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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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Aphid infested *Spirodela polyrhiza* in a pond in Gujarat, India

Cover page

Aphid infested *Spirodela polyrhiza* in a pond in Gujarat, India

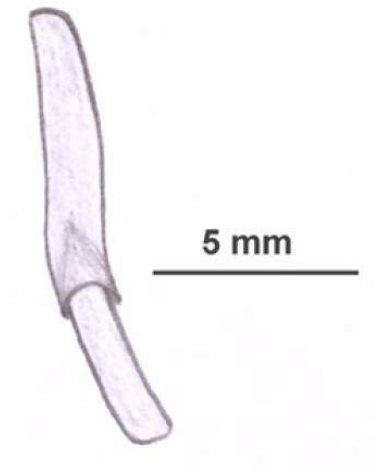
A pond in Gujarat, India covered by a mixed culture of *Spirodela polyrhiza* and *Wolffia globosa*. Of interest is the widespread infestation of *S. polyrhiza* fronds with an insect pest, aphids. One of the species could be identified as Waterlily aphids, *Rhopalosiphum nymphaeae* (https://www.mobot.org/jwcross/duckweed/duckweed-pests.html#Waterlily_Aphid). This might be of particular concern to farmers and application specialists who are trying to grow *S. polyrhiza* or other duckweed species on a large scale in open facilities. This problem could equally attract the attention of basic researchers. Photo by: Dr. K. Sowjanya Sree, Central University of Kerala, India.

In this issue

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

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

Science meets art: *Wolffiella denticulata* (Hegelm.) Hegelmaier



Wolffiella denticulata (Hegelm.) Hegelmaier was first described in1895. This species is endemic to Mozambique and South Africa. The fronds grow to less than 7 mm length and less than 0.8 mm in width. Fronds are submerged, with the basal part near the surface of the water and the tip bent downwards. The species flowers very rarely. In contrast to the opinion of C.F. Hegelmaier, E. Landolt showed that the stipe, after abscission of the daughter frond from the mother frond, remains in the pouch of the mother frond. The colonies consist of 2 to 7 fronds and have a star-like, i.e. stellate appearance. Urbanska (1980) reported chromosome numbers of n=20 and n>40. Most probably, diploid and tetraploid forms exist. In all stock collections, presently only one clone (8221) of this species exists. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor

Dear Duckweed Community,

Spring is here in the Northern Hemisphere and on behalf of all the members of our Steering Committee, I like to send our warm greetings to all readers of our community newsletter. Time truly flies as we already reached the twenty-fifth issue of the Duckweed Forum (DF). I sincerely believe that this newsletter has played an important role in sharing useful ideas and information in the working space of duckweed research and applications over the years since the second International Conference of our community at Rutgers University in 2013. I would like to thank everyone who has contributed to this newsletter for their time and dedication. With your input and continual support, I hope this important forum will continue to facilitate the growth of our community in the future.

As the commercial sector of duckweed applications gains momentum in rising numbers of start-up and the first duckweed products became available in mainstream market venues, issues confronting the methods of duckweed farming and integrated management became key factors for success. Appropriately, the Cover Photo for this issue of the Duckweed Forum (DF) illustrates potential insect pests such as aphids that can plaque open pond systems for duckweed production. Similarly, in the Discussion Corner, our Steering Committee members Klaus and Sowjanya commented that knowledge of affordable commercial fertilizer that could be used to effectively grow duckweed is urgently needed to enable adoption of economically viable duckweed farming for human food production. Thus, applied research in these areas should be important endeavors for long term sustainability of our effort to domesticate duckweed for bioproducts production. These may even find applications in the more futuristic vision of harnessing duckweed's unique qualities for space exploration, as illustrated by Space Lab Technologies, LLC in this issue. It cannot be overstated that cross-feeding of ideas and resources between basic and applied research in the duckweed community will be critical for overcoming the challenges, both biological and technical, that we will confront as different approaches are being taken to cultivate these plants as a crop at different scales.

In this issue of the DF, you will also find the current program for the upcoming International Conference at Rehovot, Israel, in September 2019. It features many excellent topics that are of interest to the duckweed community from fundamental research on basic biology of duckweed to characterization of duckweed's quality for human nutrition. This promises to be an excellent meeting indeed! Aside from the Science meets art contribution by Sowjanya and the Database by Klaus, both from our Steering Committee, we also have a fine article by Paul Ziegler to discuss his ideas about using duckweed as a system to produce a "Biomarker Bank" that may eventually be used to identify the types of contaminants that are present in water samples. A Student Spotlight by Hassana Ghanem from Lebanon and a Useful Methods article on the antibiotic cefotaxime by my laboratory round out the topics that you will find in this issue of DF. I hope you will find them all to be interesting and helpful to your endeavors.

Finally, I would like to invite you to check out the two requests for nominees to elect members for the next Steering Committee or hosting the next International Conference (ICDRA-2021). I hope that you will consider participating in both of these important activities in any way that you could, since community involvement is key for our mission to serve the general membership. Your contribution will be vital.

Best wishes to all,

Eric Lam, Chair of the ISCDRA

5th ICDRA: Scientific Program

Conference content: Talks and poster presentations on recent advances in duckweed genomics, physiology, microbiomes, ecosystems, ecotoxicology, nutrients, natural products, biomass production and other commercial applications.

Venue: All activities will be held at the Lopatie Conference Centre of the Weizmann Institute of Science. The Weizmann Institute is one of the world's leading multidisciplinary basic research institutions in the natural and exact sciences. It is located in the university town of Rehovot, between Tel Aviv and Jerusalem. The Weizmann Institute educates a substantial proportion of Israel's scientific leadership. There are 238 research groups and 1480 graduate students and postgraduate fellows at the Institute. A campus map can be viewed at https://map.weizmann.ac.il

Register at: http://www.weizmann.ac.il/conferences/DRA2019/registration. Fee includes admission to all sessions, conference kit, coffee breaks, lunch and dinner on conference days, half day trip, transport from and to airport. **Registration closes on 9th July, 2019**

Spotlight on posters: All delegates are urged to submit a poster. Those doing so will benefit from a reduction in registration fee. Poster viewing and poster talks will be featured at the conference and a best poster prize will be awarded. Posters will remain hung for the entire conference period and be situated at a strategic, central location. **Details for poster preparation at Registration site**

Accommodations: Details at **Registration site**

Travel: There are a multitude of direct and connecting international flights to Israel's Ben-Gurion International airport (TLV). See https://www.touristisrael.com/full-list-fliQhts-tel-aviv-isrqel/12331

Questions? Conference Secretariat talias@weizmann.ac.il

Conference WEB SITE: http://www.weizmann.ac.il/conferences/DRA2019

All Conference activities take place at the

David Lopatie Conference Centre, Weizmann Institute Campus

PROGRAM OUTLINE

Monday, Sept 9

14:00-17:30 *Registration*

David Lopatie Conference Centre, entrance lobby

Poster hanging

David Lopatie Conference Centre, main hall, poster area

Opening Session

Lectures (* Keynotes)

David Lopatie Conference Centre, Kimmel Lecture Hall

17:30-17:45	**Welcome**	
17:45-18:30	**Marcel Jansen***	Using duckweeds to resolve basic questions in ecosystem biology

18:30-20:30 *Mixer/buffet dinner*

David Lopatie Conference Centre, main hall, dining area

Tuesday, Sept 10

08:30-	*Registration, Poster hanging*	
	Lectures (* Keynotes)	
	David Lopatie Conference Centre, Kimmel Lecture Hall	
09:15-10:00	Asaph Aharoni*	Metabolic insights from duckweed metabolomics
10:00-10:30	Nikolai Borisjuk	Duckweed surface cuticle
10:30-11:00	*Coffee*	
11:00-11:30	Masaaki Morikawa	Bacteria and growth promotion of duckweed
11:30-12:00	Eric Lam	Duckweed microbiomes
12:00-13:15	*Lunch*	
13:15-14:45	Poster session 1	
14:45-15:30	K. Sowjanya Sree*	Flowering in duckweed
15:30-16:00	Ingo Schubert	Genome evolution among duckweeds
16:00-16:30	*Coffee*	
16:30-17:15	Tokitaka Oyama*	Circadian rhythms and single-cell analysis in duckweed
17:15-17:45	Uwe Heinig	Isoprenoid metabolism in duckweed - *Lemna* as a model for flux analysis in plants
17.45-18:15	*ISCDRA meeting*	
19:00-21:00	*Dinner*	Colloquium: WIS: Centre of duckweed research

Wednesday, Sept 11

Lectures (* Keynotes)

David Lopatie Conference Centre, Kimmel Lecture Hall

09:15-10:00	Todd Michael*	New technologies for genome mapping in duckweed
10:00-10:30	Shuqing Xu	Genetic variation and mutation rate in *Spirodela*
10:30-11:00	*Coffee*	
11:00-11:30	Hongwei Hou	Gene transformation protocols in duckweed
11:30-12:00	Sergey Dolgov	*Wolffia arrhiza* & *Lemna minor* as expression platforms for pharmaceutical & veterinary substances
12:00-13:00	*Lunch*	
13:00-14:30	Poster session 2	
14:30	*Trip to Jerusalem*	Guided tour Sound & Light show
	Dinner (Jerusalem)	

Thursday, Sept 12

Lectures (* Keynotes)

David Lopatie Conference Centre, Kimmel Lecture Hall

09:30-10:00	**Iris Shai**	Protein bioavailability in duckweed
10:00-10:30	**Klaus Appenroth**	Nutritional value of duckweeds
10:30-11:00	*Coffee*	
11:00-11:30	**Jurriaan Mes**	Health impact of eating *Lemna minor*
11:30-12:00	**Hans Derksen**	*Lemna* protein concentrate in human food products
12:00-13:00	*Lunch*	
13:00-14:30	**Poster session 3**	
14:30-16:00	**Poster talks**	Six 15 min talks
16:00-16:30	*Coffee*	
16:30-17:15	**Jay Cheng***	Phytoremediation & energy production using duckweed
17:15-17:45	**Yubin Ma**	Starch accumulation in duckweed: molecular mechanisms
17:45-18:15	**Rob Martienssen**	Duckweed genomics and genome engineering for sustainable biofuel
19:00-21:00	*Banquet*	Best poster award, Wrap up

Autonomous Duckweed Growth Chambers & Growth Optimization Research for Space Applications

Space Lab Owners
Adam & Christine Escobar

Space Lab Technologies, LLC (Space Lab) is a minority owned small business based in Boulder, Colorado offering research and development for space exploration technology. Founders Adam Escobar and Christine Escobar have strong industry experience in the development of support systems and payloads for small satellites, high altitude balloons, and sub-orbital rockets. They also have specialized expertise in space habitat design and crew life support systems.

Space Lab develops innovative space hardware for both flight and ground systems, including science instrumentation, communications, attitude determination, power, vacuum, structures & deployment, space habitat architectures, and environmental control and life support (ECLSS). Space Lab also provides design services including research, feasibility studies, systems architecting & analysis, electrical & mechanical engineering, test planning and test system development, integration testing & operations support, and post-flight data analysis.

So, what does space exploration and flight hardware have to do with duckweed? Historically, stabilized, dehydrated, packaged meals have been the sole source of food for space missions. This is cost effective, but they don't always taste very good and they have a limited shelf life. As we venture into more long-term space missions, there is only so much food that we can carry with us in a spacecraft. Constantly bringing new food and supplies from Earth will become too expensive. We will need a more sustainable and healthy food source for our crew, requiring space farming.

Duckweed has all "*the right stuff*" for a space food crop. Historically, plants have not been grown in space as a food source, because of high resource costs (energy for lighting, water consumption, equipment mass, and volume). All those resources are heavy and launching mass into space is expensive. Hence, a good space crop is one that minimizes the use of mass, volume, or energy consumed, as well as crew time for crop management. It should have the capacity to recycle a lot of carbon dioxide from the cabin, take up little space for support hardware, grow very fast, and propagate easily. It should be 100% edible so that energy is not wasted on growing parts that the

crew cannot eat. Of course, the plants should also be nutritious and taste good. Duckweed checks all the boxes for the perfect space vegetable:

- ✓ *100% Edible:* With *no* inedible biomass, all sequestered CO_2 goes back to the crew diet.
- ✓ *Can be Eaten Raw:* Can be consumed as a fresh vegetable right after harvest.
- ✓ *High Growth Rate:* High yield/area means more food production in a small volume
- ✓ *Vegetative Propagation:* No crew time needed to facilitate pollination and flowering.
- ✓ *Thrives in Elevated CO_2 Found in a Spacecraft Cabin*
- ✓ *Grows in 24-Hour Light:* With no dark period required, CO_2 can be continuously consumed.
- ✓ *Grows in Shallow Water:* Smaller volume needed for growth means higher mass efficiency.
- ✓ *Tolerates Wide Range of Growing Conditions:* Duckweed is robust to a spacecraft environment.
- ✓ *Palatable:* Duckweed is consumed by humans in some Asian countries as a vegetable.
- ✓ *Heterotrophic Growth:* Can grow in the dark w/ sucrose, allowing survival during power failure.
- ✓ *Preferential Uptake of Ammonia-N:* Bestows capacity for human wastewater treatment.
- ✓ *Have Been Grown in Low Earth Orbit,* indicating tolerance to radiation and microgravity.
- ✓ *Nutrition Packed:* Up to 45% high quality protein, low ratio of omega 6 to omega 3 fatty acids, and a source of essential micronutrients.

Many micronutrients (like vitamins A, C, E, and antioxidants) are not manufactured by the human body, and hence must be present in the diet. Because they have a limited shelf life, it makes sense to produce them on-board the spacecraft.

Space Lab is working with the University of Colorado at Boulder to develop an autonomous, environmentally controlled growth chamber for duckweed production in microgravity, called µG-LilyPond™. The company was awarded funding through the NASA SBIR/STTR program, to develop the growth chamber concept and will be testing a benchtop prototype next year.

µG-LilyPond™ includes shallow stacked growing trays that maintain a stable thin film of water, and close canopy LED lighting that allows even light distribution from a short distance. This enables very high production rates in a small volume. It has a rotary sieve to separate the nutrient solution from the duckweed when it is time to harvest the crop. Once this chamber is built, Space Lab's next task will be to define optimal growing conditions for both high yield and nutritional quality.

In order to make the most of this plant's genetic potential for use in space, we need to know what growth conditions the µG-LilyPond™ growth chamber should provide. In collaboration with Dr. Barbara Demmig-Adams at the University of Colorado at Boulder (CU Boulder), Space Lab is investigating environmental modifications *to increase nutrient content while maintaining high volumetric yield and CO_2 sequestration in duckweed biomass during a space mission.* This research is supported by the Translational Research Institute through NASA grant NNX16AO69A. The overarching research goal is to environmentally (rather than genetically) modify duckweed plants for superior yield, nutritional density, and energy-use efficiency (biomass/antioxidants produced per energy input), at spacecraft-relevant CO_2 levels (up to 1%).

The research team will design and test an innovative pulsed lighting technique to avoid the problem of *Yield-Vitamin Tradeoff*. Although duckweed has tremendous potential for both productivity and nutritional value, high biomass yield in any plant can come at the cost of poor micronutrient quality (especially antioxidant vitamins), and *vice versa*. The environmental conditions that tend to maximize yield can actually reduce micronutrient production. This is especially true for antioxidants like zeaxanthin which are produced in defense to excess light, as a way to dissipate heat. Unfortunately, that same excess light intensity that stresses the plant to produce vitamins also results in photosynthetic inhibition and slower growth rates. The application of short periods of high light intensity stimulates micronutrient production (especially zeaxanthin). But if the light pulse duration is short, growth rate should not decrease. It's as if the plants prepare for an oncoming storm, but the storm never comes and so they have no need to slow down. This research will also investigate the effects of spectral density (or quality) on energy-use efficiency. Though red light is the more efficient driver of photosynthesis, blue LEDs are more power efficient. Therefore, the most energy efficient color combination for either continuous or pulsed lighting remains unclear.

In the 2-year research effort, Space Lab and CU Boulder will conduct a series of targeted growth experiments inside of an environmental chamber with controlled temperature and CO_2 concentrations. Space Lab is designing and building a custom test rig and close canopy LED panels. The panels supply irradiation <1.5" from the growing surface at high light uniformity, while allowing spectral tuning.

Space Lab is very excited to take part in this research and looks forwarding to sharing the results with the science community!

Can duckweed be used to identify toxic water contaminants?

Duckweed has long been established as a model organism for detecting water-borne toxicity to aquatic higher plants (aquatic ecotoxicology). Duckweeds exposed to soluble or finely dispersed toxic substances exhibit growth inhibition and/or chlorophyll depletion that can readily be registered and quantified ("overall toxicity"). Guidelines for the use of *Lemna minor* and *Lemna gibb*a in this regard have been set down by several national and international organizations such as Environment Canada, the Organization of Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO) (Mkandawire et al. 2014, Ziegler et al. 2016). **These tests do not, however, in themselves provide any information as to the identity of the substances resulting in toxicity or as to the mechanisms by which these substances cause their detrimental action.**

Numerous studies have investigated the developmental, anatomical, physiological, biochemical and molecular effects of duckweed exposure to known toxic water contaminants that include nutrients, heavy metals and a great variety of organic xenobiotics. **These investigations have led to the identification of numerous *biomarkers of effect* that describe specific phenomena elicited in duckweeds by toxic substances** and aid in understanding how these substances exert their deleterious effects on aquatic plants (Brain and Cedergreen 2009, Ziegler et al. 2016, Ziegler et al. 2018: the review under discussion here).

Our present knowledge of duckweed biomarker/toxicant relations has thus come from observing deleterious effects of exposure to known water contaminants. **Could a reverse approach also be applicable, i.e. screening for biomarkers to identify toxic substances in water?** This would entail incubating a duckweed with an unknown (uncharacterized) water sample, testing the duckweed for overall toxic response, detecting biomarkers associated with any such response, and identifying the substance(s) responsible for the toxicity by drawing upon knowledge of specific correspondence between the biomarkers and the presence of particular water contaminants. This would be of interest for situations in which the suitability of uncharacterized water for aquatic higher plant growth is at issue, and is the focus of a recent review by Paul Ziegler, Sowjanya Sree and Klaus-Jürgen Appenroth (Ziegler et al. 2018), the Abstract for which was recently included in "From the database" on page 32 of Volume 7(1), issue 24 of the Duckweed Forum.

Even though ever more substances are being tested for toxicity-related biomarkers for duckweed (see Ziegler et al. 2018 and every new issue of the Duckweed Forum thus far!), there are still many water contaminants for which no biomarkers have been established, and most of the observed biomarkers of effect are not specific for particular contaminants. Some biomarkers (e.g., oxygen radical production) may be thought as being characteristic of exposure to certain types of water contaminants (in this case heavy metals), but they are often observed upon exposure to multiple water contaminants (oxygen radicals can result from exposure to NH_4^+, rare earth metals, natural organics, herbicides, fungicides and various pharmaceuticals, in addition to numerous heavy metals).

Since only biomarkers that are absolutely specific for particular water contaminants can be of use in identifying those contaminants precisely, there is a need to establish such specificities to a significant extent. Furthermore, as many biomarkers as possible should be identified for as many water contaminants as possible (ideally for all known toxic water contaminants). In addition to the traditional developmental, anatomical, biochemical and physiological approaches to identifying biomarkers of effect, modern developments in the fields of genomics, transcriptomics, proteomics and metabolomics can greatly expand the possibilities for compiling comprehensive biomarker catalogues corresponding to water contaminants. The comparison of these catalogues may identify individual biomarkers or sets of biomarkers that correspond *specifically* to particular toxic water contaminants; the observation of such biomarkers upon toxic exposure of duckweeds to unknown water samples could then point accurately to the water-borne substances actually responsible for the toxicity. The experimental investment required to set up and exploit such comprehensive biomarker/water contaminant catalogues is not likely to be made in the near future. At present, however, the compilation and systematic application of available and shortly forthcoming data related to the toxic effects of water contaminants on duckweeds can make biomarker-based identification a reality for some discrete water toxins and several categories of these substances.

References:

Brain R.A. and N. Cedergreen (2009) Biomarkers in Aquatic Plants: Selection and Utility. *Reviews of Environmental Contamination and Toxicology* 198: 49-109.

Mkandawire M., J.A, Teixeira da Silva and E.G. Dudel (2014) The *Lemna* bioassay: contemporary issues as the most standardized plant bioassay for aquatic ecotoxicology. *Critical Reviews in Environmental Science and Technology* 44:154–197.

Ziegler P., K.S. Sree and K.-J. Appenroth (2016) Duckweeds for water remediation and toxicity testing. *Toxicological & Environmental Chemistry* 98(19): 1127-1154.

Ziegler P., K.S. Sree and K-J. Appenroth (2018) Duckweed biomarkers for identifying toxic water contaminants? *Environmental Science and Pollution Research* DOI:10.1007/s11356-018-3427-7.

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Useful methods: Cefotaxime: a useful antibiotic for duckweed culture management

Eric Lam and Kenneth Acosta

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Biology is complicated! This expression of amazement is exemplified by the deceivingly simple duckweed plants and the complex task of caring for a living collection of over 800 clones (or strains) in the Rutgers Duckweed Stock Cooperative (RDSC). In order to maintain a germplasm stock for duckweed that will have consistent and reliable characteristics and performance, we endeavor to keep them as gnotobiotic cultures that are not complicated by bacteria or fungal endophytes. While this can be done for the majority of clones in our collection, some strains and species can be particularly recalcitrant to purging their resident microbes. These will require sequential sterilization using various concentrations of sodium hypochlorite (the active ingredient in bleach) that can take a lot of patience, dexterity and time. Once a gnotobiotic culture has been achieved, however, duckweed clones can also easily become "reinfected" with bacterial or fungal endophytes during their subculture. In many cases, while the infection *per se* often does not cause an overt pathological phenotype in appearance at first, the compromised strains are more prone to turn yellow in their fronds upon long term culture as well as displaying slow growth and death in later stages. To counter some of these challenges, we have found that the antibiotic cefotaxime is a very useful agent in helping us to manage the large collection of duckweed strains in the RDSC (see picture to the right). We mainly utilize this antibiotic to help remove difficult bacteria from duckweed during the sterilization phase, and to suppress reinfection of gnotobiotic duckweed plants by new bacteria strains.

Some important characteristics of cefotaxime are thus important to appreciate by the user. Cefotaxime is a β-lactam antibiotic, related to penicillin, and can inhibit both Gram-negative and Gram-positive bacteria. However, it is important to note that it is apparently not active against *Pseudomonas* and *Enterococcus* species. Like Penicillin, cefotaxime inhibits bacteria cell wall biosynthesis that eventually causes lysis of the bacteria. In addition, it can inhibit cell division in cyanobacteria as well as organellar division in glaucophytes and bryophytes. Interestingly, cefotaxime has very low toxicity in vascular plants and is thus often used in plant tissue culture. For duckweed cultures, we typically apply cefotaxime at a final concentration of 100 mg/L in order

to destroy or inhibit multiplication of any remaining bacteria in bleach-treated duckweed tissues during their recovery from the harsh treatment. We also routinely maintain our strains in the RDSC on multiple types of medium for long term (~3 month) storage, a couple of which contains cefotaxime. These are 0.5X Schenk and Hildebrandt (SH) salts, cefotaxime, ± 0.1%(W/V) sucrose. However, we note that for some strains in the *Wolffia* and *Wolffiella* genera, heightened sensitivity to cefotaxime may occur and lower concentrations of the antibiotic could be needed.

For the more recalcitrantly infected duckweed clones, cefotaxime-containing agar plates could be one way to help purge the resident microbes using a dilution-by-division approach. In this method, we will spot a few clusters of bleach-treated duckweed fronds onto an SH plate with cefotaxime (SH-cef plates) and let the fronds regenerate from the protected meristems (see example in picture below for fronds from a *Lemna* species). These will be transferred to new SH-cef plates after 2 to 3 weeks and wait for new clusters to form. Fronds from the edge of the new clusters are then plated onto another fresh SH-cef plate and grown again to a cluster before repeating this process one more time. Finally, new fronds at the edge of the clusters are transferred onto SH-sucrose plates to promote more rapid plant growth. When new clusters are formed, fronds are then checked for bacteria presence by plating on LB and TSB agar plates. In this time-consuming approach, we reason that as the plant divides in the presence of cefotaxime, the remaining bacteria present deep within the meristem pocket(s) of the duckweed will be sequentially diluted since their division will be inhibited even if they are recalcitrant against lysis. Thus, after several rounds of subculturing with this procedure, one may be able to obtain gnotobiotic fronds located away from the original mother frond, which could still contain dormant bacteria. It should be noted that cefotaxime is not very stable at 25^{o}C, decreasing in activity by ~20% after 5 days (1). Thus, transfer to fresh plates will be necessary after 2 to 3 weeks under most plant culture conditions in order to maintain its efficacy.

In summary, we hope this Discussion topic is of interest to the general community as well as helpful to duckweed researchers and application specialists for maintaining their own culture collections. For convenience, we have appended at the end of this article a detailed protocol that we use to include cefotaxime in our culturing media.

1. Behin S, Punitha ISR, and Krishnan S (2012) Physical and Chemical Stability Studies on Cefotaxime and its Dosage Forms by Stability Indicating HPTLC Method. *Int. J. Pharma. Chem. and Biol. Sci*. 2(4): 517-523. ISSN: 2249-9504

PROTOCOL FOR PREPARING MEDIA WITH CEFOTAXIME

(Preparation of Cefotaxime Stock Solution)[1]

1. Add 1 g of cefotaxime (GoldBio; Catalog # C-104) to 10 mL sterile H_2O. Dissolve completely.
2. Filter sterilize solution using 0.22 µm syringe filter.
3. Aliquot into 1 mL centrifuge tubes.
4. Store at -20^{o}C until use.

[1] https://www.goldbio.com/documents/1036/Cefotaxime+Stock+Solution.pdf

(Preparing Agar Media With Cefotaxime)

1. Autoclave agar media at 122^{O}C for 30 minutes.
2. Let agar media cool until it's warm to the touch.
3. Thaw cefotaxime stock solution.
4. Add 500 µL cefotaxime stock solution (100 mg/mL stock; 1,000X) to 500 mL agar media for a final concentration of 100 µg/mL.
5. Pour plates. Plates are left overnight in laminar flow hood to solidify and dry.
6. Store plates the following day at 4^{O}C until use.

(Preparing Liquid Media With Cefotaxime)

1. Thaw cefotaxime stock solution.
2. Add 500 µL cefotaxime stock solution (100 mg/mL stock; 1,000X) to 500 mL liquid media for a final concentration of 100 µg/mL.

Student Spotlight: Hassana Ghanem

Beirut Arab University, Beirut, Lebanon (Email: hassanaghanem@hotmail.com)

Since Childhood, I had an interest in nature and plants. I started to engage myself in environmental projects at school. As a continuation, I graduated as an Agricultural Engineer from the faculty of Agronomy, Lebanese University. The research projects and teaching skills I went through since my graduation had encompassed and intensified my experience in plant and environmental research and developed my spectrum of knowledge. My broadening academic responsibilities include tutoring practical courses for the junior and senior students in the college, as well as coaching and supervising the postgraduate scholars in their diverse research projects. The reason why I have opted to pursue my studies in environmental field comes mainly from my professional background as a researcher and lecturer in the Faculty of Agronomy, Lebanese University. During the course of my research, I have become aware of the ever-rising pollution levels in Lebanon due to industrial effluent discharge and recognize its damaging effects on life and human beings. This led me to develop a study that aims to find eco-friendly solutions for lowering pollution from Lebanese ecosystems.

A view of a pond completely covered with duckweeds

Lebanon is quite rich in plant diversity and important indigenous species. I noticed that there weren't any study carried out on the use of duckweeds in phytoremediation of polluted waterbodies in Lebanon. Hence, I decided to continue my research on duckweed as they are remarkable plants with potential environmental and economical benefits. Duckweed is an aquatic plant that has been utilized in both fundamental and applied sciences. It is distinguished by its simple morphological structure that is sensitive to changes in the chemical composition of the aquatic environment. Its ability to grow in polluted and adverse conditions has to be particularly emphasized. The study of the potential of duckweed in phytoremediation of the ecological state of the environment is extremely relevant in the current scenario of increasing anthropogenic impact on the natural environment.

My research is aimed at investigating the phytoremediation potential of the Lebanese clones of *Lemna minor* and *Lemna gibba,* growing naturally in Lebanese surface waterbodies, to remove and bioaccumulate heavy metals from polluted estuaries. The field monitoring included water analysis and assessment of other environmental parameters as well as studying the bioconcentration abilities of duckweed for *in situ* phytoremediation of heavy metals. In addition, the ability of *Lemna* sp. to grow under *in vitro* conditions was assessed in order to determine its capacity to uptake heavy metals from water under laboratory conditions. Furthermore, heavy metal toxicity, oxidative stress responses and tolerance capacity of Lebanese clones of *Lemna* sp. were studied.

My work has been presented at various national conferences. The main achievement for me was my participation in the 4th International Conference on Duckweed Research and Applications, at Kerala, India in October 2017. This conference revealed to me the fantastic opportunities to meet experts who have been leading the duckweed research and applications. I was so pleased to get many useful discussions and comments for my future work in the field of duckweed research. I had the chance to meet and discuss my work with Prof. Klaus Appenroth (Friedrich Schiller University of Jena, Germany), Prof. Eric Lam (Rutgers, The state University of New Jersey, New Brunswick, USA) and Dr. K. Sowjanya Sree (Central University of Kerala, India).

At work: Culturing duckweeds in sterile conditions

During my Ph.D. I acquired a deep knowledge of duckweed physiology and gained experience in culturing these plants both in laboratory as well as under field conditions. It is now my wish to use my expertise that I have gained, to address environmental and economic problems that affect the Lebanese water ecosystems. I believe there are a lot of work to be done on duckweeds in Lebanon. My ultimate target is to provide outcomes for the benefit of both the society and nature. I always follow duckweed-related articles, papers, symposia and conferences curiously. I think there is great opportunity to take advantage of duckweed plants for both research and applications in Lebanon, because they are still unfamiliar for phytoremediation purposes there. I hope that our study will help spread awareness of duckweed plants and their special qualities and potential into the Lebanese society.

Discussion Corner: What do we need in the near future?

Klaus-J. Appenroth[1] & K. Sowjanya Sree[2]
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In this forum, we would like to share our opinion about the following fields in which we need to progress:

1. Often, people who are interested in large-scale cultivation of duckweeds, search for information regarding the use of fertilizers as a source of nutrients for the growth of plants. This is not trivial because there are certain differences between the nutrient availability in agriculture with soil as the substrate and in aquaculture. As an example, it is for sure necessary to add trace elements to the medium because after a few harvests of duckweed the water will be devoid of them. In contrast to laboratory work with highly purified chemicals, what can be used as an effective fertilizer in the field cannot be deduced easily. In many cases, practitioners try to cultivate duckweeds without having any prior experience and stop soon thereafter, being frustrated with negative results because of the poor information about fertilizing the aquatic medium and its management. Therefore, we urgently need publicly available recommendations regarding the type of commercial fertilizers and their quantities that could be used for large-scale production of duckweeds. We would like to invite researchers and the application specialists in the field to contribute to this effort.
2. Over the years, quite a number of methods for genetic transformation of different species of duckweeds have been published. Recently, we found an interesting paper "*Agrobacterium rhizogenes*-mediated transfer of tuberculosis antigens ESAT6 and AG85B genes to *Lemna minor* L." describing the genetic transformation. This paper was published in the Ukrainian journal "Biotechnologija" in Ukrainian language. We have cited this paper just to demonstrate that there are quite a lot of reports over time about this important topic. Several decades ago, both Marvin Edelman (Weizmann Institute of Science, Rehovot, Israel) and A.M. Stomp (North Carolina State University, USA) published already transformation methods in several duckweed species. It would be very helpful for the duckweed community, to have a publicly available review about the published methods of genetic transformation of duckweeds. A contribution of a critical review in this area would be of high interest.
3. Counting the number of publications in different fields of duckweed research, those of phytotoxicity are by far the highest in number. Unfortunately, in this field there is not much progress in the last few years (see the contribution of Paul Ziegler in this issue). In many articles, the heavy metal used is different and similar, or only slightly different, parameters are investigated. One of the directions for further progress was pointed out by A. van Hoeck et al. in *Biotechnol. Biofuels* 8: 188 (2015). The most frequently used duckweed species in phytotoxicity studies are *Lemna minor* and *Lemna gibba*. In order to support further work related to stress physiology, we need high quality genome drafts of these two duckweed species. We have been informed that these have been completed by Robert Martienssen and group at CSHL, USA as reported at the recent PAG 27 meeting in San Diego. The availability of high quality genome drafts of other duckweed species as well would contribute to the use of locally available duckweeds for the purpose of phytotoxicity studies and for their use in phytoremediation.

The above mentioned points are just to begin with. We would like to invite both researchers and application specialists working with duckweeds to share and discuss their thoughts about other or related areas that could find a place under this topic and have not been covered by this article.

Announcing Call for ISCDRA Nominees

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

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

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

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

2) Anyone who has previously attended any of the ICDRA or will be attending it this year, or receives the ISCDRA Newsletter can suggest potential candidates including themselves up to 6 weeks before the meeting. Candidates should have attended at least one of the three previous ICDRA meetings. Suggestions may be sent to the present Chair of the ISCDRA- Dr. Eric Lam, Email: ericL89@hotmail.com up to 6 weeks before the start of this year's conference at Rehovot, Israel on September 9th: the deadline for submission of candidate names will thus be 29th of July 2019.

3) The voting procedure will be announced in the next Duckweed Forum issue, scheduled to be available in early July, 2019.

4) The five newly elected members will be notified by email and they will elect the head of the committee before the ICDRA.

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

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

Request for Applications to Host ICDRA- 2021

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

The applications should briefly introduce the proposed venue, its benefit/attractions, relevance to duckweed research and/or applications, and the responsible organizer's credentials as well as experience. The list of all applications will be send out to the community with the next issue of "Duckweed Forum" (in early July, 2019) before the ICDRA and decided during the "General Assembly" at the end of the conference on the 9th – 12th September 2019, in the Weizmann Institute of Science, Rehovot, Israel.

From the database

Highlights

Post-transcriptional adaptation of the aquatic plant *Spirodela polyrhiza* under stress and hormonal stimuli

Fourounjian, P; Tang, J; Tanyolac, B; Feng, Y; Gelfand, B; Kakrana, A; Tu, M; Wakim, C; Meyers, BC; Ma, J, Messing J (2019) The Plant Journal DOI:10.1111/tpj.14294

The Lemnaceae family comprises aquatic plants of angiosperms gaining attention due to their utility in wastewater treatment, rapid production of biomass that can be used as feed, fuel, or food. Moreover, it can serve as model species for neotenous growth and environmental adaptation. The latter properties are subject to post-transcriptional regulation of gene expression, meriting investigation of how miRNAs of *Spirodela polyrhiza*, the most basal, and most thoroughly sequenced member of the family, are expressed under different growth conditions. To further scientific understanding of its capacity to adapt to environmental cues, we measured miRNA expression and processing of their target sequences under different temperatures, and in the presence of abscisic acid, copper, kinetin, nitrate, and sucrose. Using two small RNA and one degradome sequencing experiments, we can provide evidence for 108 miRNAs. Sequencing cleaved mRNAs validated 42 conserved miRNAs with 83 targets and 24 novel miRNAs regulating 66 targets and created a list of 575 predicted and verified targets. These analyses revealed condition-induced changes in miRNA expression and cleavage activity, and resulted in the addition of stringently reviewed miRNAs to miRBase. This combination of small RNA and degradome sequencing provided not only high confidence predictions of conserved and novel miRNAs and targets, but also a view of the post-transcriptional regulation of adaptations. A unique aspect is the role of miR156 and miR172 expression and activity in its clonal propagation and neoteny. Additionally, low levels of 24nt sRNAs were observed, despite the lack of recent retrotransposition.

An integrated approach for efficient conversion of *Lemna minor* to biogas

Kaur, M; Srikanth, S; Kumar, M; Sachdeva, S; Puri, SK (2019) ENERGY CONVERSION AND MANAGEMENT 180: 25-35

Aquatic weed, *Lemna minor* was evaluated for its potential as a feedstock for gaseous fuel production (biohythane) in an integrated strategy. Three approaches viz., acidogenic fermentation (H_{AP}), electrohydrogenesis (H_{MEC}) and methanogenesis (M_{AD}), were evaluated in single stage as well as in different combinations of two stage (H_{AF} -> H_{MEC}, H_{AP} -> M_{AD}) and three stage (H_{AF} -> H_{MEC} -> M_{AD}, H_{AF} -> M_{AD} -> H_{MEC}) to tap the maximum feasible energy. Compared to single and two -stage operations, three -stage operation evidenced higher biogas (H_2 + CH_4) yield with remarkable total organic carbon (TOC) reduction. Irrespective of the integration sequence, H_{AF} in first stage depicted the possibility of harnessing higher energy by accumulation of volatile fatty acids (WA) along with H_2 production. Similarly, integration of M_{AD} in second stage showed the possibility of tapping higher energy rather than H_{MEC} due to higher carbon loss as CO_2 coupled to more H_2 fraction in biogas in case of H_{MEC}. Among three -stage integrations, higher biogas yield and energy recovery was observed in H_{AF} -> M_{AD}. -> H_{MEC} (38.77 mol biogas/kg TOCR; 25,415 KJ/kg TOCR) as compared to H_{AP} -> H_{MEC} -> M_{AD} (37.79 mol biogas/kg TOCR; 15,416 KJ/kg TOCR). Along similar lines, analysis

of organic carbon flow exhibited significant substrate degradation in three stage integrations (72.5-81.4%) as compared to second (66.2-70%) and first stage (39.7-56.5%).

Ecology

The effects of glyphosate-based herbicide formulations on *Lemna minor*, a non-target species

Sikorski, L; Baciak, M; Bes, A; Adomas, B (2019) Aquatic Toxicology 209:70-80

Research into plants plays an important role in evaluations of water pollution with pesticides. *Lemna minor* (common duckweed) is widely used as an indicator organism in environmental risk assessments. The aim of this study was to determine by biological *Lemna* test and chemical methods the effect of glyphosate (GlyPh) concentrations of 0-40 μM on duckweed, an important link in the food chain. There are no published data on glyphosate's effects on the activity of enzymes of the amine biosynthesis pathway: ornithine decarboxylase, S-adenosylmethionine decarboxylase, tyrosine decarboxylase, lysine decarboxylase and arginine decarboxylase, and the content of shikimic acid and glyphosate residues in the tissues of common duckweed. It was found that glyphosate was taken up by duckweed. In plants exposed to 3 μM of glyphosate for 7 days, glyphosate content exceeded the acceptable Maximum Residue Level (MRL) 10-fold. Glyphosate accumulation in plant tissues exerted toxic effects on duckweed by decreasing its growth and yield, inhibiting the synthesis of chlorophyll a and b and carotenoids, and decreasing the photochemical activity of photosystem II (PSII). However, glyphosate increased the concentration of shikimic acid in the tested plants. The activity of ornithine decarboxylase increased 4-fold in plants exposed to 20 muM of the herbicide. As a water pollutant, glyphosate increased the content of biogenic amines tyramine, putrescine, cadaverine, spermidine and spermine. The activity of peroxidase and catalase was highest in duckweed exposed to 20 muM and 7 muM of the herbicide, respectively. The predicted toxic units were calculated based on glyphosate content and the computed EC values. The mean effective concentration calculated for all morphological and biochemical parameters of duckweed was determined at EC_{10}=1.55, EC_{25}=3.36, EC_{50}=6.62 and EC_{90}=14.08 μM of glyphosate. The study demonstrated that glyphosate, the active ingredient of Roundup Ultra 360 SL herbicide, induces morphological and biochemical changes in non-target plants and exerts toxic effects on aquatic ecosystems even during short-term exposure.

Whole angiosperms *Wolffia columbiana* disperse by gut passage through wildfowl in South America

Silva, GG; Green, AJ; Weber, V; Hoffmann, P; Lovas-Kiss, A; Stenert, C; Maltchik, L (2018) BIOLOGY LETTERS14: Article Number: 20180703. DOI: 10.1098/rsbl.2018.0703

For the first time to our knowledge, we demonstrate that whole angiosperm individuals can survive gut passage through birds, and that this occurs in the field. Floating plants of the genus Wolffia are the smallest of all flowering plants. Fresh droppings of white-faced whistling duck *Dendrocygna viduata* (n = 49) and coscoroba swan *Coscoroba coscoroba* (n = 22) were collected from Brazilian wetlands. Intact *Wolffia columbiana* were recovered from 16% of *D. viduata* and 32% of Coscoroba samples (total = 164 plantlets). The viability of plants was tested, and asexual reproduction was confirmed. *Wolffia columbiana* is an expanding alien in Europe. Avian endozoochory of asexual angiosperm propagules may be an important, overlooked dispersal means for aquatic plants, and may contribute to the invasive character of alien species.

Feed & Food

Protein bioavailability of *Wolffia globosa* duckweed, a novel aquatic plant, - A randomized controlled trial

Kaplan, A; Zelicha, H; Tsaban, G; Yaskolka, MA; Rinott, E; Kovsan, J; Novack, L; Thiery, J; Ceglarek, U; Burkhardt, R; Willenberg, A; Tirosh, A; Cabantchik, I; Stampfer, MJ; Shai, I (2019) Clinical nutrition DOI:10.1016/j.clnu.2018.12.009

While the world is extensively looking for alternatives to animal protein sources, it is not clear which plant sources can provide the requisite full complement of essential amino acids (EAAs). *Wolffia globosa* is an aquatic, edible duckweed, the smallest plant on earth, and it offers all nine EAAs, dietary fibers, polyphenols, iron, zinc and B12 vitamin. This work was designed to evaluate Mankai (a newly developed high-protein strain of *W.globosa*) as an optional bioavailable source of EAAs for humans (primary outcome), and of further nutrients such as vitamin B12, in comparison to well-established animal and plant protein sources; cheese and peas, respectively. 36 men, subjected for 3 days to a stable diet and subsequent overnight (12h) fast, were randomized to consume one of three iso-protein (30g) based test-meals (soft cheese, green peas, Mankai). Blood samples were collected at 0, 30, 90 and 180min. The 3h blood concentrations of the EAAs: histidine, phenylalanine, threonine, lysine, and tryptophan, triggered by intake of Mankai, was essentially significant as compared to baseline ($p<0.05$) and similar to that of soft cheese and pea changes ($p>0.05$ between groups). Although branched-chain-amino-acids (leucine/isoleucine, valine) increased significantly by Mankai within 3h ($p<0.05$ vs. baseline), the change was relatively higher for cheese as compared to Mankai or peas ($p<0.05$ between groups). The increase in vitamin B12 by Mankai was higher as compared to changes induced by either cheese ($p=0.007$) or peas ($p=0.047$, between groups).

Interaction with microorganisms

Microbial community succession and pollutants removal of a novel carriers enhanced duckweed treatment system for rural wastewater in Dianchi Lake basin

Chen, GK; Huang, J; Fang, Y; Zhao, YG; Tian, XP; Jin, YL; Zhao, H (2019) BIORESOURCE TECHNOLOGY 276: 8-17

Carriers strengthened duckweed treatment system (CDW), duckweed treatment system (DW) and water hyacinth treatment system (WH) were developed to treat rural wastewater in Dianchi Lake basin. Results showed that adding microbial carrier did not affect the growth and biomass components of duckweed. The following features were discovered in the CDW system. First, the NO3--N and TN removal efficiencies were the highest among three systems, reaching 80.02% and 56.42%, respectively. Secondly, Illumina sequencing revealed the highest microbial diversity. Thirdly, a distinct succession of microbial community was observed. *Rhodobacter*, Bacteria vadinCA02, C39 and *Flavobacterium* dominated in the start- up stage, and contributed to biofilm formation and pollutants degradation. *Acinetobacter*, *Planctomyces* and *Methylibium* significantly increased in the stable stage, and contributed to nitrogen removal. Finally, highly abundant plant growth- promoting bacteria were found. Comprehensive analysis indicated that the functional bacteria community was closely related to the pollutant removals, plant growth and system operating status.

Colonization and competition dynamics of plant growth-promoting/inhibiting bacteria in the phytosphere of the duckweed *Lemna minor*

Ishizawa, H; Kuroda, M; Inoue, K; Inoue, D; Morikawa, M; Ike, M (2019) Microbial ecology 77: 440-450

Despite the considerable role of aquatic plant-associated bacteria in host plant growth and nutrient cycling in aquatic environments, the mode of their plant colonization has hardly been understood. This study examined the colonization and competition dynamics of a plant growth-promoting bacterium (PGPB) and two plant growth-inhibiting bacteria (PGIB) in the aquatic plant *Lemna minor* (common duckweed). When inoculated separately to *L. minor*, each bacterial strain quickly colonized at approximately 106 cells per milligram (plant fresh weight) and kept similar populations throughout the 7-day cultivation time. The results of two-membered co-inoculation assays revealed that the PGPB strain *Aquitalea magnusonii* H3 consistently competitively excluded the PGIB strain *Acinetobacter ursingii* M3, and strain H3 co-existed at almost 1:1 proportion with another PGIB strain, *Asticcacaulis excentricus* M6, regardless of the inoculation ratios (99:1-1:99) and inoculation order. We also found that *A. magnusonii* H3 exerted its growth-promoting effect over the negative effects of the two PGIB strains even when only a small amount was inoculated, probably due to its excellent competitive colonization ability. These experimental results demonstrate that there is a constant ecological equilibrium state involved in the bacterial colonization of aquatic plants.

Assessment of in vitro multiplication of *Lemna minor* in the presence of phenol: Plant/bacteria system for potential bioremediation - Part I

Radulovic, O; Petric, M; Raspor, M; Tadic, V; Jovanovic, P; Zecevic, V (2019) POLISH JOURNAL OF ENVIRONMENTAL STUDIES 28: 803-809

The aim of this work was to examine the multiplication of the common duckweed (*Lemna minor*), an aquatic plant species widespread in European stagnant waters, in two different media (Murashige - Skoog and Hoagland) with and without phenol supplementation. In order to quantify plant multiplication we have used relative growth rate and tolerance indices on both tested media and at five phenol concentrations (10, 15, 20, 30 and 100 mg/L). Furthermore, we examined the possibility of phenol removal from aqueous media containing different phenol concentrations, by using plant/bacteria system consisting of the duckweed and its naturally occurring microbial populations. After 7 days, number of newly formed fronds was approximately four times higher than at the beginning of the experiment on both tested media. The most important result in this study was removal of 70% of phenol from the highest initial concentration of 100 mg/L, in mixed cultures of duckweed and bacteria. By comparison, aseptic duckweed cultures removed approximately 50% of phenol at the same initial concentration. Our duckweed specimen showed a fast reproduction rate, high tolerance to phenol and a possible cooperation with rhizosphere-associated bacteria. All of these traits can be ultimately utilized for bioremediation purposes.

Culture-dependent analysis of 16S rRNA sequences associated with the rhizosphere of *Lemna minor* and assessment of bacterial phenol-resistance: Plant/bacteria system for potential bioremediation - Part II

Radulovic, O; Petric, M; Raspor, M; Stanojevic, O; Janakiev, T; Tadic, V; Stankovic, S (2019)

POLISH JOURNAL OF ENVIRONMENTAL STUDIES 28: 811-822

In this work, we demonstrate that the rhizosphere of common duckweed (*Lemna minor*) is inhabited with various phenol-resistant bacterial strains. Based on 16S rRNA sequencing, we have identified 60 rhizosphere-associated bacterial isolates belonging to 10 different bacterial genera (*Pseudomonas, Hafnia, Serratia, Enterobacter, Micrococcus, Stenotrophomonas, Xanthomonas, Bacillus, Staphylococcus* and *Klebsiella*). All isolates have been tested for phenol resistance and ability to utilize phenol as the sole carbon source. 70% of all isolates survived high doses of phenol (≥ 200 mg/L) and at least 27% can be potentially acclimatized by gradual increase of phenol concentration. Finally, based on high phenol resistance, ability to utilize phenol as the sole carbon source and documented low pathogenicity, we propose 5 strains as potentially excellent candidates for bioremediation. These 5 strains taxonomically correspond to *Klebsiella* sp., *Serratia* sp., and *Hafnia* sp., respectively. To the best of our knowledge, this is the first attempt to assess decontamination capacity of *Serratia nematodiphila* and *Hafnia* sp. in the context of bioremediation of phenol-contaminated aqueous media. Although additional analyses are needed, interaction between the common duckweed and the selected bacterial strains may be utilized in future bioremediation strategies.

Molecular Biology

Gene coexpression analysis reveals dose-dependent and type-specific networks responding to ionizing radiation in the aquatic model plant *Lemna minor* using public data

Fu, LL; Ding, ZH; Kumpeangkeaw, A; Sun, XP; Zhang, JM (2019) JOURNAL OF GENETICS

98, Article Number: 9, DOI: 10.1007/s12041-019-1063-8

Ionizing radiations (IRs) are widespread damaging stresses to plant growth and development. However, the regulatory networks underlying the mechanisms of responses to IRs remains poorly understood. Here, a set of publicly available transcriptomic data (conducted by Van Hoeck et al. 2015a), in which *Lemna minor* plants were exposed to a series of doses of gamma, beta and uranium treatments was used to perform gene coexpression network analysis. Overall, the genes involved in DNA synthesis and chromatin structure, light signalling, photosynthesis, and carbohydrate metabolism were commonly responsive to gamma, beta and uranium treatments. Genes related to anthocyanin accumulation and trichome differentiation were specifically downregulated, and genes related to nitrogen and phosphate nutrition, cell vesicle transport, mitochondrial electron transport and ATP synthesis were specifically upregulated in response to uranium treatment. While genes involved in DNA damage and repair, RNA processing and RNA binding were specifically downregulated and genes involved in calcium signalling, redox and degradation of carbohydrate metabolism were specifically upregulated responding to gamma radiation. These findings revealed both dose-dependent and type-specific networks responding to different IRs in *L. minor*, and can be served as a useful resource to better understand the mechanisms of responses to different IRs in other plants.

Physiology

Use of a duckweed species, *Wolffiella hyalina*, for whole-plant observation of physiological behavior at the single-cell level

Isoda, M; Oyama, T (2018) PLANT BIOTECHNOLOGY 35: 387-391

We developed a new model system to analyze physiological behavior at the single-cell level in whole plants. *Wolffiella hyalina* is a species of rootless duckweed, which has a thin and very small structure and can grow rapidly on the surface of culture medium. Epidermal and mesophyll cells were transfected with a reporter gene using particle bombardment and were observed at the single-cell level in the whole living plant. An EM-CCD camera system with a macro zoom microscope was used to capture time-lapse images of bioluminescence, and we successfully detected circadian rhythms in individual cells that expressed a luciferase gene under the control of a circadian promoter. We also detected individual S-phase cells in meristematic tissues of intact *W. hyalina* plants by using a 5-ethyny1-2'-deoxyuridine (EdU)-labeling assay. Our observations indicated that low-molecular-weight compounds could access the inside of the plant body. Thus, *W. hyalina* showed the experimental characteristics suitable for single-cell analyses that could be combined with whole-plant observations and/or pharmacological analyses/chemical biology.

Creation of culture media for efficient duckweeds micropropagation (*Wolffia arrhiza* and *Lemna minor*) using artificial mathematical optimization models

Khvatkov, P; Chernobrovkina, M; Okuneva, A; Dolgov, S (2019) PLANT CELL TISSUE AND ORGAN CULTURE 136: 85-100

Recently, computer technologies have provided the researchers with the new approaches for modeling and better understanding the role of the factors that are involved in plant growth in vitro. To develop new models for the optimization of growth conditions, it is reasonable to use plants with a high speed of vegetative in vitro reproduction, such as duckweed (Lemnaceae family). This article focuses on the trophic levels of the two types of duckweeds (*Wolffia arrhiza* and *Lemna minor*). Using the development of the optimal modeling of the biological processes we have obtained the prescriptions for individually-balanced culture medium that enable 3.0 higher yields of the total soluble protein from each of the populations for both types of Lemnaceae.

Demographic senescence in the aquatic plant *Lemna gibba* L. (Araceae)

Chmilar, SL; Laird, RA (2019) AQUATIC BOTANY 153: 29-32

Senescence is progressive, age-related bodily deterioration, accompanied at the population level by declines in average survival and fecundity (i.e., 'demographic senescence'). Demographic senescence of plants has been investigated in only a few species, including small, floating macrophytes in the genus *Lemna* (family Araceae, subfamily Lemnoideae - the 'duckweeds'). Unlike most plant species, *Lemna* ramets exhibit determinate growth, potentially rendering them more likely to experience demographic senescence. Here, our objective was to investigate senescence in a *Lemna* species not previously studied in this context, *L. gibba* L., toward the long-term goal of conducting cross-species comparative analyses. In a longitudinal lab study, we investigated a cohort of 334 individual *L. gibba* fronds, whose survival and reproduction we followed daily from birth (defined by the date a focal frond detached from its parent) to death (defined by the date a focal

frond's last daughter detached). We fit survival data to exponential, Weibull, Gompertz, and logistic models, the first of which represents 'no senescence'. The logistic model was found to have the greatest support (AIC(C) weight > 0.99), indicating strong age-related declines in survival. We fit reproduction data using a generalized estimating equation approach, which showed a significant age-related decline in the predicted probability of daily reproduction - from 0.61 at age 3 days to 0.23 at age 52 days (i.e., after excluding the first two days of reproduction data to account for the initial, pre-reproductive phase of the *L. gibba* lifecycle). These age-related declines provide strong evidence that *L. gibba* does exhibit demographic senescence, consistent with evidence from congeneric species.

Phytoremediation

Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: State and future perspective

Ekperusi, AO; Sikoki, FD; Nwachukwu, EO (2019) Chemosphere 223:285-309

Over the past 50 years, different strategies have been developed for the remediation of polluted air, land and water. Driven by public opinion and regulatory bottlenecks, ecological based strategies are preferable than conventional methods in the treatments of chemical effluents. Ecological systems with the application of microbes, fungi, earthworms, plants, enzymes, electrode and nanoparticles have been applied to varying degrees in different media for the remediation of various categories of pollutants. Aquatic macrophytes have been used extensively for the remediation of pollutants in wastewater effluents and aquatic environment over the past 30 years with the common duckweed (*L. minor*) as one of the most effective macrophytes that have been applied for remediation studies. Duckweed has shown strong potentials for the phytoremediation of organic pollutants, heavy metals, agrochemicals, pharmaceuticals and personal care products, radioactive waste, nanomaterials, petroleum hydrocarbons, dyes, toxins, and related pollutants. This review covers the state of duckweed application for the remediation of diverse aquatic pollutants and identifies gaps that are necessary for further studies as we find pragmatic and sound ecological solutions for the remediation of polluted environment for sustainable development.

Phytoextraction of Ni, Pb and, Cd by duckweeds

Bokhari, SH; Mahmood-Ul-Hassan, M; Ahmad, M (2019) International Journal of Phytoremediation DOI:10.1080/15226514.2019.1566882

Heavy metals phytoextraction potential of swollen duckweed (*Lemna gibba* L.) and lesser duckweed (*Lemna aequinoctialis* Welw.) was determined under greenhouse conditions by exposing to untreated industrial/municipal effluent for a period of 21 days. The nickel (Ni), lead (Pb), and cadmium (Cd) concentrations in water samples were measured weekly and in plant biomass at the termination of experiments. Significant differences ($p<0.05$) between initial and final physicochemical parameters and in heavy metal concentrations of plant and water samples were observed. Periodically measured metal concentrations in media revealed that removal percentage was dependent on initial Ni (2.15mg L^{-1}), Pb (1.51mg L^{-1}), and Cd (0.74mg L^{-1}) concentrations. The final metal removal percentages were in the sequence of Ni (97%) > Pb (94%) > Cd (90%) when treated with *Lemna gibba* L. as compared to control (9-12% reduction). High biomass production of *Lemna gibba* L. resulted in a large metal reduction in the growth medium and the total plant metal contents were in the sequence of Ni (427g) > Pb (293g) > Cd (105g). The lesser duckweed did not

survive under experimental conditions. Based on these results, we concluded that *Lemna gibba* L. is a good candidate for phytoremediation of wastewater.

Effect of duckweed plants on removal of heavy metals from paper-based packaging

Elmas, GM; Oral, D; Akburak, S (2019) FRESENIUS ENVIRONMENTAL BULLETIN 28: 473-479

There are many scientific studies conducted by using duckweed to remove heavy metals from waste water. Duckweed plants have been used first time with this study for the removal of heavy metals from the paper-based packaging used as raw materials in recovered paper production. Objective of this study was to use an environmental friendly method to remove or decrease heavy metals from paper, paperboard and corrugated board. A mixture of *Lemna minor*, *L. gibba* and *Spirodela polyrhiza* from duckweed taxa was used for the phytoremediation applications. The phytoremediation application was carried out on paper-based samples for 7 days in a batch reactor system to remove heavy metals such as lead, zinc, cadmium, nickel, copper, chrome and aluminum from the samples. Results showed that the duckweed plants can be used during the removal or decrease in heavy metal concentrations in the paper based packaging. It was determined that there was no removal or reduction heavy metals in the surface treated paper packages with low grammage.

Bioaccumulation of cadmium and thallium in Pb-Zn tailing waste water by *Lemna minor* and *Lemna gibba*

Sasmaz, M; Obek, E; Sasmaz, A (2019) APPLIED GEOCHEMISTRY 100: 287-292

The present study investigated the removal ability to phytoremediate cadmium and thallium from tailing wastewater of *Lemna gibba* and *Lemna minor*. These plants were separately adapted to the reactors, placed in the water and daily collected during the eight days. During the study, the plant and water samples were taken daily and the pH, temperature and electric conductivity of the tailing wastewater were daily measured in situ. *Lemna minor* and *L. gibba* were firstly washed, dried in and then ashed at 300 °C for 24 h in an oven. Both ashed plant and water samples were analyzed by ICP-MS to find out the concentrations of cadmium (Cd) and thallium (Tl). Although Cd and Tl are at low values (11.4 +/- 0.5 mu g L-1 for Cd and 2.85 ± 0.5 μg L^{-1}) in tailing waste water, the Cd and Tl were accumulated at the highest amounts by *L. minor* (31.08 mg L^{-1} for Cd and 13.43 mg L^{-1} for Tl) and *L. gibba* (38.9 mg L^{-1} for Cd and 17.18 mg L^{-1} for Tl). Our study on the fourth day showed that *L. minor* accumulated more removal abilities of Cd (94.56 times) and Tl (7.33 times) than in *L. gibba* L. (25.89 times on the third day for Cd and 6.16 times on the fourth day for Tl) but *L. gibba* accumulated higher Cd and Tl concentrations (38.9 mg Cd kg^{-1})and 17.18 mg Tl kg^{-1} than in *L. minor*. Therefore, these plants can use to remove Cd and Tl in tailing waste water polluted by Cd and Tl.

Phytotoxicity

Effects of ZnO nanoparticles on the toxicity of cadmium to duckweed *Lemna minor*

Sun, SQ; Li, XL; Sun, C; Cao, WX; Hu, CW; Zhao, YJ; Yang, AA (2019) SCIENCE OF THE TOTAL ENVIRONMENT 662: 697-702

Release of nanoparticles into the aquatic environment will inevitably influence the behavior and toxicities of other existing pollutants. In the present study, 10 mg/L of nano-ZnO (diameter 20-30 nm) was used to evaluate its impacts on cadmium(Cd) toxicity on duckweed *Lemna minor* based on IC_{50} values and four biological parameters including percent inhibition of growth rate (I_r), ratio of chlorophyll/pheophytin (D665/D665a), antioxidant enzymes, and H+-ATPase. Results of the 96-h IC_{50} values of Cd with or without nano-ZnO indicate no additional toxicological effects of nano-ZnO to plants. Further examinations using two Cd concentrations (0.1 and 1 mg/L) showed that nano-ZnO did not influence the inhibitory effect of 0.1 mg/L Cd, but significantly ($P < 0.05$) reduced the stress of 1 mg/L Cd to the duckweed. The index D665/D665a reflected that the toxic effect of 1 mg/L Cd was significantly ($P < 0.05$) suppressed by nano-ZnO. H+-ATPase was also sensitive to reveal the protective effects of nano-ZnO on the duckweed under Cd exposure. However, the responses of the antioxidant enzymes SOD and CAT failed to reflect the effects of nano-ZnO on Cd toxicity. Hysteretic addition of nano-ZnO for 24 h showed that the protective effects of nano-ZnO were weakened. Our results suggest that the adsorption of Cd to nano-ZnO may result in lower Cd uptake by *L. minor*, thus reducing its toxicity.

Evaluation of pharmaceutical toxic effects of non-standard endpoints on the macrophyte species *Lemna minor* and *Lemna gibba*

Alkimin, GD; Daniel, D; Frankenbach, S; Serodio, J; Soares, AMVM; Barata, C; Nunes, B (2019) SCIENCE OF THE TOTAL ENVIRONMENT 657: 926-937

In the last years the environmental presence of pharmaceuticals has gained increasing attention. Research data show that these compounds can cause toxicological effects in different species of fish, mollusks and macroinvertebrates. However, the literature is scarce in terms of ecotoxicity data especially focusing on plants as test organisms. Ecotoxicological plant-based tests following the standard OEDC guideline 221 (OECD, 2006) are strongly restricted due to the recommended end-points: growth and yield of plants. It is necessary to develop and validate alternative macrophyte-based tests (non-standard endpoints), more sensible and providing additional information about the chemical contamination effects in plants. To attain this purpose, species from the *Lemna* genus were selected. Thus, the aim of this study was to analyze the toxic effects of pharmaceuticals in non-standard endpoints on two macrophyte species, *Lemna minor* and *Lemna gibba*. To this purpose an acute assay (96 h) was performed with *L. minor* and *L. gibba* exposed to chlorpromazine (CPZ), paracetamol (APAP), and diclofenac (DCF), in the following concentration ranges: 0 to 20 μg/L, 0 to 125 μg/L, and 0 to 100 μg/L, respectively. The analyzed endpoints were: levels of chlorophyll a and b, total chlorophyll, carotenoids, anthocyanins; chlorophyll fluorescence; and catalase activity. In general, higher concentrations of the tested pharmaceuticals caused significant effects on both *Lemna* species in terms of the different endpoints analyzed. In conclusion, acute exposures to CPZ, APAP, and DCF differently affected the defensive system of the tested species; among chlorophylls, chlorophyll b content was more affected, but pharmaceutical exposure was not able to cause alterations on chlorophyll fluorescence.

Environmental variations mediate duckweed (*Lemna minor* L.) sensitivity to copper exposure through phenotypic plasticity

Roubeau Dumont, E; Larue, C; Pujol, B; Lamaze, T; Elger, A (2019) Environmental Science and Pollution Research International DOI:10.1007/s11356-019-04630-3

Environmentally mediated sensitivity of *Lemna minor* to copper (Cu) was evaluated for the first time in three experiments: the effects of two levels of nutrient concentration, light irradiance or Cu pre-exposure were tested. Various Cu concentrations (ranging from 0.05 to 0.25mg/L) were used to assess the sensitivity of *L. minor* to this metal, using one common strain previously acclimatized to two different levels of light intensity, nutrient enrichment and Cu pre-exposure. Our results showed a phenotypic plastic response of the relative growth rates based on frond number and fresh mass production, and maximum quantum yield of photosystem II (Fv/Fm). Growth was affected by the three environmental conditions both prior and during Cu exposure, whereas Fv/Fm was mostly affected during Cu exposure. Copper significantly influenced all the parameters measured in the three experiments. Environmental conditions significantly modified *L. minor* sensitivity to Cu in all experiments, with up to twofold difference depending on the treatment. Growth rate was the parameter that was most impacted. Our study revealed for the first time the existence of phenotypic plasticity in *L. minor* sensitivity to chemical contamination, and implies that environmental context needs to be taken into account for a relevant risk assessment.

Evaluating the toxic Impacts of cadmium selenide nanoparticles on the aquatic plant *Lemna minor*

Tarrahi, R; Movafeghi, A; Khataee, A; Rezanejad, F; Gohari, G (2019) MOLECULES 24: Article Number: 410.

Cadmium selenide nanoparticles (CdSe NPs) were synthesized by an easy and simple method and their properties were assessed by XRD, TEM and SEM techniques. The effects of CdSe NPs as well as Cd^{2+} ions on *Lemna minor* plants were investigated. The absorption of CdSe NPs by the plants had some adverse consequences that were assessed by a range of biological analyses. The results revealed that both CdSe NPs and the ionic form of cadmium noticeably caused toxicity in *L. minor*. Morphological parameters as well as peroxidase (POD) activity were deteriorated. In contrast, the activities of some other antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) as well as the contents of total phenol and flavonoids went up. Taken all together, it could be implied that CdSe NPs as well as Cd^{2+} were highly toxic to plants and stimulated the plant defense system in order to scavenge produced reactive oxygen species (ROS).

Toxicity of pure silver nanoparticles produced by spark ablation on the aquatic plant *Lemna minor*

Minogiannis, P; Valenti, M; Kati, V; Kalantzi, OI; Biskos, G (2019) JOURNAL OF AEROSOL SCIENCE 128: 17-21

The increasing penetration of nano-products to the market is raising big concerns about the potential toxic and environmental effects of their constituent engineered nanoparticles (ENPs). Contradictory toxicity test results reported in the literature thus far can be explained by differences in the ENP production methods, which can strongly affect nanoparticle purity and therefore the outcome of the tests. In this paper we investigate the toxicity of Ag nanoparticles (AgNPs) produced by spark ablation - a gas-phase technique that can deliver well-defined nanoparticles of high purity on *Lemna minor*. Our results show that AgNPs exhibit a toxic behavior at concentrations as low as 5 $\mu g\ L^{-1}$, which is considerably lower compared to the threshold concentrations reported in other studies. This difference can be attributed to the purity of the ENPs used in our measurements, which can release higher concentrations of toxic Ag+ ions upon dilution in the test solutions.

Inhibitory effects of silver nanoparticles on photosystem II performance in *Lemna gibba* probed by chlorophyll fluorescence

Dewez, D; Goltsev, V; Kalaji, HM; Oukarroum, A (2018) CURRENT PLANT BIOLOGY 16: 15-21

Toxic effects of silver nanoparticles (AgNPs) on photosynthetic processes and biomass were investigated in aquatic plant *Lemna gibba*, and the plants were exposed to different concentrations of AgNPs for 7 days. At 7 day of exposure, a direct negative impact on the physiological state of plant was indicated by the decrease of chlorophyll (Chl) synthesis, the deterioration of photosynthetic activity and the change in biomass. When *L. gibba* was treated to 1 mg L^{-1} of AgNPs, total Chl content and the biomass decreased respectively by 66 and 51% compared to the control ($P < 0.05$). Under this condition, the alteration of photosystem II (PSII) photochemical reactions was indicated by a significant change compared to control of different studied fluorescence parameters value: F_V/F_M (35%), ET/RC (63%), VJ (61%), q_P (57%) and NPQ (102%). Therefore, Chl a fluorescence measurements showed strong evidence of inhibitory effects on energy transfer from light harvesting complexes to reaction centers, the deterioration of the PSII water splitting system and the inactivation of PSII reaction centers at 1 mg L^{-1}. In conclusion, our results demonstrated that AgNPs induced an inhibitory mechanism on photosynthetic processes and biomass of *L. gibba* plant.

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Links for further reading

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

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

http://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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