the members of the International Steering Committee for Duckweed Research and Applications (ISCDRA). I hope this new issue of the *Duckweed Forum*, now the 31st edition already, will find everyone in good health and coping alright with the global health crisis over the past months. As we are nearing the end of the year 2020, it is sobering to reflect on all that has transpired in the past ten months to begin this new decade with. Our world has changed so much in such a short time during this pandemic and the future yet remains uncertain to this day. However, as a scientist, it is at least heartening to witness the ability of technologies that are now at our disposal to tackle this invisible disease agent and can hope for an effective vaccine before long. In addition, the availability of online tools allowed us to quickly adopt alternatives to keep our society functioning in new ways and adapt to the new constraints. Indeed, as this pandemic demonstrated, I believe the resilience of humanity to global challenges such as future health crisis and climate change will significantly depend on technological advances that we make today. As a highly versatile plant family, I believe duckweed-based platforms for bioproducts can have important impact in these scenarios in the not-so-distant future. Our community will have a significant role to play in bringing this promise to reality.

In this issue of the *Duckweed Forum*, a contribution from two young university students in Switzerland strived to identify the variance in consumer preference for acceptance of duckweed in their diet, using *Wolffia arrhiza* as their model. This type of marketing survey would be an important step toward a more data-driven approach for the identification of successful strategies to bring duckweed-based food to commercial deployment. An interesting item in this DF is the account of the history of *Lemna minor* 9441, the oldest known duckweed isolate that has been in continuous culture since the end of WWII almost 75 years ago. It is indeed fitting the proverb of "Necessity is the Mother of Invention" that the lack of adequate space and financial resources in post-war Germany was what prompted Prof. Pirson to isolate and culture this strain of duckweed in Marburg to enable him to carry on biological research. In addition to a nice *Student Highlight* from the Oyama lab in Kyoto, we also bring you the *Database* section by Klaus Appenroth to bring you abstracts for duckweed-related papers over the past quarter. Last but not least is an important announcement in this DF issue by Prof. Ingo Schubert (Gatersleben, Germany) to postpone the next ICDRA meeting by 1 year to May of 2022 due to issues for traveling internationally during this pandemic. To coordinate with this change, all members of the current ISCDRA agreed to serve until the ICDRA meeting in 2022, when the newly appointed members by the community will be inaugurated.

Finally, the ISCDRA and I hope you will enjoy this issue of the DF. I especially like to acknowledge all contributors for their time and effort to share interesting ideas and knowledge with others in our community. We look forward to hearing from you in the near future.

Sincerely,

Eric Lam

Chair, ISCDRA

6th ICDRA 2022: 2nd Circular

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6th ICDRA 2022: 2nd Circular

The 6th International Conference on Duckweed Research and Applications (6th ICDRA) will be held from **May 29 - June 01, 2022** in Gatersleben, Germany, at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)

(postponed from the original date of May 2021 due to the pandemic)

The **Conference website** will be available soon under: **icdra-2022.ipk-gatersleben.de** and open **for registration.** (Student: 100.- €, Academic: 350.- €, Industry: 700.- €)	**Abstract submission** Authors may indicate their preference for short talk or poster presentation; the final decision will be made by the organizers in April 2022.

The **registration and abstract submission deadline is March 31, 2022.**
A reminder will be sent out in March 2022.

If you need an official invitation or any other support, please, do not hesitate to contact the organisation committee.

We hope to meet you at the 6th ICDRA in Gatersleben.

The ISCDRA and the local organizers

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Acceptance of *Wolffia arrhiza* as Novel Food in Switzerland and China

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1. Introduction and objective of the project

Fig 1: Picture of fresh *Wolffia arrhiza* (provided by LemnaPro)

Wolffia (shown in Fig.1) is a promising food source. It is a fast-growing plant with relatively low environmental impact and is also especially attractive to consider due to its high nutritional value. Wolffia could be a good source of the essential omega-3 fatty acids and provides important fibers. In addition, it contains all essential amino acids making it a valuable, alternative plant-based protein source (Appenroth et al., 2018; van der Spiegel et al., 2013). Wolffia has already been served as food for human in several Southeast Asian countries (Saengthongpinit, 2017). For European and Chinese consumers, however, Wolffia is a novel, unfamiliar food. According to the literature the acceptance of novel foods is influenced by various factors such as cultural habits, social norms, general demographic factors in addition to the price, expected taste, quality and nutritional value (Barrena and Sanchez, 2013; Rozin and Vollmecke, 1986).

The general objective of our project was to investigate the acceptance of *Wolffia arrhiza* as a novel food and the possible influencing factors. A cross-cultural comparison was performed between Switzerland and China to investigate the influence of culture, positive information about Wolffia and meal context on its acceptance, willingness to try and the expected preference.

2. Experimental set-up

A 10-minute online questionnaire was designed to investigate the acceptance of Wolffia as a novel food in Switzerland and China. The questionnaire was peer-reviewed by 10 students of the master course “Selected Topics in Sensory Science” at ETH Zurich. The final questionnaire, consisting of 5 parts, was translated into Chinese.

In Part 1, participant’s attitude towards novel foods and the importance of nutritional value and environmental impact was assessed for foods in general. The participants chose their familiarity with Wolffia in Part 2, followed by Part 3 where they were asked about the willingness to try Wolffia before and after being exposed to its positive information. In Part 4, the impact of meal contexts on the respective expected preference was assessed. The participants were invited to an imaginary serving of 7 dishes according to their dietary preferences (plant-based/meat-based, no allergens, etc.): Sushi with Wolffia as filling, cooked vegetables with Wolffia, Wolffia as an ingredient of a fresh salad, pasta made from Wolffia; Burger patty with Wolffia; chocolate specialty with Wolffia filling and

a green smoothie based on Wolffia juices. The participants were asked to indicate how much their expected preference is for the dishes in random order on the hedonic 9-point preference scale. The dietary pattern and some demographic questions were investigated in the final part of the questionnaire.

3. Results and discussion

Data was collected in March 2020 and consisted of 32 ETH Zurich food science bachelor students and 35 Chinese participants. Similar to the Swiss sample cohort, most of the Chinese respondents were also food science students in universities but living in China instead of Switzerland. The participants' detailed information profile is shown in the Table 1. The results were analyzed by performing statistical analysis such as ANOVA and t-tests with the software IBM SPSS 25.0 and R-studio. The table below summarizes the data collected using our online questionnaire from both Swiss and Chinese cohorts.

Table 1: Demographics, dietary pattern, food neophobia, general food choice, category of Wolffia considered by respondents and percentage of respondents who previously were unfamiliar with Wolffia in the Swiss (N=32) and Chinese (N=35) cohorts.

	Range	Switzerland		Range	China	
		M[1] or %	SD		M or %	SD
Age (years)	21-31	24.0	2.07	21-25	23.3	1.19
Gender (female)		68.8%			62.9%	
Food neophobia	10-36	21.5 (MD[2])	7.47	16-49	32 (MD)	8.20
Dietary pattern						
Omnivore		47%			94%	
Flexitarian		28%			6%	
Pescatarian		6%				
Vegetarian		6%				
Vegan		13%				
Wolffia unfamiliarity previously		65.6%			94%	
Country of residence (Europe)		100%			14%	
China					86%	
Category of Wolffia						
Vegetable		16%			14%	
Superfood		78%			37%	
Herbs					26%	
Salad					23%	
Food choice questionnaire						
Taste		6.8	0.49		6.4	0.85
Price		5.4	0.91		5.5	1.00
Environmental impact		5.3	1.27		4.5	1.36
Nutritional value		5.5	1.33		5.2	1.15
Willingness to try Wolffia						
Before information		6.5	0.76		5.3	1.29
After information		6.8	0.51		5.7	1.22

[1] Mean value: the average value of all results from the participants in the study.
[2] Median value of food neophobia scale from the participants in the study.

Influence of information: The two-way mixed ANOVA revealed a significant main effect of information ($F(1,65) = 21.3$, $p < 0.001$). As assumed, exposing participants to advantageous nutritional and environmental facts about Wolffia increased the acceptability of Wolffia as a novel food. These findings were consistent with previous studies showing that positive communication leads to a better evaluation of Wolffia by Dutch consumers (de Beukelaar et al., 2019).

Influence of culture: There was also a significant effect of country ($F(1,65) = 21.0$, $p < 0.001$), which showed that Chinese participants had more conservative attitude towards trying Wolffia. It was consistent with the results that Chinese participants had a higher food neophobia score, meaning a lower willingness to try new foods. This observation was in agreement with literature (Johns et al., 2011) reporting that Asians, especially Chinese, are generally more neophobic than Europeans.

Influence of meal context: The paired-sample t-test of the Swiss sample revealed a significant effect of the addition of Wolffia regarding to cooked vegetables ($p = 0.009$), chocolate specialty ($p < 0.001$), and fresh salad ($p = 0.002$). These findings indicate that the presence of Wolffia in these three types of meal contexts significantly decreased the respondents' expected preference. In the Chinese cohort, there was a significant effect from the introduction of Wolffia in terms of cooked vegetables ($p = 0.006$) and burger ($p = 0.031$). These findings suggest that the Chinese participants' expected liking of cooked vegetables and burger were significantly lower due to the addition of Wolffia. Therefore, smoothie, sushi, burger and pasta may be more appealing routes of application for Wolffia in the Swiss food sector, while sushi, chocolate specialty, fresh salad and pasta could be combinations that are better appreciated for Chinese consumers.

4. Outlook

As summarized in the table above, the willingness to try Wolffia varied significantly among the participants with different dietary patterns. Vegans might be the potential target group of Wolffia consumption. However, the specific influence of dietary pattern on the acceptability and the correlations of acceptability of Wolffia with other factors such as demographics, general food choice and changing residence of country need to be further investigated in view of the limited and unevenly distributed sample. Moreover, sensory characteristics like taste and flavor may also influence consumers' behavior towards Wolffia. Therefore, the combination of online questionnaire with sensory test about Wolffia should be conducted in further research. Independent of the product, we would suggest sticking an information card with the description of the potential benefits of Wolffia on the food packaging. Furthermore, educating potential consumers about these benefits by advertisement can serve to increase its familiarity and consequently also its acceptance.

Acknowledgement

We would like to thank Prof. Dr. Jeannette Nuessli Guth and Prof. Dr. Sandrine Gouinguené for their guidance through this interesting project and for the implementation of the online questionnaire in Compusense. Also, many thanks go to our supervisors Melanie Binggeli and Cyrill Hess from LemnaPro for introducing us to the world of Wolffia, giving us useful feedback throughout the project and supporting us with the statistical analysis.

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Historical Account: *Lemna minor*, clone 9441 – the oldest living duckweed clone in culture

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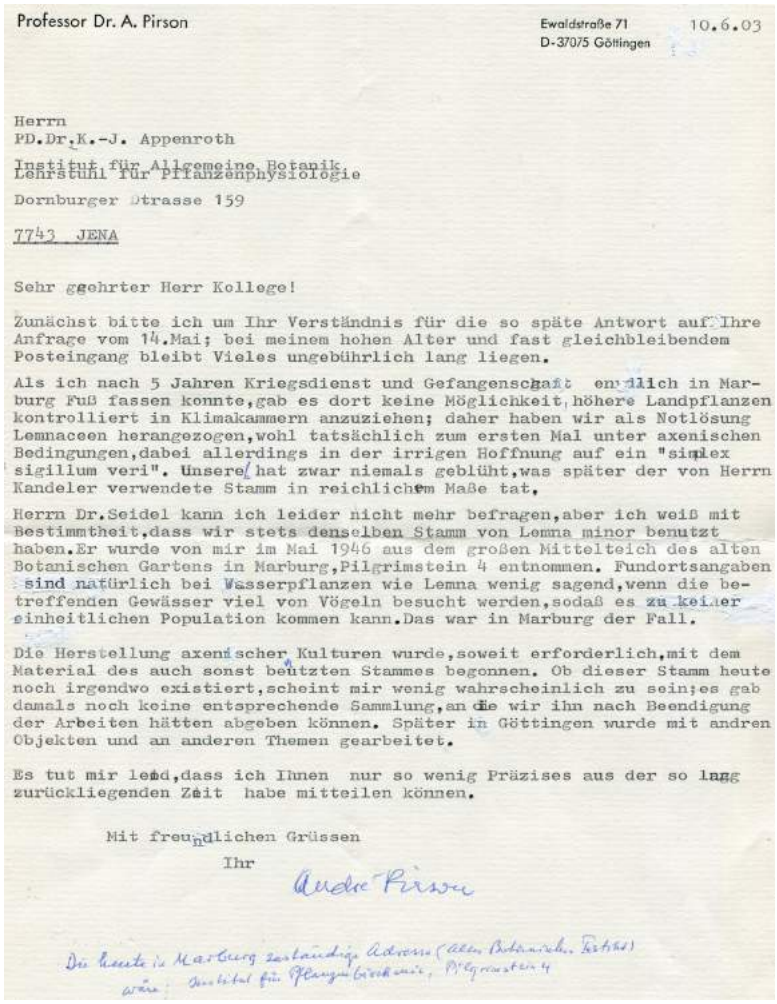
The *Lemna minor* clone 9441 was isolated more than seven decades ago in 1946 from Marburg, Hesse, Germany by André Pirson (1910-2004). It is noteworthy that this was just one year after the end of the second world war. In a letter written by Professor Pirson to one of us (KJA) in 2003 (Figure), less than a year before he passed away (7th of February, 2004), he wrote "When after 5 years of service in the war and being a prisoner of war I could finally establish myself in Marburg, there was no opportunity to grow higher land plants under controlled conditions in a climate chamber. So, as a less than ideal solution, we have cultivated duckweed indeed for the first time under axenic conditions, however under the misguided hope of "The simple is the seal of the true" (Pirson, 2003). Pirson was the Director of the Botanical Institute in Marburg. Lacking sufficient space in his Institute, he could not start his research on crop plants and decided to work with the less space-demanding duckweeds. Together with his PhD student, Franz Seidel, he isolated a clone of *L. minor* in May, 1946 from a pond (so-called "Mittelteich") in the old Botanical Garden of the University of Marburg, Pilgrimstein 4, Germany. Seidel defended his thesis in December 1950 under the title "Contribution to the cell and metabolic characterisation of potassium and calcium deficiencies" (Seidel, 1950) and this research was



Professor Dr. A. Pirson
Ewaldstraße 71
D-37075 Göttingen
10.6.03

Herrn
PD.Dr.K.-J. Appenroth
Institut für Allgemeine Botanik
Lehrstuhl für Pflanzenphysiologie
Dornburger Strasse 159
7743 JENA

Sehr geehrter Herr Kollege!

Zunächst bitte ich um Ihr Verständnis für die so späte Antwort auf Ihre Anfrage vom 14.Mai; bei meinem hohen Alter und fast gleichbleibendem Posteingang bleibt Vieles ungebührlich lang liegen.

Als ich nach 5 Jahren Kriegsdienst und Gefangenschaft endlich in Marburg Fuß fassen konnte, gab es dort keine Möglichkeit, höhere Landpflanzen kontrolliert in Klimakammern anzuziehen; daher haben wir als Notlösung Lemnaceen herangezogen, wohl tatsächlich zum ersten Mal unter axenischen Bedingungen, dabei allerdings in der irrigen Hoffnung auf ein "simplex sigillum veri". Unsere hat zwar niemals geblüht, was später der von Herrn Kandeler verwendete Stamm in reichlichem Maße tat.

Herrn Dr.Seidel kann ich leider nicht mehr befragen, aber ich weiß mit Bestimmtheit, dass wir stets denselben Stamm von Lemna minor benutzt haben. Er wurde von mir im Mai 1946 aus dem großen Mittelteich des alten Botanischen Gartens in Marburg, Pilgrimstein 4 entnommen. Fundortsangaben sind natürlich bei Wasserpflanzen wie Lemna wenig sagend, wenn die betreffenden Gewässer viel von Vögeln besucht werden, sodaß es zu keiner einheitlichen Population kommen kann. Das war in Marburg der Fall.

Die Herstellung axenischer Kulturen wurde, soweit erforderlich, mit dem Material des auch sonst benutzten Stammes begonnen. Ob dieser Stamm heute noch irgendwo existiert, scheint mir wenig wahrscheinlich zu sein; es gab damals noch keine entsprechende Sammlung, an die wir ihn nach Beendigung der Arbeiten hätten abgeben können. Später in Göttingen wurde mit andren Objekten und an anderen Themen gearbeitet.

Es tut mir leid, dass ich Ihnen nur so wenig Präzises aus der so lang zurückliegenden Zeit habe mitteilen können.

Mit freundlichen Grüssen
Ihr
André Pirson

Die heute in Marburg zuständige Adresse (alten Botanischen Instituts) wäre: Institut für Pflanzenbiologie, Pilgrimstein 4

published in the same year (Pirson and Seidel, 1950). In 1958, Pirson moved to the University of Goettingen, Hesse, Germany, where he worked with even smaller organisms than duckweed, algae, until his retirement (Winkler and Pirson, 1980). Most probably he passed this *L. minor* clone to Reinhard Bornkamm at the University of Goettingen.

As clone 9441 was isolated in 1946, it is the oldest living duckweed clone in culture known to us (Cover photo). Elias Landolt, working in the ETH Zurich, started his duckweed collection much later, during his post-doctoral stay from 1953-1955 in several Institutes in California (Laemmler and Bogner, 2014) and published his first duckweed related research paper in 1955 (Landolt, 1955). Moreover, with this *L. minor* clone, Pirson and Seidel established axenic cultures of duckweeds for the first time. They called this clone St, most probably meaning "Sterile". The nomenclature of the clone changed from St to 9441 when Elias Landolt agreed in 2011 to use his four-digit clone ID for all the clones under his international duckweed stock collection (Zhao et al., 2012).

Lemna minor clone 9441 has been kept under axenic conditions in the stock collection of the University of Jena since 1969 under the responsibility of Helmut Augsten who was the Director of the Botanical Institute since March 1966. This clone is now available in many international duckweed stock collections. Tracing back the travels of this clone, the manager of the duckweed stock collection in that time at the University of Jena, Fritz Jungnickel, received this plant in 1969 from Erich Ohmann, Institute of Biochemistry of Plants ADW, Halle/ Saale, Saxonia-Anhalt, Germany. Ohmann communicated that he received this clone from R. Bornkamm, University of Goettingen (Jungnickel, 1978). Since then, this clone has been used for several research purposes under the name 'St' (Naumann et al., 2007) and more recently under the revised clone identity of 9441 (Ziegler et al., 2015). This clone was characterised by molecular taxonomy (Bog et al., 2010), growth rate measurements (Ziegler et al., 2015) and was used as a model system for phytotoxicity tests according to the standard protocol ISO20079 (Naumann et al., 2007). Meanwhile, the whole genome of this clone has recently been sequenced but the results are yet to be published (Todd P. Michael, The Salk Institute, California and Eric Lam, Rutgers University, New Brunswick, NJ).

Soon, in 2021, the 75-year 'Diamond Jubilee' can be celebrated for isolation of this clone, 9441 *Lemna minor* L. and all the research that it had enabled over this time.

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Announcement of upcoming special issue in the journal 'Plants"

Plants: ISSN 2223-7747

Title of the special issue: **"Duckweed: Research Meets Applications"**

The aim of this Special Issue is to provide a comprehensive update of the current progress in duckweed research and applications. Contributions in forms of original research papers, reviews and short communications from a broad scope of disciplines related to duckweed research and applications (e.g., morphology, taxonomy, and ecology including ecological interactions, ecotoxicology, environmental monitoring and remediation, physiology, biochemistry, genetics, omics, biotechnology, biomass production and its uses, etc.) are welcome. We hope that this overview will be of interest to those involved in basic research or potential applications of duckweeds, and will also attract readers from other fields.

Keywords: Duckweed, Lemnaceae, Physiology, Genetics, Omics, Phylogenetics, Ecology, Ecotoxicology, Remediation, Biomass

This special issue is now open for submission.

Deadline for manuscript submissions: 31 May 2021

Manuscript Submission Information

Manuscripts should be submitted online at www.mdpi.com by registering and logging in to this website. Manuscripts can be submitted until the deadline. All papers will be peer-reviewed.

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We look forward to your article submissions.

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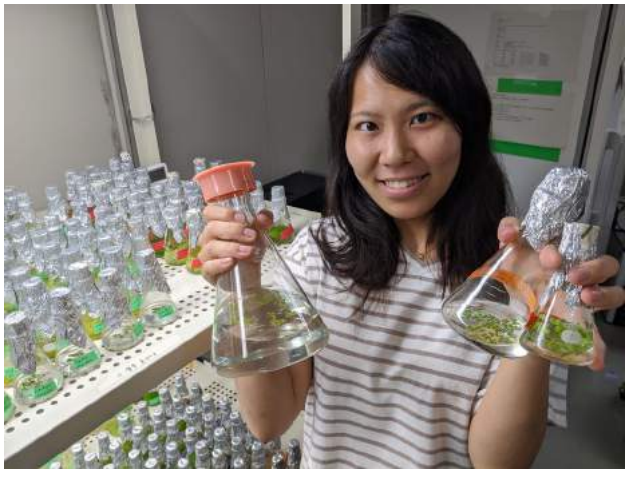
When I was a child, I lived in the countryside near Chiba. My playground was mostly rice fields, forests, and small rivers. I often spent my free time finding wild berries in the field, catching insects in the forests near shrines or temples, or fishing for crayfish in the river. There were so many chance for me to be interested in nature, but I was not. Nature was too familiar to me at that time that I took it for granted. Nature surrounded my life like air; hence, I did not really think about it.

The situation changed when I entered High School. My Biology teacher in High School was great at teaching, and her lecture made me intrigued by Biology. I became curious about what exactly happens in nature at the molecular level, and I started developing an interest in it. When my family moved to the urban city of Tokyo, I realized how I love being in nature and how much precious time I had spent in there. My curiosity about Biology continued to grow; therefore, I decided to study Biology at the University.

After I entered the University, I took a class named Biological Rhythms. I was extremely intrigued by the knowledge that almost all organisms have a biological clock to adapt them to the 24-hour cycle of environmental changes caused by the rotation of the earth. I enquired the professor of my class, Prof. Okamura, started a biological clock experiment at the lab using mice, and it seemed as if everything was going well. Unfortunately, two years later, I developed an allergy to mice and could not continue the experiment. I needed to change my lab, but I wanted to continue studying biological clock. Luckily, I found a lab where biological clock is being studied using plants. That was the first time I met Prof. Tokitaka Oyama. When he showed me around his lab, I came across wonderful plants called duckweed. I loved duckweed the minute I saw them, and my favorite one is *Wolffiella hyalina*.



My research theme is based on the mechanism of flowering in duckweed, especially *W. hyalina*. The reason I am interested in this theme is that the flowering mechanism is controlled by a biological clock. Plants sense seasonal changes in environments to synchronize their flowering to maximize fitness. The integration of external light information into biological clock-controlled mechanisms enables plants to precisely measure day length changes in the environment. When it comes to some duckweed plants, salicylic acid (SA) is important for flowering. According to previous studies, *W. hyalina* 7378 strain does not normally flower when grown on a basal medium under either long- or short-day photoperiodic schedule; however, flowering is induced under a short-day photoperiodic schedule when SA is added to the medium. These results suggest that there are photoperiodic and SA-induced pathways in *W. hyalina* flowering system; however, the relationship between these two pathways and their flower induction mechanisms have not been analyzed at the molecular level.

Therefore, we approached the photoperiodic- and SA-induced flowering pathway of *W. hyalina*. Next, to investigate the relationship between the two pathways, we have created *W. hyalina* cDNA library using de novo transcriptome assemblies derived from normalized RNA-Seq. We identified two flowering-related genes, *FLOWERING LOCUS T* homolog (*WhFT*) and a *14-3-3* homolog (*Wh14-3-3*). We analyzed the expression of *WhFT* and *Wh14-3-3* under flowering inductive/non-inductive conditions using *W. hyalina* 7378. It has been reported that FT acts as florigen and 14-3-3 protein GF14c acts as intracellular receptors for florigen in shoot apical cells in rice. FT interacts with 14-3-3 protein and binds to the FD1 transcription factor, and the 14-3-3 - FT complex induces flowering. In my study, *WhFT* mRNA was found to be upregulated by SA treatment under both long-day and short-day conditions, suggesting the involvement of *WhFT* only in the SA-induced pathway. *Wh14-3-3* mRNA was upregulated by SA treatment only under the short-day condition, suggesting the involvement of *Wh14-3-3* in the downstream flowering pathway of SA and photoperiod signals. According to these results, the WhFT-Wh14-3-3 complex might be necessary for flowering. Three days after the addition of SA, the flowering-related genes, including WhFT and Wh14-3-3, were upregulated under short-day conditions, suggesting that the plants may already be in a state of readiness for flowering before the addition of SA. I hypothesize that biological clock may prime the plants to a state that is more likely to induce flowering. To verify this hypothesis, we are now analyzing the differences in the variation of expressed genes when SA is added under short- and long-day conditions. I look forward to sharing my results with the community soon.

Sometimes, aside from working in the laboratory, we go on short trips to find duckweed. When I did not know about duckweed, rice fields were just rice fields. But now, I can find some beautiful things there. I am so grateful that I have a chance to know duckweed, and I will continue trying to do my best to elucidate the unclear mechanism of flowering control in duckweed.

From the Database

Highlight

Preference, performance, and impact of the water-lily aphid on multiple species of duckweed

Subramanian, SK; Turcotte, MM (2020) Ecological Entomology DOI: 10.1111/een.12932

The role of herbivores in driving the structure of freshwater macrophyte communities remains poorly understood in comparison with terrestrial ecosystems. For instance, although duckweed (subfamily Lemnoideae) are globally distributed, can be locally highly abundant and ecologically dominant, and are of growing economic importance, their interactions with herbivores remain understudied. To address how herbivores may impact duckweed species composition, we here experimentally quantify the preference and performance of a common duckweed herbivore, the water-lily aphid (*Rhopalosiphum nymphaeae*) on four widespread duckweed species. Our two-way choice experiments reveal that aphids display a preference for *Spirodela polyrhiza* > *Landoltia punctata* = *Lemna minor*> > *Wolffia brasiliensis*. These results are rarely influenced by natal host species. By evaluating the growth of aphid populations on each duckweed species, we find that preference may be adaptive in certain ecological conditions. Quantifying the population growth rate of duckweed growing in the presence and absence of aphids revealed differential tolerance of herbivory across duckweed species. This study shows that aphids, through preferential feeding and significant differential effects on duckweed growth, can have a significant impact on duckweed population dynamics and potentially community composition.

Biotechnology

Expression of LamB vaccine antigen in *Wolffia globosa* (Duckweed) against fish vibriosis

Heenatigala, PPM; Sun, ZL; Yang, JJ; Zhao, XY; Hou, HW (2020) Frontiers in Immunology 11: 1857

Vibriosis is a commonly found bacterial disease identified among fish and shellfish cultured in saline waters. A multitude of Vibriospecies have been identified as the causative agents. LamB, a member of outer membrane protein (OMPs) family of these bacteria is conserved among all Vibriospecies and has been identified as an efficient vaccine candidate against vibriosis. Rootless duckweed (Wolffia) is a tiny, edible aquatic plant possessing characteristics suitable for the utilization as a bioreactor. Thus, we attempted to express a protective edible vaccine antigen against fish vibriosis in nuclear-transformed Wolffia. We amplified LamB gene from virulent *Vibrio alginolyticus* and it was modified to maximize the protein expression level and translocate the protein to the endoplasmic reticulum (ER) in plants. It was cloned into binary vector pMYC under the control of CaMV 35S promoter and introduced into *Wolffia globosa* by Agrobacterium-mediated transformation. Integration and expression of the LamB gene was confirmed by genomic PCR and RT-PCR. Western blot analysis revealed accumulation of the LamB protein in 8 transgenic lines. The cross-protective property of transgenic Wolffia was evaluated by orally vaccinating zebrafish through feeding fresh transgenic Wolffia and subsequently challenging with virulent *V. alginolyticus*. High relative percent survival (RPS) of the vaccinated fish (63.3%) confirmed that fish immunized with transgenic Wolffia were well-protected from Vibrioinfection. These findings suggest that Wolffia expressed LamB could

serve as an edible plant-based candidate vaccine model for fish vibriosis and feasibility of utilizing Wolffia as bioreactor to produce edible vaccines.

Effects of reaction conditions on products and elements distribution via hydrothermal liquefaction of duckweed for wastewater treatment

Chen, G; Yu, Y; Li, W; Yan, B; Zhao, K; Dong, X; Cheng, Z; Lin, F; Li, L; Zhao, H; Fang, Y (2020) Bioresource Technology 317:124033

Wastewater treatment by duckweed is a naturally sustainable technology. However, its development is limited due to the lack of a follow-up treatment of duckweed. The duckweed was proposed for the treatment of rural domestic wastewater and agricultural wastewater, and it was further processed to produce bio-oil via hydrothermal liquefaction at various temperatures (250°C-370°C) and residence times (15-60min). The highest bio-oil yield of 35.6 wt% was obtained at 370°C, 45min. The higher heating value of bio-oil was 40.85 MJ kg^{-1}, and the H/C ratio (1.72-1.98) was similar to that of petroleum (1.84). The gas chromatography-mass spectrometry analysis results revealed that the bio-oil mainly consisted of N-heterocycles, cyclic ketones, esters, amides, long-chain hydrocarbons, phenols, and aromatic intermediates. Valuable compounds (3-pyridinol, 2-pyrrolidinone, and its analogues) of high concentration were identified in the water-soluble organic matter. Compared with other materials, this study produced higher-quality bio-oil and water-soluble organic matter.

Nutrient starvation and light deprivation effects on starch accumulation in *Landoltia punctata* cultivated on anaerobically digested dairy manure

Kruger, K; Chen, L; He, BB (2020) Journal of Environmental Quality 49:1044-1053

Duckweed has been recognized for its potential of producing biomass on nutrients from waste streams. Our research has shown that strains of duckweed can be successfully cultivated on anaerobically digested (AD) dairy manure under controlled levels of nitrogen (N) and phosphorus (P). The objective of this study was to explore the maximization of starch accumulation in *Landoltia punctata* (Mey.) Les & Crawford strain 0128 under different cultivation conditions using AD dairy manure as the nutrient source. Experimental results have shown that the most influential factors for starch accumulation in *L. punctata* were the nutrient concentration and the appropriate scheduling of nutrient starvation at the right growth stages. In our study, nutrient starvation for starch accumulation in *L. punctata* was achieved by incorporating nutrient addition of appropriately diluted AD dairy manure sequentially in a controlled manner under a constant light intensity of 3,000 lx. It was observed that a starch concentration of 30% (w/w) within the *L. punctata* biomass was achieved with an initial total N of 57.1mg L^{-1} and a total P of 6.7mg L^{-1} after a 30 d cultivation. Under the abovementioned cultivation conditions, the duckweed *L. punctata* recovered 16.3% (±4.0%) of total N and 25.9% (±6.6%) of total P from AD manure into its biomass. It is concluded that *L. punctata* can be successfully cultivated on nutrients from dairy manure for starch production, which would achieve well-being for dairy farmers by producing a feedstock for biofuels while treating dairy wastewater in an environmentally friendly manner.

Ecology

Latitudinal variation in norms of reaction of phenology in the greater duckweed *Spirodela polyrhiza*

Hitsman, HW; Simons, AM (2020) Journal of Evolutionary Biology DOI: 10.1111/jeb.13678

Variable environments may result in the evolution of adaptive phenotypic plasticity when cues reliably indicate an appropriate phenotype-environment match. Although adaptive plasticity is well established for phenological traits expressed across environments, local differentiation in norms of reaction is less well studied. The switch from the production of regular fronds to overwintering 'turions' in the greater duckweed *Spirodela polyrhiza* is vital to fitness and is expressed as a norm of reaction induced by falling temperatures associated with the onset of winter. However, the optimal norm of reaction to temperature is expected to differ across latitudes. Here, we test the hypothesis that a gradient in the length and predictability of growing seasons across latitudes results in the evolution of reaction norms characterized by earlier turion production at higher latitudes. We test this by collecting *S. polyrhiza* from replicate populations across seven latitudes from Ontario to Florida and then assessing differentiation in thermal reaction norms of turion production along a common temperature gradient. As predicted, northern populations produce turions at a lower birth order and earlier; a significant latitude-by-temperature interaction suggests that reaction norm differentiation has occurred. Our results provide evidence of differentiation in reaction norms across latitudes in a phenological trait, and we discuss how the adaptive significance of this plasticity might be further tested.

Greenhouse gases emissions from duckweed pond system treating polyester resin wastewater containing 1,4-dioxane and heavy metals.

Osama, R; Awad, H M; Zha, S; Meng, F; Tawfik, A (2020) Ecotoxicology and Environmental safety 207:111253

Phytoremediation of polyester resin wastewater containing 1,4-dioxane and heavy metals using *Lemna gibba* (*L. gibba*) was enhanced by incorporation of perforated polyethylene carrier materials (PCM) onto the duckweed pond (DWP) system. The DWP module was operated at a hydraulic retention times (HRTs) of 2, 4 and 6 days and as well as 1,4-dioxane loading rate of 16, 25 and 48g $m^{-3}.d^{-1}$. The maximum removal efficiency of 54±2.5% was achieved for 1,4-dioxane at an HRT of 6 days and loading rate of 16 g 1,4-dioxane $m^{-3}.d^{-1}$. Similarly, the DWP system provided removal efficiencies of 28.3±2.1, 93.2±7.6, 95.7±8.9 and 93.6±4.9% for Cd^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} at influent concentration of 0.037±0.01, 1.2±0.9, 27.2±4.7 and 4.6±1.2 mg L^{-1} respectively. The structural analysis by Fourier-transform infrared spectroscopy (FTIR) clearly displayed a reduction of 1,4-dioxane in the treated effluent. A strong peak was detected for *L. gibba* plants at frequency of 3417.71 cm^{-1} due to N-H stretching, which confirm the proposed mechanism of partially conversion of 1,4-dioxane into amino acids. Glycine, serine, aspartic, threonine and alanine content were increased in *L. gibba* by values of 35±2.2, 40±3.2, 48±3.7, 31±2.8, and 56±4.1%, respectively. The contribution of DWP unit as a greenhouse gases (GHG) emissions were relatively low (1.65 g CO_2 kg^{-1} BOD removed d^{-1}, and 18.3 g CO_2 kg^{-1} biomass d^{-1}) due to photosynthesis process, low excess sludge production and consumption of CO_2 for nitrification process (1.4 g CO_2 kgN removed.d^{-1}). Based on these results, it is recommended to apply such a technology for treatment of polyester resin wastewater containing 1,4-dioxane and heavy metals at a HRT not exceeding 6 days.

Modifying effects of leaf litter extracts from invasive versus native tree species on copper-induced responses in *Lemna minor*

Karitonas, R; Jurkoniene, S; Sadauskas, K; Vaiciuniene, J; Manusadzianas, L (2020) PEERJ 8: e9444

Invasive plant species tend to migrate from their native habitats under favourable climatic conditions; therefore, trophic and other relationships in ecosystems are changing. To investigate the effect of natural organic matter derived from native *Alnus glutinosa* tree species and from invasive in

Lithuania *Acer negundo* tree species on copper toxicity in *Lemna minor*, we analysed the dynamics of Cu binding in aqueous leaf litter extracts (LLE) and plant accumulation, morphophysiological parameters, and antioxidative response. The results revealed that *A. glutinosa* LLE contained polyphenols (49 mg pyrogallol acid equivalent (PAE)/g DM) and tannins (7.5 mg PAE/g DM), while *A. negundo* LLE contained only polyphenols (23 mg PAE/g DM). The ability of LLE to bind Cu increased rapidly over 1.5-3 h to 61% and 49% of the total Cu concentration (6.0 ± 0.9 mg/L), respectively for *A. glutinosa* (AG) and *A. negundo* (AN), then remained relatively stable until 48 h. At the same time, *L. minor* accumulated 384, 241 or 188 µg Cu/g FW when plants were exposed to Cu (100 µM $CuSO_4$), Cu with 100 mg/L dissolved organic carbon (DOC) from either AG LLE or AN LLE, accordingly. Catalase (CAT) and guaiacol peroxidase (POD) played a dominant role in hydrogen peroxide scavenging when plants were exposed to Cu and 10 or 100 mg/L DOCAG mixtures in both the first (up to 6h) and the second (6-48 h) response phases. Due to functioning of oxidative stress enzymes, the levels of the lipid peroxidation product malondialdehyde (MDA) reduced in concentration-dependent manner, compared to Cu treatment. When combining Cu and DOCAN treatments, the most sensitive enzymes were POD, ascorbate peroxidase and glutathione reductase. Their activities collectively with CAT were sufficient to reduce MDA levels to Cu induced in the initial, but not the second response phase. These data suggest that leaf litter extracts of different phenolic compositions elicited different antioxidant response profiles resulting in different reductions of Cu stress, thus effecting *L. minor* frond and root development observed after seven days. The complex data from this study may be useful in modelling the response of the aquatic ecosystem to a changing environment.

Assessing the effects of tropical wood leachate to *Desmodesmus subspicatus*, *Lemna minor* and *Daphnia magna*

Sackey, LNA; Koci, V (2020) HELIYON 6: e04268

Ghana has a long history as a major supplier of high-value hardwood timber and wood products to many countries. The research seeks to assess the effects of tropical wood leachates to aquatic organisms. Hence, five wood samples were selected; Mahogany (*Khaya ivorensis*), Cedrela (*Cedrela odorata*), Emire (*Terminalia ivorensis*), Wawa (*Triplochiton scleroxylon*) and Ceiba (*Ceiba pendandra*) from Oboyow forest reserve in Eastern Region Ghana to assess their toxicity to aquatic organisms. Toxicity tests: Algal (*Desmodesmus subspicatus*) Duckweed (*Lemna minor*) and crustacean (*Daphnia magna*) were carried out using exposures to concentrations of 20, 30, 45, 67 and 100% v/v wood leachate in control media. The high levels of phenols measured in the various wood leachates was the main cause of toxicity. The percentage median Inhibition Concentration (%IC50) of the various wood leachate, ranged from 21.5-55.6% with mahogany exhibiting the highest toxicity and wawa the lowest. All the wood leachates were toxic to the aquatic organisms. The %IC50 showed both confirmed and potential toxicity among the various wood leachates and established that there was significant difference between various wood leachate toxicity.

Native Growth and Conservation of Duckweed (Lemnaceae) in Jordan

Shammout MW (2020) Pakistan Journal of Biological Sciences 23: 1055-1059

Duckweeds are the world's smallest flowering plants. Its existence is affected by the water quality and availability. The increased water demand and water scarcity in most of the Mediterranean countries as Jordan have caused remarkable lowering in the water-table which reduced floods and disappearance of the seasonal lakes. Due to this, aquatic plants that grow on good quality of fresh water have disappeared totally. Therefore, this paper highlights the native growth and conservation of duckweed (Lemnaceae) in Jordan. It also focuses on the identification of water sources for

duckweed's movement. Field surveys were conducted to identify water source for water bodies that contain duckweeds. These surveys included visits to the Zarqa river, dams, irrigation ponds and Jordan valley. The source of water for duckweed movement and availability was determined. Water samples from the targeted areas were analyzed for selected parameters as pH, EC, NO_3, PO_4, BOD5, Zn, Pb, Cd and Cu. The results showed that King Abdullah Canal and Zarqa river are the water sources that encourage the duckweeds growth. In Sukhnah, Jerash and Jordan valley areas, water analysis ranges (mg L^{-1}) are: NO_3 0.7-38, PO_4 0.3-7.6 and BOD5 0-20. The pH range value is 7.1-8.1 and EC range value is 1.62-2.5 mS cm-1.The heavy metals values are, Zn is <0.02 mg L^{-1}, Pb is <0.01 mg L^{-1}, Cd is <0.002 mg L^{-1} and Cu is <0.01 mg L^{-1}. It can be concluded that mitigation measures are highly needed to overcome and altering the quality of the natural water flowing and participation of governmental institutions is also required for the success of applying mitigation measures to preserve the aquatic plants.

Feed & Food

Use of *Lemna minuta* Kunth. for composition of sustainable diets and influence on hydrochemical, technological and blood biochemical parameters in common carp (*Cyprinus carpio* L.) cultivated in aquaponics

Velichkova, K; Sirakov, I; Veleva, P (2020) Bulgarian Journal of Agricultural Science 26: 674-679

The purpose of this study was to investigate the influence of aquaponically grown *L. minuta* used as a part of biofilter in recirculation aquaculture systems, with its subsequent inclusion in the composition (50 and 100% content of daily feed ratio) in sustainable diets on hydrochemical, biochemical blood and technological parameters in common carp (*Cyprinus carpio* L.) fingerlings cultivated in aquaponics recirculation systems. At the end of the experiment were calculated average final weight, specific growth rates, feed conversion ratio, meat quality and blood biochemical parameters. The inclusion of up to 100% duckweed (*L. minuta*) in feed for common carp fingerlings decreases the quantity of ammonium, nitrite, nitrate and phosphate accordingly with 66%, 71.4%, 38.8% and 44.1% compared with the quantities of these parameters found for experimental groups fed with feed without inclusion of *L. minuta* . The carps fed with inclusion of up to 50% duckweed showed better growth of fish, SGR and FCR compared with fish fed with a diet without substitution of duckweed.

Interaction with other organisms

Effect of treated sewage characteristics on duckweed biomass production and microbial communities

Iwano, H; Hatohara, S; Tagawa, T; Tamaki, H; Li, YY; Kubota, K (2020) Water Science and Technology 82:292-302

Duckweed biomass production in a duckweed pond fed with three differently treated sewage (i.e. sewage treated by primary sedimentation (PS); conventional activated sludge process (CAS); and downflow hanging sponge process (DHS)) was evaluated in order to assess the effects of water quality on biomass yield. Higher and stable biomass production was observed when the duckweed pond was fed with PS or DHS-effluent than with CAS-effluent, evidently due to the difference in

nutrient loads. Availability of nutrients, especially phosphorus, affected the biomass production rate: higher the nutrient, faster the production. Microbial community analysis revealed that the members of Rhizobiales were likely to contribute to stable and high biomass growth. From the results of the study, a sewage treatment system consisting of a primary sedimentation followed by a duckweed pond and a tertiary treatment unit can be proposed to maximize biomass production without compromising treatment objectives. Size and operational parameters of the duckweed pond should be determined primarily based on the nutrient availability in the influent water to maximize duckweed growth.

Comparative analysis of microbial communities in fronds and roots of three duckweed species: *Spirodela polyrhiza*, *Lemna minor*, and *Lemna aequinoctialis*

Iwashita, T; Tanaka, Y; Tamaki, H; Yoneda, Y; Makino, A; Tateno, Y; Li, Y; Toyama, T; Kamagata, Y; Mori, K (2020) Microbes and Environments 35: DOI:10.1264/jsme2.ME20081

The microbial communities inhabiting the fronds of duckweeds have not been investigated in as much detail as those on the roots. We herein examined the microbial communities in three duckweed species using 16S rRNA amplicon sequencing and compared them to those on the roots. The microbial compositions of the fronds were distinct from those of the roots in the three species. Various types of taxonomic bacteria, including rarely cultivated phyla, Acidobacteria, Armatimonadetes, and Verrucomicrobia, were also isolated from the fronds, but at a slightly lower abundance than those from the roots. These results suggest that duckweed fronds are an alternative source for isolating rare and novel microbes, which may otherwise be recalcitrant to cultivation using conventional strategies.

Molecular Biology

Estimation of the SNP mutation rate in two vegetatively propagating species of duckweed

Sandler, G; Bartkowska, M; Agrawal, AF; Wright, S (2020) G3 (Bethesda, Md.).

DOI:10.1534/g3.120.401704

Mutation rate estimates for vegetatively reproducing organisms are rare, despite their frequent occurrence across the tree of life. Here we report mutation rate estimates in two vegetatively reproducing duckweed species, *Lemna minor* and *Spirodela polyrhiza*. We use a modified approach to estimating mutation rates by taking into account the reduction in mutation detection power that occurs when new individuals are produced from multiple cell lineages. We estimate an extremely low per generation mutation rate in both species of duckweed and note that allelic coverage at de novo mutation sites is very skewed. We also find no substantial difference in mutation rate between mutation accumulation lines propagated under benign conditions and those grown under salt stress. Finally, we discuss the implications of interpreting mutation rate estimates in vegetatively propagating organisms.

Transcriptional analysis reveals potential genes and regulatory networks involved in salicylic acid-induced flowering in duckweed (*Lemna gibba*)

Fu, L; Tan, D; Sun, X; Ding, Z; Zhang, J (2020) Plant Physiology and Biochemistry 155: 512-522.

Duckweed is a simple aquatic floating plant having great potential in sewage treatment and bioenergy production. Duckweed rarely flowers in nature, which greatly limits its germplasm collection, conservation, and heterosis usage. Salicylic acid (SA) can efficiently induce flowering of duckweed (e.g., *Lemna gibba*); however, the related genes and regulatory networks remain unclear. In this work, we demonstrated that *L. gibba* flowering induced by SA was photoperiod-dependent, stress-involved, and abscisic acid (ABA)-disrupted. Totally 202, 78, and 413 differentially expressed (DE) genes were up-regulated, while 429, 72, and 307 were down-regulated at flower induction, flower initiation, and flowering stages, respectively. At the flower induction stage, the down-regulated genes were mainly involved in cell wall, auxin and ABA, light reaction, and abiotic stress, while the up-regulated genes were involved in development, brassinosteroid, major CHO metabolism, and redox. At the flower initiation stage, the down-regulated genes were enriched in light reaction and lipid metabolism, whereas the up-regulated genes were enriched in starch degradation and Ca^{2+} signaling. At the flowering stage, the down-regulated genes were significantly enriched in photosynthesis, gibberellic acid, starch synthesis, nitrogen metabolism, and redox, while the up-regulated genes were enriched in cell wall, jasmonic acid, secondary metabolism, and Ca^{2+} signaling. Besides, 46 transcription factors and 13 flowering-related DE genes were identified. Finally, a possible floral pathway, where *LgTEM*1, *LgSVP*, and *LgFT*1 might play critical roles in SA-induced flowering in *L. gibba*, was discussed. These findings provide a useful foundation for further investigation of genes and regulatory networks of SA-induced flowering in duckweed.

Physiology & Stress

Effect of the growth medium composition on nitrate accumulation in the novel protein crop *Lemna minor*

Devlamynck, R; Fernandes de Souza, M; Bog, M; Leenknegt, J; Eeckhout, M; Meers, E (2020) Ecology and Environmental Safety 206:111380

Duckweed is a potential alternative protein source for food and feed. However, little is known about the nitrate accumulation in this plant. A high nitrate level in vegetables can indirectly lead to an elevated intake of nitrites and N-nitroso compounds, increasing the risk of diseases for humans and animals. This research hypothesizes that the nitrate accumulation of *Lemna minor* differs between growing media. Additionally, it evaluates whether legal safety levels of nitrate for human and animal intake are exceeded. The duckweed was grown on (i) rainwater, and (ii) three synthetic media containing different nutrient levels. Furthermore, (iii) biological effluent of swine manure treatment and (iv) aquaculture effluent from pikeperch production were used, as these are potential media for closing nutrient loops in the agriculture sector. It was found that nitrate levels increased with the increasing availability of macronutrients in the water, and pH showed a particularly strong negative correlation with the nitrate levels in the plant. Nevertheless, nitrate content never exceeded 530 mg NO_3 kg^{-1} fresh weight. To conclude, *L. minor*'s nitrate content was below safety limits for human consumption in all tested growing media; however, a potential risk for ruminants was observed as these are more sensitive to nitrate conversions in their gastro-intestinal track.

Enhanced biomass production and pollutant removal by duckweed in mixotrophic conditions

Sun, Z; Guo, W; Yang, J; Zhao, X; Chen, Y; Yao, L; Hou, HW (2020) Bioresource Technology 317: 124029

Duckweed is a potential biomass source for alternative energy production. This work reports the effects of trophic modes on growth rates, biomass accumulation, and removal rates of pollutant by duckweed. Glucose, fructose, galactose, sucrose, and maltose all supported heterotrophic and mixotrophic growth of duckweed. The mixotrophic growth rate was 4.98 and 6.22 times higher than those in heterotrophic and photoautotrophic conditions, respectively. Notably, mixotrophy produced more biomass than the simple sum of the biomass accumulation during heterotrophy and photoautotrophy. Mixotrophy was also superior in starch and protein production, as well as in removal rates of nutrients and organic carbon from the growth medium. However, the starch content of duckweed grown heterotrophically was 2.06 times higher than in mixotrophy, suggesting a combination of mixotrophy and heterotrophy as an effective strategy for starch-rich biomass production. This study thus provides a paradigm for future studies supporting duckweed-based biomass production and organic wastewater treatment.

Phytoremediation

Microalgal and duckweed based constructed wetlands for swine wastewater treatment: A review

Li, X; Wu, S; Yang, C; Zeng, G (2020) Bioresource Technology 123858

Constructed wetlands for swine wastewater treatment have been one of the most exciting research topics. Usually hydrophytes based constructed wetlands could not adapt well to high concentration of ammonia nitrogen in swine wastewater, while microalgal and duckweed based constructed wetlands are promising for the nutrient removal. In this critical review, the important roles of microalgae and duckweeds played in wastewater treatment in constructed wetlands were first summarized. Performances including biomass growth, nutrient removal capacities and mechanisms of microalgal and duckweed based constructed wetlands were reviewed for swine wastewater treatment. Challenges for the applications of constructed wetlands including microalgal and duckweed based ones were discussed which includes a better understanding and utilization of synergistic effects among microalgae and duckweeds, difficulty and costs in harvesting biomass, applications in various field conditions including low temperatures, and selections of various types of microalgal and duckweed species. Future research needs were also proposed accordingly.

Lemna minor bioassay evaluation using computer image analysis

Haffner, O; Kucera, E; Drahos, P; Ciganek, J; Kozakova, A; Urminska, B (2020) Water 12: 2207

This article deals with using computer vision in the evaluation of the *Lemna minor* bioassay. According to the conventional method, the growth of *Lemna minor* mass is determined from the number of leaves grown. In this work, instead of counting individual leaves, we propose measuring the area occupied by the leaves using computer vision and compare the new approach with the conventional one. The bioassay is performed according to the ISO 20079 standard as a 168 h growth inhibition test; the aim of the experiment was to quantify the negative effects on the vegetative growth using two parameters - the number of leaves and the area occupied by the leaves. The method based on image processing was faster and also more precise since it enabled us to detect the negative effect of the tested substance on leave size, not only on their number. It can be concluded that the toxic effect has shown to be more significant when considering the leaves area rather than the number of leaves. Moreover, mistakes caused by human factor during leaves counting are eliminated using the computer vision based method.

Tolerance of *Landoltia punctata* to arsenate: an evaluation of the potential use in phytoremediation programs

Canatto, RA; de Oliveira, JA; da-Silva, CJ; Albino, BES (2020) International Journal of Phytoremediation DOI: 10.1080/15226514.2020.1797630

Plants used in phytoremediation should accumulate and tolerate a specific pollutant. Here, we aimed at evaluating possible arsenic (As) accumulation and mechanisms of tolerance against As-induced damage in *Landoltia punctata* to explore this species for phytoremediation. Plants were subjected to increasing As levels. As absorption was higher with increasing As levels. The activity of superoxide dismutase and glutathione reductase as well as anthocyanin levels increased with As levels. Catalase and peroxidase activities increased in plants subjected to As levels up to 1.0 mg L^{-1} and decreased at higher levels. Due to the antioxidant system, higher levels of reactive oxygen species were restrained in plants under low levels of As. However, the levels of superoxide anion, hydrogen peroxide, and lipid peroxidation increased in response to the impaired antioxidant system induced by the highest As levels. Biomass decreased in plants exposed to As and scanning electron microscopy revealed root structural damage in the root cap of plants under 3.0 mg L^{-1} As. This work highlights that *L. punctata* can be considered a hyperaccumulator species and has potential for As phytoremediation when levels are lower than 1.0 mg L^{-1} - a concentration 100-fold higher than that recommended for drinking water.

Improving the efficiency of wastewater treatment plants: Bio-removal of heavy-metals and pharmaceuticals by *Azolla filiculoides* and *Lemna minuta*

Bianchi, E; Biancalani, A; Berardi, C; Antal, A; Fibbi, D; Coppi, A; Lastrucci, L; Bussotti, N; Colzi, I; Renai, L; Scordo, C; Del Bubba, M; Gonnelli, C (2020) The Science of the Total Environment 746: 141219

In this study, we investigated the removal of Fe(III), Cr(VI), Al(III), diclofenac, and levofloxacin from treated wastewater in the presence of the free-floating plants *Azolla filiculoides* and *Lemna minuta*, to understand whether these species can be effectively used in a surface flow constructed wetland as wastewater refining treatment. Fe and Al were selected owing to their wide use as coagulant agents in wastewater treatment plants for promoting clariflocculation processes, whilst Cr was chosen due to its common use in industry. Diclofenac and levofloxacin, two molecules belonging to the most widely used pharmaceutical classes in the world, were studied as representative anti-inflammatory drugs and antibiotics, respectively. The study was performed at laboratory scale, exposing the plants separately to each individual contaminant at the concentrations of 5 mg L^{-1} for the metals (i.e. 2.5-5 times higher than the European limits concerning discharge into surface water), and 1 μg L^{-1} for the pharmaceuticals (concentration levels commonly found in wastewater). Depending on the plant species and contaminant tested, the range of different effects observed included low toxicity (i.e. Cr, Fe and diclofenac in *L. minuta*) and even a stimulatory effect on plant growth (i.e. for *A. filiculoides* with Al and for *L. minuta* with Al and levofloxacin). Moreover, both species proved to be very effective in the removal of Fe, Al and levofloxacin, with *A. filiculoides* showing the best performance (removal efficiency of 92%, 96%, and 60%, respectively), whereas for Cr and diclofenac the removal was always less than 10%. The higher removal capacity of *A. filiculoides* compared to *L. minuta* can be attributed to its superior tolerance of the contaminants, probably in turn related to the presence of nitrogen-fixing microorganism in its fronds.

Dual function of *Lemna minor* and *Azolla pinnata* as phytoremediator for palm oil mill effluent and as feedstock

Abd Kadir, A; Abdullah, SRS; Othman, BA; Abu Hasan, H; Othman, AR; Imron, MF; Ismail, NI; Kurniawan, SB (2020) CHEMOSPHERE 259: 127468.

In this study, two native duckweeds (*Lemna minor* and *Azolla pinnata*) were cultivated in Palm Oil Mill Effluent (POME) to extract nutrients from the effluent. Five grams of *A. pinnata* and 2 g of *L. minor* were transferred to 2 L POME (Initial concentrations: 198 mg/L COD, 4.3 mg/L nitrates, pH 9.53, 4 mg/L phosphate, 2.98 mg/L ammonia) with four different dilutions (2.5%, 5%, 10%, 15%) under greenhouse conditions. Samples of POME were taken every two days up to 10 days. Growth parameter, phosphate, ammonia, nitrates, pH, and COD were monitored within 10 days to select the most suitable growth medium for both plants. Results showed that 2.5% POME dilution had positive effect on *L. minor* growth and *A. pinnata* (wet weight increased by 8.7 g and 9.8 g, respectively), with all plants able to survive until the final day of exposure. The highest removal of ammonia was accomplished in 5% POME dilution by *A. pinnata* (98%) and *L. minor* (95.5%). The maximum phosphate removal was obtained in 10% POME dilution with 93.3% removal by *A. pinnata* and 86.7% by *L. minor*. Significant COD removal in 15% POME was obtained by *L. minor* (78%) and *A. pinnata* (66%). Both plants responded positively to the phytoremediation process, especially for *A. pinnata* which showed significant decreases in all parameters. The nutrient extraction by both plants from POME showed a positive effect on growth parameter, which has further promising potential to be used as animal feedstock.

DF comment: *Azolla pinnata* does not belong to the family of duckweed.

Municipal wastewater treatment by combining in series microalgae *Chlorella sorokiniana* and macrophyte *Lemna minor*: Preliminary results

Kotoula, D; Iliopoulou, A; Irakleous-Palaiologou, E; Gatidou, G; Aloupi, M; Antonopoulou, P; Fountoulakis, MS; Stasinakis, AS (2020) Journal of Cleaner Production 271: 122704

Conventional treatment of municipal wastewater requires large inputs of energy, and high operation and maintenance costs. On the other hand, the use of microalgae and macrophytes on wastewater treatment offers an alternative approach that combines removal of pollutants with production of valuable biomass. An integrated sequencing batch reactor system that combines in series microalgae *Chlorella sorokiniana* UTEX 1230 and macrophyte *Lemna minor* was tested for municipal wastewater treatment in the current study. The average removal of COD, TKN, NH_4-N and PO_4-P was 99%, 88%, 90% and 91%, respectively, while the concentrations of major pollutants in the effluents complied with European Directive 91/271 for wastewater discharge to the aquatic environment as well as with national legislation for wastewater reuse for limited irrigation. The microalgae reactor was capable of fully removing COD and partially removing N and P nutrients, while *Lemna minor* reactor contributed mainly to nitrogen removal. Batch experiments with *Chlorella sorokiniana* and different types of municipal wastewater (raw sewage, anaerobically treated wastewater and aerobically treated wastewater) with no pH adjustment and CO_2 addition showed that the optimal microalgae growth and the highest pollutants removal were achieved when raw sewage were used. Comparison of mixotrophic and heterotrophic conditions showed highest biomass growth under mixotrophic conditions. Except for nitrates, the higher removal of other pollutants was also achieved under mixotrophic conditions. Further experiments should be conducted to examine the characteristics of produced microalgae and macrophyte biomass and the alternative ways of their valorization under the frame of circular economy.

Lemna minor influence in the treatment of organic pollution of the industrial effluents

Alvarado, K; Esenarro, D; Rodriguez, C; Vasquez, W (2020) 3C Tecnologia 9: 77-96

The purpose of the research was to determine the influence of industrial wastewater treatment using the Lemna Minor aquatic plant. Certain varieties of macrophyte plants can absorb or retain various contaminants. Thanks to this, it has been determined that the variety known as Lemna Minor presents this type of property. Three treatment trials were carried out varying the amounts of Lemna Minor (100, 200, and 300g). They are keeping constant the retention time of 10 days that were analyzed at 3, 6, and 10 days after the treatment and with a constant volume of the residual effluent. The results indicate that in terms of the parameters that determine organic contamination, BOD was reduced by (61 %); COD was reduced by (68 %) and the concentration of total suspended solids by (61 %).

Uncoupling growth from phosphorus uptake in Lemna: Implications for use of duckweed in wastewater remediation and P recovery in temperate climates

Paterson, JB; Camargo-Valero, MA; Baker, A (2020) Food and Energy Security Article Number: e244

Phosphorus (P) is an essential nutrient for crop growth and the second most limiting after N. Current supplies rely on P-rich rocks that are unevenly distributed globally and exploited unsustainably, leading to concerns about future availability and therefore food security. Duckweeds (Lemnaceae) are aquatic macrophytes used in wastewater remediation with the potential for nutrient recycling as feed or fertilizer. The use of duckweeds in this way is confined to tropical regions as it has previously been assumed that growth in the colder seasons of the temperate regions would be insufficient. In this study, the combined effects of cool temperatures and short photoperiods on growth and P uptake and accumulation in Lemna were investigated under controlled laboratory conditions. Growth and P accumulation in Lemna can be uncoupled, with significant P removal from the medium and accumulation within the plants occurring even at 8 degrees C and 6-hr photoperiods. Direct measurement of radiolabeled phosphate uptake confirmed that while transport is strongly temperature dependent, uptake can still be measured at 5 degrees C. Prior phosphate starvation of the duckweed and use of nitrate as the nitrogen (N) source also greatly increased the rate of P removal and in-cell accumulation. These results form the basis for further examination of the feasibility of duckweed-based systems for wastewater treatment and P recapture in temperate climates, particularly in small, rural treatment works.

Exploring the efficacy of powered guar gum (*Cyamopsis tetragonoloba*) seeds, duckweed (*Spirodela polyrhiza*), and Indian plum (*Ziziphus mauritiana*) leaves in urban wastewater treatment

Pandey, N; Gusain, R; Suthar, S (2020) Journal of Cleaner Production 264: 121680

The efficiency of aqueous extract of powdered guar gum seed (*Cyamopsis tetragonoloba*), duckweed fronds (*Spirodela polyrhiza*), and Indian plum leaves (*Ziziphus mauritiana*) was tested to remove nitrate, sulphate, phosphate, and chemical oxygen demand from urban wastewater in this study. Coagulants were applied in four different doses (1, 2, 3, and 5 mL L^{-1}) without adjusting the pH of it in wastewater treatment and changes in wastewater parameters were measured. The crude extract of guar gum seed, Indian plum leaves, and duckweed exhibited the removal activities 71.7-95.03%

(nitrate), 78.3-95.9% (phosphate), and 83.1-99.6% (sulphate). Nitrate removal was highest (93.5%), followed by phosphate (83.4%) and sulphate (77.7%). The 3 mL L^{-1} dose of coagulant showed the best results of wastewater treatment, followed by 2 mL L^{-1}, 1 mL L^{-1}, and 5 mL L^{-1}. Chemical oxygen demand reduction was also in a good range (73.2-79.08%) with 3 mL L^{-1} dose for all studied coagulants. pH reduced significantly in all setups of wastewater spiked with plant coagulants. The chemical analysis of Fourier transforms infrared (FTIR) spectroscopy of aqueous extract and plant powder indicates the presence of functions groups like - OH, -COOH, -NH, C=O, C-C R-CHO, C=C-CO-OH, C-H, and R-NH2, in raw coagulants. Overall, results indicate the possible utility of guar gum seed, duckweed, and Indian plum leaf powder as low-cost natural materials for treatment of urban wastewater. However, few high doses of coagulants showed a slight increase in chemical oxygen demand in wastewater, which can be moderated by purifying the active compounds responsible for coagulation activities.

Bioremediation of *Landoltia punctata* to *Microcystis aeruginosa* contaminated waters

Li, S; Le, SX; Li, GL; Luo, M; Wang, R; Zhao, Y (2020) Water 12: 1764

Microcystis aeruginosa is one of the dominant algae in the "phytoplankton bloom" phenomenon. A high density of microcystins (MCs) is produced when algae have explosive growth, which can damage the water environment and pose a great threat to aquatic animals, plants, and human health. Duckweed (*Landoltia punctata*) is a morphologically highly degraded flowering plant with a short growth cycle and wide environmental adaptability. Importantly, duckweed can grow in eutrophic water and has great potential in water remediation. The present study aims to analyze the physiological and biochemical changes of *L. punctata* when co-culturing with *M. aeruginosa* in the laboratory. Our results showed that all the biomass, chlorophyll content, antioxidant enzyme activities, and amylase activity of *L. punctata* increased in 2 x 10^8 cells/L and 4 x 10^8 cells/L for *M. aeruginosa*, and also significantly reduced in 1.6 x 10^9 cells/L for *M. aeruginosa*, while cytotoxic substance (malondialdehyde (MDA)) content showed a completely opposite trend. After co-culturing, it was found that the MC content in *L. punctata* reduced to 138.87 g/g, and the MC removal rate was 29.48%. These results indicate that *L. punctata* can grow normally in high-density *M. aeruginosa*, which paves the way for *L. punctata*'s bioremediation of water polluted by *M. aeruginosa*.

Phytoremediation efficiencies of *Spirodela polyrhiza* and *Brassica oleracea* in removing nutrients from treated sewage effluent

Mun, NK; Mohamed, RMSR; Miswan, MS; Al-Gheethi, A; Kassim, AHM; Mishima, Y (2020) Desalination and Water Treatment 187: 87-92

The study investigates the capacity of phytoremediation as a post-treatment step for the nutrient-rich-treated sewage effluent from Saga City sewage treatment plant, Saga, Japan. Phytoremediation in the context of this study is the removal of nutrients such as ammoniacal nitrogen, nitrate nitrogen and phosphorus from the nutrient-rich-treated sewage effluent by plants. In this study, *Spirodela polyrhiza* (*S. polyrhiza*) and *Brassica oleracea* (*B. oleracea*) were used to phytoremediate the treated sewage effluent collected from the Saga City Sewage Treatment Plant under laboratory scale. Plants were grown in polypropylene planter box supplied with 8 L treated sewage effluent under indoor environment and full water retention throughout the experimental studies. The removal efficiency and daily absorption of nutrients by phytoremediation plants were determined. It was found that the most optimal removal efficiency and average daily nutrient removal rate by *S. polyrhiza* throughout the experiment were 92.42% ±1.29% or 15.4 mg/L/d for ammoniacal nitrogen achieved in day 1,

78.69% ± 10.31% or 2.68 mg/L/d for nitrate-nitrogen achieved in day 4, and 93.45% ± 3.26% or 0.51 mg/L/d for phosphorus in day 3 of an experiment. On the other hand, the removal efficiency and average daily nutrient removal rate by *B. oleracea* throughout the experiment gave a total of 8 d where 76.07% ± 10.38% or 1.68 mg/L/d for ammoniacal nitrogen, 78.38% ± 0.40% or 1.19 mg/L/d for nitrate-nitrogen and 67.40% ± 10.91% or 0.10 mg/L/d for phosphorus. The overall findings demonstrated that phytoremediation by S. polyrhiza was far more effective in removing nutrients from the nutrient-rich-treated sewage effluent compared to *B. oleracea*. The significance of the study includes reducing the possibility of eutrophication outbreak caused by the disposal of treated sewage effluent, advocating less dependency on global demand for non-renewable phosphorus resources in the agriculture sector, and solving food demand due to the increasing world population.

Degradation studies of selected bisphenols in the presence of beta-cyclodextrin and/ or duckweed water plant

Kaleniecka, A; Zarzycki, PK (2020) Journal of AOAC International 103: 439-448

This research reports a multivariate experiment enabling observation of the potential application of macrocyclic compound [beta-cyclodextrin (beta-CD)] and/or duckweed organisms as the active factors for elimination of selected bisphenols A, B, and S from water samples. Target bisphenols selection was based on observation that such components can be present in food or environmental samples (e.g., vegetable/fruit juices, milk, drinking water, or treated wastewater). Biological research was carried out using aquatic organisms containing chlorophyll, particularly duckweed (*Lemna minor* L), that may work as an active biomass for the elimination or extraction of bisphenols micropollutants from water. Using such a system, we studied the potential encapsulation effect and removal efficiency of nontoxic macrocyclic oligosaccharide (beta-cyclodextrin) acting as an encapsulation reagent to promote the removal of selected bisphenols from liquid phase both with and without the presence of duckweed biomass. Experimental data have revealed that beta-CD or combined beta-CD/duckweed system has an effect on bisphenols elimination from water. The initial data set obtained from this preliminary experiment (and combined with supramolecular complex formation data calculated from chromatographic experiments, published previously) enables designing of further experiments focusing on the development of green chemistry technology. It is hoped that this may be used for the efficient removal of low-molecular-mass micropollutants using classical technological wastewater treatment processes modified by biomass and macrocyclic additives. This process needs to be optimized, but the results presented have revealed that such green chemistry technology, if successful, may be an interesting alternative for the selective removal of the micropollutants investigated from wastewater using classical adsorbents (e.g., carbons and carbon-related nanomaterials), particularly in terms of the worldwide problem with microplastic pollutants in the environment and food products.

Phytotoxicity

Ecotoxicological effects of the azole antifungal agent clotrimazole on the macrophyte species *Lemna minor* and *Lemna gibba*

de Alkimin, GD; Santos, J; Soares, AMVM; Nunes, B (2020) Comparative Biochemistry and Phyiology C-Toxicology and Pharmacology 237: 108835

Pharmaceuticals are a large and diverse group of compounds used to treat, prevent and diagnose disease. Among these, a group that has been recently detected in the aquatic environment is that of the azole compounds, commonly used as antifungals. Clotrimazole (CLO) is a nonbiodegradable

persistent azole compound, with broad-spectrum antifungal activity for which virtually no toxicological data are available, especially towards aquatic plants. The few existent data point to a documented interference with cytochrome P450 system of exposed organisms. Therefore, the aim of this paper was to evaluate the ecotoxicological effects of the fungicide CLO on two aquatic macrophyte species, namely, *Lemna minor* and *Lemna gibba*. To attain this purpose, an acute assay (96 h) was performed with both species being exposed to CLO, in a concentration range of 0 to 5 µg L^{-1}. The analyzed endpoints were levels of chlorophyll a and b, total, carotenoids, catalase (CAT) and glutathione-transferases activities (GSTs). In general, CLO exposure caused some minor alterations in *L. minor* and *L. gibba* pigment contents. Antioxidant enzymes exhibited a different pattern in both species, since the highest concentrations of CLO caused an increase on CAT activity, and a decrease on GSTs activity in *L. minor*, and the opposite in *L. gibba*, reflected by a decrease on CAT activity and an increase on GSTs activity in all tested concentrations. These results demonstrate that CLO exposure resulted in potential deleterious effects on macrophytes, namely with the involvement of the antioxidant defense mechanisms that were likely deployed to cope with pro-oxidative conditions established by CLO.

Individual and combined effects of amoxicillin, enrofloxacin, and oxytetracycline on *Lemna minor* physiology

Gomes, MP; Brito, JCM; Rocha, DC; Navarro-Silva, MA; Juneau, P (2020) Ecotoxicology and Environmental Safety 203: 111025

We investigated individual and combined effects of environmentally representative concentrations of amoxicillin (AMX; 2 µg L^{-1}), enrofloxacin (ENR; 2 µg L^{-1}), and oxytetracycline (OXY; 1 µg L^{-1}) on the aquatic macrophyte *Lemna minor*. While the concentrations of AMX and ENR tested were not toxic, OXY decreased plant growth and cell division. OXY induced hydrogen peroxide (H_2O_2) accumulation and related oxidative stress through its interference with the activities of mitochondria electron transport chain enzymes, although those deleterious effects could be ameliorated by the presence of AMX and/or ENR, which prevented the overaccumulation of ROS by increasing catalase enzyme activity. *L. minor* plants accumulated significant quantities of AMX, ENR and OXY from the media, although competitive uptakes were observed when plants were submitted to binary or tertiary mixtures of those antibiotics. Our results therefore indicate *L. minor* as a candidate for phytoremediation of service waters contaminated by AMX, ENR, and/or OXY.

Copper and mercury induced oxidative stresses and antioxidant responses of *Spirodela polyrhiza* (L.) Schleid.

Singh, H; Kumar, D; Soni, V (2020) Biochemistry and Biophysics Reports 23:100781

Duckweed is recognized as a phytoremediation aquatic plant due to the production of large biomass and a high level of tolerance in stressed conditions. A laboratory experiment was conducted to investigate antioxidant response and mechanism of copper and mercury tolerance of *S. polyrhiza* (L.) Schleid. To understand the changes in chlorophyll content, MDA, proline, and activities of ROS-scavenging enzymes (SOD, CAT, GPOD) during the accumulation of Cu^{+2} and Hg^{+2}, *S. polyrhiza* were exposed to various concentrations of Cu^{+2} (0.0-40 µM) and Hg^{+2} (0.0-0.4 µM). antioxidant activity initially indicated enhancing trend with application of 10 µM Cu^{+2}; 0.2 µM Hg^{+2} (SOD), of 20 µM Cu^{+2}; 0.2 µM Hg^{+2} (CAT) and of 10 µM Cu^{+2};0.2 µM Hg^{+2} (GPOD) and then decreased consistently up to 40 µM Cu^{+2} and 0.4 µM Hg^{+2}. In the experiment chlorophyll and frond multiplication initially showed increasing tendency and decreased gradually with the application of increased metal concentration. Application of heavy metal has constantly enhanced proline and MDA content while the maximum

increase was observed with the application of 40 µM Cu; 0.4 µM Hg for proline and MDA respectively. The upregulation of antioxidant enzymes and proline reveals that *S. polyrhiza* has strong biochemical strategies to deal with the heavy metal toxicity induced by the accumulation of Cu^{+2} and Hg^{+2}.

Growth inhibition and recovery patterns of common duckweed *Lemna minor* L. after repeated exposure to isoproturon

Varga, M; Zurga, P; Brusic, I; Horvatic, J; Moslavac, M (2020) Ecotoxicology

DOI: 10.1007/s10646-020-02262-9

Aquatic non-targeted organisms are more likely to be exposed to herbicides in multiple pulse events then long continuous exposure. The potential of an organism to recover between exposures has an important role in the overall effects of the toxicant. Common duckweeds show high potential for recovery after a single exposure to isoproturon. To evaluate the growth patterns and recovery potential between multiple exposures, *L. minor* plants were exposed to isoproturon in three repetitive 7-day treatment cycles in three time-variable exposure scenarios with equivalent time-weighted average concentrations. The growth was significantly inhibited during each exposure phase with significant cumulative effects in every subsequent treatment cycle resulting in a cumulative decrease in biomass production. However, inhibitory effects were reversible upon transferring plants to a herbicide-free nutrient solution. These results indicate that *L. minor* plants have a high recovery potential even after multiple exposures to isoproturon. Observed cumulative decrease in biomass production, as well as the potential for fast and efficient recovery from repeated herbicide exposure, might affect the competitiveness of *L. minor* in surface water communities. The observations made during each exposure period, recovery patterns, and the resulting cumulative effects over time may contribute to further development, calibration and validation of mechanistic toxicokinetic/toxicodynamic models for simulating the effects of pesticides on aquatic plants populations in the laboratory and environmental conditions.

Assessing potential aquatic toxicity of airport runoff using physicochemical parameters and *Lemna gibba* and *Aliivibrio fischeri* bioassays

Calvo, OC; Quaglia, G; Mohiley, A; Cesarini, M; Fangmeier, A (2020) Environmental Science and Pollution Research DOI: 10.1007/s11356-020-09848-0

A critical problem derived from airport operations is the environmental impact of runoff water. Airport runoff includes a complex mixture of pollutants, e.g., from deicing agents, that may affect negatively natural water bodies. This study assesses the spatial and temporal aquatic ecotoxicity of runoff water and possible aeroplane drift in a German airport. Over winter 2012-2013, from November to May, water samples were collected within the airport and surrounding area. These samples were analyzed using traditional physicochemical analysis and biotests with two aquatic organisms from different trophic levels, *Lemna gibba* and *Aliivibrio fischeri*. Overall, the samples examined in this study were relatively non-toxic to the tested organisms. The physicochemical parameters were mainly influenced by the sampling period being higher in colder months. In contrast, the ecotoxicity was influenced by the sampling site. For sites within the airport, a high correlation between the physicochemical parameters (EC and TOC) and toxicity in *L. gibba* was found. These correlations were not evident in samples taken outside the airport or when *A. fischeri* was used as a bioindicator. However, a pronounced seasonality has been observed, linked to the coldest months with average inhibition values of 50% in *L. gibba* and 25% *in A. fischeri*, particularly in

January. Both biotests yielded differing results; therefore, more biotests should be included. However, *L. gibba* showed a good response with this type of water samples to be included in future studies together with detailed chemical analysis. The present study provides data to assess the potential ecotoxicological effects of airport runoff affected by winter operations.

The toxicity of BDE-47 to the photosystem of *Lemna minor* fronds

Qiu, NW; Zhang, WR; Yan, XH; Wang, RJ; Tian, L; Han, GL; Zhou, F (2020) Biologia Plantarum 64: 591-597

To elucidate the toxicity of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) on photosynthetic primary processes, in vivo and in vitro treatments of BDE-47 were performed. The 20-d treatment in vivo (5 - 20 μg dm^{-3}) suppressed the reproduction of duckweed (*Lemna minor*) and led to decline in chlorophyll (Chl) content of fronds. The most obvious features of BDE-47-treated fronds included a Chl a fluorescence rise at the J phase and a depression at the G phase, whereas significant fluorescence rises at the L, K, and J phases were found on the Delta V-t curve of thylakoid membranes treated with 10 - 15 mg dm^{-3} BDE-47 for 4 h (in vitro). In both in vivo and in vitro experiments, the BDE-47 treatments significantly reduced the density of the active reaction centers (RC/CSo), affected the efficiency and speed of photosynthetic electron transfer [the maximum quantum yield of photosystem (PS) II photochemistry - F-v/F-m, quantum yield for electron transport (at t = 0) - phi(Eo), electron transport flux per excited cross section - ETo/CSo, and net rate of reaction centers closure at 300 and 100 mu s - dV/dt(o) and dVG/dt(o), respectively], and increased energy dissipation [quantum yield for energy dissipation (at t = 0) - phi(Do), dissipated energy flux per reaction center - DIo/RC, and dissipated energy flux per excited cross section - DIo/CSo]. The BDE-47 at 5 - 15 mg dm^{-3} had no impact on the minimum (initial) fluorescence (Fo) and total electron carriers per reaction center (S-m) of the thylakoid membranes, but PS II units were less tightly grouped (a positive L-band). On the contrary, there was no positive L-band on the difference between relative fluorescence intensities of the normalized induction curves from 50 μs to 300 μs (W-K) of each BDE-47 treatment and control (Delta W-K), and F-o and S-m increased after the treatment with BDE-47. The above results indicate that BDE-47 not only affected the permeability of thylakoid membranes, but also relaxed the structure of PS II, thereby affecting the function of PS II. In addition, BDE-47 could induce secondary damage to the PSs in duckweed fronds.

Image analysis of *Spirodela polyrhiza* for the semiquantitative detection of copper

Nesan, D; Chieh, DCJ (2020) Journal of Environmental Chemical Engineering 8: UNSP 103043

Digital image analysis is a processing technique that allows users to extract quantifiable data from digital images. In this study, digital camera photography was used in the determination of leaf chlorophyll content. By analyzing the degree of color change, image analysis served as a method for fast, inexpensive and non-destructive measurement of overall plant health. This study applied image analysis methods on *Spirodela polyrhiza* plantlets which were exposed to copper, to determine if the rate and degree of leaf color change is proportional to the concentration of copper present in the growth medium. Within 1 day, chlorophyll concentrations of plantlets grown in 2.5 mg/L and 5mg/L CuSO4 were 0.52 and 0.47 mg/g compared to a control of 0.64 mg/g. Additionally, higher copper concentrations in the growing medium resulted in higher measured mean colour distance, Delta E*ab. Plantlets grown in 2.5 mg/L and 5 mg/L CuSO4 solutions showed a Delta E*ab divergence of 0.2 and 0.25 from the control. It was concluded that the leaf color change can be used as a measure of copper concentration within the range of 1.25 mg/L and 5 mg/L. Lower concentrations of copper

did not produce a consistent measurable effect on the plantlets, while higher concentrations exceeded the uptake ability of the plant and could not be accurately distinguished from one another.

Selenate and selenite uptake, accumulation and toxicity in *Lemna minuta*

Li, J; Loi, G; Otero-Gonzalez, L; Du Laing, G; Ferrer, I; Lens, PNL (2020) Water Science and Technology 81: 1852-1862

The kinetics of Se uptake and toxicity to Lemna were studied over a period of 14 days of exposure to Se(IV) or Se(VI). The growth of Lemna stopped immediately after exposure to 5.0 mg/L of Se(IV) or Se(VI). The content of chlorophyll and phaeopigments of Lemna exposed to 5.0 mg/L of Se(IV) was two to three times less than in the control after 3 d exposure. Lemna took up Se rapidly within the first 3 d. The Se content in Lemna along with the exposure time fitted well the two-compartment and the hyperbolic model, which demonstrates that the mechanism of Se(IV) and Se(VI) uptake in Lemna is not only through passive diffusion, but also through other processes such as ion channel proteins or transporters. The kinetic bioconcentration factors (BCFs) were 231 and 42 for 0.5 mg/L Se(IV) and Se(VI) exposure, respectively. The uptake rate of Lemna reached 263 mg/kg/d and 28 mg/kg/d in the Se(IV) and Se(VI) treatments, respectively. This study showed that Se(IV) has a faster accumulation rate than Se(VI), but a higher toxicity, indicating Lemna could be a good candidate to remove Se(IV) from water, producing Se-enriched biomass which may eventually also be considered for use as Se-enriched feed supplement or fertilizer.

Toxicity of the herbicide flurochloridone to the aquatic plants *Ceratophyllum demersum* and *Lemna minor*

Zhou, JA; Wu, ZH; Yu, D; Yang, L (2020) Environmental Science and Pollution Research 27: 3923-3932

As a new and efficient selective pre-emergence herbicide, flurochloridone (FLC) has been widely promoted in recent years but readily results in residues in nature. As the primary producers and restorers of the water environment, aquatic plants are at risk of FLC exposure. In the present research, we studied the phytotoxicity of FLC in *Lemna minor* and *Ceratophyllum demersum*. The physiological and growth responses of these two aquatic plants exposed to different concentrations of FLC (0, 20, 100, 300, 1000, and 2000 μg/L) were measured. The results showed that FLC (≥ 20 μg/L) could cause serious photosynthesis pigment damage and bleaching in *C. demersum* and *L. minor*. Significant oxidative damage was observed in *L. minor* at 20 μg/L FLC, while there was no severe oxidative damage in *C. demersum*. At 100-300 μg/L FLC, peroxidase (POD) and superoxide dismutase (SOD) were activated to scavenge free radicals in *L. minor*, while POD acted as a protective enzyme in C. demersum. At higher concentrations of FLC ≥ 1000-2000 μg/L*), L. minor* reached less than healthy stability through the regulation of the antioxidant enzyme system and the chlorophyll a/b value. POD, SOD, and protein content returned to normal levels, and the growth parameters increased. However, in *C. demersum*, the enzymes POD and SOD and soluble protein were damaged, and oxidative stress reached the highest level at 1000-2000 μg/L FLC. Taken together, our results suggested that when treated with FLC, *L. minor* was more sensitive at lower doses (20 μg/L) and more adaptive at higher doses (1000-2000 μg/L) than *C. demersum*.

Phytoremediation processes of domestic and textile effluents: evaluation of the efficacy and toxicological effects in *Lemna minor* and *Daphnia magna*

de Alkimin, GD; Paisio, C; Agostini, E; Nunes, B (2020) Environmental Science and Pollution Research 27: 4423-4441

Phytoremediation has been proposed as a potential biotechnological strategy to remediate effluents before their release into the environment. The use of common aquatic plant species, such as macrophytes (e.g., Lemna spp.) as a clean-up solution has been proposed decades ago. However, the effectiveness of such processes must be assessed by analyzing the toxicity of resulting effluents, for the monitoring of wastewater quality. To attain this purpose, this work intended to quantify the efficacy of a Lemna-based wastewater phytoremediation process, by analyzing toxicological effects of domestic and textile effluents. The toxic effects were measured in *Lemna minor* (same organisms used in the phytoremediation process, by quantifying toxicological endpoints such as root length, pigment content, and catalase activity) and by quantifying individual parameters of *Daphnia magna* (immobilization, reproduction, and behavior analysis). Phytoremediation process resulted in a decrease of chemical oxygen demand in both effluents and in an increase in root length of exposed plants. Moreover, textile effluent decreased pigments content and increased catalase activity, while domestic effluent increased the anthocyanin content of exposed plants. *D. magna* acute tests allowed calculating a EC_{50} and Toxic Units interval of 53.82-66.89%/1.85-1.49, respectively, to raw textile effluent; however, it was not possible to calculate these parameters for raw and treated domestic effluent (RDE and TDE). Therefore, in general, the acute toxicity of effluent toward *D. magna* was null for RDE, and mild for the treated textile effluent (TTE), probably due to the effect of phytoremediation. Exposure to textile effluents (raw and treated) increased the total number of neonates of *D. magna* and, in general, both textile effluents decreased *D. magna* distance swim. Moreover, although both effluents were capable of causing morphological and physiological/biochemical alterations in *L. minor* plants, organisms of this species were able to survive in the presence of both effluents and to remediate them.

Genotoxicity and repeated-dose toxicity evaluation of dried *Wolffia globosa* Mankai

Kawamata, Y; Shibui, Y; Takumi, A; Seki, T; Shimada, T; Hashimoto, M; Inoue, N; Kobayashi, H; Narita, T (2020) Toxicology Reports 7:1233-1241

Wolffia is a genus of protein-rich aquatic plants. Mankai, a cultivated strain of *Wolffia globosa*, contains more than 40 % protein based on dry matter evaluation. Furthermore, Mankai is nutritionally excellent as a food material, and is expected to be applicable to various products as a substitute for animal protein. A battery of toxicological studies was conducted on the dried product of Mankai (Dry Mankai), with the expectation to utilize it as a raw material for food applications. Dry Mankai was not genotoxic in a bacterial reverse mutation test and in vitro micronucleus assay. In the subchronic toxicity study, rats were provided Dry Mankai in the diet at levels of 0 %, 5 %, 10 %, or 20 % (w/w), equivalent to 0, 3.18, 6.49, and 13.16 g/kg/day for males and 0, 3.58, 7.42, and 15.03 g/kg/day for females, respectively. No adverse effects that could be attributable to treatment were observed in clinical observations, body weight, food consumption, ophthalmology, hematology and blood chemistry, urinalysis, and macroscopic and microscopic findings. According to the repeated-dose study in rats, the no observed adverse effect level of Dry Mankai was 20 % (w/w) for both sexes (13.16 and 15.03 g/kg/day for males and females, respectively).

Taxonomy

Key to the determination of taxa of Lemnaceae: an update

Bog, M; Appenroth, KJ; Sree, KS (2020) Nordic Journal of Botany 38: e02658

Species of Lemnaceae have a high potential for fast biomass production, and this is increasingly gaining attention among researchers in basic plant sciences as well as among entrepreneurs for feed, food and energy production. Hence, the correct identification of the species being used for different duckweed research and applications is becoming indispensable. Here, we present an updated identification key based on morphological markers to the currently accepted 36 species of duckweeds, considering all taxonomic revisions since the publication of the previous key by E. Landolt in 1986. We also provide supplementary morphological characterization and the geographical occurrence of each species of Lemnaceae.

Taxonomic identity of *Landoltia punctata* (Araceae, Lemnoideae) in Korea

Lee, Y; Choi, HJ; Shiga, T (2020) Journal of Asia-Pacific Biodiversity 13: 494-498

The duckweed species, *Landoltia punctata* (G. Meyer) D. H. Les & D. J. Crawford (family, Araceae; subfamily, Lemnoideae; common name, dotted duckmeat) was found in artificial wetlands in Jeju Special Self-Governing Province, Republic of Korea; however, it has no formal record in Korea. We identified this species based on morphological characteristics and DNA sequences. We also checked Lemnoideae specimens in the Korean and Japanese herbaria but found no specimens of *La. punctata* collected in Korea previously. The chloroplast DNA sequence (atpF-atpH) of the species collected from Jejudo was identical to the other *La. punctata* sequences in the DNA Data Bank of Japan (DDBJ)/The European Molecular Biology Laboratory (EMBL)/GenBank. Their habitat environments and our herbarium research suggest that this species has recently become distributed in Korea. This study also provides a key to closely related taxa in the subfamily Lemnoideae, with descriptions of these species

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:

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

Title:

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- 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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Image resolution and submission:

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

Links for Further Reading

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

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

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

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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