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DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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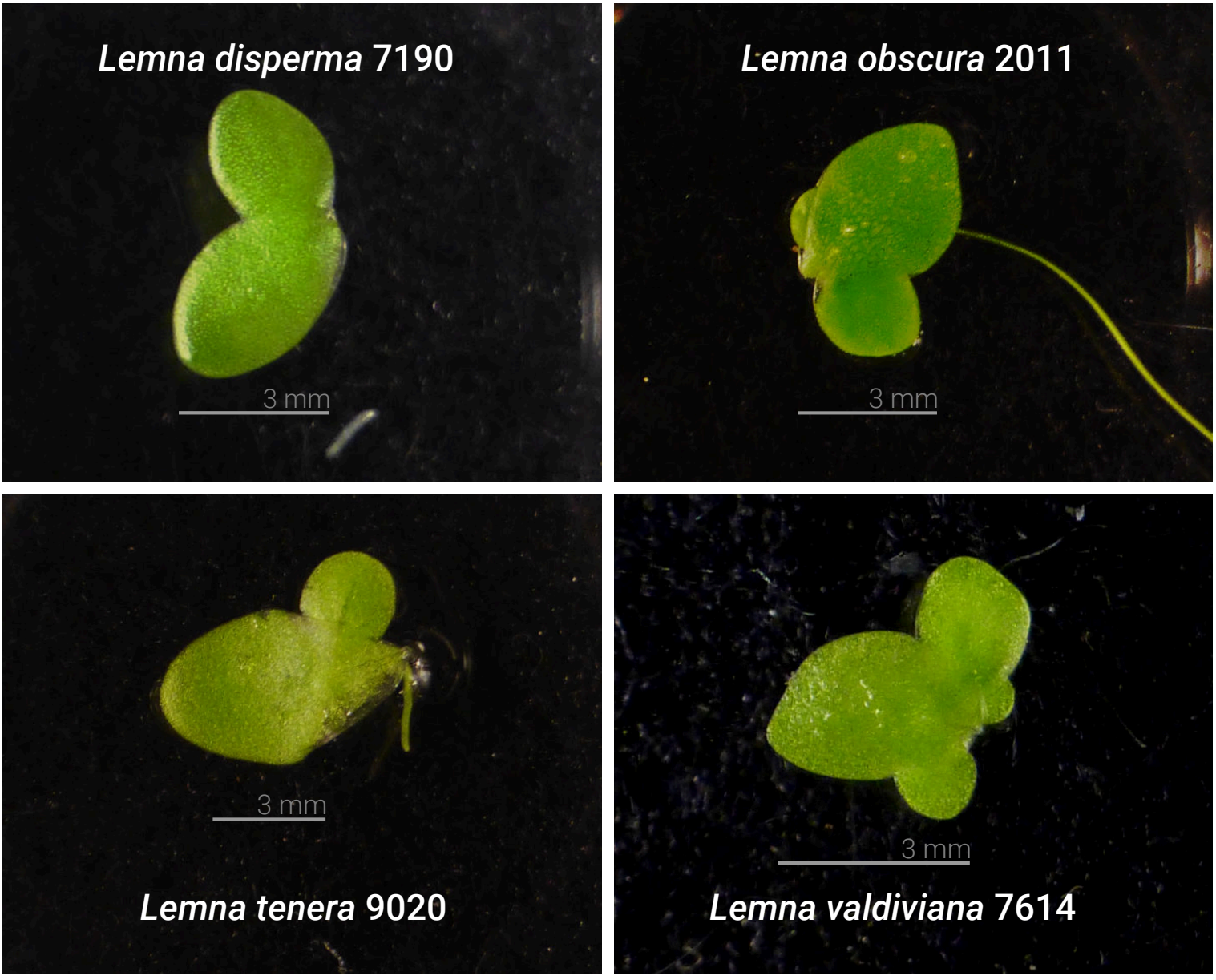


The cover of this issue of the Duckweed Forum features four Lemna species that are endemic to different regions of the world. ***Lemna disperma*** (clone 7190 from Melbourne, Australia), is distributed in the lowlands of Southern Australia and New Zealand. ***Lemna obscura*** (clone 2100 from the Pineland Nursery, NJ, USA), can be found over a large area covering the Southeast regions of North America. It was likely introduced to Hawaii in the 1970's. *L. dispermah*as often been mis-identified as *L. obscura* (and sometimes with *L. gibba*) since their morphological traits are quite similar. Clear separation of these species are enabled with introduction of molecular techniques by Les et al. in 2002, and later on by others using comprehensive barcoding and AFLP strategies. In contrast to *L. disperma*, ***Lemna tenera*** (clone 9020 from the Northern Territory, Australia) can be found in tropical regions of Southeast Asia extending to Northern Australia. It appears to be a rare species. In contrast to the narrow distribution of *L. tenera*, ***Lemna valdiviana*** (clone 7614 from Lake Titicaca, Puno, Peru) is a species that is widely distributed in the warmer regions of the Americas. Currently, it remains difficult to distinguish this species from *L. minuta* (formerly*L. minuscula*) and *L. yungensis*, even when applying barcoding approaches.
Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

In this issue

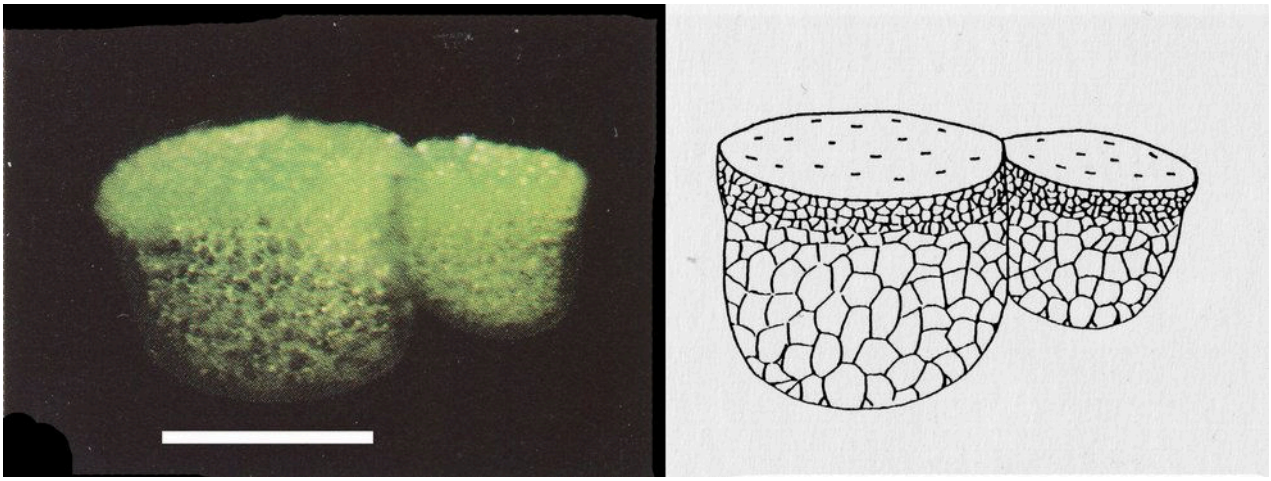
International Steering Committee on Duckweed Research and Applications Members

- **Chair: PD Dr. Klaus-J. Appenroth**, University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Eduardo Mercovich**, MamaGrande, Rosario, Argentina; eduardo@mamagrande.org
- **Louis Landesman**, Duckweed49.com, USA; landesman49@yahoo.com
- **Prof. Eric Lam**, Rutgers, the State University of NJ, New Brunswick, USA; ericL89@hotmail.com
- **Tamra Fakhoorian**, International Lemna Association, Mayfield, KY, USA; tamraf9@gmail.com

Information about the ISCDRA: http://lemnapedia.org/wiki/ISCDRA

All prior Duckweed Forum issues: http://lemnapedia.org/wiki/Duckweed_Forum

Science meets art: *Wolffia australiana* (Benth.) Hartog & Plas



E. Landolt published in his paper in Ber. Geobot. Inst. ETH 60: 137-151 (1994) both microphotographs and drawings of seven species of the genus *Wolffia*. This makes the difference between science and art very obvious: Whereas science presents the world as well as the present (always limited) technical tools permit it, art tries to show the soul of nature. There is no competition but only complementarity to each other. Bar: 1 mm.

Letter from the editor

Dear friends of duckweed research and applications,

These days we are entering the second half of the year 2017 – the part of the year when we will have our 4th International Conference on Duckweed Research and Applications (ICDRA-2017). I hope we will exceed previous record concerning the number of participants and meet as many of you as possible in Kerala, India. The following page will inform you how to register and whom to contact in case you have any questions. The next page thereafter summarizes some deadlines for abstract submission, conference registration, nomination of candidates for the election of members for the "International Steering Committee on Duckweed Research and Applications" (ISCDRA), and finally the voting. Rules were published already in the previous issue of our Newsletter "Duckweed Forum". You may check it at http://lemnapedia.org/wiki/Duckweed_Forum or http://www.ruduckweed.org.

There is much, much more in this issue. Marvin Edelman and colleagues volunteer to organize the 5th ICDRA-2019 and suggest the Weizman Institute in Rehovot, Israel as the place. John Cross, USA, well-known in the duckweed community from his famous homepage "The Charms of Duckweed" (http://www.mobot.org/jwcross/duckweed/duckweed.htm) writes about the role of roots in duckweed. Ingo Schubert from the Leibnitz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany, submitted an opinion paper about duckweed genome research in the present and future. And Guenther Theissen from the University of Jena, Germany confesses that he and some of his co-workers are MADS about duckweed - but what he writes sounds nevertheless very interesting.

Of course, we have our standard contributions such as the duckweed photos from Eric Lam of the Rutgers Duckweed Stock Cooperative on the cover page, and something about "Science meets art", this time with a drawing from the late Elias Landolt. The chapter "Student Spotlight" comes from Kyoto, Japan, and again the newsletter is appended by the recent literature on Lemnaceae in the chapter "From the database".

We feel that we need more contribution from the readers of the "Duckweed Forum". We would like to remind you of our "Discussion corner", which awaits your contribution about all possible duckweed-related topics. Moreover, perhaps it would be interesting to learn more about the history of duckweed research. We suspect there is someone out there who knows something about duckweed research pioneers such as W.S. Hillman or D.L. Jacobs or any other interesting duckweed researcher from the past. Please, write to us and let's share your ideas.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),

Klaus-J. Appenroth, Chair

ICDRA 2017 in Kerala, India

Would you like to talk to the leading experts of duckweed research and applications? Would you like to learn about the state of art? Would you like to present your results and discuss them with the duckweed community?

Topics: Duckweed: Basic biology, Duckweed: Genetics and molecular biology

Duckweed- Microbe interactions, Practical applications of duckweeds.

Registration is open online: http://icdra2017.cukerala.ac.in/Home.html

The chairs of the conference are

Dr. K. Sowjanya Sree, Assistant Professor, Dept. of Environmental Science

Central University of Kerala, Padanakkad, India.

Prof. Dr. Jitendra P. Khurana, Director, Univ. of Delhi South Campus, Dept. of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India.

If you have further questions, please, contact the chair Dr. K. Sowjanya Sree, Ph.: +91 9999672921, Email: ksowsree9@cukerala.ac.in; ksowsree@gmail.com

ICDRA important appointed days

15th August:	Deadline for abstract submission
23rd August:	Last opportunity for registration
8th September:	Last chance to suggest candidates for the new "International Steering Committee on Duckweed Research and Applications"
12th September to 2nd October:	Voting for the candidates of the Steering Committee.
23rd to 26th of October	4th International Conference on Duckweed Research and Applications Periye, Kerala, India; Central University of Kerala http://icdra2017.cukerala.ac.in/Home.html

1st proposal for the next "International Conference on Duckweed Research and Applications" in 2019

Asaph Aharoni, Avi Levy and Marvin Edelman have suggested hosting the 5th International Conference of Duckweed Research and Applications in 2019 at the Weizmann Institute of Science in Rehovot, Israel.

The Weizmann Institute of Science is a world-class basic research institution set in a lushly landscaped campus in the university town of Rehovot, 25 min. from Tel Aviv and 45 min. from Jerusalem. It is host to 240 experimental and theoretical research groups across five faculties — Biology, Biochemistry, Chemistry, Mathematics/Computer Science, and Physics— and to 1400 advanced degree students and postdoctoral fellows.

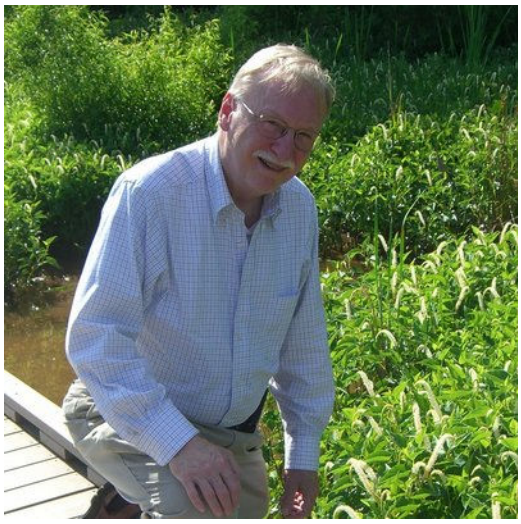
Accommodations for visitors and conventions exist on campus and a leading hotel is located in the Rehovot Science Park, 5 min. walk from campus.

Left to right: Asaph Aharoni, Marvin Edelman, Avi Levi

Duckweed Roots: their role in vegetative dispersal

John W. Cross (jw.cross@verizon.net)

The manuscript was originally conceived when the author was visiting in the Department of Biology, University of San Francisco, California.



Summary

The roots of duckweeds (Lemnaceae) are sticky when they dry. The physiological literature indicates that the roots of these aquatic species are not the major route for uptake of dissolved nutrients, nor do they conduct sufficient water to hydrate the plant when the plant thallus is removed from direct contact with water. Several species of Lemnaceae lack roots entirely. I propose that an important function of roots in these organisms is in dispersal. Attachment of the roots to fur or feathers would allow animals and birds to transport these plants to distant bodies of fresh water. This hypothesis explains the wide geographic dispersal of the Lemnaceae without assuming human intervention.

Introduction

1. Geographic dispersal of the duckweeds

The duckweeds are small aquatic plants found in many parts of the world. Maps published by Landolt (1986) show that that the range of many species of Lemnaceae is extremely broad, in several cases, covering more than one continent (*Spirodela polyrhiza*, *Lemna gibba*, *Lemna minor*, etc.). It is generally accepted that these wide distributions are the result of transport of the plants by animal and human activity (Landolt, 1986, Armstrong, 1989). However, the specifics of how the plants are carried has not been addressed.

2. The functions of roots in duckweeds

The literature (reviewed by Landolt, 1986, especially see Vol. 1, pp 69-79.]) shows that the roots of duckweeds are not essential for the uptake of water and nutrients. In Lemna, the underside of the frond itself absorbs most of the nutrients. Furthermore, the water supplied from the roots is not sufficient to keep the plant hydrated (Gorham, 1941). If the frond is removed from direct contact with water with the root tip still immersed, the frond suffers a water deficit[1].

Two genera of Lemnaceae (*Wolffiella* and *Wolffia*) lack roots entirely, and the roots of Spirodela and Lemna plants occasionally drop off (Landolt, 1986). One species of Lemna does not always form roots. Furthermore, root branching and formation of root hairs are unknown in this family.

Landolt (1986) believes that the major functions of roots in the Lemnaceae are as organs of stabilization. The roots help individual plants to maintain their upright position relative to the surface of the water. They also serve as "sea anchors", minimizing the effects of wind on the exposed fronds. Finally, the roots of adjacent plants become tangled, so that the plants form a cohesive mat across the surface of the water. Such a mat allows groups of plants to resist the disruptive action of

1 Editor's note: This may be explained in part by the fact that stomates on the surfaces of the fronds are permanently open in duckweed, thus transpiration rate would be expected to be rather high when the fronds are separated from direct contact with water and the root may not be able to keep up.

small surface waves and currents.

Some of those Lemnaceae lacking roots have downward-facing protuberances or appendages which may serve in place of roots for stabilizing the plants. Thus, Landolt proposes that the ribbon-shaped appendages of *Wolffiella hyalina* serves as a flotation stabilizer.

Observations

The roots of Lemna are sticky.

In physiological and biochemical experiments with *Lemna gibba*, it is often necessary to manipulate the plants without damage. I have found that these operations are made tedious by the adherence of the roots to surfaces onto which they are placed. This is particularly true once the bulk water has been removed from the surface by blotting. The roots will attach not only to porous surfaces like paper, but also to smooth surfaces like the sides of glass vessels and stainless steel spatulas.

The roots first bind to the surface by capillary action, but the bond becomes stronger as the root dries onto the surface. Even when still moist, it is difficult to move the roots with gentle rinses of water. Once a root dries onto a surface, it may be necessary to break it off to remove the plant. It appears that the root (and also possibly the underside of the frond) possess a sticky surface, which quickly acts to glue the root to dry materials.

Stickiness in fresh roots of other species has been reported before, and is known to be produced by surface charge (Tanada, 1978). That effect is under phytochrome control. The effect reported here may not be related to those effects, since the roots of Lemna show no adhesive properties until the bulk water has been removed and they are drawn into contact with a surface by capillary action.

Proposal

I propose that an important function of Lemnacean roots (and possibly the root-like appendages in some members) is to aid dispersal. The sticky surface of these organs should promote their attachment to fur or feathers of animals and birds they contact. Their natural resistance to desiccation (Landolt, 1986) would then allow these plants to be transported to distant bodies of fresh water. At the new site mechanical action or prolonged soaking would then free the plant.

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Opinion paper: Duckweed genome research at present and in future

Ingo Schubert

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, D-06466 Stadt Seeland, Germany

With the option to use the nearly cosmopolitan, fast growing and easy-to-harvest aquatic duckweeds in an industrial scale for livestock feeding, wastewater remediation, biofuel generation and even for human food, an increasing interest emerged in genetics and genomics of this group of monocot plants.

Genetic maps are not available and difficult to be established for most of the duckweed clades that propagate mainly or exclusively via vegetative proliferation. Promoting clonal selection and improvement of duckweeds as crops for aquaculture and biotechnology urgently needs understanding of their genomic composition. Several efforts are undertaken to sequence duckweed genomes. Draft genomes are available for *Spirodela polyrhiza* (Wang et al. 2014; Michael et al. 2017), and for *Lemna minor* (Van Hoeck et al. 2015), or are in pr,ogress, e.g. for *S. intermedia* (our laboratory) and for further species in other labs. Furthermore, genotyping-by-sequencing is ongoing in the Appenroth lab for unambiguous identification of all *Lemna* species. Correct genome assembly requires genetic maps as a reference or alternative approaches for mutual refinement of results. Serial multicolor fluorescent in situ hybridization (mcFISH) is such an approach which has successfully been applied for validation and chromosomal integration of assembled supercontigs of *S. polyrhiza* (Cao et al. 2016). Another alternative approach is optical mapping (BioNano, Michael et al. 2017). In near future a combination of these techniques will provide further improvement of the chromosomally integrated genome map of *S. polyrhiza*. Having sufficient anchor points, derived from a BAC tiling path, comparative cross-hybridization into a related species, for which genomic, genetic and chromosomal data are lacking, is possible and provides insight into chromosome homeology and linkage group relations between the compared species. This is demonstrated in our lab for the second Spirodela species *S. intermedia*.

Genomic data are not only essential for potential economic applications. Also under evolutionary aspects duckweed genomes are a bonanza. The *S. polyrhiza* genome revealed two ancestral whole genome duplications (Wang et al. 2014) and the seven ancestral homeologous gene blocks could be assigned to 19 of the 20 extant *S. polyrhiza* chromosome pairs (Cao et al. 2016). Moreover, the less than 20,000 genes reflect the neotenic reduction of organismic complexity and the aquatic life style. A copy number reduction of genes involved in water transport, lignin biosynthesis and cell wall organization by 28% compared to the *Arabidopsis thaliana* genome of similar size (157 Mbp/1C) was found (Wang et al. 2014). A very low copy number of 45S rDNA units were reported by Michael

et al. (2017).

Another peculiar feature of duckweeds is that genome size and genome size range increased with the degree of neoteny from about 160 Mbp/1C for the two Spirodela species (Bog et al. 2015) towards >1,800 Mbp/1C in the minute Wolffia arrhiza (Wang et al. 2011). Neoteny and genome size increase paralleled by a decrease in body size. First it will be of interest to find out via genome sequencing or via chromosome counting and cross-in situ hybridization with single copy sequences, whether the genome size variation by more than one order of magnitude is based on whole genome duplication(s) or rather on genome expansion by repeat amplification after duckweed radiation. In case of neo- or mesopolyploidy, single copy probes should yield more than one pair of FISH signals, while diploid genomes that expanded mainly by retroelement spreading, should display only one signal pair. A prerequisite for the success of such investigations is a cross-hybridization of single copy sequences between genomes of different genera as reported for Brassicaceae (e.g. Lysak et al. 2006). In this context it is remarkable that the so far studied plant species revealed a positive correlation between genome size, nucleus size and cell size (Jovtchev et al. 2006). If true also for duckweeds, one could expect an evolutionary progressing reduction of cell number per organisms towards the phylogenetically younger duckweed clades *Wolffiella* and *Wolffia*. Thus, exciting insights are expected to emerge from comprehensive cytogenomic and cytological investigation of duckweeds.

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This is a revised version of the article as by mistake the list of references was not published in the original issue of Duckweed Form No. 18.

MADS about duckweeds

Günter Theißen (guenter.theissen@uni-jena.de)

Department of Genetics, Friedrich Schiller University, Jena, Germany.

Duckweeds (Lemnaceae) are the smallest and morphologically simplest of flowering plants (angiosperms); they all lack a stem and many species even roots (Wang et al., 2014). As a geneticist working on the field of evolutionary developmental biology (evo-devo, for short) I am deeply impressed by the dramatic simplification and miniaturization that the angiosperm body plan must have undergone during the origin of the duckweeds.

According to the evo-devo rationale changes in adult body plans of multicellular organisms are brought about by changes in developmental processes from the zygote to the adult. Since developmental processes are largely under genetic control, studies on changes in developmental control genes are crucial for an understanding of the proximate mechanisms that generate morphological novelties (Theißen et al., 2000). Thus the intriguing question arises as to which mutations in which developmental control genes have brought about the dramatic morphological transitions on the way to duckweeds.

Employing the detailed knowledge about the genetics of developmental control in model angiosperms such as *Arabidopsis thaliana,* quite a number of reasonable candidates can easily come to mind. In plants, many (if not most) genes that control or fine-tune development are members of large and often evolutionary ancient gene families encoding transcription factors (TF) or microRNAs (miRNAs). For example, different types of homeodomain (HD) TFs are involved in stem cell maintenance in the shoot and in the root (members of the WUSCHEL-related (WOX) protein family), and appear to be master regulators of embryonic apical fate (HD-Zip III proteins); many MIKCc-group MADS-domain TFs control meristem and floral organ identities; some TCP TFs control cell proliferation in developing tissues such as axillary meristems and thus affect plant shape by controlling branching and apical dominance; YABBY TFs may have an ancient function in promoting lamina outgrowth along an adaxial-abaxial boundary (for a review about the TFs that "shape the plant", see e.g. Melzer and Theißen, 2011). Likewise, the miRNAs miR156 and miR172, targeting the mRNAs encoding other types of TFs (SPL and AP2-like proteins, respectively), are conserved key regulators of various phase transitions in angiosperms, including the juvenile-to-adult vegetative transition (Huijser and Schmid, 2011).

Given the extreme deviation of duckweed development and morphology from that of ordinary angiosperms, changes in genes that control phase transitions, promote stem cell maintenance, meristem or organ identity appear almost unavoidable during duckweed origin and diversification. Thus the types of genes mentioned above are prime candidates for loci that have undergone relevant mutations during duckweed evolution. For instance, there is evidence that the frond of duckweeds represents a juvenile tissue (somewhat resembling a floating cotyledon that generates new cotyledons) that originated by neoteny (the prolongation of juvenile traits) from an ordinary Araceae ancestor (Wang et al., 2014). In line with this, sequencing of the first duckweed genome, that of *Spirodela polyrhiza*, revealed in comparison to *Arabidopsis thaliana* increases in gene numbers ("copy numbers") of genes encoding repressors of the transition from juvenile to adult phase, and a reduction of components of gene regulatory networks (GRNs) that enhance the progression through the adult phase and the onset of inflorescence meristem formation (Wang et al., 2014). In line with this, the number of genes that promote the juvenile phase was also found to be increased (Wang et al., 2014). However, complete loss or new gains of specific types of developmental control genes were not reported.

We used the first known duckweed genome sequence for some preliminary investigations on our favorite gene family, the MIKCc-group MADS-box genes. Previous investigations had identified 17 clades of highly conserved MIKCc-group genes that very likely existed already in the most recent

common ancestor (MRCA) of extant angiosperms (Gramzow et al., 2014). Most of these clades have never been lost in any sequenced plant genome, reflecting the important role that the clade members play in angiosperm development and evolution. In the *Spirodela polyrhiza* genome, however, we did not find even a single representative of four clades, termed AGL9-like (also known as SEP3-like), AGL12-like, FLC-like and OsMADS32-like genes. Thus the *Spirodela polyrhiza* genome revealed the highest number of missing MIKCc-group gene clades observed in any angiosperm genome (Gramzow and Theißen, 2015). It was tempting to speculate that there is a causal relationship between the extreme simplification of the duckweed body plan and loss of some MIKCc-group gene clades – the simplest assumption being that some genes were just not needed anymore in an organism as simple as *Spirodela* (Gramzow and Theißen, 2015). One should note, however, that the genome version that we had investigated covered only about 90% of genic sequences, so that some genes may have been missed (Wang et al., 2014; Gramzow and Theißen, 2015). However, more clones of *Spirodela polyrhiza* (including clone 9509 that had been collected at a site near my current hometown; Michael et al., 2017), and also other species of duckweeds (*Lemna gibba*, *L. minor*) are being sequenced, so that much more rigorous analyses can be done in the near future. Considerable gene loss in duckweed genomes seems not to be restricted to MIKCc-group genes, since *Spirodela polyrhiza* has 28% less predicted protein-coding genes than *Arabidopsis thaliana*, and even 50% less than *Oryza sativa* (rice) (Wang et al., 2014).

Despite the loss of some clades of MIKCc-group genes we found representatives of all the different classes of floral homeotic genes, except for a subfamily of class E genes (AGL9-like genes). Taking the considerable redundancy among class E genes in many angiosperm species into account, this finding suggests that *Spirodela polyrhiza* has still a set of floral homeotic genes for specifying all floral organ identities. Indeed, duckweeds, despite the juvenile character of their fronds and the simplicity of their body plan, still flower, some even with surprising frequency (Sree et al., 2015).

It would be very exciting to test the functional importance of the genes encoding TFs and miRNAs that potentially control duckweed development and evolution. The fact that some duckweed species (*Landoltia punctata*, *Lemna gibba*, *L. minor*) are amenable to transformation, together with the versatility of modern genome editing tools such as CRISPR-Cas9, make such studies appear feasible. However, one should not think about duckweed evolution only in terms of miniaturization, simplification and neotenous reduction. Some duckweeds have even evolved novel structures, such as in case of *Wolffia microscopica* a protrusion on the ventral surface termed a “pseudoroot” (Sree et al., 2015). To investigate how preexisting GRNs have been recruited and modified during the origin of such evolutionary novelties would be an additional attractive goal for future evo-devo studies on duckweeds.

Acknowledgements

I thank Klaus Appenroth for his kind invitation to write this little article, and him and K. Sowjanya Sree for resurrecting my interest in duckweeds and for valuable discussions. Many thanks also to Lydia Gramzow for believing me (and other biologists) that duckweeds are really flowering plants and including them into her MADS phylogenomics studies.

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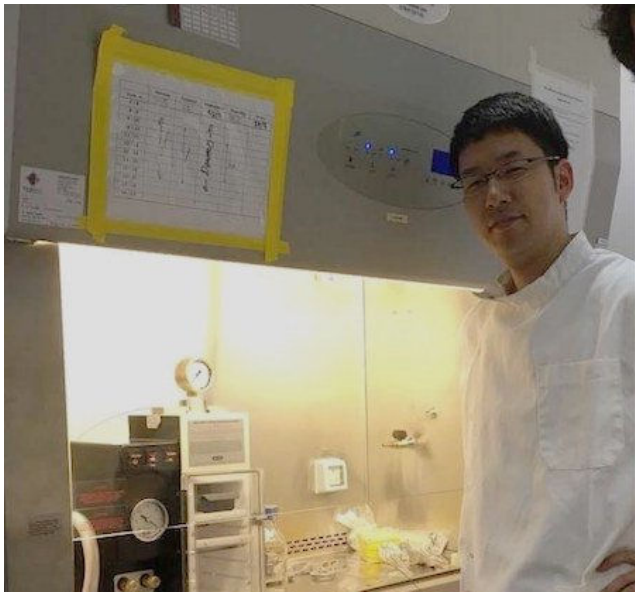
The majority of current members of the Theißen lab. Almost everyone of us is MADS about something, including duckweeds (and it shows)…

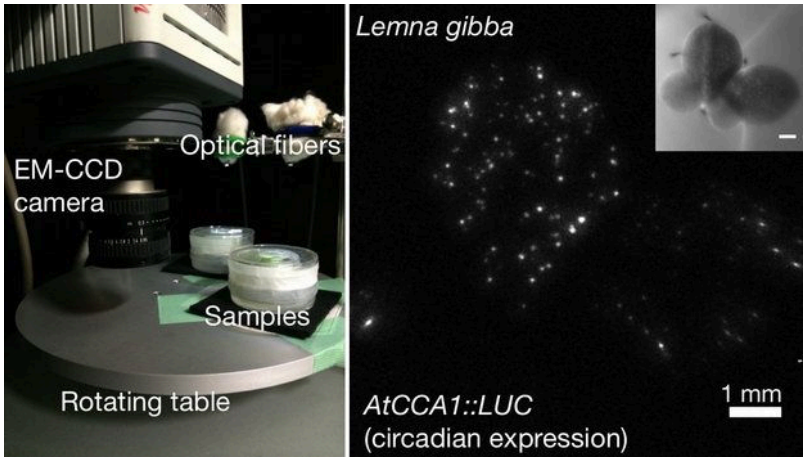
Student Spotlight: Mr. Masaaki Okada

I had some opportunities to study the latest research about the circadian rhythm during a master's course in Tokyo University of Science (2011–2012), in which I studied defense responses and autophagic dynamics in plants using tobacco BY-2 cells. Studies of the circadian clock, which is a basic cellular mechanism that is conserved in biological organisms, really appealed to me. Thus in 2012 I visited Prof. Tokitaka Oyama, who was researching on circadian rhythms using duckweed plants and cyanobacteria as model systems at the Department of Botany at Kyoto University, Japan. To be honest, I did not know duckweed plants and I've never paid attention to such tiny plants before I looked at his huge collections of duckweeds in the cultivation room. At that moment, in his laboratory two projects were ongoing using duckweed plants: comparison of circadian properties among various duckweeds and characterization of cellular circadian rhythms at the whole plant level. Since I had an interest in the circadian behavior of individual cells and interactions between cells that compose a plant body, I joined the Oyama laboratory started my PhD work there in April 2013.



In the Oyama laboratory, we investigate circadian rhythms by monitoring bioluminescence of duckweed plants transfected with a circadian-regulated luciferase reporter. The reporter gene is transfected to plants using a particle bombardment apparatus and only transfected cells emit bioluminescence. Thus, we can analyze properties of circadian rhythms at the single cell level. Since Lemna plants are flat and grow horizontally, they are ideally suitable for long-term monitoring using our custom-made microscope platform.

For my PhD thesis research, my results revealed that circadian clocks show heterogeneous properties among cells even in the same frond of *Lemna gibba*, when they are synchronized to light/dark cycles with a short period (see article cited in the Database section of this Duckweed Forum issue). In May 2017, I have just finished my PhD, and at present I'm working as a postdoctoral researcher in the same laboratory. Now we are trying to apply the single-cell monitoring method to other duckweed species and *Arabidopsis thaliana*, and to compare the property of the circadian clock system between plant species.



In August 2013, I fortunately got an opportunity to participate in The Second International Conference on Duckweed Research and Applications at Rutgers, New Jersey, USA. I was very impressed by a rich diversity of studies using duckweeds. Also, I was happy to meet many colleagues again and to discuss with them at the 3rd ICDRA held at our university in July 2015.

That experience of engaging with researchers from diverse locations in the world, especially around the same age, is motivating me. I think there is great opportunity to take advantage of duckweed plants for both research and applications in Japan, because they are still unfamiliar here for most people. I hope that our study can help spread awareness of duckweed plants and their special qualities and potential into Japanese society.

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.

Lemna minor plants chronically exposed to ionising radiation: RNA-seq analysis indicates a dose rate dependent shift from acclimation to survival strategies

Van Hoeck, A; Horernans, N; Nauts, R; Van Hees, M; Vandenhove, H; Blust, R

PLANT SCIENCE 257: 84-95 (2017)

Ecotoxicological research provides knowledge on ionising radiation-induced responses in different plant species. However, the sparse data currently available are mainly extracted from acute exposure treatments. To provide a better understanding of environmental exposure scenarios, the response to stress in plants must be followed in more natural relevant chronic conditions. We previously showed morphological and biochemical responses in *Lemna minor* plants continuously exposed for 7 days in a dose-rate dependent manner. In this study responses on molecular (gene expression) and physiological (photosynthetic) level are evaluated in *Lemna minor* plants exposed to ionising radiation. To enable this, we examined the gene expression profiles of irradiated *L. minor* plants by using an RNA-seq approach. The gene expression data reveal indications that *L. minor* plants exposed at lower dose rates, can tolerate the exposure by triggering acclimation responses. In contrast, at the highest dose rate tested, a high number of genes related to antioxidative defense systems, DNA repair and cell cycle were differentially expressed suggesting that only high dose rates of ionising radiation drive *L. minor* plants into survival strategies. Notably, the photosynthetic process seems to be unaffected in *L. minor* plants among the tested dose rates. This study, supported by our earlier work, clearly indicates that plants shift from acclimation responses towards survival responses at increasing dose rates of ionising radiation.

Evaluation of environmental bacterial communities as a factor affecting the growth of duckweed *Lemna minor*

Ishizawa, H; Kuroda, M; Morikawa, M; Ike, M

BIOTECHNOLOGY FOR BIOFUELS 10: Article Number: 62, DOI: 10.1186/s13068-017-0746-8 (2017)

Duckweed (family Lemnaceae) has recently been recognized as an ideal biomass feedstock for biofuel production due to its rapid growth and high starch content, which inspired interest in improving their productivity. Since microbes that co-exist with plants are known to have significant effects on their growth according to the previous studies for terrestrial plants, this study has attempted to understand the plant-microbial interactions of a duckweed, *Lemna minor*, focusing on the growth promotion/inhibition effects so as to assess the possibility of accelerated duckweed production by modifying co-existing bacterial community. Co-cultivation of aseptic *L. minor* and bacterial communities collected from various aquatic environments resulted in changes in duckweed growth ranging from -24 to + 14% compared to aseptic control. A number of bacterial strains were isolated from both growth-promoting and growth-inhibitory communities, and examined for their co-existing effects on duckweed growth. Irrespective of the source, each strain showed promotive, inhibitory, or neutral effects when individually co-cultured with *L. minor*. To further analyze the interactions among these bacterial strains in a community, binary combinations of promotive and inhibitory strains were co-cultured with aseptic *L. minor*, resulting in that combinations of promotive-promotive or inhibitory-inhibitory strains generally showed effects similar to those of individual strains. However, combinations of promotive-inhibitory strains tended to show inhibitory effects while only *Aquitalea magnusonii* H3 exerted its plant growth-promoting effect in all combinations tested. Significant change in biomass production was observed when duckweed was co-cultivated with environmental bacterial communities. Promotive, neutral, and inhibitory bacteria in the community would synergistically determine the effects. The results indicate the possibility of improving duckweed biomass production via regulation of co-existing bacterial communities.

Biotechnology

Abscisic Acid-induced starch accumulation in bioenergy crop duckweed *Spirodela polyrhiza*

Wang, XZ; Cui, WH; Hu, WW; Feng, CP

BIOENERGY RESEARCH 10: 417-426 (2017)

Spirodela polyrhiza, a fast-growing duckweed with high starch and low lignin content, shows promise as a feedstock for bioenergy. Abscisic acid (ABA) is a biological hormone that controls plant growth and stress response. The effects of different ABA concentrations (0, 1.0 x 10(-5), 1.0 x 10(-4), 1.0 x 10(-3), 1.0 x 10(-2), and 1.0 x 10(-1) mg/L) on duckweed biomass growth, carbon dioxide fixation, formation of photosynthetic pigments (Chlorophyll a (Chla), Chlorophyll b (Chlb), and carotenoids), the activities of soluble starch synthase (SSS) and starch branching enzyme (SBE), and the starch content of biomass were investigated in this study. ABA at concentrations lower than 1.0 x 10(-3) mg/L promoted carbon dioxide fixation, whereas it inhibited carbon dioxide fixation at concentrations over 1.0 x 10(-3) mg/L. ABA enhanced SSS and SBE activities at concentrations lower than 1.0 x 10(-2) mg/L. ABA treatment increased the content of Chla, Chlb, and carotenoids and resulted in the enhancement of starch content. Chla content gradually increased with the increasing concentration of ABA (1.0 x 10(-5) to 1.0 x 10(-2) mg/L). After culturing for 10 days, starch content in 1.0 x 10(-2) mg/L ABA medium reached 35.3% of dry weight (DW), which was the highest level in this study. This suggests that there is a great potential to develop a technology to increase starch accumulation in duckweed which can be used as an alternative to corn, sugarcane, or other food crops as a starch source.

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 CH4 yield (mL CH4 g(-1), VS) ranged between 60 and 468 for pre-treated, and 9 and 76 for non-pre-treated. The maximum CH4 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 CH4 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 (NO3--N)-76.5%; orthophosphate (PO4-3-P)-76%; sulphate (SO4-2)-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.

An assessment of duckweed as a potential lignocellulosic feedstock for biogas production

Yadav, D; Barbora, L; Bora, D; Mitra, S; Rangan, L; Mahanta, P

INTERNATIONAL BIODETERIORATION & BIODEGRADATION 119: 253-259 (2017)

Due to the complicated structure of lignocellulosic plant cell wall, their utilization for biogas production via anaerobic digestion has not been widely adopted. Alternative to this is to use aquatic plant materials as feedstock for biogas production. In this context, duckweed, an aquatic plant may

prove to be a promising new energy source for bioenergy as well efficient CO2 sequestration. This study entails a detailed characterization of duckweed to evaluate their potential as an alternate feedstock to cattle dung for biogas production. The duckweed was characterized for volatile matter, moisture content, ash content and carbon, hydrogen, and nitrogen (CHN) content. Property analysis of duckweed was also done by Fourier transform spectroscopy and thermogravimetric analysis. The volatile matter of duckweed was found to be 84.24 +/- 0.2% with a lignin content of 12.2%, which is very encouraging for biogas production. Co-digestion of duckweed (DW) with cattle dung (CD) in varying ratios (DW:CD = 90:10, 75:25, and 50:50 respectively) in batch type anaerobic digesters was performed at 37 degrees C temperature for 55 days. The cumulative biogas production for CD (100%), DW/CD (90:10), (75:25) and (50:50) was found to be 11,620, 305, 11,695, and 12,070 mL, respectively, which indicated that duckweed can be a potential lignocellulosic feedstock when co-digested with cattle dung at an optimum ratio of 1:1. Methane content of the biogas from co-digested feedstock is comparable to the biogas from cattle dung alone.

Evidence for organic phosphorus activation and transformation at the sediment-water interface during plant debris decomposition

Zhang, WQ; Zhu, XL; Jin, X; Meng, X; Tang, WZ; Shan, BQ

SCIENCE OF THE TOTAL ENVIRONMENT 583: 458-465 (2017)

The processes and mechanisms through which phosphorus (P) is released from sediment and organic P is transformed, induced by the decomposition of plant (duckweed (*Lemma minor* L)) debris, were studied experimentally. In the simulation experiments, the dissolved oxygen concentration, pH, and oxidation-reduction potential at the water-sediment interface first decreased rapidly. The lowest oxidation-reduction potential reached was 225.4 mV, and the solution became weakly acidic (pH 5.14) and anoxic (dissolved oxygen concentration 0.17 mg. L-1). The dissolved oxygen concentration, pH, and oxidation-reduction potential then became stable. The soluble reactive P, total dissolved P, and total P concentrations in the overlying water all increased rapidly because of the particulate P and dissolved organic P released as the plant debris decomposed. P-31 NMR analysis of the solution showed that orthophosphate monoesters were the main organic P compounds in the sediment. The orthophosphate monoester and orthophosphate diester concentrations were higher during the first 7 d of the experiment (at 71.2 and 153 mg.kg(-1), respectively) than later (60.8 and 14.6 mg.kg(-1), respectively). The decomposition of the duckweed could have mineralized the orthophosphate monoesters and orthophosphate diesters to give orthophosphate. The results indicated that the decomposition of aquatic plant debris is a key factor in the release of P from sediment even when external P is excluded. It is therefore necessary to remove plant debris from freshwater ecosystems to control the release of P from plant debris and sediment.

Comparative study of compounds of primary exchange duckweed (*Lemna minor* L.), trisulki duckweed (*Lemna trisulca* L.) and spirodela (*Spirodela polyrhiza* L. Schleid.)

Nikiforov, LA; Fursa, NS; Krivoshchekov, SV; Kurkin, VA; Belousov, MV

BYULLETEN SIBIRSKOY MEDITSINY 16: 59-64 (2017)

The purpose of the paper is to study qualitative composition and quantitative content of primary exchange compounds in duckweed (*Lemna minor L.*), trisulki duckweed (*Lemna trisulca L.*) and Spirodela (*Spirodela polyrhiza* (L.) Schleid). The subject of the study was air-dried samples of grass collected during their 20102011 growing season in low-flow and stagnant water bodies of Kozhevnikovsky and Tomsk districts of the Tomsk region. The concentration of free monosaccharides was determined by direct-phase high-performance liquid chromatography. The concentration of the bound sugars was determined by capillary electrophoresis using Applied Biosystem 273T (Thermophischer Ltd., USA). To obtain data on the qualitative composition and quantitative content of amino acids, the amino acid analyzer Hitachi 835 (Japan) was used. It was

found out that the least amount of amino acids contained in the water extract from *Lemna trisulca* - 96,14 mg, which is 2 times less than in extracts of *Lemna minor* and *Spirodela polyrhiza* (205,65 and 208,38 mg, respectively). In duckweed the minimum content of free and bound monosaccharides was determined to be 10,54%, while in the *Lemna trisulca* and *Spirodela polyrhiza* their content was 14,30% and 15,35%, respectively. This study showed the qualitative and quantitative differences of free and bound monosaccharide and amino acid composition between previously mentioned species.

Ecology

"Step by step": high frequency short-distance epizoochorous dispersal of aquatic macrophytes

Coughlan, NE; Kelly, TC; Jansen, MAK

BIOLOGICAL INVASIONS 19: 625-634 (2017)

Aquatic macrophytes can successfully colonise and re-colonise areas separated by space and time. The mechanisms underlying such "mobility" are not well understood, but it has often been hypothesised that epizoochory (external dispersal) plays an important role. Yet, there is only limited, and mostly anecdotal, evidence concerning successful epizoochorous dispersal of aquatic macrophytes, particularly in the case of short-distance dispersal. Here we examine *in situ* and *ex situ* dispersal of aquatic macrophytes, including three invasive alien species. A high frequency of *Lemna minor* Linnaeus dispersal was observed in situ, and this was linked to bird-mediated epizoochory. We concluded that wind had no effect on dispersal. Similarly, in an ex situ examination *Lemna minuta* Kunth and *Azolla filiculoides* Lamarck, were found to be dispersed with a high frequency by mallard ducks (*Anas platyrhynchos*). No dispersal was measured for *Elodea nuttalli* (Planchon) H. St. John. It is concluded that short-distance or "stepping-stone" dispersal via bird-mediated epizoochory can occur with high frequencies, and therefore can play an important role in facilitating colonisation, range expansion and biological invasion of macrophytes.

Evaluation of some physiological parameters of *Lemna minor* L. exposed to different hypertrophic phosphate levels

Sivaci, ER; Sevgiler, Y; Duman, S; Yardimci, M; Eroglu, S; Sivaci, A

FRESENIUS ENVIRONMENTAL BULLETIN 26: 1589-1594 (2017)

The aim of this study was to determine the response of *Lemna minor* L. to 6, 16, and 60 mg.L-1 phosphate concentrations, which are resembled in hypertrophic lake types, for 24, 48, 96, and 144 hours in laboratory conditions. Glutathione reductase (GR; EC 1.8.1.7) and catalase (CAT; EC 1.11.1.6) activities, thiobarbituric acid reactive substances (TBARS), total protein, chlorophyll a and b, carotenoids, and total phenolics contents in *L. minor* were determined spectrophotometrically in different phosphate concentrations at different time intervals. GR showed the maximum decrease at the minimum phosphate concentration at 24(th) hour. CAT also decreased at 24(th), 48(th), and 96(th) hours, especially at high phosphate levels. TBARS decreased at 6 mg.L-1 and 60 mg.L-1 phosphate concentrations at 144(th) and 24(th) hours, respectively. Total protein content decreased at 96(th) and 144(th) hours, especially at the highest phosphate concentration. Chlorophyll a decreased at 48(th) and 144(th) hours especially at the highest phosphate level, whereas chlorophyll b content increased at 48(th) and 96(th) hours at the same concentrations. The contents of phenolics and carotenoids were not affected except for a decrease in carotenoids at 48(th) hour. As a result, phosphate at its hypertrophic levels lead to pronounced effects in *L. minor*.

Distributions of vascular plants in the Czech Republic. Part 3

Kaplan, Z; Danihelka, J; Lepsi, M; Lepsi, P; Ekrt, L; Chrtek, J; Kocian, J; Prancl, J; Kobrlova, L; Hrones, M et al.

PRESLIA 88: 459-544 (2016)

The third part of the publication series on the distributions of vascular plants in the Czech Republic includes grid maps of 105 taxa of the genera *Acorus*, *Amelanchier*, *Asplenium*, *Calla*, *Cerastium*, *Ceratophyllum*, *Eichhornia*, *Hieracium*, *Hippuris*, *Hottonia*, *Lemna*, *Limosella*, *Peplis*, *Pistia*, *Pontederia*, *Sorbus*, *Spirodela*, *Symphytum*, *Trapa*, *Valerianella* and *Wolffia*. The maps were produced by taxonomic experts based on all available herbarium, literature and field records. Three of the studied genera include Czech endemics, which are confined to small geographic areas, mostly have small population sizes and thus are of conservation concern. These maps resulted from detailed fieldwork and herbarium revisions by monographers of the respective groups and for many of these endemics they are first available maps. The endemic species of *Hieracium* occur mainly in the subalpine habitats in the Krkonose, Kralicky Sneznik and Hruby Jesenik Mts. By contrast, a great majority of *Sorbus* endemics are found mainly in thermophilous open broad-leaved and pine forests on rocky habitats at middle altitudes. *Cerastium alsinifolium* is confined in its total distribution to serpentine outcrops in western Bohemia. *Asplenium* is another ecologically specialized group, which includes petrophytes, some of which are restricted to specific substrates, such as siliceous, limestone, basalt or serpentine rocks. The plants studied include 53 taxa classified in the Red List of vascular plants of the Czech Republic, some of which have shown remarkable declines. *Symphytum bohemicum*, distributed mainly in central Europe, is confined to calcareous fens in the lowlands. There are many endangered and vulnerable species amongst aquatic plants, which are threatened mainly by fish-farming intensification, eutrophicalion and habitat destruction. Populations of some of the most endangered and attractive aquatics, including *Hippuris vulgaris* and *Trapa natans*, have been lost and locally replaced by plants of unknown provenance purchased in garden stores, which causes a potential threat of genetic erosion of native populations. Attractive appearance is the reason why alien aquatics, such as *Eichhornia crassipes*, *Pistia stratiotes* and *Pontederia cordata*, are sometimes planted not only in garden pools but also in wetlands in the countryside; each has been recorded at about a dozen such sites during the past 25 years. *Lemna turionifera*, by contrast, has been introduced and dispersed by waterfowl and is now widespread in the country. The histories of the introduction and subsequent spread are also described and analysed for the widespread neophyte *Acorus calamus* and for the alien species of *Amelanchier* and *Symphytum*. Spatial distributions and temporal dynamics of individual species are shown in maps and documented by records included in the Pladias database and available in electronic appendices. The maps are accompanied by comments, which include additional information on the distribution, habitats, taxonomy and biology of the species.

Molecular Biology

Synchrony of plant cellular circadian clocks with heterogeneous properties under light/dark cycles

Okada, M; Muranaka, T; Ito, S; Oyama, T

SCIENTIFIC REPORTS 7, Article Number: 317, DOI: 10.1038/s41598-017-00454-8 (2017)

Individual cells in a plant can work independently as circadian clocks, and their properties are the basis of various circadian phenomena. The behaviour of individual cellular clocks in *Lemna gibba* was orderly under 24-h light/dark cycles despite their heterogeneous free-running periods (FRPs). Here, we reveal the entrainment habits of heterogeneous cellular clocks using non-24-h light/dark cycles (T-cycles). The cellular rhythms of AtCCA1:: LUC under T = 16 h cycles showed heterogeneous entrainment that was associated with their heterogeneous FRPs. Under T = 12 h cycles, most cells showed rhythms having similar to 24-h periods. This suggested that the lower limit of entrainment to the light/dark cycles of heterogeneous cellular circadian clocks is set to a period longer than 12 h, which enables them to be synchronous under similar to 24-h daily cycles without being perturbed by short light/dark cycles. The entrainment habits of individual cellular clocks are likely to be the basis of the circadian behaviour of plant under the natural day-night cycle with noisy environmental fluctuations. We further suggest that modifications of EARLY FLOWERING3 (ELF3) in individual cells

deviate the entrainability to shorter T-cycles possibly by altering both the FRPs and light responsiveness.

Physiology

Temporal dynamics in photosynthetic activity of *Spirodela polyrhiza* turions during dormancy release and germination

Olah, V; Hepp, A; Meszaros, I

ENVIRONMENTAL AND EXPERIMENTAL BOTANY 136: 50-58 (2017)

Turions (vegetative propagules) of aquatic plants functionally resemble seeds but in fact they are overwintering buds having the ability to photosynthesize. They spend long periods in dormant state in the dim and cold bottom layer of natural waters during winter of temperate regions. In this study photosynthetic acclimation of *Spirodela polyrhiza* (L.) Schleiden (giant duckweed) turions to contrasting environments was assessed in laboratory experiments by means of chlorophyll fluorescence induction and O-2-evolution methods and photosynthetic pigment content. Photosynthetic performance of turions had been monitored for 5 weeks in dark and cold (after-ripening) and then for 48 h under continuous irradiation at room temperature (germination induction). Photosynthetic activity of turions displayed biphasic acclimation during after-ripening. A gradual decrease in O-2-evolution, variable fluorescence and relative fluorescence decrease was observed during the first 3 weeks with a parallel increase in chlorophyll fluorescence parameters indicative of excitation energy dissipation. Most interestingly later, while turions were still kept in dark and cold, their photosynthetic activity increased again to the level of newly-formed turions. This temporal pattern suggests that changes in photosynthetic performance might be regulated in close connection with dormancy release of turions. After transferring after-ripened turions to warm and illuminated conditions their photosynthetic acclimation took place rapidly. All parameters reflecting photosynthetic efficiency of germinating turions reached or even exceeded the respective levels of newly formed control turions within 24-48 h.

High flavonoid accompanied with high starch accumulation triggered by nutrient starvation in bioenergy crop duckweed (*Landoltia punctata*)

Tao, X; Fang, Y; Huang, MJ; Xiao, Y; Liu, Y; Ma, XR; Zhao, H

BMC GENOMICS 18: Article Number: 166, DOI: 10.1186/s12864-017-3559-z (2017)

As the fastest growing plant, duckweed can thrive on anthropogenic wastewater. The purple-backed duckweed, *Landoltia punctata*, is rich in starch and flavonoids. However, the molecular biological basis of high flavonoid and low lignin content remains largely unknown, as does the best method to combine nutrients removed from sewage and the utilization value improvement of duckweed biomass. A combined omics study was performed to investigate the biosynthesis of flavonoid and the metabolic flux changes in *L. punctata* grown in different culture medium. Phenylalanine metabolism related transcripts were identified and carefully analyzed. Expression quantification results showed that most of the flavonoid biosynthetic transcripts were relatively highly expressed, while most lignin-related transcripts were poorly expressed or failed to be detected by iTRAQ based proteomic analyses. This explains why duckweed has a much lower lignin percentage and higher flavonoid content than most other plants. Growing in distilled water, expression of most flavonoid-related transcripts were increased, while most were decreased in uniconazole treated *L. punctata* (1/6 x Hoagland + 800 mg center dot L-1 uniconazole). When *L. punctata* was cultivated in full nutrient medium (1/6 x Hoagland), more than half of these transcripts were increased, however others were suppressed. Metabolome results showed that a total of 20 flavonoid compounds were separated by HPLC in *L. punctata* grown in uniconazole and full nutrient medium. The quantities of all 20 compounds were decreased by uniconazole, while 11 were increased and 6 decreased when grown in full nutrient medium. Nutrient starvation resulted in an obvious purple accumulation on the

underside of each frond. The high flavonoid and low lignin content of *L. punctata* appears to be predominantly caused by the flavonoid-directed metabolic flux. Nutrient starvation is the best option to obtain high starch and flavonoid accumulation simultaneously in a short time for biofuels fermentation and natural products isolation.

Phytoremediation

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 Co-operation and Development (OECD) protocol 221 and no inhibition on specific growth rate of *Lemna minor* was observed for concentrations up to 200 mu g 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.

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.

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

Iatrou, EI; Gatidou, G; Damalas, D; Thomaidis, NS; Stasinakis, AS

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

Stegemeier, JP; Colman, BP; Schwab, F; Wiesner, MR; Lowry, GV

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 AgNO3. 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 (*Spriodela 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.

Agro-Industrial effluent phytoremediation with *Lemna gibba* and *Hydrocotyle ranunculoides* in water recirculating mesocosms

Basilico, G; Magdaleno, A; Paz, M; Moretton, J; Faggi, A; de Cabo, L

CLEAN-SOIL AIR WATER 45: Article Number: 1600386, DOI: 10.1002/clen.201600386 (2017)

Treatment of agro-industrial effluents can be carried out via phytoremediation with mono-or multi-specific macrophyte crops. A 6 day phytoremediation assay was performed in mesocosms with continuous recirculation of wastewater from a poultry industry with the macrophytes *Lemna gibba* and/or *Hydrocotyle ranunculoides*. The plant effects over removal of nutrients (N, P and C), fecal contamination indicators (total coliforms and *Escherichia coli*) and the genotoxicity of wastewater by the *Allium cepa* test were evaluated. A decrease by about 97.6% of total coliforms and *E. coli*, without significant differences between treatments was observed. The highest removal rates of ammonium (77.9%), dissolved inorganic nitrogen (77.9%), soluble reactive phosphorus (47.6%), total phosphorus (60.6%), and particulate organic carbon (82.1%) were observed in mesocosms with *L. gibba*+*H. ranunculoides*, although there were no significant differences ($p>0.05$) with respect to the monospecific culture of *L. gibba*. Lower rates of nutrients removal were observed in monospecific cultures of *H. ranunculoides*. Moreover, the use of both species meant a reduction of the genotoxicity of wastewater, with no chromosomal aberrations and micronuclei in meristematic root cells of *A. cepa*. The obtained results confirm the benefits of the joint use of *H. ranunculoides* and *L. gibba* for phytoremediation of wastewater from the poultry industry, being preferable to the use of monospecific crops.

Effective adsorption of aqueous Pb2+ by dried biomass of *Landoltia punctata* and *Spirodela polyrhiza*

Tang, J; Li, Y; Wang, X; Daroch, M

JOURNAL OF CLEANER PRODUCTION 145: 25-34 (2017)

Lead contamination has become a serious issue and approaches are continuously searched for lead removal. Here, dried biomasses of *Landoltia punctata* and *Spirodela polyrhiza* were utilized as adsorbent for Pb2+ removal. Batch experiments were conducted to investigate effects of contact time, initial pH, temperature, stirring speed, adsorbent dosage, and initial Pb2+ concentrations on Pb2+ adsorption. Results showed that all these parameters had significant effect on Pb2+ removal efficiency of the adsorbents. Both the adsorption processes followed the pseudo-second-order kinetic model, and were well described by Langmuir isotherm. Spectroscopy analysis indicated the involvement of functional groups (-OH, C-N, C=O, N-H and C-O) in Pb2+ adsorption. Remarkably, the maximum Pb2+ adsorption capacity of *L punctata* and *S. polyrhiza* were 250 and 200 mg g(-1) (dry weight), respectively. Thus, it could be concluded that the dried biomass of *L. punctata* and *S. polyrhiza* are promising adsorbents for effective lead removal.

Phytoremediation applications in natural condition and in mesocosm: The uptake of cadmium by *Lemna minuta* Kunth, a non-native species in Italian watercourses

Chiudioni, F; Trabace, T; Di Gennaro, S; Palma, A; Manes, F; Mancini, L

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 19: 371-376 (2017)

Metal pollution in water and soil is an environmental and public health issue. Cadmium (Cd) is included in the list of priority hazardous substances in the European Water Framework Directive. Phytoremediation system is a cost-effective, plant-based approach that takes advantage of the ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. We studied the presence and the importance of an invasive species, such as *Lemna minuta*, in the environment and the effects of Cd pollution on this species. Growth, removal, and tolerance were evaluated for different Cd concentrations and different times of plant exposure. Overall, the results show that *L. minuta* has a good capacity of growth, metal bioconcentration, and tolerance up to 3 days of exposure at 0.5 and 1.5 mg L-1 of Cd. In particular, *L. minuta* was able to accumulate Cd up to 3771 mg kg(-1) on dry mass basis. We can conclude that *L. minuta* possesses a great capability of Cd absorption and accumulation, thus supporting a potential use of this species in designing a metal bioremediation system in phytoremediation field.

Tolerance and hyperaccumulation of a mixture of heavy metals (Cu, Pb, Hg, and Zn) by four aquatic macrophytes

Romero-Hernandez, JA; Amaya-Chavez, A; Balderas-Hernandez, P; Roa-Morales, G; Gonzalez-Rivas, N; Balderas-Plata, MA

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 19: 239-245 (2017)

In the present investigation, four macrophytes, namely *Typha latifolia* (L.), *Lemna minor* (L.), *Eichhornia crassipes* (Mart.) Solms-Laubach, and *Myriophyllum aquaticum* (Vell.) Verdc, were evaluated for their heavy metal (Cu, Pb, Hg, and Zn) hyperaccumulation potential under laboratory conditions. Tolerance analyses were performed for 7days of exposure at five different treatments of the metals mixture (Cu+2, Hg+2, Pb+2, and Zn+2). The production of chlorophyll and carotenoids was determined at the end of each treatment. *L. minor* revealed to be sensitive, because it did not survive in all the tested concentrations after 72hours of exposure. *E. crassipes* and *M. aquaticum* displayed the highest tolerance to the metals mixture. For the most tolerant species of aquatic macrophytes, The removal kinetics of *E. crassipes* and *M. aquaticum* was carried out, using the following mixture of metals: Cu (0.5 mg/L) and Hg, Pb, and Zn 0.25 mg/L. The obtained results revealed that *E. crassipes* can remove 99.80% of Cu, 97.88% of Pb, 99.53% of Hg, and 94.37% of Zn. *M. aquaticum* withdraws 95.2% of Cu, 94.28% of Pb, 99.19% of Hg, and 91.91% of Zn. The obtained results suggest that these two species of macrophytes could be used for the phytoremediation of this mixture of heavy metals from the polluted water bodies.

Phytotoxicity

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 mu M). There was a concentration dependent increase in Ce accumulation, which reached a maximum of 67 mg g(-1) of dry weight (DW) at 60 mu M 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 DI,

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). 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 environmental 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 phyto-toxic 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.

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

Gomes, MP; Goncalves, CA; de Brito, JCM; Souza, AM; Cruz, FVDS; Bicalho, EM; Figueredo, CC; Garcia, QS

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 1(-1) while hormesis occurs at the lowest concentration studied (0.75 mg ciprofloxacin 1(-1)). By impairing normal electron flow in the respiratory electron transport chain, ciprofloxacin induces hydrogen peroxide (H2O2) production. The ability of plants to cope with H2O2 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.

Electrochemical oxidation of quinoline aqueous solution on beta-PbO2 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-PbO2 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 (center dot OH) electro-generated on a beta-PbO2 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. NO3- 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 Assessment of Phosphate Recyclates from Sewage Sludges

Rastetter, N; Rothhaupt, KO; Gerhardt, A

WATER AIR AND SOIL POLLUTION 228, Issue: 4, Article Number: 171 (2017)

Sewage sludge contains valuable plant nutrients, especially phosphorus. But unfortunately, it also contains pollutants which are hazardous for the environment. Phosphorus recovery from sewage sludge and its agricultural valorisation in recycling fertilisers based or containing recovered phosphate provides opportunities to minimise negative environmental effects caused by direct sludge application or conventional fertilisation. For validation, crystallised (struvite) and thermally treated phosphate recyclates (PRs) were chemically analysed, ecotoxicologically assessed and compared with a conventional phosphate fertiliser (triple superphosphate (TSP)). Three test species covering the environmental compartments water, sediment and soil were applied to evaluate the acute toxic effects of the phosphate fertiliser samples in laboratory tests (*Lemna minor*, *Gammarus fossarum*, *Eisenia fetida*). The assessment and comparison showed that TSP was more toxic than the PRs at the higher tested concentrations, probably due to a higher water solubility and not to chemical composition. Higher concentrations of the crystallised PRs caused mostly a slightly higher negative effect on tested parameters of the duckweed and the freshwater amphipod than the thermally treated PRs. Agronomical relevant application amounts of all PRs and TSP (worst case scenario) might not have an acute toxic effect on the soil invertebrates. The PRs might have minor effects on the growth of *L. minor*, and TSP might negatively affect the survival of the freshwater amphipods. Recovered phosphate-containing materials (PRs), in particular struvite, proved to be of high quality and low hazard in a relative risk ranking; thus, it could be one of the future alternatives of phosphorus fertilisation in agriculture.

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.

Mixture toxicity of six sulfonamides and their two transformation products to green algae *Scenedesmus vacuolatus* and duckweed *Lemna minor*

Bialk-Bielinska, A; Caban, M; Pieczynska, A; Stepnowski, P; Stolte, S

CHEMOSPHERE 173: 542-550 (2017)

Since humans and ecosystems are continually exposed to a very complex and permanently changing mixture of chemicals, there is increasing concern in the general public about the potential adverse effects they may cause. Among all "emerging pollutants", pharmaceuticals in particular have raised great environmental concern. For these reasons the aim of our study was to evaluate the mixture toxicity of six antimicrobial sulfonamides (SAs) and their two most commonly identified degradation products sulfanilic acid (SNA) and sulfanilamide (SN) - to limnic green algae *Scenedesmus vacuolatus* and duckweed *Lemna minor*. The ecotoxicological data for the single toxicity of SNA and SN towards selected organisms are presented. The concept of Concentration Addition (CA) was applied to estimate the effects, and less than additive effects were observed. In general terms, it seems sufficiently precautionary for the aquatic environment to consider the toxicity of a sulfonamide mixture as additive. The Concentration Addition model proves to be a reasonable worst-case estimation. Such a comparative study on the mixture toxicity of sulfonamides and their transformation products has been presented for the first time.

Fourier-transform infrared spectroscopy as a novel approach to providing effect-based endpoints in duckweed toxicity testing

Hu, LX; Ying, GG; Chen, XW; Huang, GY; Liu, YS; Jiang, YX; Pan, CG; Tian, F; Martin, FL

ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 36: 346-353 (2017)

Traditional duckweed toxicity tests only measure plant growth inhibition as an endpoint, with limited effects-based data. The present study aimed to investigate whether Fourier-transform infrared (FTIR) spectroscopy could enhance the duckweed (*Lemna minor* L.) toxicity test. Four chemicals (Cu, Cd, atrazine, and acetochlor) and 4 metal-containing industrial wastewater samples were tested.

After exposure of duckweed to the chemicals, standard toxicity endpoints (frond number and chlorophyll content) were determined; the fronds were also interrogated using FTIR spectroscopy under optimized test conditions. Biochemical alterations associated with each treatment were assessed and further analyzed by multivariate analysis. The results showed that comparable x% of effective concentration (ECx) values could be achieved based on FTIR spectroscopy in comparison with those based on traditional toxicity endpoints. Biochemical alterations associated with different doses of toxicant were mainly attributed to lipid, protein, nucleic acid, and carbohydrate structural changes, which helped to explain toxic mechanisms. With the help of multivariate analysis, separation of clusters related to different exposure doses could be achieved. The present study is the first to show successful application of FTIR spectroscopy in standard duckweed toxicity tests with biochemical alterations as new endpoints.

Microbial Detoxification of Deoxynivalenol (DON), Assessed via a *Lemna minor* L. Bioassay, through Biotransformation to 3-epi-DON and 3-epi-DOM-1

Vanhoutte, I; De Mets, L; De Boevre, M; Uka, V; Di Mavungu, JD; De Saeger, S; De Gelder, L; Audenaert, K

TOXINS 9: Article Number: 63, DOI: 10.3390/toxins9020063 (2017)

Mycotoxins are toxic metabolites produced by fungi. To mitigate mycotoxins in food or feed, biotransformation is an emerging technology in which microorganisms degrade toxins into non-toxic metabolites. To monitor deoxynivalenol (DON) biotransformation, analytical tools such as ELISA and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) are typically used. However, these techniques do not give a decisive answer about the remaining toxicity of possible biotransformation products. Hence, a bioassay using *Lemna minor* L. was developed. A dose-response analysis revealed significant inhibition in the growth of *L. minor* exposed to DON concentrations of 0.25 mg/L and higher. Concentrations above 1 mg/L were lethal for the plant. This bioassay is far more sensitive than previously described systems. The bioassay was implemented to screen microbial enrichment cultures, originating from rumen fluid, soil, digestate and activated sludge, on their biotransformation and detoxification capability of DON. The enrichment cultures originating from soil and activated sludge were capable of detoxifying and degrading 5 and 50 mg/L DON. In addition, the metabolites 3-epi-DON and the epimer of de-epoxy-DON (3-epi-DOM-1) were found as biotransformation products of both consortia. Our work provides a new valuable tool to screen microbial cultures for their detoxification capacity.

Uranium binding on *Landoltia punctata* as a result of formation of insoluble nano-U (VI) and U (IV) phosphate minerals

Nie, XQ; Dong, FQ; Bian, L; Liu, MX; Ding, CC; He, HC; Yang, G; Sun, SY; Qin, YL; Huang, R.et al.

ACS SUSTAINABLE CHEMISTRY & ENGINEERING 5: 1494-1502 (2017)

This work investigated the binding mechanism of uranium by an indigenous *Landoltia punctata* (*La. punctata*) in the wastewater at a uranium mine. The results showed that the removal capacity of the living (healthy fronds) and the dead (dried powder) *La. punctata* toward U (VI) were 40 and 132 mg/g after 2 h at pH 5, respectively. The U (VI) removal mechanisms of *La. punctata* were dependent on the pH of wastewater. SEM images and spectroscopic analysis indicated that U (VI) was immobilized as lamellar crystal insoluble nano-U (VI) and U (IV) phosphate minerals such as chernikovite by the living *La. punctata* at acidic pH after 30 min, which might have resulted from the binding with phosphate groups that were likely released from the organophosphorus of the living cells. In a contrast, U (VI) mainly existed as amorphous on the dead *La. punctata* via the complexation with amino and hydroxyl groups. Chernikovite was reduced into UO2 after hydrothermal treatment, while the main phase of uranium was transformed into other U (VI) and U (IV) phosphate minerals after ashing treatment. This simple process of biomineralization and reduction provides a potential method for the treatment of uranium-contaminated wastewater using

the living *La. punctata*.

Combined modelled and measured uptakes of arsenic and uranium by *Lemna gibba* G3 cells

Mkandawire, M; Irwin, DJG

CHEMISTRY AND ECOLOGY 33: 375-388 (2017)

With data from in vitro and in situ investigations, we developed a mathematical model to describe cellular uptake of uranium and arsenic in solution by living *Lemna gibba* under homeostatic regulation. The model considers the ability of healthy cells to resist accumulation of toxic metal species by regulating physicochemical properties of the cell membrane. In the bulk solution, the ratio of the total amounts of bioavailable metal ions to the metal ions uptake by the cells is very high. Consequently, the main rate-limiting processes of uptake are the biosorption kinetics on both external and internal surfaces at the biological interface, and the transport of the metal ions across the cell membrane. The model prediction correlates well with uptake results from field and microcosm experiments for uranium and arsenic by *L. gibba*, a model ecotoxicological test organism.

Antioxidant activity and ultrastructural alterations in the biosensor *Lemna minor* L. exposed in bags in Sarno river (South Italy)

Conte, B; Sorbo, S; Piscopo, M; Rabbito, D; De Ruberto, F; Guerriero, G; Basile, A

FRESENIUS ENVIRONMENTAL BULLETIN 26: 225-236 (2017)

Water pollution is a major environmental and human health issue and the Sarno river is regarded as the most polluted River in the whole of Europe. This study aimed at evaluating antioxidant response to freshwater pollution in the Sarno River (Campania, South Italy), using bags containing the biosensor water plant, *Lemna minor* L. Homogeneous samples of *L. minor*, collected from the Botanical Gardens of the University of Naples, were accurately washed and disposed in nylon bags. The bags were exposed for one week, floating on the water of the Sarno River at three sites, to determine antioxidant response to pollutants. Five heavy metal concentrations, representative of pollution of the River, were measured in the three exposure sites. Ultrastructural alterations of the exposed samples were examined to confirm the environmental stress, which underwent the exposes samples. Three different methods were used to measure the antioxidant activity: Trolox equivalent antioxidant capacity, chemiluminescence, and GST activity assays. Moreover, some specimens were in vitro exposed to the same heavy metal (HM) concentrations measured in the three exposure sites of the River and the results were compared with those obtained from the bag experiment. *L. minor* reacted to pollution increasing the antioxidant activity, which gets higher from the river springs towards the flatland (see bag experiments) and is related to the heavy metal concentrations (see in vitro experiments). Furthermore, TEM observations showed that cell ultrastructure alterations increased along the river in the same way as the antioxidant activity. The results confirmed the potential use of this species as biosensor and this methodological approach both in biomonitoring and phytoremediation studies.

Systematics and Evolution

Development of a New Marker System for Identification of *Spirodela polyrhiza* and *Landoltia punctata*

Feng, B; Fang, Y; Xu, ZB; Xiang, C; Zhou, CH; Jiang, F; Wang, T; Zhao, H

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Lemnaceae (commonly called duckweed) is an aquatic plant ideal for quantitative analysis in plant

sciences. Several species of this family represent the smallest and fastest growing flowering plants. Different ecotypes of the same species vary in their biochemical and physiological properties. Thus, selecting of desirable ecotypes of a species is very important. Here, we developed a simple and rapid molecular identification system for *Spirodela polyrhiza* and *Landoltia punctata* based on the sequence polymorphism. First, several pairs of primers were designed and three markers were selected as good for identification. After PCR amplification, DNA fragments (the combination of three PCR products) in different duckweeds were detected using capillary electrophoresis. The high-resolution capillary electrophoresis displayed high identity to the sequencing results. The combination of the PCR products containing several DNA fragments highly improved the identification frequency. These results indicate that this method is not only good for interspecies identification but also ideal for intraspecies distinguishing. Meanwhile, 11 haplotypes were found in both the *S. polyrhiza* and *L. punctata* ecotypes. The results suggest that this marker system is useful for large-scale identification of duckweed and for the screening of desirable ecotypes to improve the diverse usage in duckweed utilization.

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.

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