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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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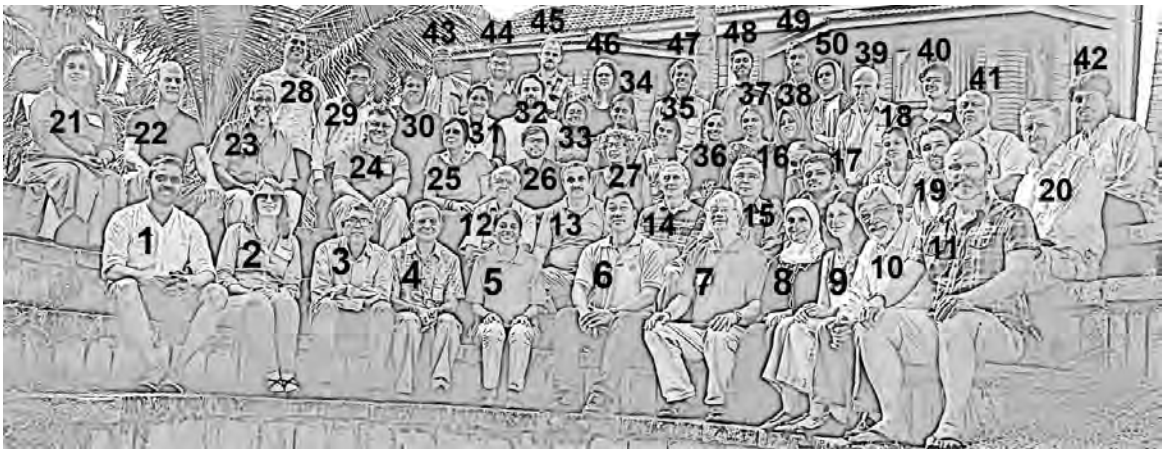
Group photo of the Fourth International Conference on Duckweed Research and Applications from 23-26 October, 2017 at the Central University of Kerala, India (Continues on next page)

Cover page



Group photo of the Fourth International Conference on Duckweed Research and Applications from 23-26 October, 2017 at the Central University of Kerala, India.

1. Mr. Prasanna Jogdeo, 2. Dr. Tsipi Shoham, 3. Dr. RN Mandal, 4. Prof. Sudhir K. Sopory, 5. Dr. K. Sowjanya Sree, 6. Prof. Eric Lam, 7. Dr. Klaus-J Appenroth, 8. Ms. Hassana Ghanem, 9. Ms. Anna Maksimova, 10. Dr. Sergio Mapelli, 11. Dr. Todd P Michael, 12. Prof. Satish C Maheshwari, 13. Dr. Autar Mattoo, 14. Prof. Marvin Edelman, 15. Prof. Jitendra P Khurana, 16. Prof. MA Vijayalakshmi, 17. Mr. JG, 18. Ms. Alsaba Khan, 19. Dr. Pavel Khvatkov, 20. Prof. Ingo Schubert, 21. Ms. Pooja Tendulkar, 22. Mr. YS, 23. Dr. Paul Ziegler, 24. Prof. Nikolai Borisjuk, 25. Prof. Halina Gabrys, 26. Mr. Evan Ernst, 27. Ms. Kyriakoula Ziegler-Skylakakis, 28. Mr. Barak Cohen, 29. Mr. Jigar K Rana, 30. Dr. Nadeem Khan, 31. Ms. Tejaswini Dash, 32. Mr. MT, 33. Ms. U Revathy, 34. Ms. B Abitha, 35. Ms. JG, 36. Ms. TK Ashitha, 37. Ms. MS Arya, 38. Ms. T Hussain, 39. Dr. Ron Vunsh, 40. Ms. AR Antony, 41. Mr. SM Sharma, 42. Mr. ML Das, 43. Mr. A Gado, 44. Mr. N Nawab, 45. Dr. Uwe Heinig, 46. Ms. Revital Ovadia-Heinig, 47. Mr. J Thomas, 48. Mr. R Vipin, 49. Mr. VP Akhil, 50. Ms. A Suhana

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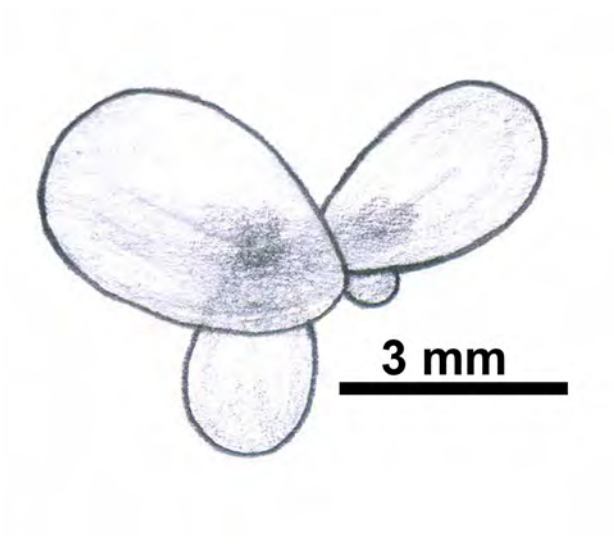
The 3rd International Steering Committee on Duckweed Research and Applications Members

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

Science meets art: *Lemna minuta* Humb., Bonpl. & Kunth



Lemna minuta belongs to the section Uninerves of the genus *Lemna* (Lemnaceae). Its synonyms (former nomenclature that is currently invalid) include *L. minuscula* and *L. minima*. After hesitating for a long time, in 1895, F. Hegelmaier distinguished the two very similar sibling species, *L. minuta* and *L. valdiviana,* from each other mainly based on the slightly different ratio between length and width of the fronds. *Lemna minuta* is often roundish at the tip. E. Landolt also accepted to distinguish these two species although in some clones he found it hardly possible to define the species. He suggested using the relative length of the single frond nerve in relation to the extension of airspaces as a morphological marker to distinguish these two species. Although only few specialists can distinguish these two species on a morphological basis, molecular taxonomic investigations confirmed that both should be treated as two distinct species. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor

Dear Duckweed Community,

On behalf of the International Steering Committee on Duckweed Research and Applications, it is my pleasure to bring you our warm greetings for the new year of 2018. In late October last year, the 4th International Conference focused on duckweed was successfully held in the Central University of Kerala at Padanakkad, India. The hosting by the local organizing committee was superb and scientists and duckweed application specialists came from the four corners of the world to exchange ideas and information. By having the Conference Participant Photo as our Cover for this 20th issue of the Duckweed Forum, we wish to highlight this event. As you can see from the attached program, a good mix of basic research from topics such as genomics and biochemistry and those more toward a focus on applications and field deployment were present. I would also like to highlight the opening ceremony of the conference that dedicated this conference to Prof. Dr. S. C. Maheshwari (Rajasthan University, India) for his truly outstanding contribution to Plant Biology (Center, photo 1). At the age of 85, his Plenary Lecture on Day 1 of the meeting recounting his journey of discovery was inspirational, to say the least. On Day 2 of the Conference, one of the members of our Steering Committee, Dr. Klaus J. Appenroth, was also honored with a Lifetime Achievement Award for his dedication and contributions to the duckweed community (Center, photo 2). An honor that he richly deserved and we are very proud of.

In addition to awards and presentations, we have also formally inaugurated the new set of members to serve as the International Steering Committee for Duckweed Research and Applications. This new cohort of members, which is the third group to serve this capacity since being established in 2013, was voted into office by open ballot. They comprised at the end six members: Klaus J. Appenroth (University of Jena, Germany); Marvin Edelman (Weizmann Institute, Israel); Eric Lam (Rutgers University, USA); Yubin Ma (Qingdao Institute of Bioenergy, China); Tsipi Shoham (GreenOnyx Ltd., Israel) and K. Sowjanya Sree (Central University of Kerala, India). By unanimous vote of the members of the Steering Committee, I was honored to be nominated to serve as the Chair of this Steering Committee for the next two years until the next International Conference to be held at Rehovot, Israel, which will be hosted by the Weizmann Institute. As introduction for this

new Steering Committee to you, I have attached their photos here (Photo 3, below). Five of the members for this committee (unfortunately Dr. Ma could not join us in India due to visa issues between China and India) were able to hold a face-to-face meeting during the Conference. We are all committed to work diligently together to facilitate the growth and success of our fledgling community.

Members of the 3rd International Steering Committee on Duckweed Research and Applications: from left to right - Yubin Ma, Klaus J. Appenroth, Tsipi Shoham, K. Sowjanya Sree, Eric Lam and Marvin Edelman.

Aside from the program of the meeting that is attached to this issue of Duckweed Forum, we have also included the Invitation from Frontiers in Chemistry for you to consider submitting a research paper for a special issue dedicated to duckweed. I would encourage you all to take up this wonderful opportunity.

Other items that I believe you will find of interest in this issue of the Forum are the more standard Science-meets-Art contribution by K. Sowjanya Sree, an Opinion piece by Klaus Appenroth on genotyping using AFLP, a discussion by the Hungarian group from the University of Debrecen on the use of turions from *Spirodela polyrhiza* for exotoxicology studies, a Student Spotlight on Sarah Gilbert from Rutgers University, and of course the useful Database section at the end contributed by Klaus Appenroth. In addition, two new items are also available for your perusal: a new piece started by Tsipi Shoham on "Practical Applications of Duckweed" in the Discussion Corner, and the award announcement for the "Knowing to Growing" award from the International Lemna Association (ILA). For the latter, both a commentary from ILA president Tamra Fakhoorian and personal accounts by the winning teams can be found.

Lastly, I want to encourage you all to think about contributing to the Duckweed Forum, a platform that is intended to produce more dialogue among its community members. To help you to do that, we have included now an instructions-to-authors page at the end of the Forum that will be found and continuously updated as needed in future issues. If you have any suggestions and/or comments, please send them to one of our committee members via email and we will be very grateful for your input.

My very best wishes to everyone,

Eric Lam, Chair of the ISCDRA

Programme Schedule of the 4th ICDRA 2017

(October 23-26), Central University of Kerala, India

Monday, October 23

13:00 – 18:00 h	Registration of participants
13:30 h	Inauguration of the ICDRA – 2017
14:15 – 16:00 h	**Plenary Session I**
Chair	**Sudhir K Sopory**, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
14:15 h	**Plenary Lecture I**: "Revisiting the Lemnaceae" **Satish C Maheshwari**, Rajasthan University, Jaipur, India Formerly University of Delhi, New Delhi, India
15:00 h	Honoring of **Prof. Dr. Satish C Maheshwari**, Rajasthan University with (Formerly University of Delhi, India, to whom the 4th ICDRA is dedicated) **LIFETIME ACHIEVEMENT AWARD** for excellence in scientific research in the area of Plant Biology
15:15 h	**Plenary Lecture II**: "The *Karma* of clones: genomics and epigenomics of oil palm and *Lemna*" **Rob Martienssen**, Cold Spring Harbor Laboratory, New York, USA
16:00 h	Networking Tea
16:30 – 18:15 h	**Session I: Phytoremediation & Stress Biology**
Chair	**Eric Lam**, Rutgers University, New Brunswick, USA
16:30 h	"Removal of pharmaceuticals by duckweed" **Tadashi Toyama**, University of Yamanashi, Yamanashi, Japan
17:00 h	"Phytoremediation efficiency of duckweed on steel waste water" **Supriya Sarkar**, Tata Steel Limited, Jamshedpur, India
17:30 h	"Uptake and bioaccumulation of certain heavy metals by aquatic weed *Lemna minor*" **Chandrima Goswami**, Jadavpur University, Kolkata, India
18:00 h	"Starch accumulation in duckweed biomass by salinity stress" **Kohei Takagi**, University of Yamanashi, Yamanashi, Japan
18:15 h	Opening of Poster presentations
19:30 h	Conference Dinner

Tuesday, October 24

09:00 – 10:00 h Chair	**Plenary Session II** **Halina Gabrys**, Jagiellonian University, Krakow, Poland
09:00 h	**Plenary Lecture III**: "Four decades of Duckweed Research: A personal view" **Klaus-J. Appenroth**, Friedrich Schiller University Jena, Jena, Germany
09:45 h	Honoring of **Dr. Klaus-J. Appenroth**, Friedrich Schiller University Jena, Germany with **LIFETIME ACHIEVEMENT AWARD** for his lifetime contribution to the field of Duckweed research
10:00 – 13:00 h Chairs	**Session II: Basic Biology, Biodiversity and Ecology of Duckweeds** **Marvin Edelman**, Weizmann Institute of Science, Rehovot, Israel **Rameshwar P Sharma**, University of Hyderabad, Hyderabad, India
10:00 h	"*Lemna trisulca*, a model plant for studying chloroplast movements" **Halina Gabrys**, Jagiellonian University, Krakow, Poland
10:30 h	"The metabolome of duckweed – Mass spectrometry based natural product identification and metabolic flux analysis" **Uwe Heinig**, Weizmann Institute of Science, Rehovot, Israel
11:00 h	Networking Tea
11:30 h	"Duckweed as indicator of ecological and geochemical state of environment" **Anna Maksimova**, Tomsk Polytechnic University, Tomsk, Russia
11:45 h	"Evaluation of plant growth promoting activity and colonization of bacterial strain SP-2-C10 on duckweeds" **Tomoki Iwashita**, University of Yamanashi, Yamanashi, Japan
12:00 h	"Intraspecific genetic variations within natural populations of duckweeds" **K. Sowjanya Sree**, Central University of Kerala, Padanakkad, India
12:30 – 14:00 h	Working lunch
14:00 h	Field visit
19:30 h	Dinner

Wednesday, October 25

09:00 h Chair	**Plenary Session III** **Autar K Mattoo**, USDA-ARS-SASL, Beltsville, USA
	Plenary Lecture IV: "Comparative Genomics and Cross-species Interactions Between Duckweed-Associated Bacteria Strains and Plants" **Eric Lam**, Rutgers University, New Brunswick, USA
09:45 – 13:00 h Chairs	**Session III: Genetics and Genomics** **Klaus-J. Appenroth**, Friedrich Schiller University Jena, Jena, Germany **Autar K Mattoo**, USDA-ARS-SASL, Beltsville, USA
09:45 h	"Cytogenomic updates for the genus *Spirodela*" **Ingo Schubert**, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
10:15 h	"Highly contiguous Lemna genomes from long read sequencing" **Evan C. Ernst**, Cold Spring Harbor Laboratory, New York, USA
10:45 h	"The *Wolffia* genome" **Todd P. Michael**, J. Craig Venter Institute, San Diego, USA
11:15 h	Networking tea
11:30 h	"Stable genome duplication of *Landoltia*" **Ron Vunsh**, Weizmann Institute of Science, Rehovot, Israel
12:00 h	"Mutagenesis of *Wolffia globosa*" **Barak Cohen**, Weizmann Institute of Science, Rehovot, Israel
12:15 h	"Comparative analysis of light signaling components in some dicots, core monocots and *Spirodela polyrhiza*" **Jitendra P Khurana**, University of Delhi South Campus, New Delhi, India
12:45 – 14:15 h	Working lunch
14:15 h	**General Assembly** • Election of the venue of 5th ICDRA symposium • Report of the 2nd ISCDRA by the Head, 2nd ISCDRA • Announcement of the results of the election of 3rd ISCDRA members • Transfer of responsibility from the 2nd ISCDRA to the 3rd ISCDRA • Announcement of ILA-Knowing to Growing Duckweed Awards

Wednesday, October 25 (Continued)

15:00 – 18:00 h Chairs	Session IV: Molecular Biology and Biotechnology Ingo Schubert, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany Jitendra P. Khurana, University of Delhi South Campus, New Delhi, India
15:00 h	"Calcium-dependent protein kinases of duckweeds" Autar K Mattoo, USDA-ARS-SASL, Beltsville, USA
15:30 h	"New growth strategies of duckweeds for biotechnological applications" Marvin Edelman, Weizmann Institute of Science, Rehovot, Israel
16:00 h	"More with less: duckweed's high growth rate with fewer genes encoding ribosomal RNAs" Nikolai Borisjuk, Huaiyin Normal University, Huaian, China
16:30 h	Networking tea
16:45 h	"Development of *Wolffia arrhiza* producer of recombinant human granulocyte colony-stimulating factor" Pavel Khvatkov, Nikita Botanical Gardens, Yalta
17:15 h	"Duckweed as a cost effective expression system for the production of recombinant biopharmaceuticals: A case study with recombinant anti-Tumor Necrosis Factor (TNF)α antibody fragment" Vijayalakshmi MA, VIT University, Vellore, India
17:45 h	Poster session First Meeting of the Third ISCDRA members
18:45 h	Open discussion on future prospects of research and application in Lemnaceae Led by Prof. Dr. Eric Lam, Rutgers University, New Brunswick, USA
19:30 h	Dinner

Thursday, October 26

09:00 – 12:00 h	**Session V: Practical Applications of Duckweeds**
Chair	**Halina Gabrys**, Jagiellonian University, Krakow, Poland
09:00 h	"Duckweeds As Key Players In Modern Food Supply and Applications – Opportunities and Challenges" **Tsipi Shoham**, Greenonyx, Tel Aviv, Israel
09:30 h	"Duckweed used as potential nourishment source for production of herbivorous fish" **RN Mandal**, Central Institute of Freshwater Aquaculture, Kolkata, India
10:00 h	"Duckweed biomarkers for identifying water toxins?" **Paul Ziegler**, University of Bayreuth, Bayreuth, Germany
10:30 h	Networking tea
11:00 h	"Duckweed aquatic plant treatment system for cleaning the liquid fraction from organic fraction of municipal solid waste (OFMSW) anaerobic digestor" **Sergio Mapelli**, National Research Council, Milano, Italy
11:30 h	"Decentralised duckweed based treatment system for domestic sewage – a case study" **Suman Chahar**, Sulabh International Social Service Organisation, New Delhi, India
12:00 h	**Close of the 4th ICDRA** Closing Remarks of the 4th ICDRA **Prof. Dr. Jitendra P Khurana**, Co-chair of the Conference University of Delhi South Campus, India
12:10 h	Valedictory Address **Prof. Dr. Eric Lam**, Rutgers University, New Brunswick, USA Head, 3rd ISCDRA
12:20 h	Felicitations
12:40 h	Vote of thanks **Dr. K. Sowjanya Sree**, Chair of the Conference Central University of Kerala, Padanakkad, India
13:00 h	Working lunch
14:00 h	Meeting adjourns

Invitation to contribute an article to the Research Topic

DUCKWEED: BIOLOGICAL CHEMISTRY AND APPLICATION
in *Frontiers of Chemistry*

Dear Colleagues,

We approached Frontiers in Chemistry about organizing a journal issue devoted to duckweed research. We are happy to relate that Frontiers has agreed to our editing a Research Topic entitled "Duckweed: Biological Chemistry and Application" in *Frontiers in Chemistry: Agricultural Biological Chemistry* (*Front. Chem. ABC*). The current Impact Factor of the journal is 3.994 while the Frontiers Group of Journals have reached 6.4 on their Impact Factors.

The scope of the Research Topic is: Duckweed biochemistry, physiology, molecular biology/molecular genetics, ecotoxicology and phytoremediation, interactions with different environments, ecosystems and climatic conditions, nutrients and natural products, biofuels and biomass production.

Researchers in the field of duckweed science are cordially invited to contribute an article to this Research Topic issue. If you accept this invitation, please send a tentative title and names of the contributing authors to marvin.edelman@weizmann.ac.il. The manuscript deadline has been extended to February 18, 2018 to allow ISCDRA Newsletters readers to participate (Frontiers requests that articles be submitted by Jan 31 if possible, however February 18 remains the final date for submissions).

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We look forward to your response.

Guest associate editors:

Marvin Edelman

Klaus-J Appenroth

K. Sowjanya Sree

Spirodela polyrhiza turions as a potential model for ecophysiology and ecotoxicology

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Duckweeds are flowering plants but usually propagate vegetatively by forming daughter fronds. Under environmental stress, however, meristems of certain duckweed species can also switch to produce dormant fronds. These so-called turions are modified vegetative organs but functionally they resemble seeds. In temperate and cold regions they are usually formed at the end of the vegetation period. Despite their ecological significance ecophysiology of turions is less discussed in the scientific literature. This under-representation drew our attention to investigate the ecophysiological aspects of turion formation and dormancy, especially in the case of giant duckweed (*Spirodela polyrhiza* (L.) Schleid.).

Many studies have shown that, under laboratory conditions, turion formation can be induced readily in *S. polyrhiza* by abscisic acid (ABA) treatment (Perry and Byrne 1969) or by limitation of certain nutrients (e.g. phosphorus) (Appenroth et al. 1996). In our laboratory a rapid switch to turion formation have also been observed under heavy metal stress. However, we have to emphasize our experience that only some metallic elements triggered prompt turion formation (e.g. Cd, Hg) while others (Ni, Cr) not (Oláh et al. 2015, Hepp et al. 2016). The underlying mechanisms of these differences have not been elucidated but a possible explanation might be the disturbed nutrient homeostasis (e.g. disorders in phosphate-metabolism). The ability of *S. polyrhiza* to form turions also seems to be a clone-dependent trait. Differences in turion yields amongst various clones have been reported earlier (Appenroth 2003). Similarly, we observed that different clones have distinct responses to heavy metal treatments in terms of turion formation (unpublished data). These results suggest that clonal differences might be significant and need to be considered when *S. polyrhiza* is used in studying endo- and exogenous regulators of dormancy and germination.

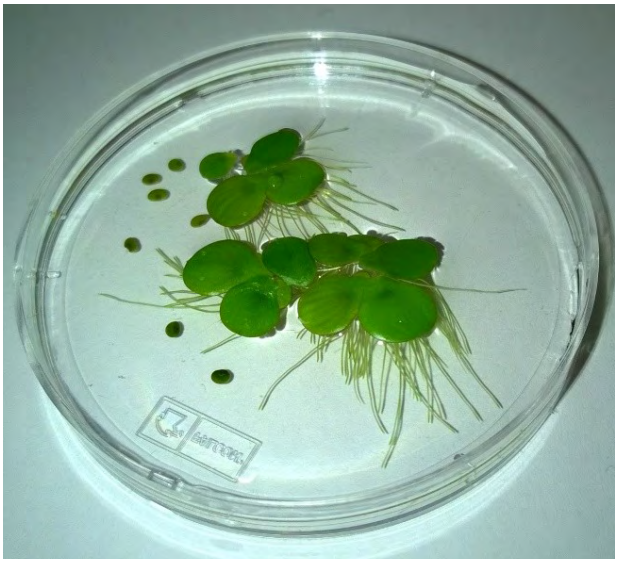
Newly formed turions are in the state of innate dormancy - that is, they cannot germinate even under favourable environmental conditions. This endogenous regulation prevents them from premature germination (e.g. during late summer). To break this state and enter the imposed dormancy – when only external factors block germination - turions require at least four weeks of after-ripening in dark and cold (Appenroth et al. 1989). Endogenous regulation of turion dormancy release is still not described thoroughly but internal ABA levels and low molecular weight carbohydrates might be involved (Appenroth et al. 2013). Germination of turions in the state of imposed dormancy is associated with the phytochrome-mediated mobilization of carbohydrate reserves (Appenroth et al. 1989). Supposedly due to the enhanced respiration a bubble is formed and buoyant turions rise to

the water surface (Newton et al. 1978). Then the activated meristems start to differentiate vegetative fronds within a few days and newly formed duckweed plants re-colonize the habitat.

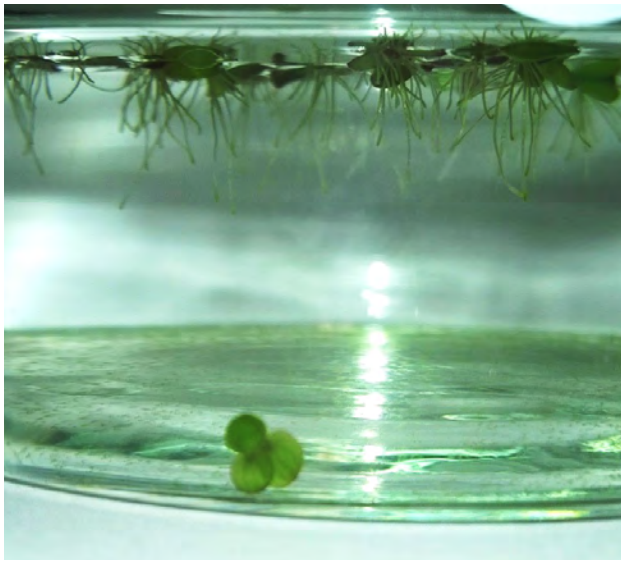
Photosynthetic properties of turions have been little studied although their green colour suggests that they also have photosynthesizing capability (Czopek 1967). Our recent study revealed that turions of *S. polyrhiza* exhibited an interestingly biphasic trend in photosynthetic activity during the consecutive developmental stages (Oláh et al. 2017). Newly formed turions have considerably lower photosynthetic efficiency as compared to mother fronds. During the shift from innate to imposed dormancy, photosynthetic activity of turions displayed a gradual decrease during the first 3 weeks but later, while turions were still kept in the dark and cold, it increased again to the level of newly-formed turions suggesting that changes in photosynthetic performance might be regulated in close connection with dormancy release of turions.

Turions can stay several months in the dormant state on the water bottom and thus it may happen that they can be exposed to contaminants in sediments for long periods. This aspect, however, has been somehow neglected in ecotoxicology. Scarce literature data indicate that heavy metals can influence both dormancy and germination (Srivastava and Jaiswal 1989, Xyländer et al. 1993). Our results with Cd, Ni and Cr suggested that turions in the state of imposed dormancy have considerably higher heavy metal tolerance than active fronds of the same clone (Oláh et al. 2015). Turions treated with environmentally relevant heavy metal concentrations have preserved their vigor and were able to germinate later in pure growth medium. Cd and Ni at high concentrations, however, disturbed both floating-up and germination.

These results directed our attention to the potential use of *S. polyrhiza* turions in ecotoxicological tests. Germination of turions consists of a series of processes including activation of metabolism, cell division and cell elongation that possibly differ in sensitivity to toxic compounds. Thus, in a subsequent work we assessed whether characteristics of turion germination can be introduced into ecotoxicological research. We elaborated a test procedure and compared its sensitivity to normal frond growth inhibition tests via Cd-treatments. Our results confirmed that turions can be used to characterize phytotoxic effects (Oláh et al. 2016). Floating up and protrusion of the first frond were less sensitive possibly because these processes are mostly based on the internal nutrient reserves of the turion. The calculated effective concentrations for subsequent frond production, on the other hand, indicated similar sensitivity to normal frond tests.



Active fronds and turions of the *S. polyrhiza* clone 5501



A turion unusually germinating at the bottom of the culturing vessel due to heavy metal treatment

Parallel to our work Baudo et al. (2015) also pointed to the advantages of using *S. polyrhiza* turions in ecotoxicology practice and developed a standardized microbiotest system. Turions can be maintained in a cost-effective way as they can be stored in small vessels for several months without loss of vigour. After-ripened turions easily germinate and are ready for use within days. Apart from physiological research routine turion phytotoxicity tests can be rapidly conducted (in 2-3 days) in small volumes (e.g. in multiwell plates) and their sensitivity is comparable to the standardized Lemna growth tests (Baudo et al. 2015). Clonal differences, however, might affect responses of these propagules in a similar way than in case of other duckweed tests, thus this fact should be also considered during the interpretation of the results.

Conclusions

Despite their importance in the life cycle of many aquatic plant species, ecophysiology of turions still has several poorly characterized aspects: exogenous and endogenous regulators of turion formation, dormancy and germination, intra- or interspecific differences in the Lemnaceae family all has interesting ecological and physiological aspects for inquiries. Besides those questions, turions - especially those of the relatively well characterized *S. polyrhiza* - are suitable model systems for ecotoxicology research. Compared to active fronds, turions are easier to store and treat with toxicants but have similar sensitivity to the standard Lemna tests and thus they are convenient for characterizing phytotoxic effects.

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Opinion paper: Fingerprinting by amplified fragment length polymorphism (AFLP) and barcoding by plastidic markers

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As a matter of fact, I do not remember exactly when I visited Professor Elias Landolt for the first time at the ETH Zurich, Switzerland. It must be shortly around the year 2000. At this time we had already exchanged quite a number of Emails. My own duckweed collection consisted of approximately 20 clones. Thus, I was very much surprised to see more than 1000 clones in Zurich at his place in the ETH (Urbanska et al., 2013). During my first visit, he tried to show me how to distinguish *Lemna minor* and *Lemna turionifera* on a morphological basis – and I gave up. I realized that I am not cut out to carry on this type of research he created and I got the impression that he is the only person who can distinguish all 37 species of duckweed. I need to mention that the paper of Les et al. (2002) was not yet published. During the train journey back from Zurich to Jena I decided to start with molecular taxonomy of duckweed. In the following year I was in a very comfortable situation: I collected many clones of duckweed in many countries, perhaps in total 100 clones, and Elias Landolt investigated them on a morphological basis and gave them a clone ID. I am very grateful to him and of his dedicated way that he did his work. However, I should mention that even he made some mistakes in typing duckweed samples because this is really difficult on a morphological basis on multiple species. To describe one example: Beside the famous *L. gibba* clone G3 (very popular for phytotoxicological investigations even today) there was a clone, *L. gibba* G1, which was collected by Prof. Riklef Kandeler from Vienna. It had some contrasting properties concerning the photoperiodic regulation of flowering. I lost the clone in my collection. Even asking all members of the German Botanical Society I could not get this clone again and I decided to collect a clone from the same place near Berlin as Riklef Kandeler described the place very precisely to me. Elias Landolt confirmed that the two samples I collected are *L. gibba* (termed by us G1A and G1B) but our molecular analysis by AFLP showed that these clones are very different from the original G1 clone. Finally, Jiaming Zhang of Hainan, China, found using molecular approaches that these clones are in fact not *L. gibba* but *L. minor*. I am telling this story to explain why I decided as a physiologist to enter the field of duckweed taxonomy. Thus, we decided to carry out molecular taxonomy of duckweed because there will be few who could duplicate the experience of Elias Landolt and even then, mistakes in identification based on morphology alone will be possible. We started with the genus *Lemna*. I was happy that Dr. Manuela Bog shared very early with my group (see Student Spotlight in DF 4(2), 248 (2016)) her competence in molecular systematics. Later, also Dr. K. Sowjanya Sree shared our

informal group and supported our further papers number 3 and 4 in duckweed taxonomy with her experience. She even developed skills in morphological delineation of duckweeds that were very helpful later in the most difficult genus of *Wolffiella*.

When we published our first paper in this series about the genus *Lemna* (Bog et al., 2010), we employed only amplified fragment length polymorphism (AFLP). In the time course of our further research we learned that AFLP has very high resolving power in distinguishing duckweed species and even intraspecific clones. However, there is one problem we could not solve: Comparison and characterisation of plant samples turned out to be possible only within one run of analysis. Beside trials of standardisations, it was not possible to integrate later analyses of other plant samples from a different experiment into the same set of evaluation. This means that AFLP is a very powerful method of fingerprinting but cannot be use as a reliable method of barcoding. Therefore, from the second paper about *Wolffia* onward (Bog et al., 2013), we always compared the results of fingerprinting by AFLP with those of barcoding by different plastidic markers. Immediately, there are two problems. The first is related to the plastidic fragments to be used. There are published guidelines by the Consortium for the Barcode of Life (CBOL) for selecting markers for sequencing but most of them turned out to be inferior in resolution for the family of Lemnaceae because of very low variability in these plastidic regions among the species. This was one reason why we received rapid rejection by the editor of the journal "Plant Biology" because we could not follow the recommendation of this consortium. Thus, we had to select those plastidic markers that are suitable to distinguish duckweed samples. The second problem is related to the genomic compartment: plastidic fragments might reflect a different evolution than nuclear fragments. Nuclear markers however have yet to be established for genotyping in plants systematically. As a consequence, we decided using further AFLP and compare the results with the most useful plastidic markers for the different genera.

Thus we published the following papers:

Part 1: *Lemna*

Bog M, Baumbach H, Schween U, Hellwig F, Landolt E, Appenroth K-J (2010) Genetic structure of the genus *Lemna* L. (Lemnaceae) as revealed by amplified fragment length polymorphism. Planta 232:609–619. https://doi.org/10.1007/s00425-010-1201-2

Part 2: *Wolffia*

Bog M, Schneider P, Hellwig F, Sachse S, Kochieva EZ, Martyrosian E, Landolt E, Appenroth K-J (2013) Genetic characterization and barcoding of taxa in the genus *Wolffia* Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). Planta 237:1–13. https://doi. org/10.1007/s00425-012-1777-9

Part 3: *Spirodela* and *Landoltia*

Bog M, Lautenschlager U, Landrock MF, Landolt E, Fuchs J, Sree KS, Oberprieler C, Appenroth KJ (2015) Genetic characterization and barcoding of taxa in the genera *Landoltia* and *Spirodela* (Lemnaceae) by three plastidic markers and amplified fragment length polymorphism (AFLP). Hydrobiologia 749:169–182. https://doi. org/10.1007/s10750-014-2163-3

Part 4: *Wolffiella*

Bog, M, Landrock, MF, Drefahl, D, Sree, KS, Appenroth, KJ (2017) Fingerprinting by amplified fragment length polymorphism (AFLP) and barcoding by three plastidic markers in the genus *Wolffiella* Hegelm. Plant Systematics and Evolution https://doi.org/10.1007/s00606-017-1482-z

Moreover, two strategies to delineate duckweed species were explained in the following paper:
Borisjuk N, Chu P, Gutierrez R, Zhang H, Acosta K, Friesen N, Sree KS, Garcia C, Appenroth KJ, Lam E (2015) Assessment, validation and deployment strategy of a two barcode protocol for facile genotyping of duckweed species. Plant Biol. (Stuttgart) 17(Suppl 1):42–49. https://doi.org/10.1111/plb.12229

When we started this strategy almost 10 years back we hoped that all problems of plant systematics in the family Lemnaceae will be solved. Therefore, we always used the highest number of clones we could manage within the projects. As a consequence, the best results of molecular taxonomy are now available as presented in the first four papers mentioned above.

However, as always in science, not all dreams were fulfilled. Here, I want to mention only a few points. In the genera *Spirodela* and *Landoltia*, the species can be determined with complete confidence; however, the clones of the different species can hardly be distinguished. In the genus *Wolffiella* (the most difficult genus for molecular taxonomy) the species *W. lingulata*, *W. oblonga*, and *W. repanda* cannot be distinguished with confidence at all although K. Sowjanya Sree can distinguish these species with complete confidence on a morphological basis. This makes the difficult relationship between classical taxonomy (i.e. on the basis of morphology) and molecular taxonomy evident: even a single gene might be responsible for the decisive morphological marker but this marker might be hardly detectible applying molecular methods that have been attempted so far. Moreover, there might be a phenotypic plasticity, perhaps dependent on the environmental condition, having a heavy impact on morphology but cannot be easily detected on the level of DNA-based markers. As an additional factor, some clones might represent hybrids, which are not easily detected by morphological markers. As a consequence, it is rather surprising that the morphological markers, partially selected already during investigation in the 19th century, have been quite successful in categorizing species. These early investigators seem to have a good feel about which markers are important for classification and which not as their conclusions are confirmed to a large degree by molecular taxonomy. As a recent example, Landolt decided in 2000 that *Lemna ecuadoriensis* LANDOLT should be combined with *L. obscura* (Landolt 2000). This decision was later completely confirmed by molecular analysis (Bog et al., 2010). On the other hand, molecular taxonomy can be more objective, reliable and can be carried out by scientists not so highly specialised in morphological taxonomy. To give another rather contrasting example: Elias Landolt decided in the very last weeks of his life in 2013 in preparation of the manuscript Bog at al. (2015) to separate *S. intermedia* and *S. biperforata* but this decision could not be confirmed by molecular analysis (Borisjuk et al. 2015).

As always in science, we have not reached the final categorisation in taxonomy of duckweeds. We will continue with more sequence-based method that have been or are being developed to rapidly type not only species but also intraspecific clones and to learn more about their evolutionary relationship. Only then we will be satisfied.

Student Spotlight: Sarah Gilbert

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I attended the University of Connecticut for my Bachelor of Science in Molecular and Cell Biology because I wanted to be in a field where there was potential to cure diseases like Parkinson's and pancreatic cancer, ones my grandparents had passed away from. While searching for a lab to join in my first year, I was fortunate to meet Dr. Gerry Berkowitz in the UConn Agriculture program. I joined his plant molecular biology lab and he mentored me throughout my undergraduate studies. I was surprised at how much I enjoyed doing plant research, but was still curious about other areas of biology research. Over the next few years I worked at various labs to determine which area of biology research was the right path for me. I volunteered at a neuroscience lab at the UConn Health Center, studied photosynthetic sea slugs at the UConn Molecular and Cell Biology department and worked at the biosafety level-3 lab, Plum Island Animal Disease Center. Although each experience was exciting in its own way, I felt most passionate about researching plants to improve human health and the environment, so after graduating with my Bachelors, I joined a small agricultural biotechnology company called Agrivida, Inc. located in Storrs, CT.

Working at Agrivida, Inc. on transgenic corn plants, I realized a PhD in Plant Biology would strengthen my plant biology background and provide me with the knowledge and skills to manage a lab. At Rutgers University, I met with Dr. Eric Lam about potentially working in his lab on duckweed. Hearing about his work on duckweed and its potential in wastewater treatment was new and exciting to me, so I wanted to become a part of the effort in improving duckweed farming.

Duckweed farming can be a sustainable practice, although large scale production remains a challenge. Recent studies have revealed that duckweed associated bacteria (DABs) can significantly improve the removal of nitrogen and phosphate from wastewater by these aquatic plants, and is correlated with their ability to promote growth of duckweeds. Ongoing work in Dr. Lam's laboratory endeavor to isolate and characterize endophytic DABs from various duckweed species in order to evaluate host specificity of the bacterial strains. Initial studies revealed that a duckweed associated *Actinobacteria* strain isolated from *Lemna* and a duckweed associated *Firmicutes* strain isolated from *Spirodela* were found to have growth promoting characteristics after the strains were inoculated onto aseptically grown *Lemna*. My goal has been to characterize DABs based on their ability to promote plant growth through auxin production. I hope that by characterizing the duckweed

microbiome, we can optimize the environmental conditions of sustainable duckweed farming for wastewater treatment.

My first objective was to determine which DABs are producing auxin compounds and what is their phenotypic effect on *Arabidopsis* plant roots. For my experiments, I use *Arabidopsis* in addition to duckweed due to the variety of auxin mutant lines available. The purpose of this aim was to select a subset of DABs to focus on for my dissertation research, based on their ability to produce auxin and potentially improve duckweed health. I am currently working on my second objective which is to perform transcriptome analysis of *Arabidopsis* and duckweed plants inoculated with DABs to determine which genes are upregulated and downregulated in both the plant and bacteria during interaction. This will help me to better understand the mechanisms of how bacteria are selected to be endophytes. My third objective is to determine how multiple bacteria interact with one another to promote plant growth. This information will be useful for building synthetic bacterial communities to improve large scale duckweed farming and potentially the growth of other plants.

I am very happy to have learned about duckweed farming from Dr. Lam and to be part of the duckweed research community. I enjoy teaching others as a Genetics Teaching Assistant at Rutgers, as well as attending conferences, giving talks to students in the New Jersey 4-H Youth program and volunteering at the Liberty Science Center in New Jersey. I hope to continue to teach others about my research after graduating and have a career in the government or industry.

During the summer of 2015, along with several undergraduate students, I collected duckweed growing at a pond owned by a New Jersey resident in the northern part of the state. The resident had contacted Dr. Lam to learn how to distinguish duckweed from algae, their effects on the ecology of the pond, and how they can manage duckweed growth.

Practical applications of duckweed - challenges and opportunities: Meeting the 21st Century's major challenge of efficient resource utilization

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Preface

Practical application is the way to attract attention and support scientific achievements, while benefiting society

Scientific discoveries that can be translated into practical applications and generate value to society create **business opportunities that attract more attention, support and success.** As such, the **field of Duckweed research** has incited an increasing interest beyond mere intellectual scientific fascination as a promising platform for various practical applications.

In this session, we will discuss new opportunities for practical applications and the key challenges in utilizing duckweed's full potential. We wish to extend the dialogue, convey ideas and share experiences that can help the field of Duckweed Research flourish and fulfill its promise.

In order to achieve these goals, this session in the coming *Duckweed Forum* issues will review Duckweed's practical applications via a specific challenge and an opportunity in the context of the following three categories:

1. Emerging challenge that can drive new opportunities
2. Prospect for further collaborations between academia and industry that can reinforce opportunities
3. Challenge in exploration and exploitation – Making an opportunity a reality.

1. The emerging 21st century challenges drive new opportunities for practical applications: The "resource efficiency" challenge

There is an urgent need to improve the efficiency in the use of finite natural resources. New approaches are required to counter the excess consumption of valuable materials and balance between resource utilization and the necessity of expanding the economic growth of emerging and developing countries. This is a key challenge for governments, businesses, and society as a whole.

Duckweed's unique natural attributes inspire and enable the advancement of various practical platforms with the potential to address these challenge. Amongst the most striking traits is the ability to provide a high ratio of biomass generation versus the required resources (e.g., water, energy, fertilizers and arable land). Targeting potential applications for duckweeds under this perspective may attract attention and provide significant differentiating advantages in comparison to alternative solutions.

2. The power of a mutually defined road map and "strategic research" topics: "Deciphering the natural photosynthetic productivity of duckweeds, targeting their potential high resource efficiency applications", could be the first scope

Academia and Industry typically operate in separate worlds. The perception is that one is leading a "basic science" approach, focusing on the theoretical and conceptual research of fundamental knowledge, whilst the other involves "applied research" and is driven by the practicalities of deadlines, goals and ultimately profits. However, intense cross-pollination between academia and industry entities can yield significant success stories. As we are all aware, basic research can lead to applied research that in turn leads to industrial development and the manufacturing of everyday products, while there are many cases in which novel technologies and innovations in applicative environments have led to advances in basic science.

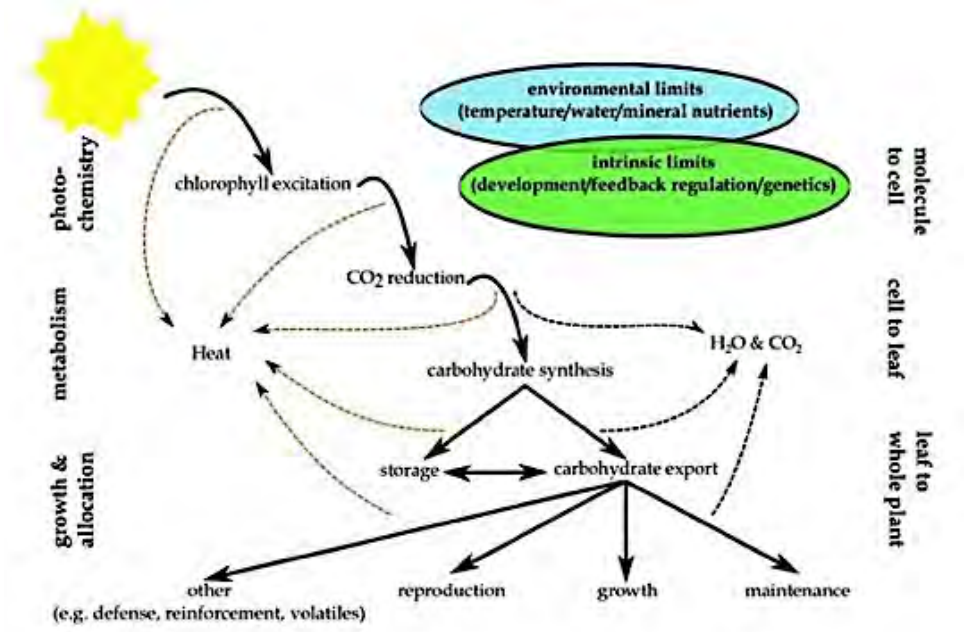
Hence, we urge to enhance our collaborative network, further linking academia and industry entities within the duckweed community. Furthermore, leading a well-defined strategy, with common objectives and a shared roadmap of "strategic research" topics, can dramatically increase our chances to thrive as the next global field for research and practical developments.

"Strategic research" is sometimes used to describe science in an intermediate category, which appears to have a high chance of relating to an application, even if its original purpose was satisfying curiosity and leading to new fundamental understandings of nature's laws. It is a kind of science that yields benefits that are general rather than specific to individual products, and therefore generate economic returns which can be captured by more than one single company or entrepreneur.

Here, I would like to propose the first potential "strategic research" inquiry: Do duckweeds standout by maximizing photosynthetic productivity? This topic could simultaneously lead to deciphering natural fundamental mechanisms alongside practical applications. As we strive to develop more productive cultures, a conclusive answer to this question may be essential.

As illustrated to the right, net photosynthetic efficiency, calculated from when light is incident on the tissue surface to its storage as a simple carbohydrate, can range between 0.1% and 10%. This large range dramatically affects biomass growth rate and yield.

Thus, while studying and scavenging for species and clones of high growth and photosynthesis rates, we could highly benefit from investigating their light-to-biomass conversion efficiency range. This may include a better understanding of the duckweeds' respiration costs - studying the ratio between the growth respiration to maintenance respiration. Furthermore, potential inter-genus, intra-genus and intra-species variations, could significantly contribute to our overall understanding of the mechanisms behind the fastest growing nature of these plants.



A simplified energy flow diagram for pondering plant productivity possibilities, locating constraints on photosynthetic productivity at the level of the cell, the leaf, or the whole-plant. In particular, these processes are conditioned by the developmental and/or acclamatory state of the plant (intrinsic limits) and are subject to environmental resources and stressors (environmental limits). (John B. Skillman et al., Photosynthetic productivity: Can plants do better? 2011)

Translating this knowledge into practical modalities that could maximize light-to-biomass conversion, may result in a significant improvement in yield performance relevant to various applicative cultivation platforms.

While strengthening the industry-academia collaboration is key for inspiring young entrepreneurship, they are yet to face major challenges in realizing their new business.

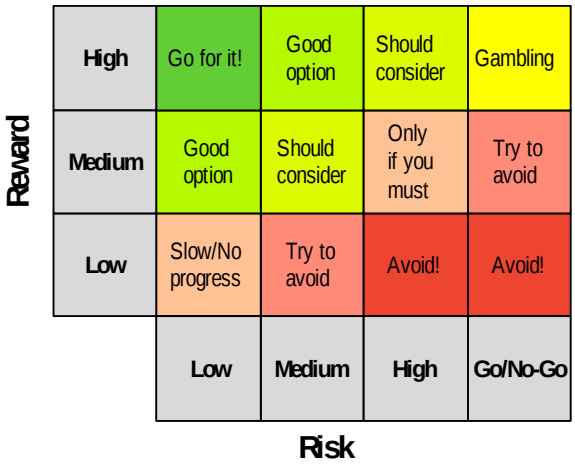
3. Challenges to exploit opportunities: dealing with the unknown can lead to "decision-making" exhaustion

Implementing a new idea, even when it has obvious advantages, is often very difficult. Entrepreneurs have to deal with various key challenges including profitability, production costs, supply stability, scaling and expansion under financial constrains; production yield while securing product safety and quality; exceeding competing alternatives; establishing a brand and accomplishing market acceptance. Under this session, we will have the opportunity to share and discuss these challenges.

In addition to these major tasks, there are other unique challenges for new entrepreneurs. Thus, if you're new in the game, or you're thinking about becoming an entrepreneur, be prepared for further hurdles. I would like to start with the one I believe is most important: "Decision-making". Believe it or not, this is probably the most stressful challenge on your list. Entrepreneurs are forced to make hundreds of decisions a day, from big, company impacting decisions, to tiny, resource allocation

ones. In order to manage your day-by-day decision-making process, you may consider applying one of the most profound tools, the **"Risk vs. Reward decision matrix"** (illustrated to the right).

Combining your risk management strategy with a decision-making matrix will allow you to maximize your venture potential. Here are a few steps to follow:



Based on Decision Matrix (know when to hold them & when to fold them) https://www.slideshare.net/dsfeiman/risk-vs-reward-decision-matrix

1. Define your challenges as risks with their potential mitigation, then categorize them as: **"Go/No-Go"** risk, means there is no anticipated mitigation and if occurs, it will terminate your venture; **High risk** – a high probability with limited mitigation options; **Medium risk**- could be at high probability but with good mitigation options; **Low risk** – at low probability and with clear and feasible mitigation plan.

2. Make your decision matrix by plotting your risks by their scores vs. the anticipated rewards

3. Guide your decision per topics in respect to their matrix location and related action recommendation

AS A CLOSING REMARK TO THIS DRAFT, I would like to invite you all to share your thoughts, questions, comments and ideas, which I'd be happy to include in the next *Duckweed Forum* issue, thus stimulating a discussion that may benefit us all. Please do not hesitate to contact me via my email: Tsipi @greenonyc.biz.

The 1st Knowing-to-Growing Duckweed Application Awards: winner and runner-up of 2017

Preface

The "Knowing to Growing Duckweed Application Award" was organized by the International Lemna Association (ILA) as an expression of gratitude for the excellent duckweed research being done around the world. We sought to recognize the academic papers that are progressing the duckweed industry the most. Journal articles written in 2017 were nominated, and then voted on by a panel of industry judges comprised of duckweed company leaders. There were many fine papers and judging proved to be a challenge. We were happy to announce that the winning team was Dr. Ishizawa, Dr. Kuroda, Professor Morikawa, and Professor Ike with their study, "Environmental bacterial community, a factor strongly affecting duckweed growth", Runner-up was Dr. Appenroth and team, "Nutritional value of duckweeds (Lemnaceae) as human food." (Appenroth et al., 2017) As a direct result of running this competition, duckweed companies were very appreciative to learn of new discoveries that they may have not have prior knowledge of. The ILA wishes to thank Paul Fourounjian for spearheading this competition and Tsipi Shoham for announcing the winners at the recent ICDRA in India.–Tamra Fakhoorian, Executive Director of the *ILA*.

Environmental bacterial community, a factor strongly affecting duckweed growth

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It is indeed a great honor to receive the "Knowing to Growing Duckweed Application Award" for our paper entitled "Evaluation of environmental bacterial communities as a factor affecting the growth of duckweed *Lemna minor*" in *Biotechnology for Biofuels*. We would like to express our sincere gratitude to the members of ILA and those who recognized and voted for our publication.

Our study was conducted to explore the possibility of enhanced duckweed production by utilizing co-existing bacteria. It is widely recognized that land plants harbor specific and diverse bacterial communities in their rhizosphere and phyllosphere, which can play very important roles in determining the growth rate of host plants. Recently, some bacterial strains were found to accelerate the growth of duckweed under certain environmental context as the first examples for aquatic plants (Yamaga et al., 2010; Tang et al., 2015). Our research project is thus an attempt to utilize such beneficial bacteria in duckweed hydroculture for increased biomass production and water treatment

performance. In this study we particularly focused on how and which bacteria affect the growth of duckweed in both the community-scale and individual strain-scale, to better understand the application of beneficial bacteria in practical environments.

Here, we reported that bacterial communities in freshwater environments can affect the growth of *Lemna minor* similarly to other environmental factors such as light and nutrient conditions (see Figure). By characterizing each member of bacterial communities formed on the surface of *L. minor*, we also found the common existence of both plant growth-promoting bacteria (PGPB) and plant growth-inhibiting bacteria (PGIB), which may cooperatively or competitively determine the effects on the host plant's growth properties. Similar studies also examined duckweed growth promotion/inhibition by bacteria from genomic and physiological aspects (Ishizawa et al., 2017a,b). Although these studies just scratched the surface of the complex plant-bacterial interactions, the results suggested that natural bacterial community often function as a comprehensive growth-regulating factor of duckweed.

Appearance of *Lemna minor* co-cultivated for 7 days with the most promotive (left) and inhibitory (right) bacterial communities.

Currently, we are trying to establish the methodology to manipulate the bacterial consortium co-existing with duckweed. Since the bacterial community associated with a plant possess far richer genetic and functional diversity than the plant itself, we believe designing this “plant secondary genome” would enable us to invest useful functions to duckweed hydroculture, such as enhanced growth, improved biomass composition, and degradation of recalcitrant compounds in wastewater (e.g. Toyama et al., 2017; Kristanti et al., 2014). We hope our studies contribute to such future technologies, and offer useful insight for further development of duckweed research and applications.

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Nutritional value of duckweeds (Lemnaceae) as human food

Klaus-J. Appenroth [a*], K. Sowjanya Sree [b], Volker Böhm[c], Simon Hammann, Walter Vetter [d], Matthias Leiterer [e], Gerhard Jahreis [c]

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Cultivation of duckweed is something that many researchers want to do. This is because these plants represent the fastest growing angiosperms that are able to produce large amounts of biomass in the shortest time. Applications are very much supported by the opportunity to cultivate duckweeds on municipal or agricultural wastewater as these resources normally can supply all the nutrients required for fast growth of duckweed. However, there is a main point: after cultivation of these fast growing plants and harvesting, the large amount of biomass must be utilized effectively for an application. What should be done with this biomass? There is a large spectrum of possible applications and we consider that the use should result in the highest possible value. Beside usage in biogas production there are possibilities as supplier of starch for the production of bioethanol or biobuthanol (degradation of starch and fermentation of the resulting sugars) or, alternatively, use of the protein content. The economic situation depends on the profit from the applications. Therefore, use for human nutrition is more profitable than feeding animals. This is the reason, why we think, that use of duckweed for human is a very good opportunity for duckweed application. As a consequence we decided to investigate duckweed quality for its possible use in human nutrition.
I think we should not explain our results here. Instead, I suggest reading our manuscript. Presently, we had a large response from local newspapers, radio stations and television presentations, that pleasantly surprised us very much. Therefore, we decided to continue our research in this direction. Whereas this published paper tries to give an overview about general situation of duckweed, we focused now our next project on plants from the genus *Wolffia*. These are species that have already been used for human nutrition in Thailand and elsewhere in Southeast Asia. We hope by this way, we will have the best possibility to contribute to the practical application of duckweed as a high value product in the near future.

From the database

Highlights

Internal versus external dose for describing ternary metal mixture (Ni, Cu, Cd) chronic toxicity to *Lemna minor*

Gopalapillai, Y; Hale, BA
ENVIRONMENTAL SCIENCE & TECHNOLOGY 51: 5233-5241 (2017)

Simultaneous determinations of internal dose ([M]tiss) and external doses ([M]tot, {M-2(+)} in solution) were conducted to study ternary mixture (Ni, Cu, Cd) chronic toxicity to *Lemna minor* in alkaline solution (pH 8.3). Also, concentration addition (CA) based on internal dose was evaluated as a tool for risk assessment of metal mixture. Multiple regression analysis of dose versus root growth inhibition, as well as saturation binding kinetics, provided insight into interactions. Multiple regressions were simpler for [M]tiss than [M]tot and {M-2(+)}, and along with saturation kinetics to the internal biotic ligand(s) in the cytoplasm, they indicated that Ni-Cu-Cd competed for uptake into plant, but once inside, only Cu-Cd shared a binding site. Copper inorganic complexes (hydroxides, carbonates) played a role in metal bioavailability in single metal exposure but not in mixtures. Regardless of interactions, the current regulatory approach of using CA based on [M]tot can sufficiently predict mixture toxicity (Sigma TU close to 1), but CA based on [M]tiss was closest to unity across a range of doses. Internal dose integrates all metal-metal interactions in solution and during uptake into the organism, thereby providing a more direct metric describing toxicity.

Reconstruction of chromosome rearrangements between the two most ancestral duckweed species *Spirodela polyrhiza* and *S. intermedia*

Hoang, PTN; Schubert, I
CHROMOSOMA 126: 729-739 (2017)

The monophyletic duckweeds comprising five genera within the monocot order Alismatales are neotenic, free-floating, aquatic organisms with fast vegetative propagation. Some species are considered for efficient biomass production, for life stock feeding, and for (simultaneous) wastewater phytoremediation. The ancestral genus Spirodela consists of only two species, *Spirodela polyrhiza* and *Spirodela intermedia*, both with a similar small genome (similar to 160 Mbp/1C). Reference genome drafts and a physical map of 96 BACs on the 20 chromosome pairs of *S. polyrhiza* strain 7498 are available and provide useful tools for further evolutionary studies within and between duckweed genera. Here we applied sequential comparative multicolor fluorescence *in situ* hybridization (mcFISH) to address homeologous chromosomes in *S. intermedia* (2n = 36), to detect chromosome rearrangements between both species and to elucidate the mechanisms which may have led to the chromosome number alteration after their evolutionary separation. Ten chromosome pairs proved to be conserved between *S. polyrhiza* and *S. intermedia*, the remaining ones experienced, depending on the assumed direction of evolution, translocations, inversion, and fissions, respectively. These results represent a first step to unravel karyotype evolution among duckweeds and are anchor points for future genome assembly of *S. intermedia*.

Biotechnology

Effects of coronatine elicitation on growth and metabolic profiles of *Lemna paucicostata* culture

Kim, JY; Kim, HY; Jeon, JY; Kim, DM; Zhou, YY; Lee, JS; Lee, H; Choi, HK
PLOS ONE 12, Article Number: e0187622 (2017)

In this study, the effects of coronatine treatment on the growth, comprehensive metabolic profiles, and productivity of bioactive compounds, including phenolics and phytosterols, in whole plant cultures of *Lemna paucicostata* were investigated using gas chromatography-mass spectrometry (GC-MS) coupled with multivariate statistical analysis. To determine the optimal timing of coronatine elicitation, coronatine was added on days 0, 23, and 28 after inoculation. The total growth of *L. paucicostata* was not significantly different between the coronatine treated groups and the control. The coronatine treatment in *L. paucicostata* induced increases in the content of hydroxycinnamic acids, such as caffeic acid, isoferulic acid, rho-coumaric acid, sinapic acid, and phytosterols, such as campesterol and beta-sitosterol. The productivity of these useful metabolites was highest when coronatine was added on day 0 and harvested on day 32. These results suggest that coronatine treatment on day 0 activates the phenolic and phytosterol biosynthetic pathways in *L. paucicostata* to a greater extent than in the control. To the best of our knowledge, this is the first report to investigate the effects of coronatine on the alteration of metabolism in *L. paucicostata* based on GC-MS profiling. The results of this research provide a foundation for designing strategies for enhanced production of useful metabolites for pharmaceutical and nutraceutical industries by cultivation of *L. paucicostata*.

Pre-treatment of duckweed biomass, obtained from wastewater treatment ponds, for biogas production

Tonon, G; Magnus, BS; Mohedano, RA; Leite, WRM; da Costa, RHR; Belli, P
WASTE AND BIOMASS VALORIZATION 8: 2363-2369 (2017)

Considering the capacity of duckweed to treat wastewater and to produce valuable biomass, the present study aimed to highlight the potential of duckweed biomass harvested from wastewater treatment plant for biogas (methane) production. In this way a pilot system, comprising an anaerobic pretreatment and two duckweed ponds designed in series (10 m^2 each), was operated with real domestic sewage. The treatment efficiency was evaluated through the monitoring of conventional physical-chemical water quality variables sach as Temperature, pH, total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), ammoniacal nitrogen (NH_4^+-N) and chemical oxygen demand (COD). Simultaneously the excess of biomass produced during the treatment was submitted to Biochemical Methane Potential test (BMP) carried out in a multi-batch reactor system. Three pretreatment approaches (fermentative, drying and alkaline) were performed in triplicate to evaluate their influence on methane production. Findings showed that the duckweed ponds removed the organic matter and nutrients from the wastewater (TN = 94%, TP = 92% and COD = 91%). Moreover, the biomass submitted to a fermentative pretreatment returned higher gas production (0.39 NmA(biogas)(3)/kgVS(fed)) compared with the anaerobic digestion (AD) of unpretreated biomass (0.25 NmA(biogas)(3)/kgVS(fed)). These results highlight the potential of duckweed ponds technologies to treat wastewater and produce clean energy simultaneously.

Potential of duckweed for swine wastewater nutrient removal and biomass valorisation through anaerobic co-digestion

Pena, L; Oliveira, M; Fragoso, R; Duarte, E
JOURNAL OF SUSTAINABLE DEVELOPMENT OF ENERGY WATER AND ENVIRONMENT SYSTEMS 4: 127-138 (2017)

Over the last decades, phytodepuration has been considered an efficient technology to treat wastewaters. The present study reports a bench scale depuration assay of swine wastewater using *Lemna minor*. The highest observed growth rate obtained in swine wastewater was 3.1 +/- 0.3 g(DW) m(-2) day(-1) and the highest nitrogen and phosphorus uptake were 140 mg N m(-2) day(-1) and 3.47 mg P m(-2) day(-1), respectively. The chemical oxygen demand removal efficiency in the swine wastewater assay was 58.9 +/- 2.0%. Furthermore, the biomass valorisation by anaerobic co-digestion with swine wastewater was assessed. Results showed a clear improvement in specific methane production rate (around 40%) when compared to mono-substrate anaerobic digestion. The highest methane specific production, 131.0 +/- 0.8 mL CH_4 g(-1) chemical oxygen demand, was obtained with a mixture containing 100 g of duckweed per liter of pre-treated swine wastewater. The water-nutrients-energy nexus approach showed to be promising for swine waste management.

Effect of thermal pre-treatment on co-digestion of duckweed (*Lemna gibba*) and waste activated sludge on biogas production

Gaur, RZ; Khan, AA; Suthar, S
CHEMOSPHERE 174: 754-763 (2017)

The duckweeds (DW) are considered as a major problem in tropical aquatic system as they grow very fast and produce enormous rich-biomass, which can be harvested for renewable energy operations. But complex lignocellulosic compounds limit their utility in process like anaerobic digestion. This batch study aimed to analyse characteristics (proximate, ultimate and physico-chemical) and possible utility of DW for anaerobic co-digestion with waste activated sludge (WAS) under mesophilic conditions for 35 d. Two sets of experiment were tested: substrate with and without thermal pre-treatment. Five combinations of DW: WAS (70:20, 60:20, 50:20, 40:20 and 30:20%) were established and biomethanation along with changes in pH, volatile solids (VS), volatile fatty acids (VFAs), and soluble chemical oxygen demand (sCOD) of digestate were recorded. The total CH_4 yield (mL CH_4 g(-1), VS) ranged between 60 and 468 for pre-treated, and 9 and 76 for non-pre-treated. The maximum CH_4 yield was 468 mL CH_4 g(-1) VS in DW: WAS (50:20). Thermally treated setups, showed about 13-, 24.1-, 21.1-, 1.4-, and 2.3-fold higher CH_4 than non-treated setups. The treated mixtures showed high reduction of sCOD (>41-96) and VS (>59-98%) in co-digesters. The high degree of Gompertz curve fitting (R-2 > 0.99) has suggested pre-treatment of substrate for optimal outputs of co-digester. Based on results obtained, it is suggested that DW (50-60% in digester) can be used as renewable energy resource for biomethanation process after thermal pretreatment.

Nutrient scaling of duckweed (*Spirodela polyrhiza*) biomass in urban wastewater and its utility in anaerobic co-digestion

Gaur, RZ; Suthar, S
PROCESS SAFETY AND ENVIRONMENTAL PROTECTION 107: 138-146 (2017)

The study aimed to investigate the biochemical up gradation of duckweed (DW)-*Spirodela polyrhiza* biomass cultivated in wastewater and then its further utility in anaerobic digestion (AD). For chemical scaling of DW biomass, a batch-scale duckweed reactor was designed using urban

wastewater (WW) and changes in WW characteristics were recorded at the end. The WW showed the significant reduction ($p < 0.05$) in pH- 16.9%; electrical conductivity (EC)-67.6%; biochemical oxygen demand (BOD)-62.6%; nitrate nitrogen (NO_3--N)-76.5%; orthophosphate (PO_4-3-P)-76%; sulphate (SO_4-2)-86.9%; sodium (Na^+)-12.0%; calcium (Ca^{+2})-75.9% and, potassium (K^+)-53.6% after duckweed treatment. After treatment, the DW biomass was harvested and analysed for biochemical properties. Results showed an increase in carbohydrate (45.5%), starch (40.8%), lipid (46.4%) and, protein (56.4%) contents. In the second stage of experiments, the harvested DW biomass was mixed (v/v) with waste activated sludge (WAS) and inoculum acclimatized anaerobic granular sludge (AAGS) to produce four anaerobic batch setups: T-1-DW/WAS/AAGS (50:10:40), T-2-DW/WAS/AAGS (40:20:40), T-3-DW/WAS/AAGS (30:30:40) and, T-4-AAGS (100%) and production of methane was recorded for 35 d. The methane production was recorded in the ranges of 3001 (T-3)-5491 (T-1) mL. The rate of methane generation in all batch reactors was in the order: T-1 (24.01) > T-2 (15.13) > T-3 (9.55). Results thus, revealed that the high content of DW in reactor caused positive effect on methane generation. During the process, soluble chemical oxygen demand (SCOD) and volatile solids (VS) also reduced 36.8-79.7% and, 42.9-70.9%, respectively. Gompertz model validates the experimental methane yield in all setups. Our study indicates that DW can be sustainable tool to solve two major problems: wastewater treatment and renewable energy production under clean development approach.

Ecology

Duckweed (*Spirodela polyrhiza*) as green manure for increasing yield and reducing nitrogen loss in rice production

Yao, YL; Zhang, M; Tian, YH; Zhao, M; Zhang, BW; Zhao, M; Zeng, K; Yin, B
FIELD CROPS RESEARCH 214: 273-282 (2017)

Increasing rice production to feed the world's growing population while protecting the environment requires more optimal use of fertilizers. In China, the current high input, high output and high reliance on synthetic nitrogen (N) fertilizer in agriculture has resulted in high N losses, especially ammonia (NH_3) emission. Urea combined with green manure (GM) might be a promising approach to improve N fertilizer management. However, few studies have evaluated duckweed in this manner. Duckweed does not require arable land for cultivation and thus avoids competition with food crops. Therefore, a field experiment was conducted for three years with five treatments (CK, no N-fertilizer; CT, conventional practice, urea alone at 300 kg N ha(-1); CTD, urea combined with duckweed at 300 kg N ha(-1); RN, urea alone at 225 kg N ha(-1); and RND, urea combined with duckweed at 225 kg N ha(-1)) in an intensive rice cropping system in the Taihu Region of China. The results for two years showed that urea combined with duckweed cover reduced NH_3 loss by 36-52% over CT. This reduction was attributed primarily to the formation of a physical barrier and the uptake of NH_4^+ by duckweed. The N-15 recovery for N-15 balance conducted for one year was 38% higher and the N-15 loss was 16% lower for CTD than that of CT. Furthermore, urea combined with duckweed increased N accumulation in the aboveground plants by 14-25% over CT for the 3 years. As a result, urea combined with duckweed achieved higher rice yield by 9-10%, and higher net economic benefit by 10-11% over CT for the 3 years; however, using the conventional rate of 300 kg N ha(-1) did not increase rice yield over using the reduced N rate of 225 kg N ha(-1), with or without duckweed. Thus, duckweed as GM combined with chemical fertilizer application provided an approach for increasing the rice yield without increasing inputs of N fertilizer and thereby provided a financially attractive option for farmers to achieve environmental integrity and ensure food security in rice production.

Impact of polyethylene microbeads on the floating freshwater plant duckweed *Lemna minor*

Kalcikova, G; Gotvajn, AZ; Kladnik, A; Jemec, A
ENVIRONMENTAL POLLUTION 230: 1108-1115 (2017)

Microplastics (MP), small plastic particles below 5 mm, have become one of the central concerns of environmental risk assessment. Microplastics are continuously being released into the aquatic environment either directly through consumer products or indirectly through fragmentation of larger plastic materials. The aim of our study was to investigate the effect of polyethylene microbeads from cosmetic products on duckweed (*Lemna minor*), a freshwater floating plant. The effects of microbeads from two exfoliating products on the specific leaf growth rate, the chlorophyll a and b content in the leaves, root number, root length and root cell viability were assessed. At the same time, water leachates from microbeads were also prepared to exclude the contribution of cosmetic ingredients on the measured impacts. Specific leaf growth rate and content of photosynthetic pigments in duckweed leaves were not affected by polyethylene microbeads, but these microbeads significantly affected the root growth by mechanical blocking. Sharp particles also reduced the viability of root cells, while the impact of microbeads with a smooth surface was neglected. It was concluded that microbeads from cosmetic products can also have negative impacts on floating plants in freshwater ecosystems.

First discovery of flowering *Wolffia arrhiza* in Central Europe

Schmitz, U; Kelm, H
AQUATIC BOTANY 143: 33-35 (2017)

Flowering of *Wolffia arrhiza* was discovered for the first time in Germany. This record is also the first observation of flowers in this species for Central Europe. The locality is described and the plants are photographically documented. Hitherto, flowering *W. arrhiza* had never been reported for the whole of Europe, with the only exception being two records from the 1980s in Istria (former Yugoslavia, South-East Europe). A further report about alleged flowering plants in the northern forelands of the Caucasus turned out to be erroneous, due to mistranslation and the wrong citation of an original publication from 1957 in Slovak language.

Interaction with microorganisms

Differential oxidative and antioxidative response of duckweed *Lemna minor* toward plant growth promoting/ inhibiting bacteria

Ishizawa, H; Kuroda, M; Morikawa, M; Ike, M
PLANT PHYSIOLOGY AND BIOCHEMISTRY 118: 667-673 (2017)

Bacteria colonizing the plant rhizosphere are believed to positively or negatively affect the host plant productivity. This feature has inspired researchers to engineer such interactions to enhance crop production. However, it remains to be elucidated whether rhizobacteria influences plant oxidative stress visa-vis other environmental stressors, and whether such influence is associated with their growth promoting/inhibiting ability. In this study, two plant growth-promoting bacteria (PGPB) and two plant growth-inhibiting bacteria (PGIB) were separately inoculated into axenic duckweed (*Lemna minor*) culture under laboratory conditions for 4 and 8 days in order to investigate their effects on plant oxidative stress and antioxidant activities. As previously characterized, the inoculation of PGPB and PGIB strains accelerated and reduced the growth of *L. minor*, respectively. After 4 and 8 days of cultivation, compared to the PGPB strains, the PGIB strains induced larger amounts of O_2^-, H_2O_2, and malondialdehyde (MDA) in duckweed, although all bacterial strains consistently increased O_2^-

content by two times more than that in the aseptic control plants. Activities of five antioxidant enzymes were also elevated by the inoculation of PGIB, confirming the severe oxidative stress condition in plants. These results suggest that the surface attached bacteria affect differently on host oxidative stress and its response, which degree correlates negatively to their effects on plant growth.

Enhanced biomass production of duckweeds by inoculating a plant growth-promoting bacterium, *Acinetobacter calcoaceticus* P23, in sterile medium and non-sterile environmental waters

Toyama, T; Kuroda, M; Ogata, Y; Hachiya, Y; Quach, A; Tokura, K; Tanaka, Y; Mori, K; Morikawa, M; Ike, M
WATER SCIENCE AND TECHNOLOGY 76: 1418-1428 (2017)

Duckweed offers the promise of a co-benefit culture combining water purification with biomass production. Acinetobacter calcoaceticus P23 is a plant growth-promoting bacterium isolated from a duckweed, *Lemna aequinoctialis*. This study quantified its growth-promoting effect on three duckweeds (*L. aoukikusa*, *L. minor*, and *Spirodela polyrhiza*) in sterile Hoagland solution and evaluated its usefulness in duckweed culture under non-sterile conditions. P23 promoted growth of three duckweeds in sterile Hoagland solution at low to high nutrient concentrations (1.25-10 mg NO_3-N/L and 0.25-2.0 mg PO_4-P/L). It increased the biomass production of *L. aequinoctialis* 3.8-4.3-fold, of *L. minor* 2.3-3.3-fold, and of *S. polyrhiza* 1.4-1.5-fold after 7 days compared with noninoculated controls. P23 also increased the biomass production of *L. minor* 2.4-fold in pond water and 1.7-fold in secondary effluent of a sewage treatment plant under non-sterile conditions at laboratory-scale experiments. P23 rescued *L. minor* from growth inhibition caused by microorganisms indigenous to the pond water. The results demonstrate that the use of P23 in duckweed culture can improve the efficiency of duckweed biomass production, and a positive effect of P23 on duckweed-based wastewater treatment can be assumed.

Molecular Biology

Salt and cadmium stress tolerance caused by overexpression of the Glycine Max Na+/H+ Antiporter (GmNHX1) gene in duckweed (*Lemna turionifera* 5511)

Yang, L; Han, YJ; Wu, D; Yong, W; Liu, MM; Wang, ST; Liu, WX; Lu, MY; Wei, Y; Sun, JS
AQUATIC TOXICOLOGY 192: 127-135 (2017)

Cadmium (Cd) pollution has aroused increasing attention due to its toxicity. It has been proved that Na+/H+ Antiporter (NHX1) encodes a well-documented protein in Na+/H+ trafficking, which leads to salt tolerance. This study showed that Glycine max Na+/H+ Antiporter (GmNHX1) improved short-term cadmium and salt resistance in *Lemna turionifera* 5511. Expression of GmNHX1 prevented root from abscission and cell membrane damage, which also can enhance antioxidant system, inhibited of reactive oxygen species (ROS) accumulation and cause a less absorption of Cd under cadmium and salt stress. The cadmium tolerance suggested that NHX1 was involved under the cadmium stress.

Enhanced duckweed alkali tolerance by overexpression of serine:glyoxylate aminotransferase (ATAGTI)

Wu, D; Han, YJ; Wu, FJ; Zhu, YR; Zhang, XL; Wei, Y; Wang, Y; Zhou, C; Yang, L
PAKISTAN JOURNAL OF BOTANY 49: 1263-1268 (2017)

Alkaline salts are more harmful to plants than neutral salts. Improvement on the alkaline tolerance in plants is important to make full use of the large area of saline-alkaline water. It has been proved that photorespiration pathway provides protection to plants under stress. In this study, Arabidopsis serine: glyoxylate aminotransferase (AtAGT/) transgenic duckweeds were studied to investigate the alkaline stress tolerance. Enhanced protection from damage to cell membrane was detected in transgenic duckweeds under Na_2CO_3 treatment. Also, in the transgenic duckweed, reactive oxygen species (ROS) accumulation was decreased, and antioxidant enzyme activities were improved with alkaline condition. These results indicated that with the enhancement of photorespiration, antioxidant enzyme activities were improved, leading to the decline of ROS content under alkaline stress. These results also revealed the function of photorespiration enzyme during alkaline stress.

Phytoremediation

Comparative transcriptome analysis of duckweed (*Landoltia punctata*) in response to cadmium provides insights into molecular mechanisms underlying hyperaccumulation

Xu, H; Yu, CJ; Xia, XL; Li, ML; Li, HG; Wang, Y; Wang, SM; Wang, CP; Ma, YB; Zhou, GK
CHEMOSPHERE 190: 154-165 (2018)

Cadmium (Cd) is a detrimental environmental pollutant. Duckweeds have been considered promising candidates for Cd phytoremediation. Although many physiological studies have been conducted, the molecular mechanisms underlying Cd hyperaccumulation in duckweeds are largely unknown. In this study, clone 6001 of *Landoltia punctata*, which showed high Cd tolerance, was obtained by large-scale screening of over 200 duckweed clones. Subsequently, its growth, Cd flux, Cd accumulation, and Cd distribution characteristics were investigated. To further explore the global molecular mechanism, a comprehensive transcriptome analysis was performed. For RNA-Seq, samples were treated with 20 uM $CdCl_2$ for 0,1, 3, and 6 days. In total, 9461, 9847, and 9615 differentially expressed unigenes (DEGs) were discovered between Cd-treated and control (0 day) samples. DEG clustering and enrichment analysis identified several biological processes for coping with Cd stress. Genes involved in DNA repair acted as an early response to Cd, while RNA and protein metabolism would be likely to respond as well. Furthermore, the carbohydrate metabolic flux tended to be modulated in response to Cd stress, and upregulated genes involved in sulfur and ROS metabolism might cause high Cd tolerance. Vacuolar sequestration most likely played an important role in Cd detoxification in *L. punctata* 6001. These novel findings provided important clues for molecular assisted screening and breeding of Cd hyperaccumulating cultivars for phytoremediation.

Removal of selected emerging PPCP compounds using greater duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland

Li, JN; Zhou, QZ; Campos, LC
WATER RESEARCH 126: 252-261 (2017)

Greater Duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland (CW) was employed for removing four emerging pharmaceuticals and personal care products (PPCPs) (i.e. DEET, paracetamol, caffeine and triclosan). Orthogonal design was used to test the effect of light intensity, aeration, *E. coli* abundance and plant biomass on the target compounds. Synthetic wastewater contaminated with the target compounds at concentration of 25 ug/L. was prepared, and both batch and continuous flow experiments were conducted. Up to 100% removals were achieved for paracetamol (PAR), caffeine (CAF) and tricolsan (TCS) while the highest removal for DEET was 32.2% in batch tests. Based on orthogonal Duncan analysis, high light intensity (240 umolmm^{-2} s^{-1}), full aeration, high plant biomass (1.00 kg/m^2) and high *E. coli* abundance (1.0 x 10^6 CFU/100 mL) favoured elimination of the PPCPs. Batch verification test achieved removals of 17.1%, 98.8%, 96.4% and 95.4% for DEET, PAR, CAF and TCS respectively. Continuous flow tests with CW only and CW followed by stabilization tank (CW-ST) were carried out. Final removals of the PPCP contaminants were 32.6%, 97.7%, 98.0% and 100% for DEET, PAR, CAF and TCS, respectively, by CW system alone, while 43.3%, 97.5%, 98.2% and 100%, respectively, were achieved by CW-ST system. By adding the ST tank, PPCP concentrations decreased significantly faster ($p < 0.05$) compared with continuous flow CW alone. In addition, after removing aerators during continuous flow CW experiments, the treatment systems presented good stability for the PPCP removals. CW-ST showed better chemical oxygen demand (COD) and total organic carbon (TOC) removals (89.3%, 91.2%, respectively) than CW only (79.4%, 85.2%, respectively). However, poor DEET removal (<50%) and high *E. coli* abundance (up to 1.7 log increase) in the final treated water indicated further treatment processes may be required. Statistical analysis showed significant correlations ($p < 0.05$) between PPCPs and water quality parameters (e.g. COD, nitrate, phosphate), and between the four PPCP compounds for the continuous flow CW and CW-ST systems. Positive results encourage further test of Greater Duckweed at pilot scale using real wastewater.

Genetic programming-based mathematical modelling of influence of weather parameters in BOD5 removal by *Lemna minor*

Chandrasekaran, S; Sankararajan, V; Neelakandhan, N; Kumar, MR
ENVIRONMENTAL MONITORING AND ASSESSMENT 189, Article Number: 607 (2017)

This study, through extensive experiments and mathematical modelling, reveals that other than retention time and wastewater temperature (T-w), atmospheric parameters also play important role in the effective functioning of aquatic macrophyte-based treatment system. Duckweed species *Lemna minor* is considered in this study. It is observed that the combined effect of atmospheric temperature (T-atm), wind speed (U-w), and relative humidity (RH) can be reflected through one parameter, namely the "apparent temperature" (T-a). A total of eight different models are considered based on the combination of input parameters and the best mathematical model is arrived at which is validated through a new experimental set-up outside the modelling period. The validation results are highly encouraging. Genetic programming (GP)-based models are found to reveal deeper understandings of the wetland process.

Removal mechanisms of benzotriazoles in duckweed *Lemna minor* wastewater treatment systems

Gatidou, G; Oursouzidou, M; Stefanatou, A; Stasinakis, AS
SCIENCE OF THE TOTAL ENVIRONMENT 596: 12-17 (2017)

The fate of five benzotriazoles (1H-benzotriazole, BTR; 4-methyl-1H-benzotriazole, 4TTR; 5-methyl-1H-benzotriazole, 5TTR; xylytriazole, XTR and 5-chlorobenzotriazole, CBTR) was studied in batch and continuous-flow *Lemna minor* systems and the role of different mechanisms on their removal was evaluated. Single and joint toxicity experiments were initially conducted using the Organization for Economic Cooperation and Development (OECD) protocol 221 and no inhibition on specific growth

rate of *Lemna minor* was observed for concentrations up to 200 ug L^{-1}. All tested substances were significantly removed in batch experiments with *Lemna minor*. Excepting 4TTR, full elimination of CBTR, XTR, 5TTR and BTR was observed up to the end of these experiments (36 d), while the half-life values ranged between 1.6 +/- 0.3 d (CBTR) and 25 +/- 3.6 d (4-TTR). Calculation of kinetic constants for hydrolysis, photodegradation, and plant uptake revealed that for all BTRs the kinetic constants of plant uptake were by far higher comparing to those of the other mechanisms, reaching 0.394 +/- 0.161 d^{-1} for CBTR. The operation of a continuous-flow *Lemna minor* system consisted of three mini ponds and a total hydraulic residence time of 8.3 d showed sufficient removal for most target substances, ranging between 26% (4TTR) and 72% (CBTR). Application of a model for describing micropollutants removal in the examined system showed that plant uptake was the major mechanism governing BTRs removal in *Lemna minor* systems.

Effects of pH, initial Pb^{2+} concentration, and polyculture on lead remediation by three duckweed species

Tang, J; Chen, CX; Chen, L; Daroch, M; Cui, Y
ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 23864-23871 (2017)

Various geographical duckweed isolates have been developed for phytoremediation of lead. The Pb^{2+} removal efficiency of *Lemna aequinoctialis*, *Landoltia punctata*, and *Spirodela polyrhiza* was investigated in monoculture and polyculture at different levels of pH and initial Pb^{2+} concentrations. *L. aequinoctialis* was not sensitive to the tested pH but significantly affected by initial Pb^{2+} concentration, whereas synergistic effect of pH and initial Pb^{2+} concentration on removal efficiency of *L. punctata* and *S. polyrhiza* was found. Although the majority of polycultures showed median removal efficiency as compared to respective monocultures, some of the polycultures achieved higher Pb^{2+} removal efficiencies and can promote population to remove Pb^{2+}. Besides, the three duckweed strains could be potential candidates for Pb^{2+} remediation as compared to previous reports. Conclusively, this study provides useful references for future large-scale duckweed phytoremediation.

Antioxidant response in duckweed after exposure to secondary effluent from municipal wastewater treatment plant, Elazig, Turkey

Tatar, SY; Obek, E; Yildirim, NC
BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 9: 399-404 (2017)

The aim of this study is to evaluate the effects of the effluent of Elazig Municipality Wastewater Treatment Plant on the oxidative defense capacity of aquatic plants (*Lemna minor L*. and *Lemna gibba* L.). For this purpose, malondialdehyde (MDA), glutathione (GSH), oxidized glutathione (GSSG), vitamin A (retinol), vitamin E (alpha-tocopherol), and vitamin C (ascorbic acid) levels were determined by the HPLC (high performance liquid chromatography) in the control groups and the groups adapting to reactors fed with discharge water. The depletion of vitamins (A, E, and C), decrease of GSH/GSSG ratio, and increase of MDA that reflect a precarious state of the cell in *L. minor* L. and *L. gibba* L. were observed after exposure to wastewater. It can be suggested that the selected biomarkers are useful in understanding the biochemical mechanisms of the secondary effluents from wastewater treatment plant in *L. minor* L. and *L. gibba* L. as early warning indicators.

Enantioselective accumulation, metabolism and phytoremediation of lactofen by aquatic macrophyte *Lemna minor*

Wang, F; Yi, XT; Qu, H; Chen, L; Liu, DH; Wang, P; Zhou, ZQ
ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 143: 186-192 (2017)

Pesticides are frequently detected in water bodies due to the agricultural application, which may pose impacts on aquatic organisms. The enantioselective bioaccumulation and metabolism of the herbicide lactofen in aquatic floating macrophyte *Lemna minor* (*L. minor*) were studied and the potential *L. minor* phytoremediation was investigated. Ultra-high performance liquid chromatography - tandem mass spectrometry (UHPLC-MS-MS) analysis for lactofen and its two known metabolites in *L. minor* was performed. The initial concentrations of racemic lactofen, R-lactofen and S-lactofen were all 30 ug L^{-1} in the growth solution. The distribution of lactofen and its metabolites in growth solution and *L. minor* was determined throughout a 5-d laboratory trial. It was observed that S-lactofen was preferentially taken up and metabolized in *L. minor*. After rac-lactofen exposure, the accumulation amount of S-lactofen was approximately 3-fold more than that of R-lactofen in *L. minor* and the metabolism rate of S-lactofen (T-1/2 = 0.92 d) was significantly faster than R-lactofen (T-1/2 = 1.55 d). *L. minor* could only slightly accelerate the metabolism and removal of lactofen in the growth solution. As for the metabolites, desethyl lactofen was found to be the major metabolite in *L. minor* and the growth solution, whereas the metabolite acifluorfene was undetectable. No interconversion of the two enantiomers was observed after individual enantiomer exposure, indicating they were configurationally stable. The findings of this work represented that the accumulation and metabolism of lactofen in *L. minor* were enantioselective, and *L. minor* had limited capacity for the removal of lactofen and its metabolite in water.

Evaluation and application of an innovative method based on various chitosan composites and *Lemna gibba* for boron removal from drinking water

Turker, OC; Baran, T
CARBOHYDRATE POLYMERS 166: 209-218 (2017)

Boron exists in various types of water environments, and it is difficult and costly to remove B with conventional treatment methods from drinking water. Clearly, alternative and cost effective treatment techniques are imperative. In the present study, an innovative and environment friendly method based on hybrid systems consisting of various chitosan composite beads and *Lemna gibba* were evaluated for removal of B from drinking water. Our results from batch adsorption experiment indicated that a plant based chitosan composite bead has a higher capacity of B removal than mineral-based chitosan composite beads. Almost 50% of total B removal was achieved using the hybrid system based on dried Lemna-chitosan composite beads and *Lemna gibba* combination in 4 days. Even at the high B concentration (8 mg B L-1), B in drinking water could be reduced to less than 2.4 mg L-1 when 0.05 g plant-based chitosan composite beads and 12 Lemna fronds were used for 50 mL test solution.

Effect of zinc and lead on the physiological and biochemical properties of aquatic plant *Lemna minor*: its potential role in phytoremediation

Jayasri, MA; Suthindhiran, K
APPLIED WATER SCIENCE 7: 1247-1253 (2017)

Plants have gained importance in situ bioremediation of heavy metals. In the present study, different concentrations of zinc (Zn^{2+}) (0.5, 5, 10, 15, 20 mg/l) and lead (Pb^{2+}) (1, 2, 4, 6, 8 mg/l) were used to evaluate metal tolerance level of *Lemna minor*. *L. minor* were exposed to metals for 4 days and tested for its dry to fresh weight ratio (DW/FW), photosynthetic pigments production and protein content. The oxidative damage was detected by measuring catalase activity. *L. minor* showed tolerance against Zn^{2+} and Pb^{2+} at a concentration of 10 and 4 mg/l, respectively. Among the metals, Pb^{2+} showed a significant toxicity at 8 mg/l. High concentration (20 mg/l of Zn^{2+} and 8 mg/l of Pb^{2+}) of the metals displayed a considerable negative effect on soluble proteins (13 fold decrease with Zn^{2+} and 4 fold decrease with Pb^{2+}) and photosynthetic pigments (twofold decrease with Zn^{2+} and one fold decrease with Pb^{2+}) and lead to a consequent reduction in number of fronds. Further, the catalase was greatly increased (twofold decrease with Zn^{2+} and six fold decrease with Pb^{2+}) under metal stress. The results indicate that *L. minor* withstands Zn^{2+} and Pb^{2+} toxicity up to the concentration of 10 and 4 mg/l, respectively. Hence, the metal tolerant property of this plant shall be exploited for bioremediation of Zinc and Lead in polluted water. Further, the detailed and wide range of heavy metal toxicity studies should be done to reveal the possible use of this plant on large scale bioremediation purpose.

Fate of antimicrobials in duckweed *Lemna minor* wastewater treatment systems

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JOURNAL OF HAZARDOUS MATERIALS 330: 116-126 (2017)

The fate of four antimicrobials (cefadroxil, CFD; metronidazole, METRO; trimethoprim, TRI; sulfamethoxazole, SMX) was studied in *Lemna minor* systems and the role of different mechanisms on their removal was evaluated. All micropollutants were significantly removed in batch experiments with active *Lemna minor*; the highest removal was observed for CFD (100% in 14 d), followed by METRO (96%), SMX (73%) and TRI (59%) during 24 d of the experiment. Calculation of kinetic constants for hydrolysis, photodegradadon, sorption to biomass and plant uptake revealed significant differences depending on the compound and the studied mechanism. For METRO, TRI and SMX the kinetic constants of plant uptake were by far higher comparing to those of the other mechanisms. The transformation products of antimicrobials were identified using UHPLC-QToF-MS. Two were the main degradation pathways for TRI; hydroxylation takes place during both phyto- and photodegradation, while demethylation occurs only in absence of Lemna minor. The operation of a continuous-flow duckweed system showed METRO and TRI removal equal to 71 +/- 11% and 61 +/- 8%, respectively. The application of mass balance and the use of published biodegradation constants showed that plant uptake and biodegradation were the major mechanisms governing METRO removal; the most important mechanism for TRI was, plant uptake.

Uptake and distribution of silver in the aquatic plant *Landoltia punctata* (Duckweed) exposed to silver and silver sulfide nanoparticles

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ENVIRONMENTAL SCIENCE & TECHNOLOGY 51: 4936-4943 (2017)

Aquatic ecosystems are expected to receive Ag-0 and Ag2S nanoparticles (NPs) through anthropogenic waste streams. The speciation of silver in Ag-NPs affects their fate in ecosystems, but its influence on interactions with aquatic plants is still unclear. Here, the Ag speciation and distribution was measured in an aquatic plant, duckweed (*Landoltia punctata*), exposed to Ag0 or Ag2S NPs, or to $AgNO_3$. The silver distribution in duckweed roots was visualized using synchrotron-based micro X-ray fluorescence (XRF) mapping and Ag speciation was determined using extended

X-ray absorption fine structure (EXAFS) spectroscopy. Duckweed exposed to Ag2S-NPs or Ag-0-NPs accumulated similar Ag concentrations despite an order of magnitude smaller dissolved Ag fraction measured in the exposure medium for Ag2S-NPs compared to Ag-0-NPs. By 24 h after exposure, all three forms of silver had accumulated on and partially in the roots regardless of the form of Ag exposed to the plants. Once associated with duckweed tissue, Ag-0-NPs had transformed primarily into silver sulfide and silver thiol species. This suggests that plant defenses were active within or at the root surface. The Ag2S-NPs remained as Ag2S, while AgNO3 exposure led to Ag-0 and sulfur-associated Ag species in plant tissue. Thus, regardless of initial speciation, Ag was readily available to duckweed.

Effects of duckweed (*Spirodela polyrhiza*) remediation on the composition of dissolved organic matter in effluent of scale pig farms

Li, L; Liu, M; Wu, M; Jiang, CY; Chen, XF; Ma, XY; Liu, J; Li, WT; Tang, XX; Li, ZP
JOURNAL OF ENVIRONMENTAL SCIENCES 55: 247-256 (2017)

The swine effluent studied was collected from scale pig farms, located in Yujiang County of Jiangxi Province, China, and duckweed (*Spirodela polyrhiza*) was selected to dispose the effluent. The purpose of this study was to elucidate the effects of duckweed growth on the dissolved organic matter composition in swine effluent. Throughout the experiment period, the concentrations of organic matter were determined regularly, and the excitation emission matrix (3DEEM) spectroscopy was used to characterize the fluorescence component. Compared with no-duckweed treatments (controls), the specific ultra-violet absorbance at 254 nm (SUVA(254)) was increased by a final average of 34.4% as the phytoremediation using duckweed, and the removal rate of DOC was increased by a final average of 28.0%. In swine effluent, four fluorescence components were identified, including two protein-like (tryptophan, tyrosine) and two humic-like (fulvic acids, humic acids) components. For all treatments, the concentrations of protein-like components decreased by a final average of 69.0%. As the growth of duckweed, the concentrations of humic-like components were increased by a final average of 123.5% than controls. Significant and positive correlations were observed between SUVA(254) and humic-like components. Compared with the controls, the humification index (HIX) increased by a final average of 9.0% for duckweed treatments. Meanwhile, the duckweed growth leaded to a lower biological index (BIX) and a higher proportion of microbial-derived fulvic acids than controls. In conclusion, the duckweed remediation not only enhanced the removal rate of organic matter in swine effluent, but also increased the percent of humic substances.

Ciprofloxacin induces oxidative stress in duckweed (*Lemna minor* L.): Implications for energy metabolism and antibiotic-uptake ability

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JOURNAL OF HAZARDOUS MATERIALS 328: 140-149 (2017)

We investigate the physiological responses and antibiotic-uptake capacity of *Lemna minor* exposed to ciprofloxacin. Ciprofloxacin (Cipro) induced toxic effects and hormesis in plants by significantly modifying photosynthesis and respiration pathways. A toxic effect was induced by a concentration >= 1.05 mg ciprofloxacin L^{-1} while hormesis occurs at the lowest concentration studied (0.75 mg ciprofloxacin L^{-1}). By impairing normal electron flow in the respiratory electron transport chain, ciprofloxacin induces hydrogen peroxide (H_2O_2) production. The ability of plants to cope with H_2O_2 accumulation using antioxidant systems resulted in stimulation/deleterious effects to

photosynthesis by Cipro. Cipro-induced oxidative stress was also associated with the ability of *L. minor* plants to uptake the antibiotic and, therefore, with plant-uptake capacity. Our results indicate that instead of being a photosystem II binding molecule, Cipro induces oxidative stress by targeting the mitochondrial ETC, which would explain the observed effects of the antibiotic on non-target eukaryotic organisms. The selection of plants species with a high capacity to tolerate oxidative stress may constitute a strategy to be used in Cipro-remediation programs.

Phytotoxicity

Effect of graphene oxide on copper stress in *Lemna minor* L.: evaluating growth, biochemical responses, and nutrient uptake

Hu, CW; Liu, L; Li, XL; Xu, YD; Ge, ZG; Zhao, YJ
JOURNAL OF HAZARDOUS MATERIALS 341: 168-176 (2018)

The wide application and unique properties of graphene oxide (GO) make it to interact with other pollutants and subsequently alter their behavior and toxicity. We evaluated the influences of GO at different concentrations (1 and 5 mg/L) on copper (Cu) stress in duckweed (*Lemna minor* L.) GO below a concentration of 5 mg/L showed no adverse effects on *L. minor*. The addition of Cu above 10 uM represented a stress condition, which was evidenced by various parameters such as frond number, percent inhibition of growth rate (I-r), total chlorophyll content, dry weight, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). When *L minor* was simultaneously exposed to GO and Cu, especially at a GO concentration of 5 mg/L and a Cu level above 10 uM, the increase of I(r) and decrease of chlorophyll content were inhibited, suggesting that the Cu stress was diminished in the presence of GO. The addition of Cu alone, ranging between 5 and 20 uM, increased Cu, B, Mn, Fe, Co, and Zn uptake, but decreased P uptake. Our results suggest that GO can lessen Cu stress in *L. minor* via Cu adsorption, thereby protecting the plants from the damaging effects of high Cu concentrations.

Comparative investigation of toxicity and bioaccumulation of Cd-based quantum dots and Cd salt in freshwater plant *Lemna minor* L.

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ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 147: 334-341 (2018)

The purpose of this study was to determine the toxicity of two different sources of cadmium, i.e. $CdCl_2$ and Cd-based Quantum Dots (QDs), for freshwater model plant *Lemna minor* L. Cadmium telluride QDs were capped with two coating ligands: glutathione (GSH) or 3-mercaptopropionic acid (MPA). Growth rate inhibition and final biomass inhibition of *L. minor* after 168-h exposure were monitored as toxicity endpoints. Dose-response curves for Cd toxicity and EC_{50}(168h) values were statistically evaluated for all sources of Cd to uncover possible differences among the toxicities of tested compounds. Total Cd content and its bioaccumulation factors (BAFs) in *L. minor* after the exposure period were also determined to distinguish Cd bioaccumulation patterns with respect to different test compounds. Laser-Induced Breakdown Spectroscopy (LIBS) with lateral resolution of 200 um was employed in order to obtain two-dimensional maps of Cd spatial distribution in *L. minor* fronds. Our results show that GSH- and MPA-capped Cd-based QDs have similar toxicity for *L. minor*, but are significantly less toxic than $CdCl_2$. However, both sources of Cd lead to similar patterns of Cd bioaccumulation and distribution in *L. minor* fronds. Our results are in line with previous reports that the main mediators of Cd toxicity and bioaccumulation in aquatic plants are Cd^{2+} ions dissolved from Cd-based Qds.

Alleviation of cadmium toxicity in *Lemna minor* by exogenous salicylic acid

Lu, QQ; Zhang, TT; Zhang, W; Su, CL; Yang, YR; Hu, D; Xu, QS
ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 147: 500-508 (2018)

Cadmium (Cd) is a significant environmental pollutant in the aquatic environment. Salicylic acid (SA) is a ubiquitous phenolic compound. The goal of this study was to assess the morphological, physiological and biochemical changes in duckweed (*L. minor*) upon exposure to 10 uM $CdCl_2$, 10 uM $CdCl_2$ plus 50 uM SA, or 50 uM SA for 7 days. Reversing the effects of Cd, SA decreased Cd accumulation in plants, improved accumulation of minerals (Ca, Mg, Fe, B, Mo) absorption, increased endogenous SA concentration, and phenylalanine ammonia lyase (PAL) activity. Chlorosis-associated symptoms, the reduction in chlorophyll content, and the overproduction of reactive oxygen species induced by Cd exposure were largely reversed by SA. SA significantly decreased the toxic effects of Cd on the activities of the superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase in the fronds of *L. minor*. Furthermore, SA reversed the detrimental effects of Cd on total ascorbate, glutathione, the ascorbic acid/oxidized dehydroascorbate and glutathione/glutathione disulphide ratios, lipid peroxidation, malondialdehyde concentration, lipoxygenase activity, and the accumulation of proline. SA induced the up-regulation of heat shock proteins (Hsp70) and attenuated the adverse effects of Cd on cell viability. These results suggest that SA confers tolerance to Cd stress in *L. minor* through different mechanisms.

Inter- and intra-specific competition of duckweed under multiple heavy metal contaminated water

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AQUATIC TOXICOLOGY 192: 216-223 (2017)

The influences of intra- and inter-species competition on ecosystems are poorly understood. *Lemna aequinoctialis* and *Spirodela polyrhiza* were used to assess the effects of exposure to different concentrations of multiple heavy metals (copper-cadmium-zinc), when the plants were grown in mixed- or mono-culture. Parameters assessed included relative growth rate (RGR), content of chlorophyll, glutathione (GSH), malondialdehyde (MDA), as well as the activity of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD). Inter-specific competition was affected by metal concentration, with results indicating that inter-specific competition significantly affected duckweed growth and metal uptake in different heavy metal exposure conditions. Inter-specific competition increased growth rate of duckweed under high metal concentrations, although when compared with intra-specific competition, it caused no obvious differences under low metal concentrations. The growth of *L. aequinoctialis* was further increased in mixed culture when exposed to high metal concentrations, with inter-specific competition increasing the content of cadmium and zinc, while decreasing copper content of *L. aequinoctialis* compared with under intra-specific conditions. Conversely, inter-specific competition increased the content of copper and cadmium of *S. polyrhiza*, without causing obvious differences in zinc accumulation under high ambient concentrations. Under high metal conditions, inter-specific competition increased antioxidant enzyme activities in duckweed species, increasing resistance to heavy metals. Results show that inter-specific competition makes duckweed develop mechanisms to increase fitness and survival, such as enhancement of antioxidant enzyme activities, rather than limiting metal uptake when exposed to high concentrations of multiple metals.

Fate of Flumioxazin in Aquatic Plants: Two Algae (*Pseudokirchneriella subcapitata*, *Synechococcus* sp.), Duckweed (*Lemna* sp.), and Water Milfoil (*Myriophyllum elatinoides*)

Ando, D; Fujisawa, T; Katagi, T
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 65: 8813-8822 (2017)

Flumioxazin separately C-14-labeled at 1,2 -positions of the tetrahydrophthalimide moiety or uniformly labelled at the phenyl ring was exposed to two algae and duckweed via the water layer and water milfoil via the water layer or bottom sediment for 14 days to investigate uptake and metabolic profiles in these aquatic plants. While C-14-flumioxazin received immediate hydrolysis through maleimide ring opening and amide bond cleavage with its hydrolytic half-life of <1 day in both water and sediment, the C-14-plant uptake was <= 4.7% of the applied radioactivity (%AR) with water exposure for all plants and 0.9%AR with sediment exposure for water milfoil. No C-14-translocation between shoot/leaves and roots occurred in water milfoil. The components of C-14 residues in plants were common among the species, which were the above hydrolysates and their transformation products, that is, dicarboxylic acid derivative metabolized via hydroxylation at the double bond of the cyclohexene ring followed by sugar conjugation with its counterpart amine derivative via acid conjugations.

Effects of exposure to nano and bulk sized TiO2 and CuO in *Lemna minor*

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PLANT PHYSIOLOGY AND BIOCHEMISTRY 119: 43-49 (2017)

Nanoparticles of TiO2 and CuO are among most commonly used nanoparticles, and elevated concentrations of them are expected to be found in all environments, including aquatic. A standard growth inhibition test ISO/CD 20079 was used to determine the toxicity of nano sized and larger micro sized (bulk) particles in the concentrations of 0.1,1, 10, 100 and 1000 μM CuO and TiO2 on common duckweed (*Lemna minor* L). Both nano and bulk CuO particles caused changes in the structure and function of treated plants. The number of fronds and colonies decreased by as much as 78%, the length of roots and fronds decreased by 99% and 14%, respectively. Furthermore, photochemical efficiency was reduced by up to 35%, and the activities of antioxidative enzymes guaiacol peroxidase, ascorbate peroxidase and glutathione reductase increased by more than 240%. The altered physiological state of the CuO exposed plants was also reflected in the elevated occurrence of necrosis and bleaching in the duckweed colonies. Nano sized particles of CuO proved more phytotoxic than bulk particles, and the effects of both studied CuO sizes were concentration dependent. On the other hand, both bulk and nano sized particles of TiO2 caused no severe phytotoxic effects, there was no concentration dependence and they could be considered as non-harmful to common duckweed.

Investigation of subcellular distribution, physiological, and biochemical changes in *Spirodela polyrhiza* as a function of cadmium exposure

Su, CL; Jiang, YJ; Li, FF; Yang, YR; Lu, QQ; Zhang, TL; Hu, D; Xu, QS
ENVIRONMENTAL AND EXPERIMENTAL BOTANY 142: 24-33 (2017)

Cadmium (Cd) is considered to be the most phytotoxic heavy metal pollutant. Duckweeds are often used in ecotoxicological investigations as experimental model systems due to their ability to accumulate toxic metals. In this study, accumulation, subcellular distribution, and alterations of metabolic fingerprinting and physiology were evaluated in *Spirodela polyrhiza* exposed to 2.5, 5, and

10 uM Cd for 4 d. The accumulation of Cd increased in a concentration dependent manner. Subcellular fractionation of Cd-containing tissues indicated that 52%-61% of the metal was localized in cell walls and 37%-46% in the soluble fraction, and lowest concentrations were found in cellular organelles. Fourier transform infrared spectrometry analysis indicated that carbonyl, hydroxyl, thiol, and amide groups might be involved in Cd uptake. Cd induced alterations in nutrient elements; for example, it significantly increased iron and calcium and reduced phosphorus and magnesium concentrations in *S. polyrhiza*. Cd-caused oxidative damage was evidenced by increased lipid peroxidation and decreased chlorophyll, protein, and unsaturated fatty acid contents - this was associated with reductions in superoxide dismutase, glutathione reductase, and catalase activities. However, *S. polyrhiza* could combat Cd-induced injury involving a mechanism of non-enzymatic antioxidants and proline and soluble sugar accumulation.

Biochemical alterations in duckweed and algae induced by carrier solvents: selection of an appropriate solvent toxicity testing

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ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 36: 2631-2639 (2017)

Carrier solvents are often used in aquatic toxicity testing for test chemicals with hydrophobic properties. However, the knowledge of solvent effects on test organisms remains limited. The present study aimed to determine the biochemical effects of the 4 common solvents methanol, ethanol, acetone, and dimethyl sulfoxide (DMSO) on 2 test species, *Lemna minor* and *Raphidocelis subcapitata*, by applying Fourier transform infrared spectroscopy (FTIR) coupled with multivariate analysis to select appropriate solvents for toxicity testing. The results showed biochemical variations associated with solvent treatments at different doses on test species. From the infrared spectra obtained, the structures of lipid membrane and protein phosphorylation in the test species were found to be sensitive to the solvents. Methanol and ethanol mainly affected the protein secondary structure, whereas acetone and DMSO primarily induced alterations in carbohydrates and proteins in the test species. The FTIR results demonstrated that methanol and ethanol showed higher biochemical alterations in the test species than acetone and DMSO, especially at the high doses (0.1 and 1% v/v). Based on the growth inhibition displayed and FTIR spectroscopy, acetone, and DMSO can be used as carrier solvents in toxicity testing.

Responses of *Landoltia punctata* to cobalt and nickel: Removal, growth, photosynthesis, antioxidant system and starch metabolism

Guo, L; Ding, YQ; Xu, YL; Li, ZD; Jin, YL; He, KZ; Fang, Y; Zhao, H
AQUATIC TOXICOLOGY 190: 87-93 (2017)

Landoltia punctata has been considered as a potential bioenergy crop due to its high biomass and starch yields in different cultivations. Cobalt and nickel are known to induce starch accumulation in duckweed. We monitored the growth rate, net photosynthesis rate, total chlorophyll content, Rubisco activity, Co^{2+} and Ni^{2+} contents, activity of antioxidant enzymes, starch content and activity of related enzymes under various concentrations of cobalt and nickel. The results indicate that Co^{2+} and Ni^{2+} (<= 0.5 mg L^{-1}) can facilitate growth in the beginning. Although the growth rate, net photosynthesis rate, chlorophyll content and Rubisco activity were significantly inhibited at higher concentrations (5 mg L^{-1}), the starch content increased sharply up to 53.3% dry weight (DW) in *L. punctata*. These results were attributed to the increase in adenosine diphosphate-glucose pyrophosphorylase (AGPase) and soluble starch synthase (SSS) activities and the decrease in alpha-amylase activity upon exposure to excess Co^{2+} and Ni^{2+}. In addition, a substantial increase in the antioxidant enzyme activities and high flavonoid contents in *L. punctata* may have largely resulted in the metal tolerance. Furthermore, the high Co^{2+} and Ni^{2+} contents (2012.9 +/- 18.8 and 1997.7 +/- 29.2 mg kg^{-1} DW) in the

tissue indicate that *L. punctata* is a hyper accumulator. Thus, *L. punctata* can be considered as a potential candidate for the simultaneous bioremediation of Co^{2+}- and Ni^{2+}-polluted water and high-quality biomass production.

Ecotoxicity of nanosized magnetite to crustacean *Daphnia magna* and duckweed *Lemna minor*

Blinova, I; Kanarbik, L; Irha, N; Kahru, A
HYDROBIOLOGIA 798: 141-149 (2017)

Along with the development of nanotechnology, an increase in production and application of nanosized magnetite (Fe_3O_4) is expected. Though magnetite is considered relatively safe, information concerning potential hazards of synthetic magnetite nanoparticles with unique physico-chemical characteristics to aquatic organisms is still limited. In this study, we evaluated the toxicity of nanosized (27.2 +/- 9.8 nm) and bulk (144.2 +/- 67.7 nm) magnetite particles to different life stages of the aquatic crustacean *Daphnia magna*. In addition, phytotoxicity of the magnetite was evaluated using duckweed *Lemna minor*. The study did not reveal any statistically significant differences between the biological effects of nanosized and bulk magnetite particles. Both forms of magnetite induced very low toxicity (EC_{50} > 100 ppm) to *D. magna* and *L. minor* in the standard acute assays. However, it was demonstrated that at acutely subtoxic magnetite concentrations (10 and 100 ppm), the number of neonates hatched from *D. magna* ephippia was decreased. Moreover, short-term (48 h) exposure of neonate daphnids to these concentrations may significantly affect the long-term survival and reproductive potential of daphnids. These results indicate that substantial contamination of aquatic ecosystems by magnetite may disrupt the stability of cladoceran populations.

Mercury induced oxidative stress, DNA damage, and activation of antioxidative system and Hsp70 induction in duckweed (*Lemna minor*)

Zhang, TT; Lu, QQ; Su, CL; Yang, YR; Hu, D; Xu, QS
ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 143: 46-56 (2017)

Mercury uptake and its effects on physiology, biochemistry and genomic stability were investigated in *Lemna minor* after 2 and 6 d of exposure to 0-30 uM Hg. The accumulation of Hg increased in a concentration- and duration-dependent manner, and was positively correlated with the leaf damage. Oxidative stress after Hg exposure was evidenced in *L. minor* by a significant decrease in photosynthetic pigments, an increase in malondialdehyde and lipoxygenase activities (total enzyme activity and isoenzymes activity). Fronds of *L. minor* exposed to Hg showed an induction of peroxidase, catalase, and ascorbate peroxidase activities (total enzyme activity and some isoenzymes activities). Exposure of *L. minor* to Hg reduced the activity (total enzyme activity and some isoenzymes activities) of glutathione reductase, and superoxide dismutase. Exposure to Hg produced a transient increase in the content of glutathione and ascorbic acid. The content of dehydroascorbate and oxidized glutathione in *L. minor* were high during the entire exposure period. Exposure of *L. minor* to Hg also caused the accumulation of proline and soluble sugars. The amplification of new bands and the absence of normal DNA amplicons in treated plants in the random amplified polymorphic DNA (RAPD) profile indicated that genomic template stability (GTS) was affected by Hg treatment. The accumulation of Hsp70 indicated the occurrence of a heat shock response at all Hg concentrations. These results suggest that *L. minor* plants were able to cope with Hg toxicity through the activation of various mechanisms involving enzymatic and non-enzymatic antioxidants, up regulation of proline, and induction of Hsp70.

Reproducibility of the effects of homeopathically potentised *Argentum nitricum* on the growth of *Lemna gibba* L. in a randomised and blinded bioassay

Majewsky, V; Scherr, C; Schneider, C; Arlt, SP; Baumgartner, S
HOMEOPATHY 106: 145-154 (2017)

A previous study reported a significant statistical interaction between experiment date and treatment effect of *Argentum nitricum* 14x-30x on the growth rate of duckweed (*Lemna gibba* L.). The aim of the present study was to investigate the stability of the test system and intra-laboratory reproducibility of the effects found. Duckweed was treated with *A. nitricum* potencies (14x-30x) as well as succussed and unsuccussed water controls. The outcome parameter area-related growth rate for day 0-7 was determined by a computerised image analysis system in two series of independent randomised and blinded experiments. Systematic negative control (SNC) experiments were carried out to investigate test system stability. Statistical analysis was performed with full two-way analysis of variance (ANOVA) and protected Fisher's Least Significant Difference (LSD) test. In the first repetition series we found a significant treatment effect ($p = 0.016$), while in the second series no effect was observed. The negative control experiments showed that the experimental system was stable. An a posteriori subgroup analysis concerning gibbosity revealed the importance of this growth state of *L. gibba* for successful reproduction of the statistically significant interaction in the original study; flat: no interaction ($p = 0.762$); slight gibbosity: no interaction ($p = 0.356$); medium gibbosity: significant interaction ($p = 0.031$), high gibbosity: highly significant interaction ($p = 0.005$). With the original study design (disregarding gibbosity status of *L. gibba*) results of the original study could not be reproduced sensu stricto. We conclude that the growth state gibbosity is crucial for successful reproduction of the original study. Different physiological states of the test organisms used for bioassays for homeopathic basic research must carefully be considered.

Response of *Spirodela polyrhiza* to cerium: subcellular distribution, growth and biochemical changes

Xu, QS; Jiang, YJ; Chu, WY; Su, CL; Hu, D; Lu, QQ; Zhang, TL
ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 139: 56-64 (2017)

Rare earth elements are new and emerging contaminants in freshwater systems. Greater Duckweed (*Spirodela polyrhiza* L.) is a common aquatic plant widely used in phytotoxicity tests for xenobiotic substances. In this study, the cerium (Ce) accumulation potential, the distribution of Ce in bio-molecules, and ensuing biochemical responses were investigated in Greater Duckweed fronds when they were exposed to Ce (0, 10, 20, 40, and 60 uM). There was a concentration dependent increase in Ce accumulation, which reached a maximum of 67 mg g^{-1} of dry weight (DW) at 60 uM Ce after 14 d. The Ce concentrations in bio-macromolecules followed the order: cellulose and pectin > proteins > polysaccharides > lipids. In response to Ce exposure, significant chlorosis; declines in growth, photosynthetic pigment and protein contents; and cell death were noted at the highest Ce concentration. Photosystem II inhibition, degradation of the reaction center protein D1, and damage to chloroplast ultrastructure were observed in Ce treated *S. polyrhiza* fronds, as revealed by chlorophyll a fluorescence transients, immunoblotting, and transmission electron microscopy (TEM). $\bullet O_2^-$ accumulation and malondialdehyde (MDA) content in the treated fronds increased in a concentration dependent manner, which indicated that oxidative stress and unsaturated fatty acids (C18:3) were specifically affected by Ce exposure. These results suggest Ce exerts its toxic effects on photosynthesis, with a primary effect on PS II, through oxidative stress.

Response of *Lemna gibba* L. to high and environmentally relevant concentrations of ibuprofen: Removal, metabolism and morpho-physiological traits for biomonitoring of emerging contaminants

Di Baccio, D; Pietrini, F; Bertolotto, P; Perez, S; Barcelo, D; Zacchini, M; Donati, E
SCIENCE OF THE TOTAL ENVIRONMENT 584: 363-373 (2017)

The increasing worldwide consumption of pharmaceuticals and personal care products such as ibuprofen (IBU) is leading to the widespread and persistent occurrence of these chemicals and their transformation products in soils and waters. Although at low concentrations, the continuous discharge of these micropollutants and the incomplete removal by the actual wastewater treatments can provoke accumulation in the environment with risks for the trophic chain. Non-target organisms as duckweed can be used for the environniental monitoring of pharmaceutical emerging contaminants. In this work, plants of *Lemna gibba* L. were exposed to high (0.20 and 1 mg L^{-1}) and environmentally relevant (0.02 mg L^{-1}) concentrations of IBU to investigate their removal and metabolization capacity. The main oxidized IBU metabolites in humans (hydroxy-IBU and carboxy-IBU) were determined in the intact plants and in the growth solutions, together with non-destructive physiological parameters and phytotoxic indicators. The IBU uptake increased with the increasing of IBU concentration in the medium, but the relative accumulation of the pharmaceutical and generation of hydroxy-IBU was higher in presence of the lower IBU treatments. Carboxy-IBU was not found in the plant tissue and solutions. The changes observed in growth and photosynthetic performances were not able to induce phytotoxic effects. Apart from a mean physical-chemical degradation of 82%, the IBU removal by plants was highly efficient (89-92.5%) in all the conditions tested, highlighting the role of *L gibba* in the biodegradation of emerging contaminants.

Electrochemical oxidation of quinoline aqueous solution on beta-PbO_2 anode and the evolution of phytotoxicity on duckweed

Ma, XJ; Bian, LX; Ding, JF; Wu, YP; Xia, HL; Li, JH
WATER SCIENCE AND TECHNOLOGY 75: 1820-1829 (2017)

Electrochemical oxidation of quinoline on a beta-PbO_2 electrode modified with fluoride resin and the comprehensive toxicity of intermediates formed during oxidation on duckweed were investigated in detail. The results showed that quinoline was initially hydroxylated at the C-2 and C-8 positions by hydroxyl radicals (•OH) electro-generated on a beta-PbO_2 anode, yielding 2(1H)-quinolinone and 8-hydroxyquinoline, then undergoing ring cleavage to form pyridine, nicotinic acid, pyridine-2-carboxaldehyde and acetophenone, which were ultimately converted to biodegradable organic acids. NO_3- was the final form of quinoline-N. The growth of duckweed exposed to the oxidized quinoline solution was gradually inhibited with the decrease in pH and the formation of intermediates. However, the growth inhibition of duckweed could be eliminated beyond 120 min of oxidation, indicating the comprehensive toxicity of the quinoline solution reduced when the amount of quinoline removed was above 80%. Additionally, the adjustment of the pH to 7.5 and the addition of nutrients to the treated quinoline solution before culturing duckweed could obviously alleviate the inhibition on duckweed. Thus, partial electrochemical degradation of quinoline offers a cost-effective and clean alternative for pretreatment of wastewater containing nitrogen-heterocyclic compounds before biological treatment. The duckweed test presents a simple method for assessing the comprehensive toxicity of intermediates.

Ecotoxicological assessments show sucralose and fluoxetine affect the aquatic plant, *Lemna minor*

Amy-Sagers, C; Reinhardt, K; Larson, DM
AQUATIC TOXICOLOGY 185: 76-85 (2017)

Pharmaceuticals and personal care products (PPCP) are prevalent in aquatic systems, yet the fate and impacts on aquatic plants needs quantification for many compounds. We measured and detected sucralose (an artificial sweetener), fluoxetine (an antidepressant), and other PPCP in the Portneuf River in Idaho, USA, where *Lemna minor* (an aquatic plant in the environment and used in ecotoxicology studies) naturally occurs. Sucralose was hypothesized to negatively affect photosynthesis and growth of *L. minor* because sucralose is a chlorinated molecule that may be toxic or unusable for plant metabolism. A priori hypotheses were not created for fluoxetine due to lack of previous studies examining its impacts on plants. We conducted laboratory ecotoxicological assessments for a large range of concentrations of sucralose and fluoxetine on *L. minor* physiology and photosynthetic function. Frond green leaf area, root length, growth rate, photosynthetic capacity, and plant carbon isotopic composition (discrimination relative to a standard; delta C-13) were measured among treatments ranging from 0 to 15000 nmol/L-sucralose and 0-323 nmol/L-fluoxetine. Contrary to our predictions, sucralose significantly increased green leaf area, photosynthetic capacity, and delta C-13 of *L. minor* at environmentally relevant concentrations. The increase of delta C-13 from sucralose amendments and an isotope-mixing model indicated substantial sucralose uptake and assimilation within the plant. Unlike humans who cannot break down and utilize sucralose, we documented that *L. minor,* a mixotrophic plant, can use sucralose as a sugar substitute to increase its green leaf area and photosynthetic capacity. Fluoxetine significantly decreased *L. minor* root growth, daily growth rate, and asexual reproduction at 323 nmol/L-fluoxetine; however, ambiguity remains regarding the mechanisms responsible and the applicability of these extreme concentrations unprecedented in the natural environment. To our knowledge, this was the first study to show aquatic plants can uptake and metabolize sucralose as a carbon source. This study further supports the common notion that *L. minor* can be useful in bioremediation of PPCP from wastewaters.

Instructions to Contributors for the Duckweed Forum

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

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

- Single-spaced text throughout.
- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
- Number all pages, including those with figures on the bottom and center of each page.

Title:

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

Authors:

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

Image resolution and submission:

It is extremely important that figures be prepared with the proper resolution for publication in order to avoid inaccurate presentation of the data. The minimum acceptable resolution for all figures is 300 dpi. Excessive file compression can distort images, so files should be carefully checked after compression. Note that figures that contain both line art (such as graphs) and RGB/grayscale areas (such as photographs) are best prepared as EPS (vector) files with embedded TIFF images for the RGB/grayscale portions. The resolution of those embedded TIFF images should be at least 300 dpi.

Original images should be submitted as a separate file to the text file. It would be helpful to insert the intended into the Word file as well, if desired, to indicate the location for it. The legend to the image/figure should be added at the end of the text file and labeled as "Legend to Figures".

Links for further reading

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

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

http://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.

http://lemnapedia.org/wiki/LemnaPedia By Eduardo Mercovich, Mamagrande, Argentina.

Note to the Reader

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