

ISCDRA

International Steering Committee on Duckweed Research and Applications

April 2015, vol 3, part 2 – Issue #8 – pages 34 to 80

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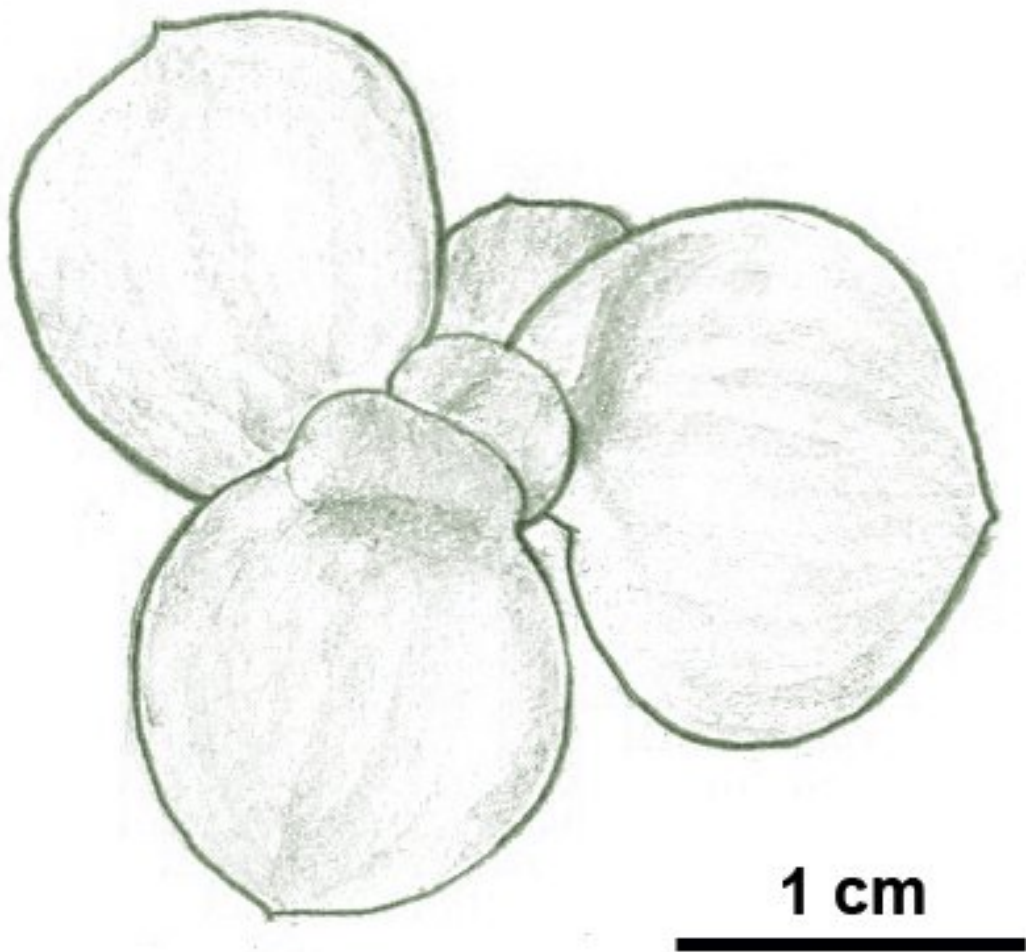
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- Prof. Zhao Hai, Chengdu Institute of Biology, Chinese Academy of Sciences, China; zhaohai@cib.ac.cn

More information about the ISCDRA and previous issues are available at

<http://lemnepedia.org/wiki/ISCDRA>

Science meets Art: *Spirodela polyrhiza* (L.) Schleid.



Spirodela polyrhiza, also called the giant duckweed, was first described by Linné in 1753 with the name *Lemna polyrhiza*. It has a worldwide distribution. This species has been extensively used for investigating developmental processes like turions formation and germination. Recently, its genome (clone 7498) was sequenced, the first one in the duckweed family. Drawing by Dr. K. Sowjanya Sree, India.



Letter from the Editor

In many parts of the world spring is arriving with an array of colours– always a reason to be happy. We also hope to present you a colourful Newsletter; Volume 3, issue 2.

The highlight is the announcement of our biannual, 3rd International Conference on Duckweed Research and Applications in Kyoto, Japan from 3rd to 6th July, 2015. More details on registration and submission of abstracts are available on the conference home page. Please note the deadline for registration: 30th of April 2015. The members for the next term of International Steering Committee on Duckweed Research and Applications have to be elected. We ask you to nominate possible candidates. The procedure and criteria of election are reported in this issue and the deadline for nominations is 22nd of May 2015.

A young research group from Qingdao, China has reported about their research in the field of duckweeds. Dr. Eric Lam, Rutgers University NJ together with some of his co-workers, visited MamaGrande in Argentina which is reported by its co-founder and one of the ISCDRA members, Mr. Eduardo Mercovich. Paul Skillicorn, known for his duckweed field-related activities, now has his emphasis more on India. In this issue he described his vision about practical applications of duckweeds. Another report comes from the Philippines: Patrick Chang, who leads a duckweed start-up project there where they presented their intentions.

We started two new serials with this issue. In one, we describe the commonly used methods in the field of duckweed research which are hopefully of use to people entering this field. The second serial will feature experiments on duckweeds designed especially for practical student courses at Colleges or Universities. With this we hope to attract the attention of teachers in using duckweed for practical student courses. Moreover, we invite contributions for these serials from around the duckweed world.

As always we introduce one species of duckweed, now under the headline “Science meets Art”. A report about the recent up-to-date publications on different aspects of duckweeds in scientific journals (“From the data base”), and some useful links for “further reading” are appended at the end.

We hope you will find this issue interesting and entertaining.

Klaus-J. Appenroth



The 3rd International Conference on Duckweed Research and Applications 2015 (ICDRA) and Call for Papers



The 3rd ICDRA will facilitate close interactions and coordination between duckweed researchers and application specialists from industries on a global scale. This workshop will be pivotal for charting a new course of development for this novel, micro-crop system by creating a new sense of teamwork and focus in the duckweed community. Working together, we can realize the remarkable potential of these plants in terms of biomass production and alleviation of urgent problems facing our planet today like waste water clean-up.



Organizers

- Chair: Tokitaka Oyama, Kyoto University
- Vice chair: Masaaki Morikawa, Hokkaido University
- Yasuyoshi Sakai, Kyoto University
- Klaus-J. Appenroth, University of Jena
- Jay Cheng, North Carolina State University
- Eric Lam, Rutgers, The State University of New Jersey
- Tamra Fakhorian, International Lemna Association, USA
- Zhao Hai, Chengdu Institute of Biology
- Eduardo Mercovich, MamaGrande

Venue: Science Seminar House of Faculty of Science, Kyoto University, Kyoto, JAPAN

Period: From 3rd to 6th July, 2015

Program (tentative)

- 3rd July: Registration and Reception
- 4th July: Scientific talks and Dinner
- 5th July: Scientific talks
- 6th July: Scientific talks, Excursion, and Closing ceremony

Call for Papers: in the conference website <http://www.duckweed2015.cosmos.bot.kyoto-u.ac.jp> there is a downloadable form and the email to send them. All submissions will be reviewed and authors promptly notified upon acceptance.

Registration fee: 40,000 yens (~116 yens/dollar, ~145 yens/euro, at Nov 17, 2014)

Housing: Kyoto Garden Palace Hotel (~8,000 yens / single night)

For a listing of past ICDRA conferences and it's presentations, please visit

http://lemnepedia.org/wiki/International_Conference_of_Duckweed_Research_and_Applications



Announcing Call for Nominees for ISCDRA

Order for the election of the members of the International Steering Committee on Duckweed Research and Applications.

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

Members of the committee cooperate with each other in order to steer duckweed research and applications. Publishing ISCDRA Newsletter is one of the obligations, among others.

- 1) The ISCDRA should consist of 5 members who should be elected before the biannual ICDRA in a secret poll.
- 2) Anyone who has previously attended any of the ICDRA or will be attending it this year, or receives the ISCDRA Newsletter can suggest potential candidates including themselves up to 6 weeks before the meeting. Candidates should have attended any of the previous 2 ICDRA meetings. Suggestions may be sent to the present head of the ISCDRA- Dr. Klaus J. Appenroth, Email: election@lemnapedia.org up to 6 weeks before the conference. For the election 2015 this will be 22nd of May 2015.
- 3) Voting will be done electronically, via email to the current ISCDRA designated members between 4 week and 2 week before the next ICDRA. Deadline: 19th of June 2015. The right of voting has everybody who is attending the present ICDRA or has attended one before. From the list of candidates, a maximum of 5 candidates could be voted for.
- 4) The five newly elected members will be notified by email and they will elect the head of the committee before the ICDRA.
- 5) In case that by chance all elected members are either from the applied field or from the research field, the elected Chair will appoint one additional member from the missing field.
- 6) At the end of the ISCDRA meeting the previous Committee reports shortly about the activities since the previous election and the duty is transferred to the newly elected ISCDRA.



Duckweed Research in Qingdao



Qingdao Institute of Bioenergy and Bioprocess Technology Chinese Academy of Sciences

Duckweed has been considered as a valuable feedstock for bioethanol production due to its high biomass and starch production. In our group, we focus on duckweed resource collect in China, special characteristics stain selection, influence factors in duckweed starch accumulation, transcriptomics investigation of duckweed starch accumulation, space-saving duckweed cultivation model development and large-scale duckweed cultivation system development using waster water from cow cultivation.

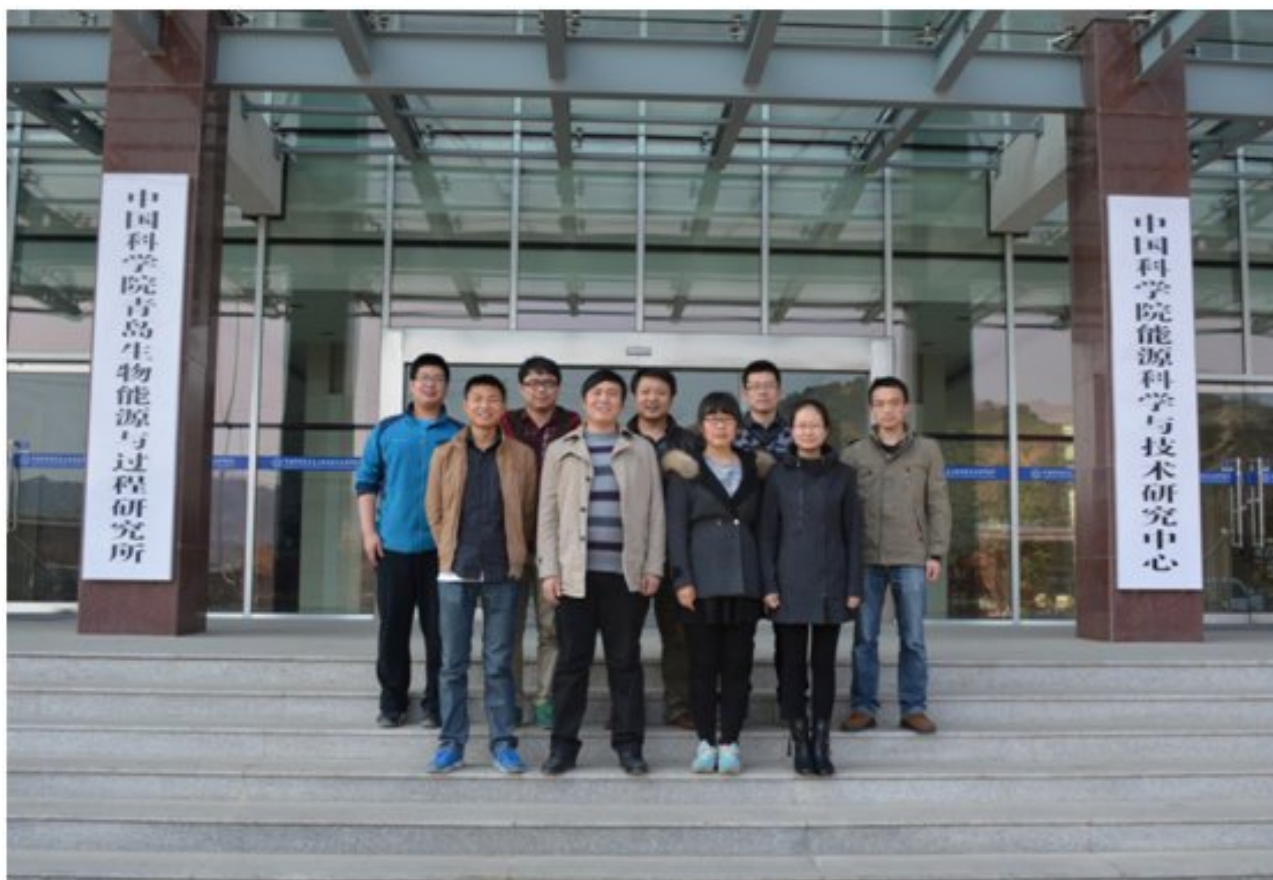
For duckweed resource and strain selection, 311 geographic isolated duckweed strains were collected from 22 provinces in China, after large-scale screening, *Lemna aequinoctialis* 6000 from Hunan province was obtained with the highest starch accumulation in SH medium. For special strain using urban sewage, *Landoltia punctata* strain 20 was obtained with the highest starch production by large-scale screening from 90 duckweed strains. Cadmium is also an important pollutant in China. In our group, 100 duckweed strains were selected to screen cadmium tolerance strain using 100 μM CdCl_2 . *Landoltia punctata* strain 52a-L was obtained with the highest cadmium tolerance ability. Furthermore, photosynthesis related data, free proline soluble phenolics and heavy ion tolerance related gene expression were determined.

As to influence factor in duckweed starch accumulation, we had investigated the starch content variation in one day, and the effect of different culture medium and salt concentration on *L. aequinoctialis* 6000 starch accumulation. To further investigate the effects of light conditions on duckweed biomass and starch production, *L. aequinoctialis* 6000 was cultivated at different photoperiods (12:12, 16:8 and 24:0 h) and light intensities (20, 50, 80, 110, 200 and 400 $\mu\text{mol m}^{-2}$



s-1) (Bioresource technology, in press). Moreover, comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol was done in our group (Plos one, 2014). Further, a highly branched galactoarabinan named DAG1 ($M_w \sim 4.0 \times 10^4$ Da) was purified and structurally analyzed from *L. aequinoctialis* 6000 (Carbohydrate polymers, 2015).

Nitrogen-starvation could induce starch accumulation in duckweed. To further investigate the starch accumulation in *L. aequinoctialis* 6000, transcriptome was analyzed after cultivated in nitrogen-starvation for 0, 3 and 7 days. Differential expression gene was investigated during starch accumulation. In addition, a few key genes in duckweed starch accumulation regulation were obtained by transcriptome sequencing. Generally, duckweed is float on the water which results in plenty of land needed in large-scale cultivation. To promote the duckweed commercialization, space-saving cultivation model was designed. Further, we established 1500 m² duckweed large-scale multilayer cultivation system using waste water from cow cultivation. The duckweed was cultivated in photovoltaic greenhouse to keep the system working in the whole year. The electricity used for supplement light of multilayer cultivation was obtained from the photovoltaic system of the greenhouse. Moreover, duckweed starch automatic isolation system was developed in our group. In the future study, our main interest is duckweed large-scale multilayer cultivation system and the mechanism of duckweed starch accumulation regulation.



Qingdao Duckweed Research Team.



Comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol

Yu, Changjiang; Sun, Changjiang; Yu, Li; Zhu, Ming; Xu, Hua; Zhao, Jinshan; Ma, Yubin; Zhou, Gongke

PLoS ONE, 17, 9(12):e115023 (2014)

Energy crises and environmental pollution have caused considerable concerns; duckweed is considered to be a promising new energy plant that may relieve such problems. *Lemna aequinoctialis* strain 6000, which has a fast growth rate and the ability to accumulate high levels of starch was grown in both Schenk & Hildebrandt medium (SH) and in sewage water (SW). The maximum growth rates reached 10.0 g DW m⁻² day⁻¹ and 4.3 g DW m⁻² day⁻¹, respectively, for the SH and SW cultures, while the starch content reached 39% (w/w) and 34% (w/w). The nitrogen and phosphorus removal rate reached 80% (SH) and 90% (SW) during cultivation, and heavy metal ions assimilation was observed. About 95% (w/w) of glucose was released from duckweed biomass hydrolysates, and then fermented by Angel yeast with ethanol yield of 0.19 g g⁻¹ (SH) and 0.17 g g⁻¹ (SW). The amylose/amylopectin ratios of the cultures changed as starch content increased, from 0.252 to 0.155 (SH) and from 0.252 to 0.174 (SW). *Lemna aequinoctialis* strain 6000 could be considered as valuable feedstock for bioethanol production and water resources purification.

Structural analysis of galactoarabinan from duckweed

Yu, Li; Yu, Changjiang; Zhu, Ming; Cao, Yingping; Yang, Haiyan; Zhang, Xu; Ma, Yubin; Gongke, Zhou

CARBOHYDRATE POLYMERS 117:807–812 (2015)

A highly branched galactoarabinan named DAG1 (Mw~4.0 ×10⁴ Da) was purified from *Lemna aequinoctialis* 6000 via 70% (v/v) ethanol extraction, followed by size-exclusion chromatography on Bio-Gel P2 and Superdex 75. Methylation analysis showed that DAG1 consisted of t-Araf, (1→5)-Araf, (1→2,5)-Araf, (1→3)-Galp, and (1→3,6)-Galp in a relative proportion of approximately 6:4:3:3:3, suggesting an arabinogalactan/galactoarabinan polysaccharide. With the aid of arabinan degrading enzymes, the structure of DAG1 repeating unit was further characterized by ELISA with specific monoclonal antibodies and Yariv reagent assay. Analyses indicated that the proposed repeating unit of DAG1 had a backbone composed of seven α-(1→5)-L-arabinofuranose residues where branching occurred at O-2 with either terminal arabinoses or arabinogalactan side chain.

The arabinogalactan side chain was composed of six β-(1→3)-D-galactopyranose residues, half of which were ramified at O-6 with terminal arabinoses and the last galactose was terminated with arabinose.

The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production

Yin, Yehu; Yu, Changjiang; Yu, Li; Zhao, Jinshan; Sun, Changjiang; Ma, Yubin; Zhou, Gongke

BIORESOURCE TECHNOLOGY, 10.1016/j.biortech.2015.03.097 (2015) (Will be published in early April)



Duckweed has been considered as a valuable feedstock for bioethanol production due to its high biomass and starch production. To investigate the effects of light conditions on duckweed biomass and starch production, *Lemna aequinoctialis* 6000 was cultivated at different photoperiods (12:12, 16:8 and 24:0 h) and light intensities (20, 50, 80, 110, 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The results showed that the duckweed biomass and starch production was increased with increasing light intensity and photoperiod except at 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Considering the light cost, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was optimum light condition for starch accumulation with the highest maximum growth rate, biomass and starch production of 8.90 g $\text{m}^{-2} \text{day}^{-1}$, 233.25 g m^{-2} and 98.70 g m^{-2} , respectively. Moreover, the results suggested that high light induction was a promising method for duckweed starch accumulation. This study provides optimized light conditions for future industrial large-scale duckweed cultivation.



Dr. Eric Lam & Team: 2nd visit to MamaGrande in Argentina

By Eduardo Mercovich, <http://MamaGrande.org>

In 2013, after the 2nd International Conference on Duckweed Research and Applications in Rutgers, Dr. Lam came to Argentina to see our first duckweed based wastewater to clean water/ high starch biomass pilot in the city of Totoras, province of Santa Fe, Argentina. In the way, he also learned why we choose a social business to do it and the power of this model to advance things for the common good.

Started during the Conference and following after on that 1st trip, we forged a strong relationship based on our mutual appreciation and common understanding of the value of Duckweed for Humankind.

So this February 2015 we had the pleasure and the honor to have Dr. Lam and some of his team again with us. This time, Philomena Chu and Ryan Gutierrez came again, along with Sarah Pfaff and Kenneth Acosta, to see how the pilot is going and see the start of our expansion to the North West of Argentina, to the provinces of Salta and Tucumán.

In Totoras

As reported previously in issue #6 of the International Steering Committee on Duckweed Research and Applications (see <http://lemnepedia.org/wiki/ISCDRA>) the Totoras pilot is working fine.

The Water Authorities of Santa Fe keep analyzing the water quality with better than legal results and we are now starting to progressively handle the operations to the Totoras Community, embodied by the Municipality.

Dr. Lam's Team reviewed the lagoon and the experimental canals while talking with the Municipal Environmental Officer.



Dr. Lam's Team reviewing one of the Totoras 8 small, ~10 m2 experimental canals.



The NW region

But the new NW region comes with a new scale and new challenges: while Totoras has 4 hectares, Salta and Tucumán sum 150 hectares of wastewater treatment lagoons (WWTL) scattered over a big area, different jurisdictions and long distances. However, climate is now our ally: tropical year round temperatures help duckweed grow



Sampling duckweeds in Totoras. It seems as a classic mix of *Lemna gibba* and *Wolfia*.

naturally. To better paint the whole picture, the whole region has suffered a serious water crisis in the last few years.

We flew to Salta (http://en.wikipedia.org/wiki/Salta_Province), visited some lagoons, tried some Argentinean cuisine (the typical "parrilla") and interviewed some actors of this public-private 3 year project to clean water and build a lactic acid biorefinery (see ISCDRA issue #7).

The Lagoons

After arriving we first went south of Salta Capital to "Laguna del Carril" (25°04'32.8"S 65°28'17.2"W), typical of the smallest WWTL that process the water for small towns: 1 hectare, quite old but well maintained, covered with plastic geomembrane, fully covered by duckweeds.



WWTL "Laguna del Carril" (<http://goo.gl/MAqRiH>).

Dr. Lam remarked how odor free was the lagoon, validating again one of the known effects of the duckweed mat, along with the concomitant reduction in mosquito larvae.



The day after we went north to the "Planta Depuradora Norte" (24°42'45.1"S 65°21'15.1"W) where we could walk other type of operation going on: only 3 years, fully geomembrane covered, 33 hectares of WWTL in 3 series, the last of them (with small canals) almost covered by duckweed. A seriously impressive view in a very beautiful valley.



Dr. Lam's Team with partial view of last third of Planta Depuradora Norte (<http://goo.gl/zSLG7U>).



Sampling duckweed on Planta Depuradora Norte. It seems *Lemna gibba* with the enlarged-bubbled parenquima typical of too densely packed duckweed.

Along the way, we sampled duckweed, chatted about the plan, priorities, machines to harvest (we already have one harvester adapted from an Archimedes screw based cereal elevator), and many other issues.

Salta is also a prime exponent of one of the classic issues with duckweed and wastewater treatment: since the water companies all over the world doesn't know what to do or how to work with the duckweed (it just doesn't fit into the classic engineering view), it grows and dies in the WWTL, creating problems with BOD and other water quality parameters. If it were for sanitation companies, just taking it out is more than enough, and that is what they try to do, usually without much success due to its high growth rate. But when they saw that it could be beneficial, their interest skyrocketed...



Leaving Planta Depuradora Norte, awed by its cleanness, operational size and the beauty of the Valley.



At Aguas del Norte

Briefly, the project have 2 objectives, with a strong synergy between them:

- to clean more water, with better quality, and lower price, and
- to produce high starch biomass, to be processed on the next biorefinery in Salta.

As you can see, both objectives are really only one: the cleaner the water, the better biomass production. So MamaGrande is partnering with both water and sanitation companies: “Aguas del Norte” on Salta, and “Sociedad de Aguas de Tucuman”... in Tucuman.

It is worth noting that Aguas del Norte in Salta (the province visited on this trip), is a public-private company: the Argentinean State is the public side, and the workers of the company the private. As such, its objective is to give the best possible service to the most people, and not to make money. That's why they educate about responsible use and work together with different communities.

Starting in Salta, Dr. Lam interviewed a few officers of Aguas del Norte: Normando Fleming (CEO), Gerardo Tarcaya (Responsible for Processes) and Darío Vaca (Responsible for Environmental issues). All of them were adamant in their interest in the project, with different points of view.

Normando, from the management vantage point, explained that the possibility to expand (both in quantity and quality) services faster that the infrastructure works seems like a dream come true. This is because the same infrastructure, with a duckweed based treatment, allows to treat more water to a better state. Also, the idea to make the whole Salta community part of this ongoing effort pays well in its employees, to work on these innovations in one of the best possible investments in the future.

Gerardo also talked excitedly about the innovation aspects of the project, but more in the technological aspects: in an industry that saw almost no new advances in the last decades, the possibility to be an active part of this endeavor filled him with pleasure and pride.

Dario, quite younger, was very serious. Environmental issues were a very small part of the company culture up until just a few years ago when the company was made public again. Now he is opening the area not only to the company-specific aspects of their operations but to a more broad general understanding of their operational impact, from before their takes (like impact on water bodies and flows) all the way to what happens after returning the water to their natural places/flows. The possibility to use a low energy, low force treatment can reduce incredibly the whole LCA operational footprint, while at the same time giving best results, making an even better gain/costs ratio than just a better treatment. Again, as with Gerardo, he saw a source of pride and professional challenge in the project that, he reported, was shared by everyone involved.

They all three agreed that water is a human right and must not be subject to other limitations and that we must use it rationally, with care and respect, to preserve it for future generations. Also, as they focus on all the things to do, they also recognize the huge positive change in the last years of the Company and see that change as a source of confidence to keep working for the better. As they say, “operations should be innovative, sustainable and replicable”



Back in Rosario

After Salta, we went to Rosario where we worked with the people on INDEAR, MamaGrande's applied research partners in the scaling up of the industrial lactic acid process.

There, Dr. Lam and his Team gave us replicates of some Argentinean ecotypes that the Duckweed Stock Cooperative (<http://www.ruduckweed.org>) had been keeping since a long time ago, along with their analysis and yields in biomass, protein and starch production. An incredible treasure,



Sarah Pfaff and Kenneth Acosta of Dr. Lam's Team (left) delivering the Argentinean ecotypes to Sebastián Lagorio (co-founder) and Sebastián Cinquini (CEO) of MamaGrande.

both in information and in applied possibilities, that we are very grateful to receive as the initial part of our own Ecotype Bank.

Closing thoughts and upcoming news

Aguas del Norte is fully committed to the project along with MamaGrande, something that shows in their behaviour, and we will start testing the bacterial consortium and the duckweed treatment in brief in selected WWTL in Salta.

Dr. Lam has been incredible in his support. As he always says, "*together we make the biggest lab*". Now our lab has 150 hectares to experiment. So please, feel yourself invited to this feast on opening possibilities to test and develop the best that duckweed can do for our Planet and our People.

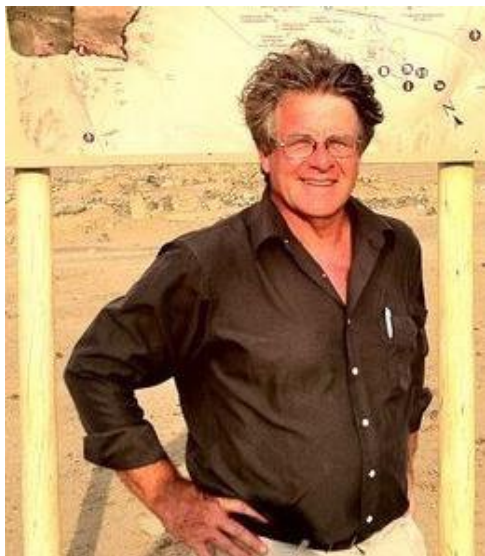
We will report back on this and other topics to the whole Duckweed Community in just 3 months, in the 3rd International Conference on Duckweed Research and Applications in Kyoto, Japan, on July 3 (more information in this same ISCDRA #8 issue). We hope to have some good news there for everyone. :)

Comments and communications

Feel free to ask whatever questions you may have. We can be found in info@mamagrande.org or in the *International Lemna Association* biweekly meetings (<http://www.internationallemnaassociation.org>), where warm, open and lively discussions and exchange are the norm.



Paul Skillicorn: Duckweed Visionary Speaks



Paul Skillicorn

Paul Skillicorn is probably best known for his groundbreaking PRISM model of duckweed-based wastewater remediation and fisheries at Mirzapur, Bangladesh

(<https://youtu.be/M93HZDqghsE>) and as co-author of "Duckweed Aquaculture: A New Aquatic Farming System for Developing Countries"¹.

We catch up with Paul, currently living in India to ask him about his personal projects and views on the global duckweed scene.

Paul writes- In the Americas, I have reluctantly abandoned a 3-year endeavor to finance a duckweed wastewater treatment plant in Olmito, a suburb of Brownsville, Texas.

The larger problem lies with the respective postures of

American engineering, construction and project finance. America is no longer a good place for starting any such new endeavor which challenges the existing status quo. America used to be the global leader in challenging the status quo. Today, unfortunately, its decision makers and attendant lawyers wield anchors rather than accelerators.

I continue to hold hope for a project based in Fabens, Texas that will demonstrate biological desalination in a profitable context. Drinking water produced from brackish groundwater is the byproduct of a project that will produce filleted fish and aquaponics-grown produce "out there in the desert." We are also planning on marketing a desert mineral water, as coming from an environment that is pristine- no pesticides, hormones or chemicals of any kind. Even though it is being planned in the United States, I believe this project has some hope because it is not challenging or threatening any existing constituency. The deserts in question lie empty and unused and the brackish water that lies under them is not being consumed -- except, perhaps, for/by the occasional oil/gas drill rig.

While I have abandoned the US, I remain convinced that Mexico is an excellent market for duckweed-based endeavors -- from wastewater treatment and nitrate stripping from groundwater to improving the nutritional status of impoverished families. We have a strong team of committed Mexican partners who are committed to engaging all these opportunities from their base in Guadalajara.

¹ http://www-wds.worldbank.org/servlet/WDSContentServer/WDSP/IB/1993/03/01/000009265_3970128103342/Rendered/PDF/multi_page.pdf



Photo courtesy BBC News; "Mexico probes mass fish death in Lake Cajititlan"

Look in particular for news on "Cajititlan," in the next several months. Cleaning up this massively polluted lake will be occupying the team's attention during the coming year.



Parque Ecologico, Barranca, Peru

In Peru, my partner Chicho continues to demonstrate both the beauty and the efficacy of duckweed in the whimsical "Parque Ecologico" overlooking the Pacific Ocean in northern Barranca. Chicho has now created the basis for a rapid expansion of duckweed-based wastewater treatment plants in Peru and neighboring Ecuador. We are assembling an "Americas Coalition" to help build and support what he's doing and then expand throughout South America from the magnificent base he's created. Offers are pouring in from Brazil, in particular.

Personally, I have committed myself to India. That is where I now live and where I intend basing myself going forward. It is here that I believe duckweed is going to explode. Duckweed can provide the minerals, vitamins and protein that India desperately needs to address what is the worst nutrition crisis in global history. It can treat the overwhelming volume of liquid (and even solid) wastes now being discharged into all of India's rivers, lakes and shoreline. More importantly, it can leverage to India's benefit the nutrients in those wastes. Let me offer a simple prediction: Prime Minister Modi is going to embrace duckweed, make it his personal plant, and use it as the engine to achieve his twin objectives of cleaning up the Ganga and improving the general disposition of India's underclasses -- its villagers.

We at Spa-Agriquatics, led by Surendra Mohnot, are working diligently to give Prime Minister Modi a flying start. To that end, we have a half dozen projects now taking shape in Gujarat, Maharashtra, the



"new state" of Andhra Pradesh, Rajasthan and Tamil Nadu. In Gujarat, we will be growing duckweed as a fertilized crop and feeding it as the new superfood to school children and lactating and pregnant women. We will also be treating the wastewater of the state's largest fertilizer factory to produce materials.



Juhu, India

In Mumbai, the Maharashtra capital and largest city in India, we've been asked to clean up a filthy drain that runs through what is the local version of Hollywood -- Juhu, home to some of Bollywood's leading stars. That project is already approved, funded and happening. We've been assured that the Bollywood stars will come out in strong support.

This coming week, our extended version of duckweed is being presented (by a leading politician -- not by us) to the Maharashtra State Assembly as "the solution" to Maharashtra's solid waste, liquid waste, water supply and renewable energy issues. State legislators are also taking the extraordinary step of recommending "their solution" to the Prime Minister -- suggesting that he embrace it as "the Modi solution."

Not wanting to be left behind, duckweed proponents in Andhra Pradesh have committed that Andhra Pradesh, led by the extraordinary CEO, Chandrababu Naidu will be where duckweed first explodes. They've asked for a comprehensive proposal. We have obliged them. I'm scheduled to engage that



particular endeavor, in person, next week. Having been a fan of CM Naidu for years, spending time with him, one-on-one, is going to be a 2015 highlight.

In Rajasthan, we've been asked to present a solution to the problem of textile factory wastewater. We have submitted a proposal and, given available time, and will be launching that particular initiative in the coming weeks. Rajasthan is also where we will be launching the Indian analog to the Fabens biological desalination project. We'll be using the brand "Thar" for our water. I say, only partly tongue-in-cheek, "look for it along side FIJI water in your favorite Whole Foods Market."

Throughout India, we are being approached by duckweed enthusiasts, duckweed scientists and experienced duckweed project managers. We hope to embrace everyone that falls within what is becoming a very expansive Venn diagram. Internationally, we hope that renowned experts such as Klaus Appenroth, and even Tamra Fakhorian will agree to work with us. There is lots of room within that Venn diagram for everyone willing to pull his/her weight.

Duckweed is happening in India. Expect PM Modi to be steering the ship as we accelerate into the future.

Five years from now, I expect duckweed to have become a relatively mature industry. There will be "the producers," and there will be the "technologists." My own ambitions lie with the former, because it is they who will ultimately hold the whip handle. Duckweed will be making a significant contribution to global nutrition -- both as a widely available superfood and as a spectrum of parsed extracts. I expect companies like Pepsi will have been persuaded to try using duckweed isolates to improve the nutrition of their ubiquitous nacho snack foods. I expect countries like India will have mandated a minimum daily allowance of duckweed in hot school lunches. I expect companies like BASF will be close to marketing advanced duckweed-based bioplastics. Most importantly, I expect, from Argentina to Sri Lanka, that national governments will have embraced duckweed as the engine for treatment of wastewater -- with Prime Minister Modi leading the charge.

Duckweed is still in its infancy at the moment. We have literally everything to learn when it comes to both production and application. Run through all the factors - light, temperature, crop density, nutrition, clone-specificity, harvesting, and a thousand specific questions come to mind. We need hundreds of focused researchers addressing all these questions. In the meantime, however, we're confident that we can now produce duckweed as a crop and a treatment agent and process the harvested product. All this with enough efficiency to make a very significant profit.

"The Bangladesh Project" was conceived and implemented in the late 80s. It has continued to function for the subsequent 25 years despite changes in management, despite two devastating floods, despite only limited supervision, and even despite a breakup among the principals. I have calculated that it has earned back its capital cost seven times over the years. Throughout, it has continued to treat a "nasty wastewater," to a higher standard than is mandated in any city in the US -- extraordinary.



One has to ask the question, "Why? How can a treatment plant that is poorly supervised and with absolutely no government oversight or treatment mandates continue to treat wastewater better than New York City, which has invested tens of billions in its treatment facilities and has both State of NY and EPA officials crawling everywhere?

The answer is: "Because it makes financial sense to treat to that level. It makes sense to extract literally everything from the waste stream because it results in a cash payoff to the plant operators -- a weekly fish harvest that depends absolutely on the quality of water treatment.

What advice can I offer the duckweed community at large? Five words: Study, learn, practice, network, and persist. Now, with things literally happening everywhere, "*It'll come to you.*"



Paul Skillicorn

Paul Skillicorn can be reached at paulskillicorn@gmail.com.



Philemna Solutions: Duckweed-based Feed Lines for Poultry and Pisciculture

In this article, we focus on an energetic duckweed startup near Manila, Philippines, led by Patrick Chang. We recently interviewed Patrick to learn more about his company.

Patrick, tell us about Philemna Solutions and its state of development.

We are currently working on a duckweed-based feed line project for poultry and pisciculture called Philemna Solutions – Wastewater Treatment to Organic Feed Conversion. It has an identical concept as the agriquatics system in Bangladesh but instead of being fed directly to a variety of fishes, the harvested duckweeds undergo processing and are mixed with other organic feed sources. Our vision is to have a duckweed-based feed product available at feed stores nationwide. The target

customers are farm owners who do not have the time, space, and extra manpower to grow and process duckweed. Right now we are using our personal funds and we are still conducting further research and product testing to make sure the product will provide the required nutrient content prior to launch.

Tell us about your background?

I finished my Bachelor of Science in Business Administration majored in Marketing Management in Colegio San Agustin – Biñan, Laguna. Before this project, our family has been involved in other business ventures. We used to own and operate a duck farm for egg production and understand the feed cost and waste accumulation from raising farm animals. This is probably one of the reasons why duckweed appeals to me.





Fresh duckweed meal

Who are the key players in your project?

The Philemna Solutions Project is being developed and funded with the combined effort of our family. We got our duckweed source, both the local and imported variety (Alaska) from the Southern Tagalog Inventors Marketing Cooperative. The project in charge for STIMC -duckweed is Ag.

Eng. Samuel Hernandez who has been promoting the importance of duckweed to local farmers along with STIMC General Manager Engr. Mario Peralta. We share the project's state of development with them every once in a while. Both have been generous in giving us our starting batch of duckweed and sharing all information that they have. For all things related to duckweed we get our source from the *International Lemna Association*, their feedback and ideas thru e-mail interaction have influenced the project model.



Patrick with Dr. Luis Cervantes Jr (wearing blue) of STIMC, with Samson Chang (gray polo shirt) , and Mercedes Chang.

How did you first become interested in duckweed as an animal feed?

It was while attending an agribusiness exhibition in Metro Manila that the idea came to mind. The participating companies showcased their feed products and were handing out brochures. I gathered and compared each product's nutritional value and also checked the ingredients. I realized the necessity of producing a duckweed-based feed line and its potential to be a market niche. During this time I was already growing duckweeds in small containers and also thinking of a product from the harvested duckweeds.



What are your biggest challenges so far and how are you overcoming them?

There aren't enough duckweed farmers in our country to exchange information and ideas with, and also since we are planning to launch it on a large scale it would be great if there are other farmers whom we can get harvested duckweeds from. Exchanging info and ideas are easy to overcome just by going to the ILA website you will get the latest in duckweed R&D as well as information you might need for your project. But getting local farmers involved in duckweed farming is much more complicated for several reasons. Most consider it to be an invasive plant that impedes rice growth when found in rice paddies. There is a lack of information about the plant and the absence of a marketable product from duckweed. We try to share the benefits of growing the plant whenever we get the chance to talk to other farmers and STIMC is also getting local farmers involved. Hopefully with the Philemna product line we will see a rise in local duckweed farmers in the next few years.

When is your projected timetable for launch?

We are still on the experimental stage of our project and are making sure we'll be providing the right nutrients as well as having a strong business model before the product launch. We are gathering data from a laboratory analyses of nutrient composition per feed type and completing a feasibility study as advised by Ag. Eng. Samuel Hernandez. If all data turns out as expected, then we will launch the product, if not, then it's back to testing for us. Probably by the 2nd quarter of 2016 is a reasonable date of launch.



Patrick Chang -duckweed water nutrient test besides oregano plants to negate bad odor.



POWDER



SHRED



PELLET



PHILEMNA

Duckweed in powder, shred, and pellet.

If you could offer advice to newly-forming duckweed companies, what would it be?

Get connected with other duckweed enthusiasts and listen to their ideas and advice. Also, make sure you learn at least one thing new about what you are working on every day.

Where can you be reached?

Feel free to share your ideas, comments, or suggestions via our email address at philemna@gmail.com.



Useful Methods 1. Determination of Growth Rates in Duckweed

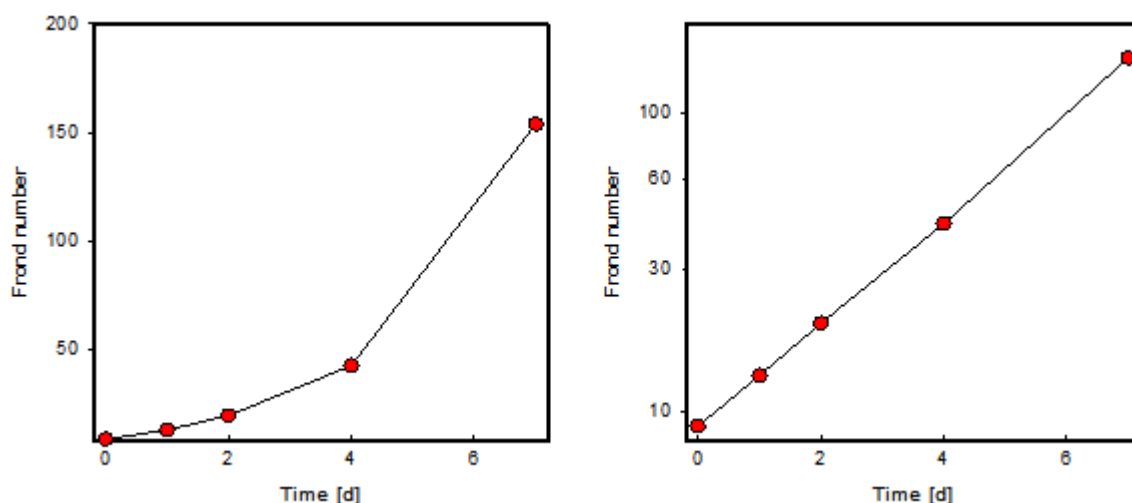
by Klaus-J. Appenroth, University of Jena, Plant Physiology, Dornburger Str. 159, 07743 Jena, Germany. Email: Klaus.Appenroth@uni-jena.de.

1. Introduction

Because of the high reproducibility of growth and developmental processes in standardized cultures of duckweed, species of this family are especially suitable for quantitative physiology. The possibility of measuring relative growth rates (RGR) is one of the great advantages of duckweed. In order to make profit out of this advantage, a high level of standardization is necessary. There is no need to start from nothing, standardized protocols already exist. This is described in ISO20079 and OECD221. While the ISO guideline was especially designed for the specific needs of environmental samples, OECD tests are focused on substances and products, used e. g. for plant protection. In both protocols *Lemna* is preferred as test organism, *Lemna minor* (e.g. clone St = 9441) in ISO20079 protocol and *Lemna gibba* (clone G3 = 7742) in the OECD protocol. When there are no specific reasons, these two species and these two clones should be used to make the results comparable with those of other labs. Here, we distinguish between (i) measurements of RGR under optimal conditions, and (ii) measurements under environment-related conditions, e.g. within the frame of phytotoxicity. Large-scale production of biomass is not treated in this short report.

2. Measurements of RGR under optimal conditions

The ISO protocol defined the following growth conditions: 25°C, continuous white light 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 7 day-test, axenic start material, and sterilized (autoclaved) nutrient medium. These are indeed not environment-related conditions but conditions that permit optimal growth. The result is the maximum growth capacity. The often used modified Steinberg medium (Naumann et al., 2007) is designed to investigate the influence of e.g. heavy metals. For normal RGR determination we suggest to increase the EDTA- Na_2 / Fe-EDTA concentrations in this medium by the factor of five. Other media like "N" (Appenroth et al., 1996) or "Schenk-Hildebrand" (Ziegler et al., 2015) are also possible. It is very important, however, that also the plant material is well standardized. We recommend to pre-cultivate the fronds under the same conditions as during the growth measurements for four weeks and to inoculate the fronds each week into new nutrient medium. Even when inorganic medium is used, plants should be free of any microbial contamination (axenic) as the interaction with the unknown contamination might result in growth inhibition or sometimes even in growth stimulation. The population of plants follows the common exponential growth as know e.g. from bacterial or yeast populations.



Exponential growth of a population of *Lemna minor*, clone 9441. (Left) Linear scale, (right) semi-logarithmic scale. Instead of frond number, fresh weight or dry weight can be used for measurements of RGR.

Basic law of exponential growth: $x_t = x_{t_0} \cdot e^{RGR \cdot t}$

x = frond number, fresh weight, dry weight or other measurements,...

Relative growth rate (d⁻¹) for two-point measurements: $RGR = (\ln x_{t_7} - \ln x_{t_0}) / (t_7 - t_0)$

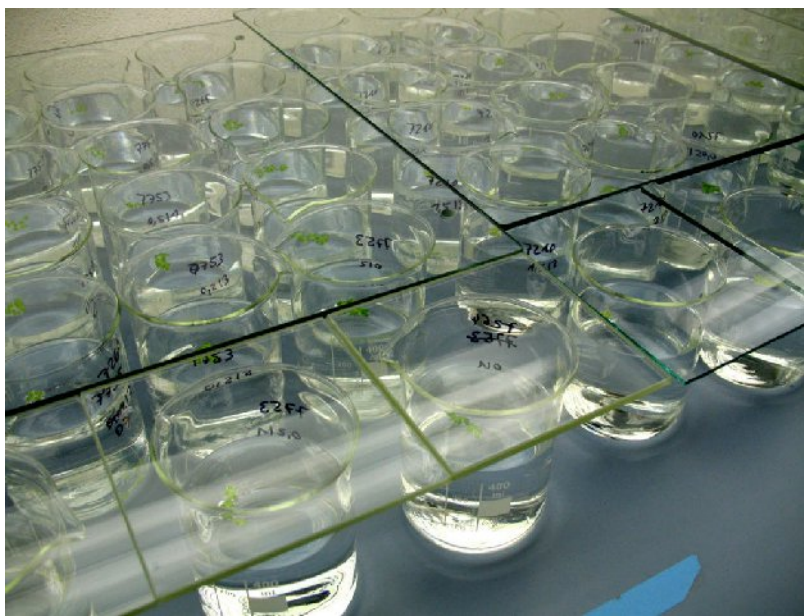
Doubling time (d): $DT = \ln 2 / RGR$

Relative weekly yield x_{t_7} (7 d⁻¹ or week⁻¹): $\ln x_{t_7} = \ln x_{t_0} + RGR \cdot (t_7 - t_0)$

The RGR (given as d⁻¹) is represented by the slope in Figure 1 (right). When parameters are measured only at the beginning of the experimental period (t_0) and at the end after 7 days (t_7), the equation simplifies as given above. Usually, 6 parallel samples have to be investigated. The meaning of the "RGR" is not easy to imagine. Therefore, often two different parameters, calculated from RGR, were used (Ziegler et al., 2015): the doubling time (DT) and the relative weekly yield (RY). The doubling time tells you, how much time is required to double the number of fronds, the fresh weight or dry weight. As long as the growth conditions are optimal and the plants are well pre-cultivated (standardized), there is no difference between these parameters used. RY tells you, how many fronds or how much fresh or dry weight is produced after 7 days starting from 1 frond or 1 g of fresh or dry weight. This parameter gives usually the best impression for people that are not so familiar with growth parameters.

The following photo gives an impression how growth rates can be determined in growth chambers. Each glass beaker (400 ml) contains 300 ml nutrient medium.

The results are given as average of



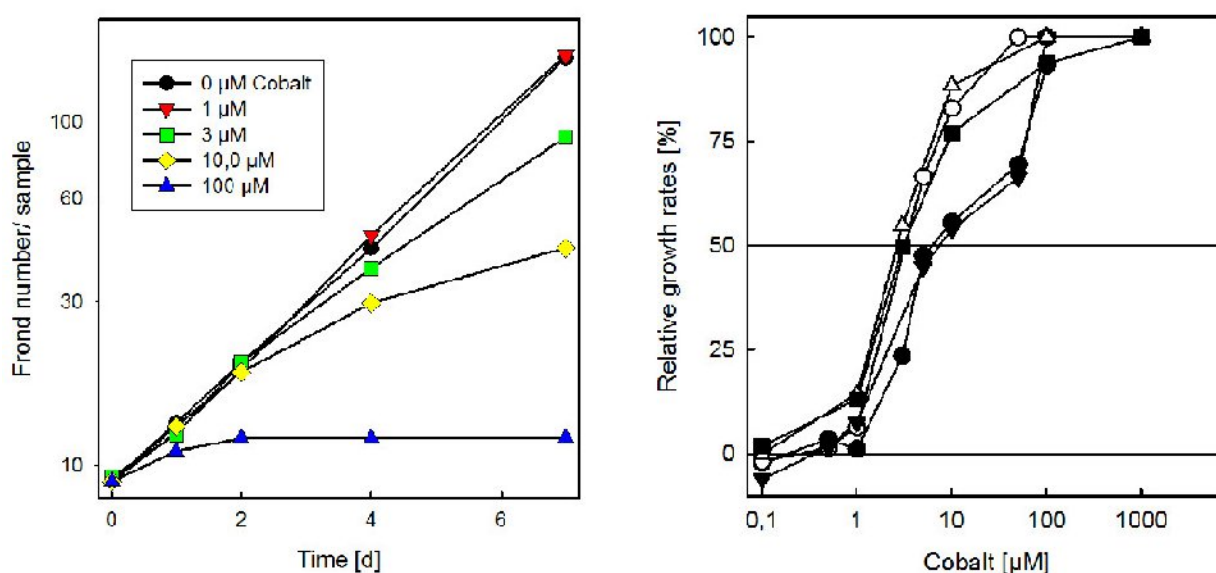


the six samples investigated plus minus standard error or standard deviation of the means. This way different clones or species could be compared or the response in different nutrient media.

3. Toxicological measurements

Such measurements are essentially carried out under some kind of stress (heavy metals, herbicides, pesticides, low temperature etc.). In this case the measuring parameters may be influenced differently resulting in different sensitivity at the level of frond number, fresh weight or dry weight (Naumann et al., XXX).

The growth test is carried out in a very similar way as described before. Different concentrations, however, might result in different growth rates. This is demonstrated with the effect of cobalt ions as an example.



The effect of Cobalt salt on the growth rates of *Lemna minor*, clone 9441. (left): Kinetics, (right) Dose-Response curves.

Such dose-response curves will be obtained for frond number, fresh weight, dry weight chlorophyll or carotenoid content (Figure 3, right). Concentrations should be selected from having practical no effects to complete inhibition. Only then the following data compressing will be possible (see below). With a suitable computer programme the concentrations can be calculated that inhibit the response (increase of frond number, fresh weight, dry weight) by a defined degree. Usually, this effective concentration (EC_x) is given at x equal to 50%, 20% and 10% - depending on the questions to be asked. Together with the parameter EC_x also an interval of confidence is calculated. Parameter and interval of confidence contain the information of how much the investigated substance or the physical parameter (temperature, UV light etc.) inhibits the process investigated. This way, risk assessment is possible with the help of duckweed.

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water to duckweed (*Lemna minor*) – Duckweed growth inhibition test. ISO TC 147/ SC 5/ WG 5. 2004.

Naumann B., Eberius M., Appenroth K.-J., (2007) Growth rate based dose-response relationships and EC-values of ten heavy metals using the duckweed growth inhibition test (ISO 20079) with *Lemna minor* L. clone St. *Journal of Plant Physiology* 164, 1656-1664.

OECD 221. Guideline for the testing of chemicals. Revised proposal for a new guideline 221. *Lemna* sp. Growth Inhibition Test. 2004.

Ziegler, P., Adelman, K., Zimmer, S., Schmidt, C., Appenroth, K.-J. 2014. Relative in vitro growth rates of duckweeds (*Lemnaceae*) – the most rapidly growing higher plants. *Plant Biology* 17, 33-41.



Experiments for Students: Part 1

Nutrition of plants- Biomonitoring

The following experiment has been used in the practical bachelor student course for many years. It can be easily modified to adapt to the local conditions.

Even as an experiment for schools it can be simplified: compare nutrient medium prepared from commercially available hydroculture with tap water as medium and distilled water as medium.

In another course we did not add glucose and worked under rather non-sterile conditions. Then the time of the experiment has to be extended accordingly.

This experiment has a meaning by far extending mineral nutrition of plants. The experimental plant species (duckweeds, Lemnaceae), especially *Lemna minor* and *Spirodela polyrhiza*, are used now in routine work for monitoring water quality.

<http://www.lemnatec.de> and <http://www.mobot.org/jwcross/duckweed/duckweed.htm>.

Materials:

- Labelled pipettes
- Labelled measuring cylinders
- Erlenmeyer flasks (100 ml)
- Cotton wool stoppers
- Glass stick, inoculation tool
- Sterile culture from duckweeds, e. g. *Spirodela polyrhiza* or *Lemna minor*.

Chemicals:

- Glucose

Equipment:

- pH-meter
- Laminar flow box, Autoclave
- Burner

Ready prepared solutions:

- Stock solutions and solutions for substitution (cf. Table and scheme)

What to do?

Start with approximately 450 ml purified water in a plastic cylinder and add (using a pipette) in the given series the required stock solutions. The concentration of glucose should be 50 mM (weigh solid substance). After adding the components (see Table), mix with the glass stick and add water to final 500 ml. Transfer 4 x 75 ml in 100 ml-Erlenmeyer flasks. Before transfer, the pH should be measured.

Because glucose is used, flasks need to be autoclaved (why?). This has to be done as early as possible in the course because plants can only be transferred into sterile nutrient media when it has cooled down to room temperature again after autoclaving.

Transfer approximately one triplet (this is one colony of three fronds) into flask. The experiment will run for 7 days at room temperature.



Start: The number of fronds has to be counted after inoculation. It is not always possible to transfer exactly a triplet. Do you know what a frond is?

| Stock solution | Complete medium | Lacking the following nutrients | | | | | | |
|-------------------------------------------------------------------------------------------------------------|-----------------|---------------------------------|-----|-----|-----|-----|-----|-----|
| | | -N | -P | -S | -K | -Mg | -Fe | -Ca |
| KH ₂ PO ₄ (100 mM) | 2.5 | 2.5 | - | 2.5 | - | 2.5 | 2.5 | 2.5 |
| Ca(NO ₃) ₂ (0,2 M) | 2.5 | - | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | - |
| KN ₃ (1,6 M) | 2.5 | - | 2.5 | 2.5 | - | 2.5 | 2.5 | 2.5 |
| MgSO ₄ (0,2 M) | 2.5 | 2.5 | 2.5 | - | 2.5 | - | 2.5 | 2.5 |
| H ₃ BO ₃ (1 mM); MnCl ₂ (2,6 mM); Na ₂ MoO ₄ (80 µM) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Fe (III) EDTA (5 mM) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | - | 2.5 |
| Substitute solution | | | | | | | | |
| KCl (1.6 M) | - | ? | ? | | | | | |
| CaCl ₂ (0.2 M) | - | ? | | | | | | |
| MgCl ₂ (1 M) | - | | | ? | | | | |
| NaH ₂ PO ₄ (0.15 M) | - | | | | ? | | | |
| NaNO ₃ (1 M) | - | | | | ? | | | ? |
| Na ₂ SO ₄ (0.2 M) | - | | | | | ? | | |
| Na ₂ EDTA (5 mM) | - | | | | | | ? | |

Table 1: Scheme to prepare the different variants (given in ml/ 500 ml). ? = please calculate how much from the substitute solution has to be added.

In the media lacking one specific ion (e.g. -N means no nitrate should be there) by withdrawing a salt, also the counter ion is missing and needs to be replaced. For that we used Na⁺ or Cl⁻ salts (why?). For the nutrient medium cf. Appenroth K.-J., Teller S., Horn M., (1996) Photophysiology of turion formation and germination in *Spirodela polyrrhiza*. *Biologia Plantarum* 38, 95-106.



| Group | complete | -N | -P | -S | -K | -Mg | -Fe | -Ca |
|-------|----------|----|----|----|----|-----|-----|-----|
| 1 | x | x | | | | | | |
| 2 | x | | x | | | | | |
| 3 | x | | | x | | | | |
| 4 | x | | | | x | | | |
| 5 | x | | | | | X | | |
| 6 | x | | | | | | x | |
| 7 | x | | | | | | | x |

Table 2: We suggest the following nutrient media for the following groups.

Evaluation during the practical student course

Please watch the development of your plants. Count the number of fronds at the beginning ($t=0$) and after one week ($t = 7d$). Evaluate also the plants. This is an important method to get a first impression and is called evaluation (good appearance, quality).

Evaluation the following weeks

Measure again the pH and count the number of fronds. For this purpose, open the flasks and put them in a bowl. Finally, the fresh weight should be measured. In case, turions (*Spirodela polyrhiza*) have formed, count them and calculate the number of turions formed per number of fronds. This step requires normally 3 weeks.

Protocol

All numerical data should be given (frond number, fresh weight) and also the result of the visual evaluation (original data). The student's t-Test may help you to evaluate significant differences between the results on the complete medium and media lacking some elements.

Using the frond number (n_1 , n_2), a given time (t_1 , t_2), the growth rate (GR) and doubling time (T_d) can be calculated as follows:

$$GR = \ln(n_2) - \ln(n_1) / (t_2 - t_1).$$

$$T_d = \ln 2 / GR \text{ (Dimension is days, if } t_1 \text{ and } t_2 \text{ were used in days).}$$

Calculate GR for each of the flask and calculate the average and standard error and use the student's t-test for a decision.



From the Data Base

Highlights

Effect of nitrogen and phosphorus deficiency on transcriptional regulation of genes encoding key enzymes of starch metabolism in duckweed (*Landoltia punctata*)

Zhao, Zhao; Shi, Hui-juan; Wang, Mao-lin; et al.

Plant Physiology and Biochemistry 86: 72–81 (2015)

The production of starch by plants influences their use as biofuels. Nitrogen (N) and phosphorus (P) regulate starch gene expression during plant growth and development, yet the role of key enzymes such as ADP-glucose pyrophosphorylase (E.C. 2.7.7.27 AGPase) in starch metabolism during N- and P-deficiency remains unknown. We investigated the effect of N- and P-deficiency on the expression of large (LeAPL1, LeAPL2, and LeAPL3) and small (LeAPS) subunits of AGPase in duckweed (*Landoltia punctata*) and their correlation with starch content. We first isolated the full-length cDNA encoding LeAPL1 (GenBank Accession No. KJ603244) and LeAPS (GenBank Accession No. KJ603243); they contained open reading frames of 1554 bp (57.7-kDa polypeptide of 517 amino acids) and 1578 bp (57.0 kDa polypeptide of 525 amino acids), respectively. Real-time PCR analysis revealed that LeAPL1 and LeAPL3 were highly expressed during early stages of N-deficiency, while LeAPL2 was only expressed during late stage. However, in response to P-deficiency, LeAPL1 and LeAPL2 were upregulated during early stages and LeAPL3 was primarily expressed in the late stage. Interestingly, LeAPS was highly expressed following N-deficiency during both stages, but was only upregulated in the early stage after P-deficiency. The activities of AGPase and soluble starch synthesis enzyme (SSS EC 2.4.1.21) were positively correlated with changes in starch content. Furthermore, LeAPL3 and LeSSS (SSS gene) were positively correlated with changes in starch content during N-deficiency, while LeAPS and LeSSS were correlated with starch content in response to P-deficiency. These results elevate current knowledge of the molecular mechanisms underlying starch synthesis.

Callus induction and regeneration in *Wolffia arrhiza* (L.) Horkel ex Wimm

Khvatkov, Pavel; Chernobrovkina, Mariya; Okuneva, Anna; et al.

Plant Cell Tissue and Organ Culture 120: 263–273 (2015)

The development of tissue-culture systems in duckweed has been limited to species of the genus *Lemna*. Here we report on a tissue-culture system (callus induction, callus growth and plant regeneration) for the rootless duckweed *Wolffia arrhiza*. We developed a two-step procedure for callus induction in *Wolffia* using Schenk & Hildebrandt (SH) medium containing glucose, mannitol and sorbitol. In the first stage, explants were precultivated in the presence of 5.0 mg l⁻¹ 2,4-dichlorophenoxyacetic acid and 0.5 mg l⁻¹ N-6-benzyladenine (BA) for 16 weeks. In the second stage, BA was replaced with 12.5 mg l⁻¹ Picloram (PCL) for 4 weeks. The resulting callus could be maintained in vitro for a long time (about 1 year) with a relatively low concentration of PCL (4.0 mg l⁻¹).



1)), or used to regenerate whole plants by transferring it to growth regulator-free SH medium. The protocols created for callus induction and regeneration were highly efficient at each stage (with the efficiency of 97 % for callus formation and 78 % for regeneration).

Biotechnology

Biogas Production from Vietnamese Animal Manure, Plant Residues and Organic Waste: Influence of Biomass Composition on Methane Yield

Cu, T. T. T.; Nguyen, T. X.; Triolo, J. M.; et al.

Asian-Australian Journal of Animal Sciences 28: 280-289 (2015)

Anaerobic digestion is an efficient and renewable energy technology that can produce biogas from a variety of biomasses such as animal manure, food waste and plant residues. In developing countries this technology is widely used for the production of biogas using local biomasses, but there is little information about the value of these biomasses for energy production. This study was therefore carried out with the objective of estimating the biogas production potential of typical Vietnamese biomasses such as animal manure, slaughterhouse waste and plant residues, and developing a model that relates methane (CH₄) production to the chemical characteristics of the biomass. The biochemical methane potential (BMP) and biomass characteristics were measured. Results showed that piglet manure produced the highest CH₄ yield of 443 normal litter (NL) CH₄ kg(-1) volatile solids (VS) compared to 222 from cows, 177 from sows, 172 from rabbits, 169 from goats and 153 from buffaloes. Methane production from duckweed (*Spirodela polyrrhiza*) was higher than from lawn grass and water spinach at 340, 220, and 110.6 NL CH₄ kg(-1) VS, respectively. The BMP experiment also demonstrated that the CH₄ production was inhibited with chicken manure, slaughterhouse waste, cassava residue and shoe-making waste. Statistical analysis showed that lipid and lignin are the most significant predictors of BMP. The model was developed from knowledge that the BMP was related to biomass content of lipid, lignin and protein from manure and plant residues as a percentage of VS with coefficient of determination (R-square) at 0.95. This model was applied to calculate the CH₄ yield for a household with 17 fattening pigs in the highlands and lowlands of northern Vietnam.

Comparative Analysis of Duckweed Cultivation with Sewage Water and SH Media for Production of Fuel Ethanol

Yu, Changjiang; Sun, Changjiang; Yu, Li; et al.

PLOS ONE Volume: 9: Article Number: e115023 (2014)

Energy crises and environmental pollution have caused considerable concerns; duckweed is considered to be a promising new energy plant that may relieve such problems. *Lemna aequinoctialis* strain 6000, which has a fast growth rate and the ability to accumulate high levels of starch was grown in both Schenk & Hildebrandt medium (SH) and in sewage water (SW). The maximum growth rates reached 10.0 g DW m(-2) day(-1) and 4.3 g DW m(-2) day(-1), respectively, for the SH and SW cultures, while the starch content reached 39% (w/w) and 34% (w/w). The nitrogen and phosphorus



removal rate reached 80% (SH) and 90% (SW) during cultivation, and heavy metal ions assimilation was observed. About 95% (w/w) of glucose was released from duckweed biomass hydrolysates, and then fermented by Angel yeast with ethanol yield of 0.19 g g⁻¹ (SH) and 0.17 g g⁻¹ (SW). The amylose/ amylopectin ratios of the cultures changed as starch content increased, from 0.252 to 0.155 (SH) and from 0.252 to 0.174 (SW). *Lemna aequinoctialis* strain 6000 could be considered as valuable feedstock for bioethanol production and water resources purification.

Coal and Biomass to Liquid Transportation Fuels: Process Synthesis and Global Optimization Strategies

Niziolek, Alexander M.; Onel, Onur; Elia, Josephine A.; et al.

INDUSTRIAL & ENGINEERING CHEMISTRY RESEARCH 53: 17002-17025 (2014)

The thermochemical conversion of coal and biomass to liquid transportation fuels from a synthesis gas intermediate is investigated using an optimization-based process synthesis framework. Two distinct types of coal (LV bituminous and coal commonly found in the province of Anhui, China) and two types of biomass (hardwood and duckweed) are considered as feedstocks. The superstructure incorporates alternative conversion pathways of synthesis gas which include methanol formation and conversion into FischerTropsch hydrocarbons. Methanol may be converted to gasoline or olefins, and the olefins may be subsequently converted to gasoline and distillate. A rigorous deterministic global optimization branch-and-bound framework is utilized to determine the optimal process topology that produces liquid fuels at the lowest possible cost. Economies of scale are evident as the refinery capacity increases and it is observed that the fuel ratios of the final liquid products have a significant impact on the optimal topology of the plant. The results suggest that liquid fuels production from coal and biomass can be competitive with petroleum-based processes.

Ecology

Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae)

By: Xu, Yaliang; Ma, Shuai; Huang, Meng; et al.

HYDROBIOLOGIA 743: 75-87 (2015)

Duckweeds are a family of aquatic flowering plants that have a high potential for environmental remediation and biofuel manufacture. Two hundred and twenty clones of duckweeds were collected in Hainan Island, China. Based on morphological and phylogenetic analyses of the chloroplast ribosomal protein S16 intron (rps16) and atpF-atpH intergenic spacer sequences, these clones were classified into four species belonging to four genera: *Lemna aequinoctialis*, *Spirodela polyrhiza*, *Wolffia globosa*, and *Landoltia punctata*. Eight community types including single-, bi-, and/or tri-species communities were observed. *L. aequinoctialis* was the most widely distributed of the four species. *W. globosa* has the highest genetic diversity followed by *L. aequinoctialis*, whereas *S. polyrhiza* and *L. punctata* did not show any significant diversity. Duckweeds collected from the south of Hainan had higher diversity than those from the north. Moreover, very high rates of transversional nucleotide



substitutions were found in the rps16 sequences of *L. aequinoctialis* and *W. globosa*, which make these duckweeds special with respect to nucleotide substitutions.

Evaluation of Performance of Full-Scale Duckweed and Algal Ponds Receiving Septage

Papadopoulos, Frantzis H.; Metaxa, Eirini G.; Iatrou, Miltos N.; et al.

WATER ENVIRONMENT RESEARCH 86 (Special Issue): 2309-2316 (2014)

The performance of duckweed and algal systems in removing fecal bacteria, organic matter, and nutrients was evaluated in three full-scale ponds operating in series. Trucks collected septage from holding tanks and discharged it into the system, daily. The inflow rates varied between the warm and the cold season. Duckweed and algae naturally colonized the ponds in two successive periods of 10 and 13 months, respectively. Environmental conditions were determined at various pond depths. Without harvesting, the duckweed system was neutral and anoxic. Alkaline and oversaturation conditions were observed in the algal system. The overall removals of 5-day biochemical oxygen demand, total suspended solids, total nitrogen removal, and orthophosphate (ortho-P₀₄₃-) ranged from 94 to 97, 62 to 84, 68 to 74, and 0 to 26%, respectively. The *E. coli* and enterococci reductions varied between 2.2 to 3.0 and 1.1 to 1.4 log units, respectively. The upper values were always associated with the algal system.

Feed & Food

Transfer of oxytetracycline from swine manure to three different aquatic plants: implications for human exposure.

Boonsaner, Maliwan; Hawker, Darryl W

Chemosphere 122: 176-82 (2015)

Little is known regarding the potential for pharmaceuticals including antibiotics to be accumulated in edible aquatic plants and enter the human food chain. This work investigates the transfer of a widely used veterinary antibiotic, oxytetracycline (OTC), from swine manure to aquatic plants by firstly characterizing desorption from swine manure to water and fitting data to both nonlinear and linear isotherms. Bioconcentration of OTC from water was then quantified with aquatic plants of contrasting morphology and growth habit viz. watermeal (*Wolffia globosa* Hartog and Plas), cabomba (*Cabomba caroliniana* A. Gray) and water spinach (*Ipomoea aquatica* Forsk.). Watermeal and water spinach are widely consumed in Southeast Asia. The OTC desorption and bioconcentration data were used to provide the first quantitative estimates of human exposure to OTC from a manure-water-aquatic plant route. Results show that under certain conditions (plants growing for 15d in undiluted swine manure effluent (2% w/v solids) and an initial OTC swine manure concentration of 43mgkg⁻¹ (dry weight)), this pathway could provide a significant fraction (>48%) of the acceptable daily intake (ADI) for OTC. While effluent dilution, lower OTC manure concentrations and not all plant material consumed being contaminated would be expected to diminish the proportion of the ADI accumulated, uptake from aquatic plants should not be ignored when determining human exposure to antibiotics such as



OTC.

Transcriptome analysis of food habit transition from carnivory to herbivory in a typical vertebrate herbivore, grass carp *Ctenopharyngodon idella*

He, Shan; Liang, Xu-Fang; Li, Ling; et al.

BMC Genomics 16. Article Number: 15 (2015)

We showed that grass carp fed with duckweed (modeling fish after food habit transition) had significantly higher relative length of gut than fish before food habit transition or those fed with chironomid larvae (fish without transition). Using transcriptome sequencing, we identified 10,184 differentially expressed genes between grass carp before and after transition in brain, liver and gut. By eliminating genes potentially involved in development (via comparing fish with or without food habit transition), we identified changes in expression of genes involved in cell proliferation and differentiation, appetite control, circadian rhythm, and digestion and metabolism between fish before and after food habit transition. Up-regulation of GHRb, Egfr, Fgf, Fgfbp1, Insra, Irs2, Jak, STAT, PKC, PI3K expression in fish fed with duckweed, consistent with faster gut growth, could promote the food habit transition. Grass carp after food habit transition had increased appetite signal in brain. Altered expressions of Per, Cry, Clock, Bmal2, Pdp, Dec and Fbxl3 might reset circadian phase of fish after food habit transition. Expression of genes involved in digestion and metabolism were significantly different between fish before and after the transition. We suggest that the food habit transition from carnivory to herbivory in grass carp might be due to enhanced gut growth, increased appetite, resetting of circadian phase and enhanced digestion and metabolism. We also found extensive alternative splicing and novel transcript accompanying food habit transition. These differences together might account for the food habit transition and the formation of herbivory in grass carp.

Interaction with other organisms

***Paenibacillus lemnae* sp. nov., an endophytic bacterium of duckweed (*Lemna aequinoctialis*).**

Kittiwongwattana, Chokchai; Thawai, Chitti

International Journal of Systematic and Evolutionary Microbiology 65: 107-12 (2015)

A Gram-stain-variable, rod-shaped and endospore-forming bacterium, designated strain L7-75, was isolated from duckweed (*Lemna aequinoctialis*). Cells were motile with a monopolar flagellum. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain L7-75(T) belonged to the genus *Paenibacillus*, and the closest phylogenetically related species were *Paenibacillus uliginis* N3/975(T) (98.5% 16S rRNA gene sequence similarity), *Paenibacillus purispatii* ES_M17(T) (98.5%), *Paenibacillus lactis* MB 1871(T) (98.2%), *Paenibacillus campinasensis* 324(T) (97.7%), *Paenibacillus glucanolyticus* S93(T) (97.7%) and *Paenibacillus lautus* ATCC 43898(T) (97.4%). Growth of strain L7-75(T) was observed at pH 7-10 and at 20-40 °C, and NaCl concentrations up to 5% (w/v) were tolerated. Major cellular fatty acids included anteiso-C15:0, C16:0 and anteiso-C17:0 that were present at 36.0%, 14.2% and 10.0% of the total cellular fatty acid profile, respectively. The major polar



lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidyl-N-methylethanolamine. MK-7 was the predominant menaquinone. The diamino acid found in the cell-wall peptidoglycan was meso-diaminopimelic acid. The DNA G+C content was 49.1 mol% (Tm). DNA-DNA relatedness values between strain L7-75(T) and its closest relatives ranged from 4.4 to 47.8%. These results indicate that strain L7-75(T) represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus lemnae* sp. nov. is proposed.

Phyllosphere Bacterial Community of Floating Macrophytes in Paddy Soil Environments as Revealed by Illumina High-Throughput Sequencing

Xie, Wan-Ying; Su, Jian-Qiang; Zhu, Yong-Guan

Applied and Environmental Microbiology 81: 522-532 (2015)

The phyllosphere of floating macrophytes in paddy soil ecosystems, a unique habitat, may support large microbial communities but remains largely unknown. We took *Wolffia australiana* as a representative floating plant and investigated its phyllosphere bacterial community and the underlying driving forces of community modulation in paddy soil ecosystems using Illumina HiSeq 2000 platform-based 16S rRNA gene sequence analysis. The results showed that the phyllosphere of *W. australiana* harbored considerably rich communities of bacteria, with Proteobacteria and *Bacteroidetes* as the predominant phyla. The core microbiome in the phyllosphere contained genera such as *Acidovorax*, *Asticcacaulis*, *Methylibium*, and *Methylophilus*. Complexity of the phyllosphere bacterial communities in terms of class number and alpha-diversity was reduced compared to those in corresponding water and soil. Furthermore, the bacterial communities exhibited structures significantly different from those in water and soil. These findings and the following redundancy analysis (RDA) suggest that species sorting played an important role in the recruitment of bacterial species in the phyllosphere. The compositional structures of the phyllosphere bacterial communities were modulated predominantly by water physicochemical properties, while the initial soil bacterial communities had limited impact. Taken together, the findings from this study reveal the diversity and uniqueness of the phyllosphere bacterial communities associated with the floating macrophytes in paddy soil environments.

Molecular Biology

Analysis of DNA methylation of *Spirodela polyrhiza* (Greater Duckweed) in response to abscisic acid using methylation-sensitive amplified polymorphism

Zhao, Z.; Shi, H. J.; Wang, M. L.; et al.

Russian Journal of Plant Physiology 62: 127-135 (2015)

Spirodela polyrhiza is an energy feedstock, which can produce a kind of dormant fronds called turions to survive cold. ABA can induce *S. polyrhiza* to form turions and is the most important hormone for plants to resist abiotic stresses. DNA methylation plays an important role in plant development by regulating gene expression. In this study, we studied DNA methylation variation in the



fronds of *S. polyrrhiza* treated with ABA and compared DNA methylation variation between fronds and turions, using the methylation-sensitive amplified polymorphism procedure. We selected 60 pairs of selective amplification primers to assess the status and levels of cytosine methylation. The results showed that ABA triggered the hemimethylation or internally full methylation of cytosine. With the prolongation of ABA treatment, the methylation of the total DNA increased. The alteration analysis of cytosine methylation showed that the number of demethylation events were much lower than those of methylation, which indicates that methylation was predominant. In addition, the methylation level in turions was higher than in the fronds. Moreover, the sequences of 14 differentially amplified DNA fragments were analyzed. According to Blast analysis, most of the 14 fragments were identified as genes or DNA involved in the abiotic stress response. The fragment M11 is homologous to ATPase. ABA may affect the methylation status of ATPase gene to regulate its expression to resist chilling. Our study showed that ABA might affect gene expression via changing the methylation status of the cytosine nucleotide.

Phytoremediation

Microbial community and removal of nitrogen via the addition of a carrier in a pilot-scale duckweed-based wastewater treatment system.

Zhao, Yonggui; Fang, Yang; Jin, Yanling; et al.

Bioresource Technology 179: 549–58 (2015)

Carriers were added to a pilot-scale duckweed-based (*Lemna japonica* 0223) wastewater treatment system to immobilize and enhance microorganisms. This system and another parallel duckweed system without carriers were operated for 1.5 years. The results indicated the addition of the carrier did not significantly affect the growth and composition of duckweed, the recovery of total nitrogen (TN), total phosphorus (TP) and CO₂ or the removal of TP. However, it significantly improved the removal efficiency of TN and NH₄(+)-N (by 19.97% and 15.02%, respectively). The use of 454 pyrosequencing revealed large differences of the microbial communities between the different components within a system and similarities within the same components between the two systems. The carrier biofilm had the highest bacterial diversity and relative abundance of nitrifying bacteria (3%) and denitrifying bacteria (24% of Rhodocyclaceae), which improved nitrogen removal of the system. An efficient N-removal duckweed system with enhanced microorganisms was established.

Biosorption of Cd²⁺ by untreated dried powder of duckweed *Lemna aequinoctialis*

Chen, Lanchai; Fang, Yang; Jin, Yanling; et al.

Desalination and Water Treatment 53: 183–194 (2015)

The duckweed *Lemna aequinoctialis* was used as a biosorbent material for Cd²⁺ adsorption in this study. Influencing factors of Cd²⁺ adsorption by *L. aequinoctialis* in aqueous solution were investigated and the process of the Cd²⁺ biosorption was optimized. The results of single-factor experiments suggested that all the factors studied except temperature had significant effects on the



removal efficiency of Cd^{2+} by *L. aequinoctialis*. Based on the results of single-factor experiments, optimization of the Cd^{2+} biosorption was performed by varying four independent parameters using the central composite design under response surface methodology. The optimal conditions for the maximum removal of Cd^{2+} were as follows: grain size of 150–200 mesh, stirring speed of 75 rpm, Cd^{2+} initial concentration of 40 mg/L, and sorbent concentration of 8 g dry matter/L. The maximum removal efficiency of 83.5% was obtained, which was in consistence with the predicted value of 83.6%. This process followed pseudo-second-order kinetics and the experimental data fitted well to Langmuir and Freundlich isotherm models. The maximum capacity of duckweed to adsorb Cd^{2+} was 33.0 mg/g, demonstrating that untreated dry powder of *L. aequinoctialis* represents a promising biosorbent for Cd^{2+} removal. Fourier transform infrared spectroscopy analysis indicated that the -OH groups of carbohydrate compounds and the -NH₂ groups of amide compounds may be the main groups involved in the adsorption of Cd^{2+} by *L. aequinoctialis*.

Phytoremediation potential of *Landoltia punctata* on petroleum hydrocarbons

Ertekin, Ozlem; Kosesakal, Taylan; Unlu, Vesile Selma; et al.

TURKISH JOURNAL OF BOTANY 39: 23–29 (2015)

In this study, the potential of *Landoltia punctata* for petroleum phytoremediation was evaluated. *L. punctata* was treated with different concentrations of Batman crude oil (BCO). The plants were grown in nutrient solutions containing 0.5%, 1%, 2%, and 3% BCO by volume in a growth chamber for 7 days. The fresh weight of the plants in the presence of BCO at a concentration of 0.5% v/v increased by 60% relative to the initial fresh weight. Furthermore, in comparison to the control, plant growth was retarded by 72% and 91% at concentrations of 2% and 3%, respectively. When plants were grown in media containing 0.5% BCO, more than 90% of C-15-C-25 n-alkane compounds were removed compared to the unplanted control (medium plus 0.5% BCO without plants). Pristane (Pr) and phytane (Ph) were both present in all samples, except in the experimental medium of 0.5% BCO application. On the other hand, Pr/Ph values obtained from all oil applications were not statistically different from those of the control samples. As a result, *L. punctata* could be used for the phytoremediation of fresh water resources contaminated with up to 1% crude oil.

Effect of combinations of aquatic plants (*Hydrilla*, *Ceratophyllum*, *Eichhornia*, *Lemna* and *Wolffia*) on arsenic removal in field conditions

Srivastava, Sudhakar; Sounderajan, Suvarna; Udas, Ambuja; et al.

Ecological Engineering 73: 297–301 (2014)

A field experiment was conducted in a combinatorial approach to evaluate the performance of five aquatic plants, singly and in all possible combinations of two plants, for As removal from 4 L of 2500 $\mu\text{g L}^{-1}$ As in 30 days. Under As stress, *Hydrilla* showed an increase in growth rate (19%) in comparison to control, while other plants demonstrated a decline. Among different combinations, *Ceratophyllum* + *Lemna*, *Hydrilla* + *Ceratophyllum* and *Hydrilla* + *Wolffia* showed higher growth in As (92%, 43%, and 33%, respectively) than under control conditions. Total As removal (μg) in 30 days



was found to be the maximum for *Ceratophyllum Lemna* (4365) followed by *Hydrilla*+ *Ceratophyllum* (3326) and *Hydrilla* + *Wolffia* (1896). In all combinations of plants, *Hydrilla* always contributed more than 50%. The study advocates the use of *Hydrilla*/*Ceratophyllum* as one plant of choice to be used in combination. It also proposes to screen for different combinations of two or more plants for better application of phytoremediation technology in field.

Integrated phytobial remediation for sustainable management of arsenic in soil and water

Roy, Madhumita; Giri, Ashok K.; Dutta, Sourav; et al.

Environment International 75: 180-198 (2015)

Arsenic (As), cited as the most hazardous substance by the U.S. Agency for Toxic substance and Disease Registry (ATSDR, 2005), is an ubiquitous metalloid which when ingested for prolonged periods cause extensive health effects leading to ultimate untimely death. Plants and microbes can help mitigate soil and groundwater As problem since they have evolved elaborate detoxification machineries against this toxic metalloid as a result of their coexistence with this since the origin of life on earth. Utilization of the phytoremediation and bioremediation potential of the plants and microbes, respectively, is now regarded as two innovative tools that encompass biology, geology, biotechnology and allied sciences with cutting edge applications for sustainable mitigation of As epidemic. Discovery of As hyperaccumulating plants that uptake and concentrate large amounts of this toxic metalloid in their shoots or roots offered new hope to As phytoremediation, solar power based nature's own green remediation. This review focuses on how phytoremediation and bioremediation can be merged together to form an integrated phytobial remediation which could synergistically achieve the goal of large scale removal of As from soil, sediment and groundwater and overcome the drawbacks of the either processes alone. The review also points to the feasibility of the introduction of transgenic plants and microbes that bring new hope for more efficient treatment of As. The review identifies one critical research gap on the importance of remediation of As contaminated groundwater not only for drinking purpose but also for irrigation purpose and stresses that more research should be conducted on the use of constructed wetland, one of the most suitable areas of application of phytobial remediation. Finally the review has narrowed down on different phytoinvestigation and phytodisposal methods, which constitute the most essential and the most difficult part of pilot scale and field scale applications of phytoremediation programs.

Phytotoxicity

Response of duckweed to various concentrations of selenite

Mechora, Spela; Stibilj, Vekoslava; Germ, Mateja

Environmental Science and Pollution Research International 22: 2416-22 (2015)

The uptake of Se(IV) and its effects on the physiological and biochemical characteristics of duckweed (*Lemna minor* L.) have been studied. Duckweed plants were cultivated in controlled conditions for 7 weeks in different concentrations of Na selenite: 0.5, 1, 2, 5 (exposed 42 days) and 10 mg Se L⁻¹



(survived 7–21 days). The addition of 1mg Se L(-1) did not negatively affect photochemical efficiency whilst respiratory potential increased in weeks 2–4 compared to control. The addition of 1mg Se(IV) L(-1) increased the amount of chlorophyll a in weeks 3 and 4 and the amount of carotenoids in weeks 1, 3 and 5. Concentrations of 2 and 5mg Se L(-1) negatively affected photochemical efficiency in weeks 3 and 4, and increased respiratory potential in comparison to the control in weeks 1–4, whilst beyond week 4, the respiratory potential decreased. Plants exposed to the highest concentration of Se(IV) had to be replaced twice during the experiment because they were dying. That was reflected in photochemical efficiency as well as in respiratory potential, which decreased in time. The content of Se in duckweed increased with the increasing concentration of Se: plants growing in 0.5mg Se L(-1) contained 0.9mg Se g(-1) DM and plants exposed to 5mg Se L(-1) contained 5.8mg Se g(-1) DM. The group of plants exposed to 10mg Se L(-1) for 21days contained 19.5mg Se g(-1) DM. Our study revealed that duckweed absorbed high amount of Se(IV) from the water.

Pb-Induced Avoidance-Like Chloroplast Movements in Fronds of *Lemna trisulca* L.

Samardakiewicz, Slawomir; Krzeszowiec-Jelen, Weronika; Bednarski, Waldemar; et al.

PLoS one 10: e0116757 (2015)

Lead ions are particularly dangerous to the photosynthetic apparatus, but little is known about the effects of trace metals, including Pb, on regulation of chloroplast redistribution. In this study a new effect of lead on chloroplast distribution patterns and movements was demonstrated in mesophyll cells of a small-sized aquatic angiosperm *Lemna trisulca* L. (star duckweed). An analysis of confocal microscopy images of *L. trisulca* fronds treated with lead (15 μ M Pb²⁺, 24 h) in darkness or in weak white light revealed an enhanced accumulation of chloroplasts in the profile position along the anticlinal cell walls, in comparison to untreated plants. The rearrangement of chloroplasts in their response to lead ions in darkness was similar to the avoidance response of chloroplasts in plants treated with strong white light. Transmission electron microscopy X-ray microanalysis showed that intracellular chloroplast arrangement was independent of the location of Pb deposits, suggesting that lead causes redistribution of chloroplasts, which looks like a light-induced avoidance response, but is not a real avoidance response to the metal. Furthermore, a similar redistribution of chloroplasts in *L. trisulca* cells in darkness was observed also under the influence of exogenously applied hydrogen peroxide (H₂O₂). In addition, we detected an enhanced accumulation of endogenous H₂O₂ after treatment of plants with lead. Interestingly, H₂O₂-specific scavenger catalase partly abolished the Pb-induced chloroplast response. These results suggest that H₂O₂ can be involved in the avoidance-like movement of chloroplasts induced by lead. Analysis of photometric measurements revealed also strong inhibition (but not complete) of blue-light-induced chloroplast movements by lead. This inhibition may result from disturbances in the actin cytoskeleton, as we observed fragmentation and disappearance of actin filaments around chloroplasts. Results of this study show that the mechanisms of the toxic effect of lead on chloroplasts can include disturbances in their movement and distribution pattern.

**Toxicological effects of copper oxide nanoparticles on the growth rate, photosynthetic pigment content, and cell morphology of the duckweed *Landoltia punctata***

Lalau, Cristina Moreira; Mohedano, Rodrigo de Almeida; Schmidt, Eder C.; et al.

Protoplasma 252: 221-229 (2015)

Recently, the application of copper oxide nanoparticles (CuO-NPs) has increased considerably, primarily in scientific and industrial fields. However, studies to assess their health risks and environmental impacts are scarce. Therefore, the present study aims to evaluate the toxicological effects of CuO-NPs on the duckweed species *Landoltia punctata*, which was used as a test organism. To accomplish this, duckweed was grown under standard procedures according to ISO DIS 20079 and exposed to three different concentrations of CuO-NPs (0.1, 1.0, and 10.0 g L⁻¹), with one control group (without CuO-NPs). The toxicological effects were measured based on growth rate inhibition, changes in the plant's morphology, effects on ultrastructure, and alterations in photosynthetic pigments. The morphological and ultrastructural effects were evaluated by electronic, scanning and light microscopic analysis, and CuO-NPs were characterized using transmission electron microscopy (TEM), zeta potential, and superficial area methods of analysis. This analysis was performed to evaluate nanoparticle size and form in solution and sample stability. The results showed that CuO-NPs affected morphology more significantly than growth rate. *L. punctata* also showed the ability to remove copper ions. However, for this plant to be representative within the trophic chain, the biomagnification of effects must be assessed.

Assessing single and joint toxicity of three phenylurea herbicides using *Lemna minor* and *Vibrio fischeri* bioassays

Gatidou, Georgia; Stasinakis, Athanasios S.; Iatrou, Evangelia I.

CHEMOSPHERE 119 (Special Issue) S69-S74 (2015)

Single and joint toxicity of three substituted urea herbicides, namely monolinuron [3-(4-chlorophenyl)-1-methoxy-1-methylurea], linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and diuron [1-(3,4 dichlorophenyl)-3,3 dimethyl urea], were studied. The duckweed *Lemna minor* and the luminescent bacterium *Vibrio fischeri* were used for the toxicity assessment and they were exposed to various concentrations of the herbicides, individually and in binary mixtures. The exposure time was 7 d for the duckweed and 30 min for the bacterium. Estimation of EC₅₀ values was performed by frond counting and reduction in light output for *Lemna minor* and *Vibrio fischeri*, respectively. *Lemna minor* was found to be much more sensitive than *Vibrio fischeri* to target compounds. The toxicity of the three herbicides applied solely was estimated to be in decreasing order: diuron (EC₅₀ = 28.3 µg L⁻¹) approximate to linuron (EC₅₀ = 30.5 µg L⁻¹) > monolinuron (EC₅₀ = 300 µg L⁻¹) for the duckweed and linuron (EC₅₀ = 8.2 mg L⁻¹) > diuron (EC₅₀ = 9.2 mg L⁻¹) > monolinuron (EC₅₀ = 11.2 mg L⁻¹) for the bacterium. Based on the environmental concentrations reported in the literature and EC₅₀ values obtained from *Lemna minor* experiments, Risk Quotients (RQ) much higher than 1 were calculated for diuron and linuron. In *Lemna minor* experiments, combination of target compounds resulted to additive effects due to their



same mode of phenylurea action on photosynthetic organisms. Regarding *Vibrio fischeri*, synergistic, additive and antagonistic effects were observed, which varied according to the concentrations of target compounds.

Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* L. plants under salt stress

Oukarroum, Abdallah; Bussotti, Filippo; Goltsev, Vasilij; et al.

ENVIRONMENTAL AND EXPERIMENTAL BOTANY 109: 80-88 (2015)

Increased rate of reactive oxygen species (ROS) is a common plant response to various environmental stresses. In chloroplast thylakoids, the reaction centers of photosystems I and II are the major generation site of ROS. In the present study, the changes of chlorophyll a fluorescence parameters, P700 absorption change and ROS production (using the fluorescent probe 2,7-dichlorofluorescein diacetate) were investigated in *Lemna gibba* plants exposed to salt stress (0-400 mM NaCl). Salt stress inhibited PSI and PSII activities and resulted in a decrease in overall activity of the electron transport chain while stimulating ROS production. When *L. gibba* plants were kept into dark condition, NaCl treatment did not show any significant change in ROS formation compared to control. However, NaCl treatment in light condition induced a strong increase in ROS formation. The production of ROS at 400 mM NaCl was 2.6 and 10 fold higher compared to the control respectively after 6 h and 24 h treatment in light. Furthermore, the correlation between ROS production and the two photosystems (PSI and PSII) activities in *L. gibba* plants was analyzed. Our data confirmed the correlation between the ROS production and PSII and PSI activities. We showed that ROS production was highly correlated to maximal quantum yield of PSII ($R^2 = 0.91$) and efficiency with which a trapped exciton can move an electron into the electron transport chain ($R^2 = 0.86$). While the correlation coefficient (R^2) value ROS formation and $\Delta I/I_0$ (820 nm) (measure of redox states of plastocyanin and P700) was 0.63.

The toxic effects of L-Cysteine-capped cadmium sulfide nanoparticles on the aquatic plant *Spirodela polyrrhiza*

Khataee, Alireza; Movafeghi, Ali; Nazari, Fatemeh; et al.

JOURNAL OF NANOPARTICLE RESEARCH 16: Article Number: 2774 (2014)

Plants play an important role in the fate of nanoparticles in the environment through their uptake, bioaccumulation, and transfer to trophic chains. However, the impacts of nanoparticles on plants as essential components of all ecosystems are not well documented. In the present study, the toxic effects of L-Cysteine-capped CdS nanoparticles on *Spirodela polyrrhiza* as an aquatic higher plant species were studied. L-Cysteine-capped CdS nanoparticles were synthesized using hydrothermal method and their characteristics were determined by XRD, SEM, HRTEM, and FT-IR techniques. The diameter of majority of synthesized nanoparticles was about 15-20 nm. Subsequently, the uptake of L-Cysteine-capped CdS nanoparticles by the plant species was confirmed using epifluorescence microscopy. The activity of peroxidase and superoxide dismutase as antioxidant enzymes was assayed and the relative frond number was calculated in the presence of different concentrations of L-



Cysteine-capped CdS nanoparticles. The obtained results revealed the toxic effects of the synthesized nanoparticles on *S. polyrrhiza*, leading to growth reduction and significant changes in antioxidant enzymes' activity.

Taxonomy and Systematics

The duckweed *Wolffia microscopica*: A unique aquatic monocot

Sree, K. Sowjanya; Maheshwari, Satish C.; Boka, Karoly; et al.

FLORA 210: 31-39 (2015)

The rediscovered species *Wolffia microscopica* (Griff.) Kurz, endemic to India, Pakistan and Bangladesh, shows several features that make it unique in comparison to other duckweeds, even to other species of the genus *Wolffia*. During vegetative propagation, the daughter fronds are produced by budding within a single pouch of the mother frond. Several generations of fronds and their initials exist in union with the parent frond at a given time. Most strikingly, almost all the fronds flower, both in nature and under controlled culture conditions. Thus, in contrast to all other duckweed species, generative propagation is as important as vegetative propagation, which opens the opportunity for artificial breeding. Moreover, flower development on both mother and daughter fronds was observed at the same time. In contrast to all other duckweed species, fronds of *W. microscopica* often possess a ventral projection of varying length ranging from an almost flat appearance of the ventral surface to a length of 4 mm. Absence of root cap, root hairs and vascular tissue demonstrate that this ventral protrusion is not a root and accordingly we name this special structure "pseudoroot". The high number of chloroplasts in the pseudoroot may result in higher capacity of photosynthesis without increasing the frond area which covers the water surface. Thus we propose that the pseudoroot serves to be advantageous to *W. microscopica* in multiplying at a faster rate in comparison to other duckweeds.

Lectotypification of the Linnaean names *Lemna arrhiza* and *L. polyrrhiza* (Araceae)

Iamonico, Duilio; Iberite, Mauro

Taxon 63: 1314-1315 (2014)

The typification of the Linnaean names *Lemna arrhiza* and *L. polyrrhiza* (Araceae) is discussed. Two illustrations from Micheli's work *Nova Plantarum Genera* are designated as lectotypes for these two names.

Fossil Araceae from the Upper Cretaceous of Patagonia, Argentina, with implications on the origin of free-floating aquatic aroids

Gallego, Julieta; Gandolfo, Maria A.; Cuneo, N. Ruben; et al.

REVIEW OF PALAEOBOTANY AND PALYNOLOGY 211: 78-86 (2014)

Herein, we describe and name a new fossil genus and species, *Aquaephyllum auriculatum*, and report on the presence of Pandaniidites pollen grains from La Colonia Formation (Maastrichtian, Upper Cretaceous), Patagonia, Argentina. The new fossil taxon is based on leaf impressions and is



morphologically similar to members of the family Araceae, subfamily Lemnoideae. The most important morphological characters comprise the adaxial venation with at least eight primary veins, the crenate margin, and the presence of an auricle with a floating function. Phylogenetic analysis places the new taxon in a close relationship within the subfamily Lemnoideae, which also includes the fossils *Limnobiophyllum scutatum*, *Cobbania corrugata*, and the extant *Pistia stratiotes*. *Aquaephyllum auriculatum* and Pandaniidites are the first fossil record of lemnaeous leaves and the oldest record for Pandaniidites pollen for the Southern Hemisphere respectively. The inclusion of *Aquaephyllum* and Pandaniidites within a phylogenetic context confirms the previously suggested hypothesis on the existence of close relationships between modern *Pistia*, and the fossils *Limnobiophyllum* and *Cobbania*.

Others

Structural analysis of galactoarabinan from duckweed

Yu, Li; Yu, Changjiang; Zhu, Ming; et al.

Carbohydrate Polymers 117: 807-812 (2015)

A highly branched galactoarabinan named DAG1 (Mw similar to 4.0×10^4 Da) was purified from *Lemna aequinoctialis* 6000 via 70% (v/v) ethanol extraction, followed by size-exclusion chromatography on Bio-Gel P2 and Superdex 75. Methylation analysis showed that DAG1 consisted of t-Araf, (1 → 5)-Araf, (1 → 2,5)-Araf, (1 → 3)-Galp, and (1 → 3,6)-Galp in a relative proportion of approximately 6:4:3:3:3, suggesting an arabinogalactan/galactoarabinan polysaccharide. With the aid of arabinan degrading enzymes, the structure of DAG1 repeating unit was further characterized by ELISA with specific monoclonal antibodies and Yariv reagent assay. Analyses indicated that the proposed repeating unit of DAG1 had a backbone composed of seven α-(1 → 5)-L-arabinofuranose residues where branching occurred at O-2 with either terminal arabinoses or arabinogalactan side chain. The arabinogalactan side chain was composed of six β-(1 → 3)-D-galactopyranose residues, half of which were ramified at O-6 with terminal arabinoses and the last galactose was terminated with arabinose.



Links for Further Reading

<http://www.duckweed2015.cosmos.bot.kyoto-u.ac.jp> Duckweed 2015 Conference site- watch for details as Kyoto, Japan conference preparations develop.

<http://duckweed2013.rutgers.edu/> Past duckweed conference papers and proceedings held at Rutgers University, New Brunswick, NJ in Aug, 2013

<http://Lemnapedia.org> Online developing compendium of duckweed research & applications, founded by the ISCDRA.

<http://InternationalLemnaAssociation.org> Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhorian

<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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