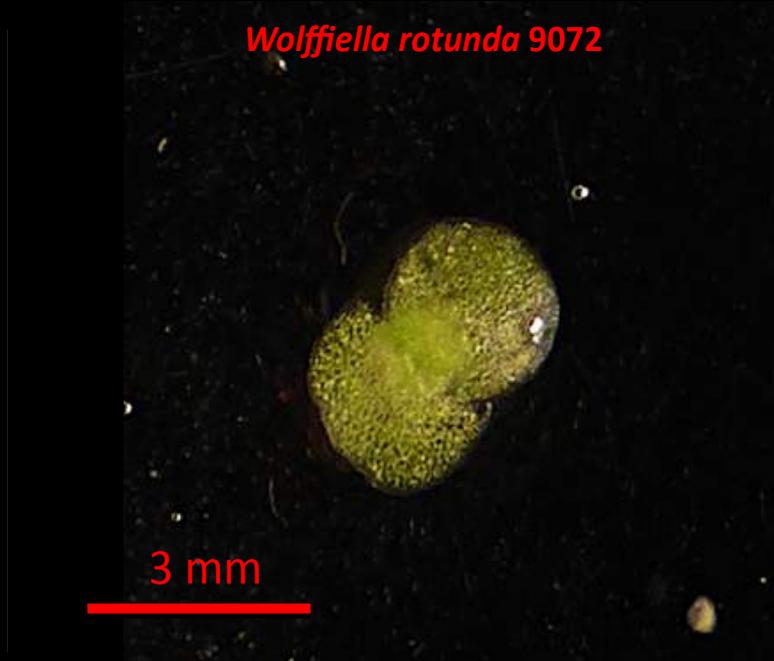
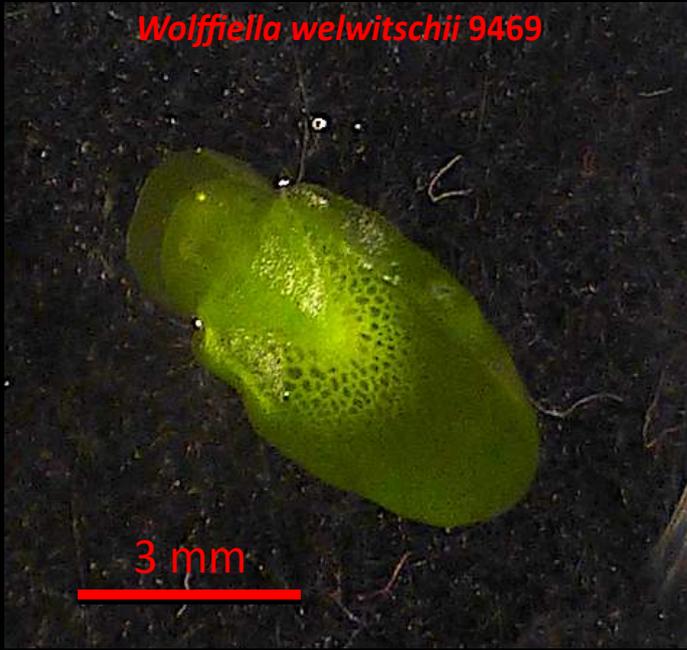


DUCKWEED FORUM



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Cover page

The cover of this issue of the Duckweed Forum features 2 *Wolffiella* and 2 *Wolffia* species that are predominantly located in warmer climates. Clockwise from top left: *Wolffiella welwitschii* (clone 9469 from Brazil), have been found in tropical regions of South and Central America and Africa. It has saddle-shaped fronds with tips bent downward into the water. This species has the unusual physiology to flower often and produce two flowers per frond. *Wolffiella rotunda* (clone 9072 from Mana Pools National Park at Zimbabwe, Africa) with rather circular fronds is only found in tropical region of Africa with dry climates and may be endangered. Similar to *W. welwitschii*, this species is known to be able to form 2 flowers per frond to some extent. Morphologically and from molecular taxonomy, *W. rotunda* resembles most closely to *W. hyalina*. *Wolffia arrhiza* (clone 8639 from Tanzania, Africa) can be found in temperate to tropical regions of Europe, Africa, western Asia and eastern Brazil. It typically displays spherical fronds. It is interesting to note that its name "*arrhiza*" literally means "rootless", which is rather redundant since all *Wolffia* strains do not have root. *Wolffia angusta* (clone 7274 from New South Wales, Australia) represents the smallest duckweed species, and likely of all angiosperms, with fronds of submillimeter in length and width. It is found in tropical and subtropical regions of Australia and southeastern Asia with humid climates. An air bubble is seen here between a mother and daughter frond. Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

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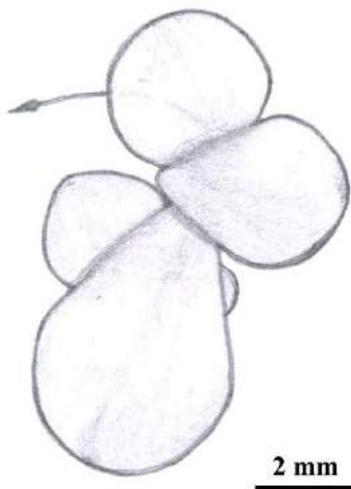
The 3rd International Steering Committee on Duckweed Research and Applications Members

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- **PD Dr. Klaus-J. Appenroth**, Friedrich Schiller University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Prof. Marvin Edelman**, Weizmann Institute of Science, Rehovot, Israel; marvin.edelman@weizmann.ac.il
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Padanakkad, India; ksowsree9@cukerala.ac.in
- **Dr. Yubin Ma**, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China; mayb@qibebt.ac.cn
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

- **External Advisor: Tamra Fakhoorian**, International Lemna Association, Mayfield, KY, USA; tamraf9@gmail.com

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

Science meets art: *Lemna minor* L.



Lemna minor L. is one of the duckweed species that has been commonly used in scientific investigations, especially in the field of phytotoxicity. This species could be confused with *Lemna gibba*, as both species have very similar morphology – except when *L. gibba* has the typical gibbous (belly-like) shape. In contrast to *L. gibba*, however, *L. minor* is more egg-shaped and occasionally has an apical and a nodal papilla. It exists also in colder regions where it might be trapped within an ice layer in freezing winters and regenerate in spring. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala.

Letter from the Editor

Dear Duckweed Community,

I hope you are all enjoying a fine July and we have already reached the half way point of 2018! Greetings from the International Steering Committee on Duckweed Research and Applications along with this new issue of our community newsletter, no. 22 of the *Duckweed Forum*.

Among the four Cover Photos, you will find the picture for *Wolffia angusta*. It has the distinction of being the smallest flowering plant that we know of. The size contrast with another species in the Wolffioideae sub-family, such as *Wolffiella welwitschii*, is rather remarkable. As always, the artistic rendering of duckweeds such as *Lemna minor* by our Steering Committee member Sowjanya Sree is a pleasure to behold. It can be nicely compared with its close relative *Lemna gibba* from the last issue of DF. For contributed articles, Tsipi Shoham (GreenOnyx, Israel) and Yubin Ma (Qingdao Inst. of Bioenergy and Bioprocess Tech., China) detailed global perspectives in the area of Duckweed Research and Application. China, the second largest economy in the world with a huge consumer base, would be a key arena to watch for development of duckweed-based applications. Yubin's article provided an R&D perspective on the current groups in China that are focusing on duckweed and their interest areas to deploy this versatile plant family to address societal needs. In Tsipi's article, she provided detailed lists for both start-ups that are involved in the front-line for exploring markets with duckweed-related technologies and products that may be able to thrive, as well as established companies that are seriously interested in adopting duckweed into their product development portfolio. It is clear from both of these articles that there are many creative minds turning their sights on duckweed worldwide and sustainable methods for biomass production and developing economically viable products from duckweed will be critical targets for the applications community. These are likely areas where close collaboration with research groups will be advantageous for all involved.

A nice contribution from Walter Laemmler, who maintains the Landolt Duckweed Collection in Zurich, Switzerland, follows. This article describes the history and current state of the legacy of Professor Elias Landolt. I had the pleasure of meeting Prof. Landolt before his passing in 2013 and he is one of the most generous scientists that I have known. By all accounts, he has always went out of his way to share his time, expertise and resources with everyone who wants to work with duckweed. I encourage anyone who may be interested to access Prof. Landolt's materials to correspond with Walter. Another interesting article is our Student Spotlight on Anna Maksimova (Tomsk, Russia), who is interested to use indigenous duckweed samples for tracking contaminants in communities. Instead of traveling to everywhere in her country to collect the necessary samples, she turned to social media to get local communities involved and deliver the necessary samples to her by mail. I think it is a wonderful illustration of how modern technologies can be creatively deployed to assist our research endeavors. To round out the issue, Klaus Appenroth delivers another well-curated list of current duckweed-related publications. I am sure you will find many interesting applications and findings associated with this remarkable plant family.

Finally, I like to thank all the contributors for their hard work and bid you all a wonderful Summer - if you reside in the Northern Hemisphere, that is. As always, we look forward to hearing from you if you have any comments and suggestions.

Eric Lam, Chair of the ISCDRA

5th ICDRA:1st Announcement

1st Announcement

5th ICDRA International Conference

Duckweed Research & Applications

at

Weizmann Institute of Science, Rehovot, Israel

September 9- 12, 2019

Save the date



The Weizmann Institute of Science is set in a lushly landscaped campus in the university town of Rehovot, 25 min. from Tel Aviv and 50 min. from Jerusalem. It is host to 240 experimental and theoretical research groups across five faculties—Biology, Biochemistry, Chemistry, Physics and Mathematics/Computer Science, and to 1400 advanced degree students and postdoctoral fellows.

Accommodations for visitors and conventions exist on campus and at a leading international hotel located 5 min. walk from campus.

Information & registration for 2nd announcement:
inbal.azoulay@weizmann.ac.il

Additional information on this event will be forthcoming in the October issue of Duckweed Forum.

Current Research and Applications of Duckweed in China

Yubin Ma (mayb@qibebt.ac.cn)

Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China

Duckweed is a promising feedstock for production of biofuels, due to their characteristics that include rapid growth and high biomass production potential, high starch accumulation capability, low lignin content and ease of harvesting and processing. Duckweed has also been widely used in environmental biotechnology, such as wastewater treatment and phytoremediation. This aquatic monocot has also been considered as a valuable plant expression platform for the production of recombinant proteins including antigens, monoclonal antibodies and exogenous enzymes. Additionally, duckweed can be used as a dietary supplement for humans and animals because of its high nutritional value. Based on the above advantages, duckweed has even been recommended as an alternative crop for the future. In China, duckweed industrialization is at its infancy, perhaps with the exception of some applications of *Spirodela polyrhiza* in traditional Chinese medicine. However, as more and more research groups are now focusing on diverse duckweed applications, it is anticipated that duckweed industrialization would rise in China in the near future. Here, I will introduce several research groups in China who are concentrating on duckweed applications as well as scientific studies with this plant family.

As many as 311 geographically isolated duckweed strains were collected from 22 provinces in China by Profs. Gongke Zhou and Yubin Ma, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. A high starch accumulating strain was identified by large-scale screening using urban sewage and livestock wastewater. The factors influencing starch accumulation in duckweed, i.e., the diurnal variations in starch content, the effects of different culture media and that of salt concentrations, were investigated. We also studied the effect of light conditions on duckweed biomass and starch production. Moreover, comparative analysis of duckweed cultivation in sewage water and SH media for production of fuel ethanol was performed. Further, a highly branched arabinogalactan named DAG1 (Mw~4.0×10⁴ Da) was purified from duckweed. It is well known that nitrogen-starvation could induce starch accumulation in duckweed. Therefore, we also carried out integrated analysis of the transcriptome and metabolites under nitrogen starvation conditions in duckweed. Cadmium (Cd) is a detrimental environmental pollutant and the molecular mechanisms underlying Cd hyperaccumulation in duckweed was investigated by our group. Duckweed CRISPR/Cas9 genome editing system was also established by our group and we are interested in value-added product biomanufacture using duckweed. To promote duckweed commercialization, space-saving cultivation model was designed. Further, we established a 1,500 m² large-scale multilayer cultivation system for duckweed using wastewater from cow farms (Fig.1). In this case, duckweed is cultivated in photovoltaic green house to keep the system working through the whole year. Moreover, an automated processing system for duckweed starch was developed by our group.



Figure 1: Large-scale duckweed cultivation system with wastewater in Qingdao.

Accumulation of starch to 76% from 6.5% in a 10 day duration was achieved by Prof. Hai Zhao and his co-workers, Chengdu Institute of Biology, Chinese Academy of Sciences. The mechanism of starch accumulation was elucidated at the physiological, enzymatic, genomic, transcriptomic, and proteomic levels. Relatively high solar conversion efficiency was also authenticated in the aspects of photosynthesis, CO₂ concentration and carbon flux. Meanwhile, the research group reported that duckweed had similar capacity to remove nitrogen (N) and phosphorus (P) from wastewater as compared to water hyacinth, after a long-term continuous operation of pilot scale wastewater treatment. Application of artificial root system in duckweed can further improve this capacity, resulting in superior performance than water hyacinth. Also, this research group had used duckweed for improvement of water from wastewater sources. Water of Grade V can be improved to Grade III, which can be a source to produce potable water, using duckweed within 3 days. So far, they have constructed pilot wastewater treatment systems using duckweed, which integrate N and P removal, CO₂ capture, and high-quality biomass production (Fig.2). It should be mentioned that the Zhao research group has been working to sequence the genome of *Landoltia punctata*. The genome size of *L. punctata* has been estimated to be 423 Mb, 95.4% of which were anchored to the chromosomes through alignment with the published *S. polyrhiza* strain 9509 using Blast.



Figure 2: Duckweed wastewater treatment system in Yunnan

Agrobacterium mediated stable genetic transformation systems for *S. polyrhiza*, *Lemna minor* and *Wolffia globosa* has been established by the group of Prof. Hongwei Hou, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan. The auxin and cytokinin distribution in *S. polyrhiza* was studied. The effect of different concentrations of mercury on the growth and chemical composition of duckweed was also investigated. The research group has set up transient and stable expression system (Bioreactor) of *Wolffia globosa* expressing edible vaccines for fishes. Genome sequencing of *W. australiana* is in progress.

Purification of wastewater from the rubber processing industry using duckweeds is being investigated by Prof. Jiaming Zhang, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences in Hainan. They are also interested in ammonia-induced senescence in duckweed. Cross-pollination between *L. gibba* strains and screening of hybrids tolerant to high ammonia containing wastewater was studied. Genetic transformation of *L. punctata* was achieved. In addition, application of duckweeds in goose and duck farming was investigated by their group.

Prof. Yong Wang from Nankai University aims to increase the starch content and stress tolerance including salt, alkali and cadmium in duckweed, making it a more robust and productive bioenergy plant. They have established the regeneration and genetic transformation system in *L. turionifera*, and they found that the photorespiratory pathway is related with starch synthesis and biomass accumulation in duckweed. Studies have shown that genetic manipulation in duckweed is an effective approach in modifying the expression of genes encoding key enzymes of starch synthesis pathway, photorespiratory pathway, and ion transport proteins. Therefore, they have generated several lines of transgenic duckweeds that show increased starch content or salt and alkali resistance or cadmium stress tolerance. The results demonstrate that genetic improvement by transformation in duckweed can clearly provide excellent resources for using duckweed as feedstocks for biofuel production and polluted water remediation. At the same time, they tested several kinds of plant regulators for their effect on the starch accumulation in duckweed and have found that maleic hydrazide could inhibit frond growth and enhance starch accumulation in *S. polyrhiza*.

Practical Applications of Duckweed: Challenges and Opportunities

On a mission to introduce duckweed as the 21st century cash crop: Current active industrial efforts

Tsipi Shoham (Tsipi@greenonyx.biz)

GreenOnyx Ltd.; Israel

Preface

Active industrial efforts on the way to realize duckweed's potential as a promising platform for various practical applications

The inherent characteristics of duckweed, revealed and demonstrated by years of scientific discoveries, have been translated into various practical applications, which could lead to significant value for society and attractive business opportunities. This review provides a summary of the current active industrial efforts that can realize duckweed's potential as a platform for various marketable applications. It aims to stimulate further investigations into practical applications and foster collaborations within the duckweed research community, thus exploiting duckweed's full potential. While the current listed practical activities are introduced within several major areas, there may be many waiting to be developed, limited only by our creativity and spirit of entrepreneurship.

Moreover, the presented lists are by no means final, and are meant to extend our dialogue. Thus, this review should be considered as a working document, while any input regarding other active efforts that should be included and/or listed efforts that are no longer operating will be highly appreciated, providing and maintaining an updated resource to be available to all.

The potential impact of the current industrial activities on exploration and exploitation of duckweed's practical use is analyzed via 2 categories:

1. Companies that lead duckweed-based new products and applications (Table 1)
2. Companies that demonstrate an interest to explore duckweeds' applicative potential (Table 2)

1. The emerging 21st century challenges drive industrial entities to launch duckweed based new products and commercial platforms

The anticipated shortage in water, food, feed and energy resources, along with the increased pollution and environmental destruction are real, immediate and interconnected. A diverse range of breakthrough technologies in duckweed practical application, including sustainable cultivation

systems along with innovative platforms for duckweed-based ingredient production, could soon be playing a role in tackling these pressing challenges and contributes to a prosperous and sustainable future. In accordance, companies that are currently active and leading these breakthrough technologies towards new products and commercial platforms, are listed in the context of the following fields of applications: Human food, Animal feed, Fish feed, Bioenergy and Aquatic ecotoxicology (Table 1). In addition, Figure 1 maps the geographical location of the current active companies, showing a high concentration in central Europe (Germany, the Netherlands, UK, Hungary and Spain) followed by the USA, Israel, Argentina and India.

Table 1: Companies that lead duckweed based new products and applications (Sorted per application, and by an alphabetical order within each application)

	Company Name	Field of application	Focused on	Country	Website
1	ABC Kroos	Human food	Plant-based protein ingredients	The Netherlands	http://abc-kroos.nl
2	Aquible	Human food & pet food	Plant-based protein & fiber ingredients	The Netherlands	http://aquible.com
3	Hinoman	Human food	Plant-based protein ingredients	Israel	www.hinoman.biz
4	GreenOnyx	Human food	Fresh green vegetables	Israel	https://www.greenonyx.biz
5	OxyGenesis GmbH	Human food	Plant-based protein ingredients	Germany	https://www.oxygenes.de/en
6	Parabel	Human food	Plant-based protein ingredients	USA	http://www.parabel.com
7	Aqua Light Ltd.	Water treatment & Air treatment	"Symbiofilter" for purifying air or water	Germany	https://symbiofilter.de/?page_id=77
8	Lyndon Water	Water treatment	Integrating water treatment with aquaculture	UK	http://www.lyndonwaterafrica.com
9	MamaGrande	Water treatment & feed	Integrating sewage treatment with bioplastics	Argentina	http://mamagrande.org
10	Sulabh Social Service Organization	Water treatment	Local waste water treatment with direct economic returns from pisciculture	India	http://www.sulabhinternational.org/duckweed-based-waste-water-treatment/
11	Agravis Raiffeisen GmbH	Animal feed	Raw material for animal feed	Germany	https://www.gw-weser-ems.de/DE/Infopool/meldungen/2016/0516_Wasserlinsen.php



12	Drygro	Fish feed	Feed ingredient for farmed fish	UK	https://www.undercurrentnews.com/2017/10/23/uk-alternative-feed-ingredient-startup-eyes-aquaculture-entry/
13	GreenSun Products, LLC	Pet food		USA	https://www.linkedin.com/company/greensun-products-llc/?originalSubdomain=il ; http://www.GreenSunProducts.com
14	Biomass Alternative Power	Bioenergy	Produce natural gas	USA	https://biomassalternativepower.com
15	Duckweed USA	Bioenergy		USA	http://duckweedusa.com/index.html
16	FMC	Aquatic ecotoxicology	Chemical manufacturing	USA	http://www.fmc.com
17	Stillmeadow	Aquatic ecotoxicology	Toxicology services: Lemna toxicity tests	USA	http://www.stillmeadow.com
18	Syntech Research	Aquatic ecotoxicology	Research, development & regulatory services	Spain	https://www.syntechresearch.com/news/new-aquatic-toxicology-services-in-europe/
19	Toxi-Coop Ltd.	Aquatic ecotoxicology	Toxicology services: Lemna growth inhibition test	Hungary	https://toxicoop.com
20	Algaecom	Duckweed growing systems	'Plug & Play' growing systems	The Netherlands	http://www.algaecom.nl
21	Bioponica	Duckweed growing systems	'Bioponics': Soilless farming & organic nutrients	USA	http://bioponica.net/growing-duckweed/ ; http://bioponica.net
22	Prosys Biorefining Systems	Duckweed growing systems	Animal feed, Human food & Bioenergy	USA	http://www.prosysbiorefining.com/products.html
23	Space Lab Tech	Duckweed growing systems	Space crop	USA	http://www.spacelabtech.com/index.html



Figure 1: Global distribution of companies active in the development of duckweed based new products and commercial platforms, demonstrating a high concentration in central Europe (Germany, the Netherlands, UK, Hungary and Spain) followed by the USA, Israel, Argentina and India.

2. A strong interest is expressed by industrial companies exploring duckweeds' applicative potential to meet the 21st century challenges

An ongoing dialog is taking place between the duckweed research community and industrial entities that are seeking to identify and develop new potential solutions that meet the above indicated 21st century challenges. As indicated by a list of companies in Table 2, these entities are involved in the fields of human food, animal health, pet food & feed, modern & future agriculture & aquaculture, regulatory (toxicity) testing, sustainable water treatment, specialty chemicals, plant-based drug discovery and bioenergy. It is interesting to note that the highest industry interest can be observed from companies that are active in the field of “modern & future agriculture” (Figure 2, right pie chart), while most companies that lead duckweed applications (Table 1) are addressing commercial opportunities relating to the human and animal food sectors (Figure 2, left pie chart).

Application in modern & future agriculture goes even further when duckweed is proposed as a potential space crop. This application, led by the Space Lab Tech group, was also highlighted recently in the agtech Hackathon #CropsOnMars in California. The team ‘Just Food’ took the win by presenting an innovative solution based on duckweed. This agtech Hackathon was initiated by Autogrow, a global solution company, that aim to merge indoor agriculture with space exploration to grow crops on Mars. In the inaugural event on Nov. 2017, the ‘Just Food’ team did an impressive job researching how they could not only grow duckweed in a challenging environment but also how it could realistically sustain life on Mars. (<https://autogrow.com/2017/11/07/winners-announced-hackathon-grow-crops-mars-sees-duckweed-take-prize-best-solution/>).

Further strengthening the industry-academia collaboration is key for inspiring entrepreneurship and for attracting leading companies and financial entities to support novel applications and new businesses. As Yubin Ma elaborates in his article on “Research and Applications of Duckweed in China”, published in this issue of DF, “as more and more research group are focusing on duckweed application it is anticipated that duckweed industrialization would rise in China in the near future”.

Table 2: Companies that demonstrate an interest to explore duckweeds’ applicative potential (Sorted per field of interest and by alphabetical order within each field)

	Company Name	Field of interest	Country	Website
1	Andrew J. Young Foundation	Human Food	USA	https://www.andrewyoung.org
2	I.E. Biolab	Human Food	Canada	https://iebiolab.com
3	Drygro	Animal health, Pet food & feed	UK	http://drygro.com
4	Fodder Solutions Uganda Ltd.	Animal health, Pet food & feed	Uganda	www.foddersolutionsafrica.com
5	Merial (part of Boehringer Ingelheim)	Animal health, Pet food & feed	USA	http://usa.merial.com
6	Autogrow	Global Ag-Tech	New Zealand	https://autogrow.com
7	Agrinos	Modern agriculture	USA	http://agrinos.com
8	Deejay Farms	Modern agriculture	India	https://deejayfarm.com
9	Heliae	Aquaculture	USA	https://heliae.com
10	Moyofarm (Private enterprise)	Modern agriculture	Malawi	https://permacultureglobal.org/users/14989-marcel-blankenstein
11	JRF Global	Regulatory testing	USA	http://www.jrfglobal.com
12	Syntech Research	Aquatic ecotoxicology	USA	https://www.syntechresearch.com
13	Venari Bio	Regulatory testing	USA	https://gust.com/companies/venari_bio
14	Carbon Clouds GmbH	Water treatment & sustainability	Germany	http://www.carboncloud.io
15	Integrated Explorations Inc.	Water treatment & sustainability	Canada	https://iebiolab.com
16	BASF	Specialty chemicals	Germany	https://agriculture.basf.com/en.html
17	Dupont	Specialty chemicals	USA	www.dupont.co
18	Fitz Chem LLC, a Nagase Group Company	Specialty chemicals	USA	https://www.nagase.co.jp/english/company/group/ ; https://www.fitzchem.com

19	Naturegenic	Plant-based drug discovery	USA	https://www.prf.org/researchpark/companies/n-companies/Naturegenic,%20Inc..html
20	Greenbelt Resources Corp.	Bioenergy	USA	http://www.greenbeltresources.com

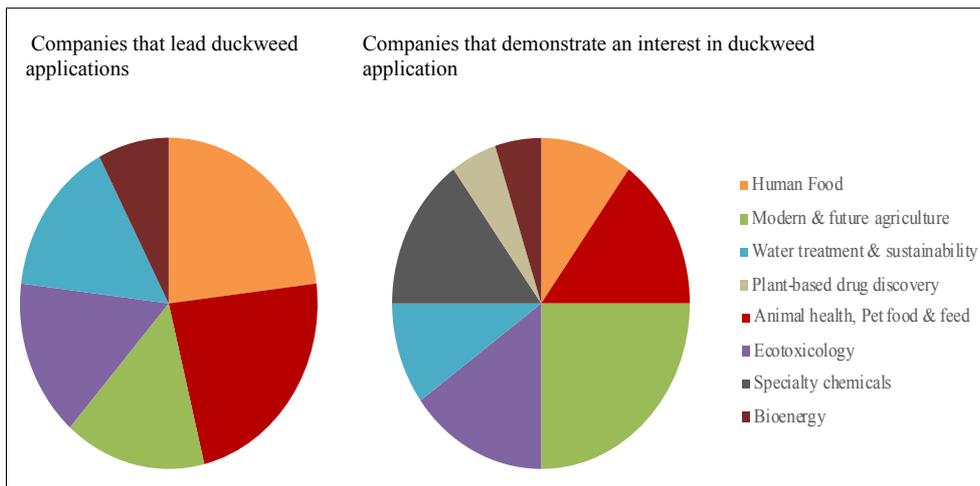


Figure 2: “Human food” and “Animal food are the leading fields of application among companies that develop new products and commercial platform based on duckweeds (left pie chart), while in the industry, the highest interest in exploring duckweed potential for practical applications comes from companies that are active in the field of “modern & future agriculture” (right pie chart).

AS A CLOSING REMARK, I would like to invite you all to share any relevant further information and to provide your feedback. I’d be happy to include your input in the next *Duckweed Forum* issue. Please contact me via my email: tsipi@greenonyc.biz.

Landolt Duckweed Collection Zurich



Walter Lämmli (wlaemmler@duckweed.ch)

1. History

The Landolt Duckweed Collection was established by Professor Dr. Elias Landolt while professor at the Swiss Federal Institute of Technology, Zurich (ETH: Eidgenössische Technische Hochschule) (Lämmli and Bogner, 2014). The collection has formed the basis for many publications on the subject of duckweed, the most important of which is “The family of Lemnaceae – a monographic study” Vol. 1 and Vol. 2. (Duckweed bibliography of Elias Landolt (Lämmli and Bogner, 2014)). In his role as Professor Emeritus, Dr. Landolt undertook expeditions all over the world studying and collecting duckweed. He was accompanied by his assistant, Walter Lämmli (Fig. 1), whose tasks included documentation, photography, and all laboratory work.

Collecting and identifying plants, especially duckweed, was Elias Landolt’s lifelong passion. In 2009, it was determined that the duckweed collection could no longer remain at the Swiss Federal Institute of Technology, Zurich. It was Walter Lämmli who had the idea to transfer it to the Landolt Duckweed Collection, a private institution at Zurich, Switzerland. Upon Elias Landolt’s death in 2013, Walter Lämmli took over the collection, and has been the curator ever since. He is assisted by Elfi Stiefel, who is responsible for maintaining the living plants in the collection.

Lämmli W., Bogner J., 2014. Elias Landolt and the Duckweeds. *Aroideana*, 37, pp. 81-88.



Figure 1: Walter Lämmli at the Landolt Duckweed Collection in Zurich.

2. Content

- Living but sterile plants of all known species of duckweed
- Approx. 500 different strains of duckweed
- Hundreds of herbarium specimens (Fig. 2; parts of them left to the United Herbaria of the University of Zurich and ETH Zurich) and coloured object slides (Fig. 3)
- More than 7,000 articles about duckweed
- Database containing over a hundred years of duckweed distribution records
- Photographs

www.duckweed.ch



Figure 2: Herbarium samples of duckweed.



Figure 3: Stained slide of *Lemna minor*.

3. Purpose

The Landolt Duckweed Collection is open to all of those interested in duckweed. It is a place designed for the storage and cataloguing of living plants, and for the exchange of knowledge. Our primary interest is in taxonomic issues, and we are able to make financial contributions to research in this field under appropriate conditions.

[If you require duckweed for a specific project, please request to see our list of available duckweed clones.](#)

4. Request

We are grateful to receive living duckweed from all over the world to expand and improve our collection. However, access to the resources of the Landolt Duckweed Collection is only possible by an e-mail to Walter Lämmli wlaemmler@duckweed.ch

Research projects are free of charge.

Contact:

Walter Lämmli
Spiegelgasse 12
CH-8001 Zürich
Switzerland

Student Spotlight: Anna Maksimova

(kyzmen44@mail.ru)

Since childhood, I had an interest in nature and different natural processes. Since the age of 13, I started to engage in environmental projects in the direction of bioindication at the Center for Environmental Education of Children (Russia, Strezhevoy). When I went to Tomsk Polytechnic University (TPU, Russia, Tomsk) at the Department of Geoecology and Geochemistry, my future scientific advisor Baranovskaya Natalia Vladimirovna (Doctor of Biological Sciences, Professor of the department) suggested that studying the bioconcentration abilities of duckweed in the Tomsk region, which is characterized by specific technogenical load, would be of high interest.

Duckweed is a water plant, that has been utilized in both fundamental and applied sciences for 50 years. It is distinguished by its simple morphological structure that is sensitive to changes in the chemical composition of the environment. It has a wide range of distribution of chemical elements. Its ability to grow under industrial conditions is to be particularly noted. The study of the chemical composition of duckweed as an indicator of the ecological and geochemical state of the environment is extremely relevant with increasing anthropogenic impact on the natural environment.

At the analytical laboratory in the Department of Geoecology and Geochemistry of TPU, starting 2013, I am investigating the chemical composition of duckweed. Elemental composition of duckweed has been studied by the following modern analytical methods: instrumental neutron activation analysis, atomic absorption spectrometry, atomic emission spectrometry and scanning electron microscopy.

At present, a collection of plants of Lemnaceae family from 64 settlements (48 regions) of The Russian Federation have been collected. And, the content of 28 chemical elements in duckweed from 56 settlements of Russia and the total mercury content of duckweed from 64 settlements has been studied. Plant samples are selected from natural ponds, mostly located in urbanized areas. It is impressive to note that the duckweed samples were collected not only by me, but also by local residents.

To collect duckweed from all over Russia, I found people who were interested in this work, using social networks. It involved ordinary citizens, school groups, environmental organizations and many others. Also, people from Karelia to Kamchatka collected samples of duckweed!

I had been regularly consulting the leading biogeochemists of my country and went to the Institute of Geochemistry and Analytical Chemistry of the Russian Academy of Sciences (Russia,





Moscow). Also, for the species identification of duckweed samples a specialist in the hydrobotanical field Kapitonova Olga Anatolievna (Russia, Izhevsk) had been actively assisting us.

My work has been repeatedly presented at various international and 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. It was extremely important for me to know the degree of study of the object in various fields of Science, and what I saw at the conference exceeded my expectations.

I anticipate that duckweed research will continue to gain momentum. I really wish that the significance of duckweed as an object for phytoremediation, toxicology, bioindication, biotechnology, as a source of starch, and many many more, will gain universal recognition and I have a lot of hope that my work will help in achieving this vision.

From the Database

Highlights

Changes in the abundance of cell wall apiogalacturonan and xylogalacturonan and conservation of rhamnogalacturonan II structure during the diversification of the Lemnoideae

Avci, U; Pena, MJ; O'Neill, MA (2018) PLANTA 247: 953-971

The diversification of the Lemnoideae was accompanied by a reduction in the abundance of cell wall apiogalacturonan and an increase in xylogalacturonan whereas rhamnogalacturonan II structure and cross-linking are conserved. The subfamily Lemnoideae is comprised of five genera and 38 species of small, fast-growing aquatic monocots. *Lemna minor* and *Spirodela polyrhiza* belong to this subfamily and have primary cell walls that contain large amounts of apiogalacturonan and thus are distinct from the primary walls of most other flowering plants. However, the pectins in the cell walls of other members of the Lemnoideae have not been investigated. Here, we show that apiogalacturonan decreased substantially as the Lemnoideae diversified since *Wolffiella* and *Wolffia* walls contain between 63 and 88% less apiose than *Spirodela*, *Landoltia*, and *Lemna* walls. In *Wolffia*, the most derived genus, xylogalacturonan is far more abundant than apiogalacturonan, whereas in *Wolffiella* pectic polysaccharides have a high arabinose content, which may arise from arabinan sidechains of RG I. The apiose-containing pectin rhamnogalacturonan II (RG-II) exists in Lemnoideae walls as a borate cross-linked dimer and has a glycosyl sequence similar to RG-II from terrestrial plants. Nevertheless, species-dependent variations in the extent of methyl-etherification of RG-II sidechain A and arabinosylation of sidechain B are discernible. Immunocytochemical studies revealed that pectin methyl-esterification is higher in developing daughter frond walls than in mother frond walls, indicating that methyl-esterification is associated with expanding cells. Our data support the notion that a functional cell wall requires conservation of RG-II structure and cross-linking but can accommodate structural changes in other pectins. The Lemnoideae provide a model system to study the mechanisms by which wall structure and composition has changed in closely related plants with similar growth habits.

Biotechnology

Sequential ethanol fermentation and anaerobic digestion increases bioenergy yields from duckweed

Calicioglu, O; Brennan, RA (2018) BIORESOURCE TECHNOLOGY 257: 344-348

The potential for improving bioenergy yields from duckweed, a fast-growing, simple, floating aquatic plant, was evaluated by subjecting the dried biomass directly to anaerobic digestion, or sequentially to ethanol fermentation and then anaerobic digestion, after evaporating ethanol from the fermentation broth. Bioethanol yields of 0.41 ± 0.03 g/g and 0.50 ± 0.01 g/g (glucose) were achieved for duckweed harvested from the Penn State Living-Filter (*Lemna obscura*) and Eco-Machine (TM) (*Lemna minor/ japonica* and *Wolffia columbiana*), respectively. The highest biomethane yield, 390 ± 0.1 ml CH₄/g volatile solids added, was achieved in a reactor containing fermented duckweed from the Living-Filter at a substrate-to-inoculum (S/I) ratio (i.e., duckweed to microorganism ratio) of 1.0. This value was 51.2% higher than the biomethane yield of a replicate reactor with raw (non-

fermented) duckweed. The combined bioethanol-biomethane process yielded 70.4% more bioenergy from duckweed, than if anaerobic digestion had been run alone.

Enhancing bioconversion potential of duckweed by acid and hydrogen peroxide pretreatment methods to improve biofuel productivity

Gonen, C (2018) SUGAR TECH 20: 474-481

One of the main triggers of the climate change is the consumption of fossil derivative fuel to satisfy the energy need, and also it is clearly known that the future of the fossil fuel supply is limited. That is why, finding and using alternative, sustainable, renewable, and eco-friendly energy sources are inevitable to fight climate change and to reduce the global warming. Biomass is a well-known renewable material for energy production and is called as biofuels. There are some limitations to utilize the biomass effectively, because of the structure of their molecular forms. For this very reason, the pretreatment pathways to increase the biomass sugar concentration potential to increase the bioconversion potential are attempted in this study. Two different pretreatment methods, i.e., hydrogen peroxide and acid, were applied to biomass, which in this case is duckweed, taken from artificial pond at Nigde, Turkey. In order to determine important factors of the processes, Plackett-Burman design was used. Chemicals dosages, timing, temperature, solid ratio, and mesh size are identified using this methodology to obtain the interested results. Box-Behnken statistical design method was applied to make the optimization of the factors chosen from the factorial design. Consequently, Box-Behnken test indicated that acid pretreatment method showed slightly better results than the hydrogen peroxide application per total sugar concentration, which are 0.60 and 0.48 g/L, respectively.

Enhanced biogas production in the duckweed anaerobic digestion process

Ren, HY; Jiang, N; Wang, T; Omar, MM; Ruan, W; Ghafoor, A (2018) JOURNAL OF ENERGY RESOURCES TECHNOLOGY-TRANSACTIONS OF THE ASME 140: Article Number: 041805.

In order to enhance biogas production in the anaerobic digestion of duckweed, and duckweed with excess sludge as single and mixed substrates, the effects of hot alkali pretreatment and variation of the ratio of substrate to inoculum were investigated. The results showed that the delayed stage of anaerobic gas generation could be shortened when the two substrates were mixed during methane production, to give a cumulative gas yield of 2963 mL, which was 11% higher than the calculated value for the complementary substrate. The methane content was 57%, which was 13% higher than that from the duckweed group and 9% higher than from the excess sludge group. Furthermore, the methane yield was improved by 8% after the duckweed was pretreated with hot alkali. When the substrate to inoculum ratio was 1:1, the maximum biogas production of 3309 mL was achieved, with a methane yield of 1883 mL which, respectively, increases of 151 mL and 304 mL compared with the worst group (1:2.5).

Large-scale screening and characterisation of *Lemna aequinoctialis* and *Spirodela polyrhiza* strains for starch production

Ma, YB; Zhu, M; Yu, CJ; Wang, Y; Liu, Y; Li, ML; Sun, YD; Zhao, JS; Zhou, GK (2018) PLANT BIOLOGY 20: 357-364

Duckweed is considered a promising feedstock for bioethanol production due to its high biomass and starch production. Selection of duckweed strains with high starch accumulation is essential for

application of duckweeds to bioethanol production. Geographic differentiation had a large influence on genetic diversity of duckweeds. Biomass production, starch content and starch amount in geographically isolated strains of 20 *Lemna aequinoctialis* and *Spirodela polyrhiza* were calculated to evaluate their potential for bioethanol production. The influence of different collection time, culture medium and NaCl concentration on starch accumulation of the best strains were analysed. The results showed that biomass production, starch content and starch production of duckweeds demonstrated clonal dependency. The best strain was *L. aequinoctialis* 6000, with biomass production of 15.381.47g m⁻², starch content of 28.68 ± 1.10% and starch production of 4.39 ± 0.25g m⁻². Furthermore, starch content of *L. aequinoctialis* 6000 was highest after 8h of light, tap water was the best medium for starch induction, and NaCl did not induce starch accumulation. This study suggests duckweed biomass production and starch production demonstrate clonal dependency, indicating that extensive clonal comparisons will be required.

Efficient production of succinic acid from duckweed (*Landoltia punctata*) hydrolysate by *Actinobacillus succinogenes* GXAS137

Shen, NK; Zhang, HY; Qin, Y; Wang, QY; Zhu, J; Li, Y; Jiang, MG; Huang, RB (2018) BIORESOURCE TECHNOLOGY 250: 35-42

A novel process of enzyme pretreatment and semi-simultaneous saccharification and fermentation (SSSF) was developed in this work to improve succinic acid (SA) productivity from duckweed (*Landoltia punctata*) and achieve low viscosity. Viscosity (83.86%) was reduced by the pretreatment with combined enzymes at 50 degrees C for 2 h to a greater extent than that by single enzyme (26.19-71.75%). SSSF was an optimal combination with 65.31 g/L of SA content, which was remarkably higher than those obtained through conventional separate hydrolysis and fermentation (62.12 g/L) and simultaneous saccharification and fermentation (52.41 g/L). The combined approach was effective for SA production. Approximately 75.46 g/ L of SA content with a yield of 82.87% and a productivity of 1.35 g/ L/ h was obtained after 56 h in a 2 L bioreactor. Further studies will focus on increasing the working scale of the proposed method.

Ecology

Competition between invasive *Lemna minuta* and native *L. minor* in indoor and field experiments

Gerard, J; Triest, L (2018) HYDROBIOLOGIA 812: 57-65

The invasion of aquatic ecosystems by introduced invasive alien species (IAS) has become a worldwide phenomenon, and often leads to competitive interactions with native species. At high-nutrient levels, native species mostly are outcompeted by the introduced species. We performed an outdoor competition experiment between IAS free-floating *Lemna minuta* and native *Lemna minor* in a eutrophicated pond to examine whether the invasive species is the better competitor. We additionally performed an indoor experiment resembling mesotrophic phosphorus (P) conditions to investigate both species' competitiveness in low P availability and compared with previous experiments at high-nutrient levels. Our results showed that in field conditions, the alien *L. minuta* was the better competitor. In the mesotrophic indoor condition, however, the native *L. minor* was the better competitor. Both species produced longer roots in the indoor experiment compared to field conditions. The species' relative growth rates were also lower in the indoor experiment. A P reduction to mesotrophic condition in the water column thus might reduce invasive *L. minuta* growth and competitive performance. Additionally, introduction and recovery of *L. minor* could reduce *L.*

minuta cover, but only following P reduction. Field experiments in mesotrophic ponds are needed to confirm these indoor findings.

Baseline isotopic data for *Wolffia* spp.: another option for tracing N-loading in freshwater systems?

Hill, JM (2018) FRESHWATER SCIENCE 37: 23-30

Delta N-15 values of aquatic plants can reflect anthropogenic N loading. Recent work suggests the duckweed *Spirodela* spp. effectively maps N loading in freshwater ecosystems, but its use may be complicated by a cyanobacterium-duckweed symbiosis that could reduce its utility in low-nutrient environments. I aimed to evaluate the potential of a 2nd duckweed species *Wolffia* spp., which lacks a cyanobacterial symbiosis, for use in pollution monitoring in freshwater ecosystems. I used a series of laboratory experiments to investigate delta N-15 equilibration rates and concentration-level effects of single-source N solutions in plant tissue over 16 d to provide baseline data for sewage-plume mapping with *Wolffia* spp. I also tested concentration-level effects in multisource solutions to investigate the effects of mixed-source inputs. *Wolffia* reflected environmental N sources with a 12- to 16-d isotopic equilibration time and showed enriched and depleted delta N-15 ratios for manure and KNO₃ solutions, respectively, but distinguished poorly between lower concentrations of manure. Fractionations at isotopic equilibrium were opposite to expectations and decreased with increasing [N]. *Wolffia* showed a consistent preference for NH₃ in mixed-source treatments, regardless of the proportion or concentration of NH₃ or NO₃-available, and a capacity for N storage, which may complicate mapping of N-loading in natural environments. *Wolffia* is likely to be a less useful bioindicator than the previously tested *Spirodela*. Future research should focus on field applications of *Spirodela* spp. to test its capacity for sewage-plume mapping of freshwater ecosystems in a natural environment.

Ecological comparison between duckweeds in central Italy: The invasive *Lemna minuta* vs the native *L. minor*

Ceschin, S; Abati, S; Leacche, I; Zuccarello, V (2018) PLANT BIOSYSTEMS 152: 674-683

The American duckweed *Lemna minuta* shows an invasive behaviour in Europe, causing weed problems in aquatic habitats there. Few studies addressed this species' ecological requirements for a suitable establishment in a site. In this paper, *L. minuta* populations were analysed through field surveys so as (1) to define the autoecology of this duckweed as regards the main environmental factors characterizing invaded habitats, and (2) to identify possible overlaps/differences in ecological requirements between the alien *L. minuta* and the common native *L. minor*, with which it is often associated and in direct competition. The occurrence/abundance of the two species and environmental data were collected from 41 wetlands in central Italy. Currently, *L. minuta* is more common and abundant than *L. minor* in the study-area, despite its recent arrival there (2007). The two species have a partially overlapped autoecology. However, *L. minuta* differs from *L. minor* since it occurs in waters which are less alkaline, slightly less warm, and richer in nitrates. It shows tolerance for environmental conditions which are limiting for most of macrophytes, including *L. minor*, such as high shading and low water oxygenation. This enables *L. minuta* to increase its invasion potentiality and thus to enlarge its distribution area.

Feed & Food

Trophic transfer of Cd from duckweed (*Lemna minor* L.) to tilapia (*Oreochromis mossambicus*)

Xue, Y; Peijnenburg, WJGM; Huang, J; Wang, DJ; Jin, Y (2018) ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 37: 1367-1377

The transfer of the toxic heavy metal Cd from duckweed (*Lemna minor* L.) to the freshwater fish tilapia (*Oreochromis mossambicus*) was investigated. Concentrations of Cd in different chemical forms in duckweed and in different tissues (gut, edible muscle, and remnants or residual) of tilapia (i.e., ethanol-extractable fraction [F-E], HCl-extractable fraction [F-HCl], and residual fraction [F-R]) were quantified, and the bioaccumulation factors (BAFs) of Cd in the tilapia body were calculated. Simple linear regression analysis was used to unravel the correlation and accumulation mechanisms of Cd along the short food chain. Our results showed that with increasing exposure concentrations of Cd (0-50 M for duckweed and 0-10 M for tilapia), the total, F-E (F-e,F-d)-, F-HCl (F-h,F-d)-, and F-R (F-r,F-d)-Cd concentrations in duckweed and different tissues of tilapia increased progressively. The Cd sources (aqueous or dietary) influenced the BAF for Cd accumulation in the whole body of tilapia. Furthermore, regression analyses yielded significant positive correlations ($R^2 > 0.96$) between the Cd concentration in duckweed and in both the 3 parts and the whole body of tilapia. This finding suggests that Cd transfer from duckweed to tilapia can be quantitatively evaluated when tilapia is exposed only to duckweed. In addition, the linear regression between Cd accumulation in whole tilapia and F-e,F-d-, F-h,F-d-, and F-r,F-d-Cd showed that particularly the correlation with F-e,F-d-Cd is statistically significant ($p < 0.001$). The accumulated Cd concentrations and chemical forms in tilapia tissues also positively correlated with Cd sources (solution or duckweed). Compared with waterborne exposure only, duckweed especially increased the accumulation of Cd in the gut of tilapia. Taken together, our findings support a strong dependence of Cd accumulation and transfer from duckweed to tilapia on its chemical forms, especially on F-e,F-d-Cd. This knowledge may expedite more accurate risk assessment of heavy metals through aquatic food chain ecosystems.

Potential impacts of dietary *Lemna gibba* supplements in a simulated ruminal fermentation system and environmental biogas production

Tirado-Estrada, G; Ramos-Mijangos, LM; Miranda-Romero, LA; Tirado-Gonzalez, DN; Salem, AZM; Mlambo, V; Medina-Cuellar, SE; Gonzalez-Reyes, M; Pliego, AB (2018) JOURNAL OF CLEANER PRODUCTION 181: 555-561

Enteric methane production from ruminants contributes to current global warming challenges faced by mankind. Supplements that improve nutritive value of diets are potential mitigating strategies that may reduce enteric methane emissions. This study was, therefore, designed to evaluate the potential of duckweed (*Lemna gibba*) supplement to reduce enteric methane emissions using an in vitro ruminal gas production technique. In the first of two experiments, *Lemna gibba* from two water bodies (LG1 and LG2), lucerne and ryegrass samples were analyzed for chemical composition and in vitro ruminal fermentation parameters. In the second experiment, the two *Lemna gibba* samples were each included in a basal diet at 5, 10, 15, 20 and 25% to create ten dietary treatments. The dietary treatments were also analyzed for chemical composition and in vitro ruminal fermentation characteristics as in the first experiment. *Lemna gibba* and lucerne fermentation resulted in similar propionate levels. The inclusion of 15% *L gibba* had no effect on the ruminal fermentation patterns (volatile fatty acids, acetate:propionate ratio, acetate, propionate and butyrate) and the dry matter and organic matter degradability. These results indicate that *L gibba* could be used in ruminant diets

as an alternative to grains or concentrates with the added advantage of possibly reducing ruminal methane emissions. Dietary supplementation with *L. gibba* in ruminant diets could be an environmentally friendly strategy to reduce feed costs and ensure sustainable production.

Insights into phytase-containing transgenic *Lemna minor* (L.) as a novel feed additive

Ghosh, M; Sharma, N; Gera, M; Kim, N; Huynh, D; Zhang, J; Min, T; Sodhi, SS; Kim, MB; Rekha, VPB ...More (2018) TRANSGENIC RESEARCH 27: 211-224

This study assessed the effect of supplementation of novel transgenic phytase on growth performance and bone mineralization in Korean native broiler chickens. The experiment was designed using four dietary groups: those with a diet supplemented with (A) recombinant phytase, (B) transgenic phytase from the plant *Lemna minor*, (C) or wild-type *L. minor* as well as (D) a control group that was supplemented with commercially available feed. Three hundred 1-day-old Korean native broiler chicks were used and divided into these four dietary treatment groups having three replicates of 25 birds each (n = 75). The results showed increases in growth performance and bone mineralization in Groups B and C; compared with Groups A and D. Hematological analyses revealed notable contrasts in erythrocyte sedimentation rate, red blood cell count, and hemoglobin levels among the experimental groups, whereas no impacts of dietary treatment were observed on total eosinophil, lymphocyte, heterophil, monocyte, and basophil levels. The relative expression profiling of candidate genes showed that the genes involved in growth response, meat quality, and P-Ca metabolism were significantly highly expressed in the phytase-supplemented groups. Hence, it is suggested that dietary supplementation with transgenic phytase plant *L. minor* for enhancing growth performance is a promising new approach in the broiler feed industry. To the best of our knowledge, we report here the most comprehensive analysis using a broiler model that provides a workable platform for further research on the cost-effective production of feed with different compositions that might be beneficial in the livestock feed industry.

Interaction with microorganisms

Duckweed diversity decreases heavy metal toxicity by altering the metabolic function of associated microbial communities

Zhao, Z; Shi, HJ; Liu, CQ; Kang, XJ; Chen, LC; Liang, XF; Jin, L (2018) CHEMOSPHERE 203: 76-82

Mono-cultured and mix-cultured duckweed species were investigated with respect to the function of their associated microbial communities in heavy metal contaminated wastewater. Results show that the carbon source utilization patterns of the *L. aequinoctialis*- and *S. polyrhiza*-associated microbial communities were different. The relationships between microbial activity, antioxidant enzyme activity (CAT, GSH, and SOD) and growth was positive and significant. The microbial activity of *L. aequinoctialis* and *S. polyrhiza* in mixture was higher than in monoculture in low and high heavy metal, respectively, thereby altering the utilization of specific carbon source types and increasing duckweed growth and antioxidant enzyme activity, when compared to the monocultured duckweed. Furthermore, results indicate that duckweed species in mixture are protected from damage through regulation of the associated bacterial communities.

Physiology

Wood ash residue causes a mixture of growth promotion and toxicity in *Lemna minor*

Lucas, JS; O'Donoghue MT; Heffernan LB; van Pelt FNAM; O'Halloran, J; Jansen MAK (2018) SCIENCE OF THE TOTAL ENVIRONMENT 625: 667-676

The use of wood as a sustainable biofuel results in the generation of residual wood ash. The ash contains high amounts of plant macronutrients such as phosphorus, potassium, calcium as well as several micronutrients. To explore the potential use of wood ash as a fertiliser, the growth enhancing properties of Sitka spruce (*Picea sitchensis* Bong.) wood ash were contrasted with the potential toxic action, using common duckweed (*Lemna minor* L.) as a model test species. The growth of *L. minor* exposed to wood bottom and fly ash solids and corresponding leachates was assessed in ultra-oligotrophic and eutrophic media. Ash solids and leachates were also tested as neutralized preparations. Suspended ash solids promoted *L. minor* growth up to concentrations of 2.5-5 g/L. Leachates promoted growth up to 10 g ash equivalents per litre, but for bottom ash only. Beneficial effects of wood ash were most pronounced on ultra-oligotrophic medium. However, on such nutrient-deficient medium severe inhibition of *L. minor* biomass and frond growth was observed at relatively low concentrations of fly ash (EC50 = 14 g/L). On standard, eutrophic medium, higher concentrations of fly ash (EC50 = 21 g/L), or neutralized fly ash (EC50 = 37 g/L) were required to impede growth. Bottom ash, or neutralized bottom ash retarded growth at concentrations of 51 g/L and 74 g/L (EC50), respectively, in eutrophic medium. It appears that phytotoxicity is due to the elemental composition of the ash, its alkaline character, and possible interactions between these two properties. Growth promotion was due to the substantial content of plant nutrients. This study underlines the importance of the receiving environment (nutrient status and pH) in determining the balance between toxicity and growth promotion, and shows that the margin between growth promoting and toxicity inducing concentrations can be enlarged through ash neutralization.

Metabolic patterns in *Spirodela polyrhiza* revealed by N-15 stable isotope labeling of amino acids in photoautotrophic, heterotrophic, and mixotrophic growth conditions

Evans, EM; Freund, DM; Sondervan, VM; Cohen, JD; Hegeman, AD (2018) FRONTIERS IN CHEMISTRY 6; Article Number: 191

In this study we describe a [N-15] stable isotopic labeling study of amino acids in *Spirodela polyrhiza* (common duckweed) grown under three different light and carbon input conditions which represent unique potential metabolic modes. Plants were grown with a light cycle, either with supplemental sucrose (mixotrophic) or without supplemental sucrose (photoautotrophic) and in the dark with supplemental sucrose (heterotrophic). Labeling patterns, pool sizes (both metabolically active and inactive), and kinetics/turnover rates were estimated for 17 of the proteinogenic amino acids. Estimation of these parameters followed several overall trends. First, most amino acids showed plateaus in labeling patterns of <100% [N-15]-labeling, indicating the possibility of a large proportion of amino acids residing in metabolically inactive metabolite pools. Second, total pool sizes appear largest in the dark (heterotrophic) condition, whereas active pool sizes appeared to be largest in the light with sucrose (mixotrophic) growth condition. In contrast turnover measurements based on pool size were highest overall in the light with sucrose experiment, with the exception of leucine/isoleucine, lysine, and arginine, which all showed higher turnover in the dark. K-means clustering analysis also revealed more rapid turnover in the light treatments with many amino acids

clustering in lower-turnover groups. Emerging insights from other research were also supported, such as the prevalence of alternate pathways for serine metabolism in non-photosynthetic cells. These data provide extensive novel information on amino acid pool size and kinetics in *S. polyrhiza* and can serve as groundwork for future metabolic studies.

The effects of photoperiod and nutrition on duckweed (*Landoltia punctata*) growth and starch accumulation

Liu, Y; Wang, XH; Fang, Y; Huang, MJ; Chen, XY; Zhang, Y; Zhao, H (2018) INDUSTRIAL CROPS AND PRODUCTS 115: 243-249

Duckweed has been considered as a renewable feedstock for bioethanol production due to its fast generation of biomass and high starch accumulation capability. In order to obtain high starch content in duckweed, *Landoltia punctata* was cultivated under 16:8 h or 24:0 h light/dark photoperiod cycles, each combined with a nutrient starvation or Hoagland's solution. The results showed that the starch percentage and starch yield under 24:0 h/ nutrient starvation was higher than other conditions, with the maximum percentage of 60.03% and 76.45 g m⁻² (dry weight) in 21 days. Moreover, the results showed that long photoperiod cycles and nutrient starvation had more positive effects on starch accumulation, but nutritional concentration has no significant effect on the biomass production in short-term culturing (7 days). Notably, the biomass yield was: 24:0 h/ Hoagland's > 24:0 h/ nutrient starvation > 16:8 h/ Hoagland's > 16:8 h/ nutrient starvation in 21 days. This study provides optimized photoperiods and nutritional conditions for future industrial large-scale duckweed cultivation.

Effects of agitating culture condition on the growth, metabolic and carotenoid profiles of *Lemna paucicostata*

Jeon, JY; Kim, SY; Kim, HY; Kim, SH; Lee, BJ; Lim, SR; Choi, HK (2018) BIOTECHNOLOGY AND BIOPROCESS ENGINEERING 23: 23-30

The effects of agitating culture conditions on the growth, metabolic and carotenoid profiles of *Lemna paucicostata* were investigated in this study using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. *Lemna paucicostata* cultivated under static and agitating conditions showed no significant difference in total plant growth. Various alcohols, amino acids, fatty acids, organic acids, sugars, and phenolic compounds were identified in the sample. The higher relative yields of ferulic acid and GABA were obtained under the agitating condition on day 28, whereas that of coumaric acid was higher under the static condition on day 42. Significantly higher relative yields of antheraxanthin and lutein were achieved under agitation condition than static condition on day 42. In addition, fucoxanthinol was identified for the first time in *L. paucicostata* culture. The results of this study suggest that static and agitating culture conditions, as well as optimal harvest timing, should be selected according to the target products including ferulic acid, GABA, coumaric acid, antheraxanthin and lutein in *L. paucicostata* culture.

Phytoremediation

The accumulation, transformation, and effects of quinnestrol in duckweed (*Spirodela polyrhiza* L.)

Geng, QQ; Li, T; Li, PL; Wang, X; Chu, WJ; Ma, YN; Ma, H; Ni, HW (2018) SCIENCE OF THE TOTAL ENVIRONMENT 634: 1034-1041

Potential risk of endocrine disrupting compounds on non- target organisms has received extensive attentions in recent years. The present work aimed to investigate the behavior and effect of a synthetic steroid estrogen quinestrol in duckweed *Spirodela polyrhiza* L. Experimental results showed that quinestrol could be uptaken, accumulated, and biotransformed into 17 alpha-ethynylestradiol in *S. polyrhiza* L. The accumulation of quinestrol had a positive relation to the exposure concentration. The bioaccumulation rate was higher when the duckweed was exposed to quinestrol solutions at low concentrations than at high concentration. While the transformation of quinestrol showed no concentration-dependent manner. Quinestrol reduced the biomass and pigment content and increased superoxide dismutase and catalase activities and malondialdehyde contents in the duckweed. The results demonstrated that quinestrol could be accumulated and biotransformed in aquatic plant *S. polyrhiza* L. This work would provide supplemental data on the behavior of this steroid estrogen compound in aquatic system.

Effects of treated industrial wastewaters and temperatures on growth and enzymatic activities of duckweed (*Lemna minor* L.)

Basiglini, E; Pintore, M; Forni, C (2018) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 153: 54-59

The efficacy of the removal of contaminants from wastewater depends on physico-chemical properties of pollutants and the efficiency of treatment plant. Sometimes, low amounts of toxic compounds can be still present in the treated sewage. In this work we considered the effects of contaminant residues in treated wastewaters and of temperatures on *Lemna minor* L. Treated effluent waters were collected, analyzed and used as duckweed growth medium. In order to better understand the effects of micropollutants and seasonal variation, the plants were grown under ambient conditions for seven days in summer and winter. Relative growth rate, pigments and phenolic compounds concentrations were determined, as well as the activities of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (G-POD) and polyphenol oxidase (PPO). The pollutant concentrations varied in the two seasons, depending on the industrial and municipal activities and efficiency of treatments. Treated waters contained heavy metals, nitrogenous and phosphorus compounds, surfactants and hydrocarbons. Compared to the control, duckweed growth of treated plants decreased by 25% in summer, while in the winter due to the lower temperatures and the presence of pollutants was completely impeded. The amounts of photosynthetic pigments of treated plants were not significantly affected in the summer, while they were higher than the control in the winter when the effluent had a high nitrogen amount. High CAT activity was registered in both seasons. Treated plants had significantly lower APX activity in the summer (53%) and winter (59%) respect to the controls. The observed inhibition of the peroxidase activities in the exposed plants, confirms the controversy existing in the literature about the variability of enzymatic response in stress condition.

Ecotoxicological evaluation of two anti-dandruff hair shampoos using *Lemna minor*

Azizullah, A; Shakir, SK; Shoaib, S; Bangash, H; Taimur, N; Murad, W; Daud, MK (2018) ENVIRONMENTAL MONITORING AND ASSESSMENT 190: Article Number: 268

Hair shampoos, a mixture of various organic and organic compounds, are commonly used personnel care products. Since shampoos are used in almost every household and beauty shop, their ingredients are common components of domestic and municipal wastewater. However, studies on the effect of shampoos to aquatic plants can hardly be found in literature. Therefore, the present study was conducted to investigate the phytotoxic effects of two commonly used anti-dandruff shampoos (named here AD 1 and AD 2) using *Lemna minor* as a biotest organism. For toxicity assessment, frond number, fresh and dry biomass, and light harvesting pigments (chlorophyll a, b

and total carotenoids) of *Lemna* were used as end points. Five different concentrations (0.001, 0.01, 0.1, 1, and 5%) of each shampoo were tested in comparison to the control. At lower concentrations of shampoos, some minor and non-significant stimulatory effects were observed in some parameters, but at concentrations above 0.01% both the shampoos significantly inhibited almost all parameters in *Lemna*. The EC50 values obtained for frond number were 0.034 and 0.11% for AD 1 and AD 2, respectively. The fresh biomass gave EC50 values of 0.07 and 0.066% for AD 1 and AD 2, respectively. Based on the preset study, it can be speculated that shampoo contamination at higher concentrations in water bodies can be a threat to aquatic organisms. This study can be used as a baseline to further investigate shampoo toxicity using other species and to explore the mechanism of shampoo toxicity in aquatic plants.

Sorption and removal of iodate from aqueous solution using dried duckweed (*Landoltia punctata*) powder

Zhang, K; Chen, T (2018) JOURNAL OF RADIOANALYTICAL AND NUCLEAR CHEMISTRY 316: 543-551

Dried duckweed (*Landoltia punctata*) powder is applied to remove IO_3^- from aqueous solutions under various conditions. The results indicate the $K(d)$ is 150 ml g^{-1} under general conditions. The sorption kinetics follow the pseudo-second-order equation, and the isotherm is well described by the Freundlich model. Hydroxyl and carbonyl groups contribute to IO_3^- sorption by ion-exchange, electrostatic attraction and redox reactions. Spectroscopic analyses prove that IO_3^- is reduced to I^- and I^0 by hydroxyl groups. These results demonstrate that duckweed (*Landoltia punctata*) is a promising biosorbent for environmental remediation of radioactive iodine pollution.

Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent

Toyama, T; Hanaoka, T; Tanaka, Y; Morikawa, M; Mori, K (2018) BIORESOURCE TECHNOLOGY 250: 464-473

To assess the potential of duckweeds as agents for nitrogen removal and biofuel feedstocks, *Spirodela polyrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* were cultured in effluents of municipal wastewater, swine wastewater, or anaerobic digestion for 4 days. Total dissolved inorganic nitrogen (T-DIN) of 20-50 mg/ L in effluents was effectively removed by inoculating with 0.3-1.0 g/ L duckweeds. *S. polyrhiza* showed the highest nitrogen removal (2.0-10.8 mg T-DIN/ L/ day) and biomass production (52.6-70.3 mg d.w./ L/ day) rates in all the three effluents. Ethanol and methane were produced from duckweed biomass grown in each effluent. *S. polyrhiza* and *L. punctata* biomass showed higher ethanol (0.168-0.191, 0.166-0.172 and 0.174-0.191 g-ethanol/ g-biomass, respectively) and methane (340-413 and 343-408 NL CH_4 / kg VS, respectively) production potentials than the others, which is related to their higher carbon and starch contents and calorific values.

Effect of cadmium and lead on nitrate and phosphate removal by the duckweed *Lemna gibba*

Aggoun, A; Benmaamar, Z; Semsari, S; Boucherit, A (2018) ANNALI DI BOTANICA 8: 17-23

In the present study, the effect of the heavy metals, such as cadmium and lead on the removal of nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) was assessed using *Lemna gibba*. Duckweed plant was cultured in N and P-rich medium, supplemented with heavy metals. A total of two initials (0.1 and 1

mg/ L) concentrations of Cd and Pb were used. Samples were taken every two days to assess plants efficiency in removing both nutrients and heavy metals over six days. Results showed that in control and in all treatments (Cd and Pb), nitrate and orthophosphate concentrations decreased markedly within the two days of initiating experiments as compared to the initial concentrations (1.76 ± 0.01 mg P/L and 850 ± 0.01 mg N/L). The highest phosphate removal efficiencies (percentage removal) were obtained on the fourth day at 1 mg Cd/ L and 1 mg Pb/ L. Whereas, nitrate removal showed maxima on the sixth day at 1 mg Cd/ L and at 0.1 mg Pb/ L. As compared to the control, the presence of Cd and Pb at 0.1 mg/ L in the culture medium had no effect on phosphate removal, while a Pb concentration of 1 mg/ L revealed a better phosphate removal. Cd and Pb at 0.1 mg/ L enhanced nitrate removal as compared to control. *Lemna gibba* was able to simultaneously remove Cd, Pb, nitrate and phosphate, major causes of contamination and eutrophication in water bodies.

Physiological and differential proteomic responses of *Lemna minor* to ammonia stress in constructed wetlands

Zhao, CC; Xu, JT; Xu, XL (2018) FRESINIUS ENVIRONMENTAL BULLETIN 27: 2026-2034

Lemna minor is widely used in constructed wetlands. However, high ammonia concentrations are toxic to *L. minor*; furthermore, the molecular mechanisms of ammonia stress and recovery remains unknown. In this study, tolerance and recovery of ammonia-exposed *L. minor* were examined by physiological and differential proteomics. Compared with the control group, soluble proteins were respectively reduced by 40% and 50% at ammonia of 80 (A-80) and 160 (A-160) mg L⁻¹, and the concentrations of potassium, calcium, and magnesium decreased as well. In contrast, phosphorus increased and reactive oxygen species (ROS) appeared. Ammonia stress evidently affected *L. minor* more when exposed to ammonia of 320 (A-320) and 640 (A640) mg L⁻¹. Although catalase and superoxide dismutase could scavenge ROS resulting in the equilibrium of antioxidant system, the plants still experienced some sort of oxidative damage. Damages in A-320 and A-640 were irreversible, some of which were fatal, while damages in A-80 and A-160 were reversible and *L. minor* could fully recover after 7 or 14 d. The tolerance mechanism of *L. minor* was also investigated by differential proteomic analysis. It was found that the proteins associated with ammonium tolerance were also involved in promoting photosynthesis, scavenging ROS, detoxifying enzymes, and inducing nitrogen.

Phytotoxicity

The influence of aquaponically grown duckweed (*Lemna minuta* Kunth) used for composition of sustainable diets on hydrochemical and technological parameters in carp (*Cyprinus carpio* L.)

Sirakov, I; Velichkova, K (2018) TURKISH JOURNAL OF FISHERIES AND AQUATIC SCIENCES 18: 1037-1044

The aim of the current study was to investigate the influence of aquaponically grown duckweed (*Lemna minuta*) used as part of a biofilter in recirculation aquaculture systems, when its included in the composition (10 and 30% content of daily feed ratio) of sustainable diets on hydrochemical and technological parameters in common carp (*Cyprinus carpio* L) fingerlings cultivated in recirculation aquaponic systems. The inclusion of *L. minuta* in diets for carp fingerlings influenced the hydrochemical parameters and decreased the quantity of nitrogen and phosphorus compounds in water of tanks where carps were fed with feed containing duckweed, but difference was statistically significant only for ammonium (0.087 ± 0.008)($P \leq 0.05$). The carps fed with a diet containing 30% (L30) duckweed of their daily feed ratio showed better survival but similar growth and FCR than fish

fed with L0 and differences were not significant ($P \leq 0.05$). The lower growth and higher FCR were measured in carps fed with L10 compared to the values of these parameters in carp's fingerlings fed with L0 diet and the differences were significant ($P \leq 0.05$). The duckweed presents cheap and easy accessible ingredients for feeding of carp. Furthermore, it could be used for treatment of wastewater in recirculating aquaculture systems this way increasing their sustainability.

Phytotoxicity of amoxicillin to the duckweed *Spirodela polyrhiza*: Growth, oxidative stress, biochemical traits and antibiotic degradation

Singh, V; Pandey, B; Suthar, S (2018) CHEMOSPHERE 201: 492-502

The increasing availability of antibiotics in wastewater has created a serious threat to non-target organisms in the environment. The aim of this study was to evaluate the potential toxicity of amoxicillin on duckweed *Spirodela polyrhiza* during a short-term exposure (7 d). The duckweed was exposed to a range of environmentally relevant (0.0001 - 0.01 mg L^{-1}) and high (0.1 and 1 mg L^{-1}) concentrations of amoxicillin. Subsequently, biomarkers of toxicity such as growth, pigments (Chl a, Chl b and carotenoids), antioxidative enzyme activity (catalase, CAT; superoxide dismutase, SOD; and ascorbate peroxidases, APX), and biochemical content (protein, lipid and starch) were analysed in their fronds. The high dose (1 mg L^{-1}) of amoxicillin caused a significant ($p < 0.05$) decrease in photopigments, protein, starch and lipid content and an increase in carotenoids/total Chl and Chl a/Chl b ratios in fronds of *Spirodela polyrhiza*. The results showed a shift in biomarkers: a decrease in frond growth and relative growth rate (RGR) (16.2-53.8%) and an increase in the activities ($\text{mmol mg protein}^{-1}$) of CAT (0.021-0.041), APX (0.84-2A9) and SOD (0.12-0.23) in fronds. The significantly ($p < 0.05$) greater reduction in amoxicillin content in duckweed setups (84.6-100%) than in the control (62.1-73%) suggested that phytodegradation is an important mechanism in removing antibiotics from water, apart from hydrolysis and photodegradation, which occur in control setups. Overall, the results suggested a toxic effect of amoxicillin on *Spirodela polyrhiza*, even at low concentrations, and nonetheless, the duckweed contributed directly to the degradation of antibiotics in the water and throughout the phytoremediation process.

Herbicidal effects of Chinese herbal medicine *Coptis chinensis* Franch. extract on duckweed (*Spirodela polyrhiza* (L.) Schleid.)

Shao, L; Li, JY; Zhang, YJ; Song, YY; Yu, KF; He, PM; Shen, AL (2018) ECOLOGICAL ENGINEERING 115: 9-14

Dense mat formed by duckweeds is a significant threat to fisheries, landscape, ecological environment, and economies around the world. Effective biological management strategies to control duckweeds are quite limited. In the present study, we determined the effect of the extract of *Coptis chinensis*, a traditional Chinese medicinal herb, on the growth of duckweed (*Spirodela polyrhiza*). The growth of *S. polyrhiza* was strongly inhibited by the *C. chinensis* extract. The number of fronds and fresh weight of the plants were significantly decreased after a 96-h treatment. Furthermore, the chlorophyll (a, b, and a + b) content was remarkably decreased by the *C. chinensis* extract. The 24-, 48-, 72- and 96-h-IC₅₀ of the *C. chinensis* extract to *S. polyrhiza* were 6.56, 1.06, 0.81, and 0.33 g DW eq. extract L^{-1} , respectively. In addition, the *C. chinensis* extract was found to be safer for the submerged macrophytes (such as *Vallisneria natans*: 96-h-EC₅₀ was 10.39 g DW eq. extract L^{-1}) and aquatic animals (such as zebrafish: 96-h-LC₅₀ was 20.35 g DW eq. extract L^{-1}). These results showed that the *C. chinensis* extract was inhibitory to the growth and reproduction of *S. polyrhiza*. Moreover, the field test results also confirmed that the inhibitory effect of *C. chinensis* extract on *S. polyrhiza*. Thus, we recommend the use of the *C. chinensis* extract as an effective and safe botanical herbicide for duckweed management in water ecosystems.

The use of computer image analysis in a *Lemna minor* L. bioassay

Mazur, R; Szoszkiewicz, K; Lewicki, P; Bedla, D (2018) HYDROBIOLOGIA 812: 193-201

Our study presents a low-cost method (no expensive hardware platforms required) of quantified biomonitoring based on computer image analysis. The negative influence of toxins on surface waters was analysed. The method was verified on widespread freshwater macrophyte *Lemna minor* to test populations treated with non-ionic detergents. We showed that the proposed automated bioassay has a broad applicability in assessing the negative impacts of aquatic toxicants. This approach enabled fast and precise evaluation of the morphometric parameters of the duckweed test population. We observed that growth rate of *L. minor* reacts to non-ionic detergents, which is reflected by the change in the surface area. The decrease in the growth of *L. minor* was revealed at high doses of detergents. This test proved to be highly useful, because it is well repeatable and fast in its implementation. Unlike classical bioassays, the proposed test allows the elimination of measurement errors, resulting from observers' subjectivity.

A comparison of growth on mercuric chloride for three Lemnaceae species reveals differences in growth dynamics that effect their suitability for use in either monitoring or remediating ecosystems contaminated with mercury

Yang, JJ; Li, GJ; Bishopp, A; Heenatigala, PPM; Hu, SQ; Chen, Y; Wu, ZG; Kumar, S; Duan, PF; Yao, LG ...More (2018) FRONTIERS IN CHEMISTRY 6; Article Number: 112

Mercury (Hg) is a toxic heavy metal that can alter the ecological balance when it contaminates aquatic ecosystems. Previously, researchers have used various Lemnaceae species either to monitor and/or remove heavy metals from freshwater systems. As Hg contamination is a pressing issue for aquatic systems worldwide, we assessed its impact on the growth of three commonly species of Lemnaceae - *Lemna gibba* 6745, *Lemna minor* 6580 and *Spirodela polyrhiza* 5543. We exposed plants to different concentrations of mercuric chloride (HgCl₂) and monitored their growth, including relative growth rate, frond number (FN), and fresh weight (FW). These data were coupled with measurements of starch content, levels of photosynthetic pigment and the activities of antioxidant substances. The growth of all three lines showed significant negative correlations with Hg concentrations, and starch content, photosynthetic pigment, soluble protein and antioxidant enzymes levels were all clearly affected. Our results indicate that the *L. gibba* line used in this study was the most suitable of the three for biomonitoring of water contaminated with Hg. Accumulation of Hg was highest in the *S. polyrhiza* line with a bioconcentration factor over 1,000, making this line the most suitable of the three tested for use in an Hg bioremediation system.

Linking mode of action of the model respiratory and photosynthesis uncoupler 3,5-dichlorophenol to adverse outcomes in *Lemna minor*

Xie, L; Gomes, T; Solhaug, KA; Song, Y; Tollefsen, KE (2018) AQUATIC TOXICOLOGY 197: 98-108

Standard chemical toxicity testing guidelines using aquatic plant *Lemna minor* have been developed by several international standardisation organisations. Although being highly useful for regulatory purposes by focusing on traditional adverse endpoints, these tests provide limited information about the toxic mechanisms and modes of action (MoA). The present study aimed to use selected functional assays in *L. minor* after exposure to 3,5-dichlorophenol (3,5-DCP) as a model to characterise the toxic mechanisms causing growth inhibition and lethality in primary producers. The results demonstrated that 3,5-DCP caused concentration-dependent effects in chloroplasts and mitochondria. Uncoupling of oxidative phosphorylation (OXPHOS), reduction in chlorophyll

(Chlorophyll a and b) content, reproduction rate and frond size were the most sensitive endpoints, followed by formation of reactive oxygen species (ROS), lipid peroxidation (LPO), reduction of carotenoid content and impairment of photosynthesis efficiency. Suppression of photosystem II (PSII) efficiency, to identify the most suitable isolates for duckweed selective breeding for bioethanol. Electron transport rate (ETR), chlorophyll (a and b) contents and oxidative phosphorylation (OXPHOS) were closely correlated while ROS production and LPO were negative correlated with ETR, carotenoid content and growth parameters. A network of conceptual Adverse Outcome Pathways (AOPs) was developed to decipher the causal relationships between molecular, cellular, and apical adverse effects occurring in *L. minor* to form a basis for future studies with similar compounds.

The response of *Lemna minor* to mixtures of pesticides that are commonly used in Thailand

Tagun, R; Boxall, ABA (2018) BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 100: 516-523

In the field, aquatic organisms are exposed to multiple contaminants rather than to single compounds. It is therefore important to understand the toxic interactions of co-occurring substances in the environment. The aim of the study was to assess the effects of individual herbicides (atrazine, 2,4-D, alachlor and paraquat) that are commonly used in Thailand and their mixtures on *Lemna minor*. Plants were exposed to individual and binary mixtures for 7 days and the effects on plant growth rate were assessed based on frond area measurements. Experimental observations of mixture toxicity were compared with predictions based on single herbicide exposure data using concentration addition and independent action models. The single compound studies showed that paraquat and alachlor were most toxic to *L. minor*, followed by atrazine and then 2,4-D. For the mixtures, atrazine with 2,4-D appeared to act antagonistically, whereas alachlor and paraquat showed synergism.

Time matters: the toxicity of zinc oxide nanoparticles to *Lemna minor* L. increases with exposure time

Chen, XL; O'Halloran, J; Jansen, MAK (2018) WATER AIR AND SOIL POLLUTION 229: Article Number: 99

The use of zinc oxide nanoparticles (nano-ZnO) has rapidly increased in recent years, and this has triggered the need for versatile toxicity tests that can be used to test a range of different exposure scenarios. Acute exposure studies, using a variety of plant species, have overwhelmingly demonstrated nano-ZnO-induced toxicity, but substantial differences in the degree of phytotoxicity are reported in different studies. Here, we analysed the role of exposure time in determining the variation in phytotoxic effects. Using the model species *Lemna minor*, the effects of short-term (24 h), standardised (1 week) and chronic (up to 6 weeks) nano-ZnO exposure were compared. Nano-ZnO effects on *Lemna minor* growth indicators (biomass growth rate, root length), chlorophyll content and photosynthetic efficiency were measured. Rapid inhibitory effects of nano-ZnO on the maximal quantum yield of photosystem II could be measured after just 24-h exposure. Standardised (1 week) experiments revealed phytotoxic effects on *Lemna minor* biomass growth. More severe inhibitory effects on growth developed gradually over 4 to 6 weeks exposure to nano-ZnO, and these were qualitatively associated with increased zinc content in the plant. Such dynamics of nano-ZnO toxicity have not been elucidated before, and this study emphasises the importance of exposure time in studies of nanoparticle toxicity. We conclude that short-term, standardised experiments can potentially underestimate the environmental phytotoxicity, which may result from chronic exposure to nano-ZnO.

Effects of TiO₂ nanoparticles on the aquatic plant *Spirodela polyrrhiza*: Evaluation of growth parameters, pigment contents and antioxidant enzyme activities

Movafeghi, A; Khataee, A; Abedi, M; Tarrahi, R; Dadpour, M; Vafaei, F (2018) JOURNAL OF ENVIRONMENTAL SCIENCES 64: 130-138

Plants are essential components of all ecosystems and play a critical role in environmental fate of nanoparticles. However, the toxicological impacts of nanoparticles on plants are not well documented. Titanium dioxide nanoparticles (TiO₂-NPs) are produced worldwide in large quantities for a wide range of purposes. In the present study, the uptake of TiO₂-NPs by the aquatic plant *Spirodela polyrrhiza* and the consequent effects on the plant were evaluated. Initially, structural and morphological characteristics of the used TiO₂-NPs were determined using XRD, SEM, TEM and BET techniques. As a result, an anatase structure with the average crystalline size of 8 nm was confirmed for the synthesized TiO₂-NPs. Subsequently, entrance of TiO₂-NPs to plant roots was verified by fluorescence microscopic images. Activity of a number of antioxidant enzymes, as well as, changes in growth parameters and photosynthetic pigment contents as physiological indices were assessed to investigate the effects of TiO₂-NPs on *S. polyrrhiza*. The increasing concentration of TiO₂-NPs led to the significant decrease in all of the growth parameters and changes in antioxidant enzyme activities. The activity of superoxide dismutase enhanced significantly by the increasing concentration of TiO₂-NPs. Enhancement of superoxide dismutase activity could be explained as promoting antioxidant system to scavenging the reactive oxygen species. In contrast, the activity of peroxidase was notably decreased in the treated plants. Reduced peroxidase activity could be attributed to either direct effect of these particles on the molecular structure of the enzyme or plant defense system damage due to reactive oxygen species.

Systematics and Evolution

Characterization of 19 polymorphic SSR markers in *Spirodela polyrrhiza* (Lemnaceae) and cross-amplification in *Lemna perpusilla*

Xu, NN; Hu, FL; Wu, JM; Zhang, WJ; Wang, MW; Zhu, MD; Ke, JW (2018) APPLICATIONS IN PLANT SCIENCES 6: Article Number: e1153

Polymorphic microsatellite primers were developed for greater duckweed, *Spirodela polyrrhiza* (Lemnaceae), to investigate genetic diversity and structure for application in a bioremediation program. A total of 401 publicly available *S. polyrrhiza* whole-genome shotgun sequences were searched for simple sequence repeat loci of two or more nucleotides. Of these, 60 primer pairs were selected to analyze 68 individuals of *S. polyrrhiza* from three populations. Nineteen polymorphic microsatellite loci were developed. A total of 108 alleles were detected with an average of 5.7 alleles per locus. The levels of expected and observed heterozygosity were 0.0511-0.8669 and 0-0.8750, respectively. Ten loci also successfully amplified in 16 individuals of *Lemna perpusilla*. The results demonstrate the broad utility of these microsatellite loci for studying population genetics in *S. polyrrhiza*.

Instructions to Contributors for the Duckweed Forum

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

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.

DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

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

Title:

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

Authors:

- All authors are responsible for the content of the manuscript.
- Provide the **complete** names of all authors.
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Links for Further Reading

<http://www.rduckweed.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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