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DUCKWEED FORUM

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A freshwater crab covered with duckweeds

Cover page

A freshwater crab covered with duckweeds, from the Northern Golan heights in Israel. Photo by Omer Viner. Contributed by Ram Viner, head of global QA/QC, GreenOnyx, Israel.

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

Letter from the Editor:

October 30th, 2022

Dear readers,

On behalf of the International Steering Committee for Duckweed Research and Applications, I bring you our warm Autumn greetings. While the terrible conflict with loss of many lives and displacement of millions continues in Europe, at least the COVID pandemic appears to be under control in large parts of the world. Let's all hope that the rate of infection and death from the virus will not surge again and that peace can return in the near future for our world.

On a brighter note, continuing with our recent successful International Conference in Gatersleben, we are happy to share some excellent news of our young community in this issue of the *Duckweed Forum*. From the application side of the duckweed community, one of our committee members, Tsipi Shoham, contributed a detailed description for the roadmap of regulatory approvals that are now in place for several kinds of duckweed products as human food. These are hopefully forerunners for many duckweed-related products that could follow in their footstep to gain a place in the market and truly begin to monetize duckweed as an agricultural crop. As a sign of the future to come, it is heartening to see two student contributors, Finn and Johannes, sharing their knowledge and ideas in the commercially relevant aspects for duckweed cultivation and utilization. I hope our readers will find this information useful as well as interesting.

Academically, two important papers were published in the last quarter that were highlighted in the Database section. Baggs *et al.* presented a careful analysis of the pathogenic response to several well-studied plant pathogens in a variety of duckweed clones from several species, all supported by excellent reference genomes. Combining genomic and transcriptomic data, the work provided support for the presence of both conserved modules as well as duckweed-specific pathways to defend against pathogenic microbes. Unraveling the novel mode of defense that have evolved in duckweeds would likely shed new light on alternative solutions for effective disease resistance in plants. In a more applied study, Liang *et al.* demonstrated the possibility of modifying the oil biosynthesis pathway in duckweed through transgenic overexpression of multiple proteins in the triacylglycerol pathway, with a key gene driven by an inducible promoter. At the end, the oil content of the transgenic duckweed can reach 8.7% of dry-weight, which is more than a hundred-fold higher oil content than background levels. With the known production potential for duckweed under field conditions, this oil content can make duckweed a serious contender as a biodiesel feedstock. While still in the experimental stage, this study nevertheless provided a critical proof-of-principle that should help jumpstart serious investment to refine this approach in the near future.

Finally, I like to share a happy news that Klaus-J. Appenroth of our committee was recently inducted as an Honorary Fellow to the German Botanical Society based on his achievements and contributions to the field of duckweed research. While it is a well-deserved recognition of the excellent science done by Klaus, it also reflects the coming of age of duckweed as a plant family that is gaining importance for the society because of its potential benefits. All good signs !

We wish you all the very best and to have a great holiday season at year's end.

Sincerely,

Eric Lam
Chair, ISCDRA

Current state of regulatory approval for duckweeds as human food

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Abstract

Regulation of novel foods is the policymaker's responsibility to assess the unknown, leading a difficult task of balancing adequate government oversight over novel human foods without stifling innovation and creativity. This article discusses how duckweeds are being regulated as human food in traditional and new global markets. It reviews some of the reasons why duckweed might be regulated as novel foods and how such regulatory goals can be accomplished. In summary, fresh *Wolffia globosa* and *Wolffia arrhiza* can be regulated as traditional food, while any other duckweed species or processed duckweed-based products will be regarded as novel food and will need to go through the complete process. Currently, along the traditional distribution and use of *Wolffia globosa* and *Wolffia arrhiza* species in southeastern Asia (e.g., in Thailand), the USA, the EU and the Israeli markets have granted regulatory approvals for the commercial sale of duckweed-based products for human consumption as follow: Fresh *Wolffia globosa* and *Wolffia arrhiza* species in USA, EU and Israel; The Mankai strain (*Wolffia globosa*) and its related processed products in USA and Israel; The Lentein powder (Lemna & Wolffia) in USA.

Background - novel food regulation

Regulation of novel foods is the policymaker's response to the unknown. Indeed, the novel food industry has grown immensely over the past decade due to the need to feed a growing world population while using less land, water, and other limited natural resources. Ultimately, policymakers have the difficult task of balancing adequate government oversight over novel foods without stifling innovation and creativity.

What are novel foods?

In most jurisdictions, new foods and ingredients introduced into the stream of commerce are generally referred to as "novel foods." Some examples of what might be considered novel foods are newly developed and innovative foods, foods produced using new technologies and production processes, or foods and food ingredients from regions where they have been in use for decades, and are now introduced to other regions where they are unknown and therefore perceived as "novel".

More than 30 years ago, the IPCS (International Programme on Chemical Safety, a joint body of UN, WHO and others), defined a novel food as "a food or food ingredient produced from raw materials not normally used for human consumption or food that is severely modified by the introduction of new processes not previously used in the production of food."[1] Currently, the Joint FAO/WHO Committee on Food Additives (JECFA), a scientific advisory body, evaluates the safety of food additives for purposes of advising the Codex Alimentarius Commission, which is the reference body for international food standards under the World Trade Organization (WTO) that produces standards to guide national legislators. Based on these international standards, novel food regulation process and legislation have been developed on a regional basis and there is a need to go through the specific process for each regional market.

1 World Health Organization (1987). International Programme on Chemical Safety [hereinafter IPCS], Environmental Health Criteria 70, Principles for the Safety Assessment of Food Additives and Contaminants in Food. http://www.inchem.org/documents/ehc/ehc/ehc70.htm#SubSectionNumber:6.2.4 (accessed 28 March 2020).

In this article, I will focus on the current main regions in which duckweeds are already commercialized as human food: The US market, the EU market, the Israeli market, Thailand traditional market and Japan.

"New food" in the US market

The US system consists of federal law, which applies throughout the country and exists concurrently with the individual regulatory frameworks of the 50 states. As federal law is usually supreme to state law, state and local legislatures are free to regulate food so long as their regulation does not contradict federal law.

The Pure Food and Drug Act (PFDA), enacted in 1906, was the first federal law to regulate food in the United States, responding to burgeoning public attention to food hygiene and safety issues. In more recent years, Congress reformed major food safety laws via the 2011 Food Safety Modernization Act; this overhaul shifted the FDA's focus from reacting to food safety issues to preventing foodborne illnesses through expanded regulatory authority.[2]

The Food and Drug Administration (FDA), which serves as the primary food regulatory agency in the USA, has no formal definition of "novel foods" as a category of food, but considers any new food ingredient either as a New Dietary Ingredient (NDI) or a new food additive. NDI refers to an ingredient that was not marketed in the United States before 1994 and is subjected to the regulation under New Dietary Ingredient Notification (NDIN) as a pre-market approval process by the FDA. Food additives, defined as "any substance that is intentionally added to food as a consumable component or will affect the characteristic of the final end product", are considered unsafe until the manufacturer can prove in a Food Additive Petition (FAP) that the ingredient is food-grade pure and meets food safety regulations. The FDA will review the scientific data and information submitted in the FAP to ensure it is "safe," which is defined as a reasonable certainty of no harm to consumers. After the FDA approves the FAP, the substance may enter interstate commerce.

An alternative is the consideration of new food ingredient as Generally Recognized as Safe (GRAS) for specific uses, to be determined independently from the FDA.[3] The GRAS pre-market approval process may rely on the opinion of "experts qualified by scientific training and experience" thus making it a self-determination (not the FDA's) that a substance's use is GRAS. Under the GRAS act is the classification of Traditional Food. A substance can be classified as GRAS if it has been used in food before 1958 and has been commonly ingested by a significant number of consumers.[4] As such, most applicants aim to list new food ingredients as self-determined GRAS where possible, to avoid the tedious FAP pre-market approval process. While there is no requirement for the FDA to corroborate the company's findings before the self-determined GRAS substance is released on the market, an applicant or a company may voluntarily submit a notification of its determination to the FDA for review before putting it on the market. The notification includes a detailed description of the substance, the conditions of use, and the basis of the determination. A "no questions" response from FDA does not mean that the FDA has approved the substance; as noted, in the GRAS process the FDA relies on the determination made by the applicant/company submitting the substance for approval.

"Novel food" in the EU market

The preeminent EU Regulation on food is The General Food Law (GFL)[5]. The GFL is applicable to EU institutions, all member state authorities, and all persons within the EU. The GFL regulatory system covers all stages "from farm to fork," starting with feed production and primary production and continuing through food processing, storage, transport, and sale, setting out an overarching framework for the development of food legislation and regulation at EU and member state levels. The GFL also introduces a science-based approach, meaning that no decision on foods can be made without scientific evidence being available.

The Novel Food Regulation (NFR),[6] which was substantially revised and came into force in its current form on January 1, 2018, defines novel foods as foods that have not been used for human consumption to a significant

2 Debra M Strauss; An analysis of the FDA Food Safety Modernization Act: protection for consumers and boon for business; Food Drug Law J. 2011;66(3):353-76

3 Magnuson, B., Munro, I., Abbot, P. et al. (2013). Review of the Regulation and Safety Assessment of Food Substances in Various Countries and Jurisdictions. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 30 (7): 1189. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3725665/ (accessed 28 March 2020).

4 21 C.F.R. 170.3(f).

5 GFL Arts. 1(3) and 4(1).

6 Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods [hereinafter NFR] Article 3(2)(a), amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (text with EEA relevance), OJ L 327, 11.12.2015, p. 1-22.

degree within the EU before May 15, 1997. One of the major revisions is the inclusion of a new category of traditional foods, defined as "agricultural products from third countries" [7]: If a food has been in continual use in a non-EU country (a "third country"), it is not considered "novel" if its safety has been confirmed with compositional data. Continual use is defined here as use for at least 25 years in the customary diet of a significant number of people residing within at least one non-EU country.

An applicant who intends to place on the EU market a novel food should submit an application to the European Commission. After having verified its validity, the European Commission makes it available to the Member States and mandates EFSA (the European Food Safety Authority) for a scientific assessment.[8]

Genetically modified (GMO) foods are not subject to the novel foods regime in the EU. Rather, a special regulatory regime requires authorization and traceability of GMOs before they are released into the environment, introduced into the market, or used in food for human beings or animal feed. Furthermore, EU law foresees certain liability requirements for damage caused by GMOs.[9]

"Novel food" in Israel

A "Novel Food" approval must be obtained from the National Food Service at the Israeli Ministry of Health. Novel Food is defined as food that had not been consumed to a significant degree by humans in Israel before 19 Feb 2006, when the first Regulation on novel food in Israel came into force. 'Novel Food' can be newly developed, innovative food, food produced using new technologies and production processes. Food which is or has been traditionally eaten outside of Israel is also defined as "Novel food" and there is no specific process for traditional food.

Why Regulate Novel Foods?

At the most fundamental level it is about regulating and assessing the unknown, generally consisting of testing, data, and scientific evaluation of potential adverse effects on human health, animal, plants and the environment. Considering human health, evaluation is mainly of the nutritional composition, presence of known toxins or anti-nutrients, allergenicity (e.g., of proteins) as well as the potential health impact of introducing the novel food into the human diet.
As such, policymakers weighing and choosing regulatory approaches to find a balance between protection and innovation, between financing of research and reaping of societal benefits, and between the freedom and rights of individuals, industry and organizations versus the necessity of government oversight for the public good.

Whereas the United States' regulatory approach requires scientific proof of harm, Europe requires regulators to take action when, after assessment of available information, a possibility of harmful effects on health is identified and scientific uncertainty persists.[10] Precaution is a key principle.

How Are Novel Foods Regulated?

Regulation of novel foods can be categorized based on the phase of market introduction of the product, conceptually divided into those regulating market access (e.g., pre-market measures such as approvals) and those regulating products already on the market (e.g., post-market measures such as liability).

Are duckweeds "novel food"?

In many markets in the world, duckweed plants will be considered as a new produce, "novel food" or a New Dietary Ingredient (NDI), thus will need to gain a local regulatory status. This regulatory status may be related to the traditional consumption of duckweed as human food for some regions, which could be a much shorter process, or as a complete novel food application. The eligibility to be classified as traditional food will depend on the following:

7 EU Reg. 2015/2283 *relating to novel foods,* article 3, paragraph 2
8 https://www.efsa.europa.eu/en/applications/novel-food-traditional-food
9 https://food.ec.europa.eu/plants/genetically-modified-organisms/gmo-legislation_en
10 General Food Law [hereinafter GFL], Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, Art. 7(1).

- **The duckweed species and strains:** only species and strains that are known as traditionally edible vegetables (*Wolffia globosa* and *Wolffia arrhiza*) are eligible, while any other duckweed species (e.g., *Lemna minor*) and strains will be evaluated as novel foods.
- **Proprietary strains vs. Natural strains:** A hold or an ownership of special strains may be granted for any distinct and new variety of plant, including cultivated seeds, mutants, or hybrids, as long as the plant is invented or discovered through breeding or other human efforts, all aimed to improve the plant found in nature. Thus, the claimed strain cannot be one that is found growing in the wild and the improved plant must be new and non-obvious.[11,12] As such, novel proprietary duckweed strains may be regulated as "novel food" while natural strains that are known as traditional strains can be regulated under the traditional food category.
- **Intended use:** Since traditionally, *Wolffia globosa* and *Wolffia arrhiza* are marketed as fresh produce, only fresh produce can be regulated under the traditional food process while any processed products (dried, frozen) or modified ingredients will be analyzed as novel food. In accordance, the marketing will be limited by the amount not higher than to achieve its intended purpose and not exceeding its traditional consumption levels.

As listed in table 1, the overall applications for regulatory approval of duckweed as human food in different geographic areas reflects the above classification to either traditional food or complete novel food category.

The current regulatory and legislative status of duckweeds for human food

In Thailand – Wolffia species are recognized by the authorities as traditional human food, listed among formally recognized edible plants

As confirmed by the Ministry of Agriculture and Cooperatives in Thailand, via the Department of Fisheries, selected duckweed species, namely *Wolffia globosa* and *Wolffia arrhiza* are known as traditional human food in Thailand, consumed in northern and northeastern Thailand for many generations by local native populations. In some local dialects in Thailand, Wolffia vegetables are called "Pum" or "Kain-Nam" (Water Meal). The governmental authority in Thailand further confirmed that *Wolffia globosa* and *Wolffia arrhiza* are native to Thailand, as to many other Asian regions, such as India, Nepal, Pakistan, Bangladesh, Myanmar (Burma), Laos, Cambodia, Vietnam, Indonesia, the Philippines, Malaysia, China, Korea, and Japan. Studies have demonstrated that *Wolffia arrhiza* was found in eastern Thailand, mainly in the provinces of Sa Kaeo, Prachinburi, Nakhon Nayok, and Chachoengsao, while both *Wolffia globosa* and *Wolffia arrhiza* are found in northeastern Thailand in the provinces of Nongkhai, Sakon, Nakhon and Loei. Till today, most of the water meal can be harvested from natural ponds and local farms, having the highest productivity towards the end of the rainy season (October to November), and distributed via local markets as fresh produce. Various traditional cuisines are known as Khaeng Om Khai (Northern Thai spice soup with water meal), Khaeng Hoi Sai Khai Pum (cockle curry with water meal) or Khai Toon Khai Pum (steamed egg with water meal).

In all other regions (e.g., US & EU), where the duckweed plants are not known to be consumed as human food, a pre-market regulatory approval process is required for any duckweed based commercial product. Accordingly, and as summarized in table 1, several applications have been submitted per specific targeted markets as follow:

In the USA market

On August 2018, Parabel USA (now renamed Lemnature Aquafarm) has received a GRAS (generally recognised as safe) no objections letter from the US Food and Drug Administration (FDA) for its water lentil (a polyculture crop consisting of species from the Lemna genus (70–100%) and the Wolffia genus (0–30%)) based Lentein plant protein.[13] The GRAS Notice was submitted by Parabel on November 2017[14], informing the FDA

11 https://www.uspto.gov/patents/basics/types-patent-applications/general-information-about-35-usc-161
12 https://www.justia.com/intellectual-property/patents/types-of-patents/plant/
13 https://www.foodnavigator-usa.com/Article/2018/08/28/Parabel-USA-receives-FDA-no-objections-letter-for-LENTEIN-plant-protein
14 https://www.fda.gov/media/113614/download

administration, in accordance with the FDA regulation 21 CFR §170 Subpart E[15], that Parabel's lentein®, Complete and Degreened products, are GRAS when consumed as a nutritional ingredient in commercial food products. Thus, pursuant to the regulatory and scientific procedures established by the FDA regulation at 21 CFR §170, the intended use of Parabel's lentein®, Complete and Degreened products, are exempt from premarket approval requirements of the United States Federal Food, Drug and Cosmetic Act, because it was determined that such use is GRAS.

GreenOnyx set a strategy that is focused first on the known edible duckweed strains that can apply via the regulation into the markets as traditional food. Thus, in 2014, GreenOnyx completed GRAS affirmation status for any natural strain and clone that belong to the *Wolffia arrhiza* or *Wolffia globosa* species, in accordance with the FDA regulation 21 CFR §170 Subpart E, thus enabling their introduction to the USA market as fresh produce. In the GreenOnyx dossier, the eligibility for classification as GRAS and a Non-NDI regulatory status was based on the ability to demonstrate traditional use in other countries: presenting a long history of safe food use, the consumption in more then one country outside of the USA, and the consumption as a part of the local customary diet for a significant number of people. Following the development of market acceptance, GreenOnyx may bring other edible duckweed strains of interest into the multiple target markets, yet under the novel food process.
On August 2015, Hinoman has completed the self-affirmed GRAS status for its Mankai, a cultivated proprietary strain of the *Wolffia globosa* species.[16] The GRAS designation was for the use of Mankai in functional foods and beverages. In 2020, Hinoman initiated sales of Mankai in the US market. However, 2 years later, in January 2022, these efforts have come to an end, a decision that was made by Hinoman's investors and is in no way a reflection of the product itself, as stated by Hinoman.[17]

In the EU market

Approvals

The European Commission authorized the placing on the market of fresh plants of *Wolffia arrhiza* and/or *Wolffia globosa* as a traditional food from a third country under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470.[18]

- On 7 September 2020, GreenOnyx Ltd. submitted a notification to the Commission of their intention to place fresh plants of *Wolffia arrhiza* and *Wolffia globosa* on the European Union market as a traditional food from a third country in accordance with Article 14 of Regulation (EU) 2015/2283.
- GreenOnyx requested that fresh plants of *Wolffia arrhiza* and *Wolffia globosa* be used as such by the general population.
- The data presented by the applicant demonstrate that fresh plants of *Wolffia arrhiza* and/or of *Wolffia globosa* as such have a history of safe food use in Southeast Asia, in particular at Laos, Myanmar and Thailand.
- No duly reasoned safety objections to the placing on the market within the EU of fresh plants of *Wolffia arrhiza* and *Wolffia globosa* were submitted to the Commission by the Member States or the Authority.
- **On 30 June 2021, the EFSA Authority published its 'Technical Report on the notification of fresh plants of *Wolffia arrhiza* and *Wolffia globosa* as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283 ' (4). The Authority concluded that the available data on the composition and the history of use of the fresh plants of *Wolffia arrhiza* and *Wolffia globosa* and the data provided on the traditional food cultivated under the conditions of vertical farming as described in the notification do not raise safety concerns.**
- The plants are strongly influenced by the environmental conditions of the waters where they are grown and may contain heavy metals, microcystins, microorganisms, trace elements and pesticides. Thus, maximum levels for a number of substances in the plants, including Manganese, are therefore established in the EU list of the authorised fresh plants of *Wolffia arrhiza* and *Wolffia globosa*.[19]

15 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.225
16 https://www.ingredientsnetwork.com/hinoman-mankai-gets-gras-status-news037281.html
17 https://www.facebook.com/EatMankaiUSA/
18 https://eur-lex.europa.eu/legal-content/en/TXT/PDF/?uri=CELEX:32021R2191&from=en
19 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32021R2191

Rejected applications

The European Commission did not authorize the placing on the market of *Lemna* and or *Wolffia* species-based powder products, stating that the safety of these products cannot be established

On 15 November 2018, the company Parabel Ltd submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/22831 to place water lentil powder from Lemnaceae on the Union market as a novel food (NF).[20]

- Thermally washed and dried water lentils, which are produced as a polyculture crop consisting of species from the Lemna genus (70–100%) and the Wolffia genus (0–30%).
- The NF is intended for human consumption as a food ingredient in a number of foods (e.g. herbs, spices and seasonings, sauces, soups and broths, protein products, dietary food for weight control) in different maximum levels, depending on the food category, and as a food supplement.
- The NF is intended for the general population. Moreover, the NF is intended for use in food supplements targeting only adults.
- The Panel considers that, based on the protein concentration, the consumption of the NF may trigger allergic reactions. The Panel concluded that an increase in manganese intake from the NF used as food ingredient or food supplements is of safety concern and the safety of the NF cannot be established.

On 24 September 2019, the company 'Hinoman' submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/22831 to authorise the placing on the market of *Wolffia globosa* powder as a novel food (NF).[21] The applicant requested to authorise the use of *Wolffia globosa* powder as ingredient in various food categories including food supplements.

- The target population is the general population except for food supplements which are intended to be consumed exclusively by adults.
- The NF is produced by cultivation of *Wolffia globosa* plants under controlled conditions, washing with hot water and drying.
- Based on the protein concentration, the Panel considers that the consumption of the NF may trigger allergic reactions. The Panel concluded that an increase in manganese intake from the NF used as food ingredient or food supplements is of safety concern and the safety of the NF cannot be established.

Pending applications

An application for authorizing *Lemna minor* and *Lemna gibba* whole plant material as a novel food, in accordance with Regulation (EU) 2015/2283, which was submitted by Wageningen Plant Research of Wageningen University & Research, is currently under evaluation.[22] As indicated in the application dossier, "Both plants species are targeted to be used as either fresh vegetable, frozen vegetable or as part of composed products like ready-to-eat dishes, smoothies or others. The plants are cultivated in closed settings like a greenhouse or indoor/vertical farming system for which cultivation, handling and processing is performed according to HACCP principles. After harvesting, the plants are immediately processed to the water lentil (based/containing) products. The products will comply with the general food law principles, food contaminants and microbiological requirements laid in Regulation (EC) No 178/2002, Regulation (EC) No 1881/2006 and Regulation (EC) 2073/2005, respectively. Thus, like any other vegetable crop, regular quality control for microbial and other contaminants will be required."

An application for authorizing protein concentrates from a mixture of *Lemna gibba* and *Lemna minor,* in accordance with Regulation (EU) No 2015/2283, which was submitted by Rubisco Foods (ABC Kroos), is currently under evaluation.[23] The mixture of *Lemna gibba* and *Lemna minor* is cultivated in a closed

20 https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6845
21 https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6938
22 https://food.ec.europa.eu/system/files/2020-07/novel-food_sum_ongoing-app_2020-1757.pdf
23 https://food.ec.europa.eu/system/files/2019-05/novel-food_sum_ongoing-app_2018-0801.pdf

environment and harvested afterwards according to the HACCP principles. After harvesting, the plants are immediately processed to the water lentil protein concentrate considered in this application. Duckweed protein concentrate is intended for the general population excluding infants and will be sold as a protein rich ingredient with a broad application in a variety of food and beverages categories. Analyses and scientific studies show no safety concerns for heavy metals, mycotoxins, cyanotoxins, pesticides, and lysino-alanine. The antinutritional factors calcium oxalate (oxalic acid), polyphenols (tannins) and trypsin inhibitor are also known to be present in only relatively low concentrations. The microbial data does not exceed the limits as set in Regulation (EC) No 2073/2005. When maximum use levels are observed, no adverse nutritional effects are expected when water lentil protein concentrate are habitually used in the European dietary context. Rubisco Foods expects to receive the Novel Food approval to market duckweed protein concentrate on the EU market in 2022.

In Israel

Approvals

In May 2019, Hinoman's application was approved and the plant *Wolffia globosa*, (registered variety PBR#2661), also known as Mankai, was allowed for use as novel food in the Israeli market, subject to the details below:

- The *Wolffia globosa* cultures must be free from any pollutants.
- Using only commercial fertilizers that are allowed to be used for growing plants for human consumption.
- Following the harvest, tap water or distilled water will be used for washing the biomass.
- Meeting the allowed maximum levels of heavy metals, pesticide, Mycotoxins and microbial load in the product as defined

In September 2020, GreenOnyx submitted an application for the registration of the plants *Wolffia globosa* and *Wolffia arrhiza* grown in a vertical growing system, under controlled conditions, as a novel food ingredient, to the Food Control Services, Israeli Ministry of Health. According to the procedure for registering novel food in Israel (procedure number 004-08), which is in effect from February 19, 2006, and the recommendation of the Novel Food Committee, in July 2022, *Wolffia globosa* and *Wolffia arrhiza* were approved for use as a novel food in Israel under the following conditions[24]:

- The cultivation of the *Wolffia globosa* and *Wolffia arrhiza* plants will be carried out in a sealed vertical growing system that match food grade criteria and under controlled conditions.
- The growing medium will contain water and commercial fertilizers that are allowed to be used for growing plants for human consumption, and will not contain pesticides.
- The harvesting and washing of the fresh plants will be done automatically or semi-automatically, while tap water or distilled water will be used to wash the biomass.
- Meeting the allowed maximum levels of heavy metals, pesticide, Mycotoxins and microbial load in the fresh plant as defined.

Table 1: The regulatory approval application status for duckweeds as human food

REGULATORY APPROVAL STATUS	APPLICANT	REGION / MARKET	TYPE	TYPE	COMPANY / APPLICANT
APPROVALS	Mix Lemna & Wolffia	USA	GRAS affirmation	Powder ingredient	Parabel
	Wolffia globosa *Wolffia arrhiza*	USA	GRAS affirmation Traditional food	Fresh produce Processed ingredient	GreenOnyx
	Wolffia globosa	USA	GRAS affirmation	Powder ingredient Dried frozen	Hinoman
	Wolffia globosa	EU	EFSA, Traditional food	Fresh produce	GreenOnyx

24 https://www.gov.il/BlobFolder/dynamiccollectorresultitem/fcs-regulation-652646522_gowollfia/he/files_databases_fcs_regulations_652646522_gowollfia.pdf

	Wolffia arrhiza				
	Wolffia globosa	Israel	Novel food Ministry of Health	Powder ingredient Dried frozen	Hinoman
	Wolffia globosa *Wolffia arrhiza*	Israel	Novel food Ministry of Health	Fresh produce	GreenOnyx
REJECTIONS	Mix Lemna & Wolffia	EU	EFSA	Powder ingredient	Parabel
	Wolffia globosa	EU	EFSA	Powder ingredient	Hinoman
PENDING APPLICATIONS	*Lemna minor* *Lemna gibba*	EU	EFSA	Fresh produce	Wageningen University
	Lemna minor *Lemna gibba*	EU	EFSA	Concentrates Protein ingredient	Rubisco Foods

Based on the above approvals and in accordance with the regulatory conditions that are part of these approvals, the applicants and companies initiated commercial activities in the targeted and restricted regions. The current commercial activities are summarized in table 2 below.

Table 2: Following the regulatory approvals, current duckweeds commercial activities as human food.

REGION / MARKET	DUCKWEED STRAIN	PRODUCT	TYPE	COMPANY	COMMERCIAL ACTIVITY
USA	Polyculture of Lemna species & Wolffia species	Complete & Degreened lentein®	Powder ingredient	Parabel	Initiated in 2018
	Wolffia globosa *Wolffia arrhiza*	Wanna greens®	Fresh produce	GreenOnyx	Initiated in 2022
	Wolffia globosa	Mankai®	Powder ingredient Dried frozen	Hinoman	Initiated in 2020 Ended - Jan 2022
EU	*Wolffia globosa* *Wolffia arrhiza*	Wanna greens®	Fresh produce	GreenOnyx	Initiated in 2022
ISRAEL	*Wolffia globosa* *Wolffia arrhiza*	Wanna greens®	Fresh produce	GreenOnyx	Initiated in 2022
	Wolffia globosa	Mankai®	Dried frozen	Hinoman	Operation and sales are detained
THAILAND	*Wolffia globosa* *Wolffia arrhiza*	Khai-Nam (Traditional name)	Fresh produce	Local farms Local market	Traditional food - Many generations
JAPAN	*Wolffia globosa*	Mankai®	Powder ingredient	Hinoman	Sales are detained

In conclusion, fresh *Wolffia globosa* and *Wolffia arrhiza* can be regulated and sold as traditional food, while any other duckweed species or processed duckweed-based products will be regarded as novel food and will need to go through the complete process. Currently, along the traditional distribution and use of *Wolffia globosa* and *Wolffia arrhiza* species in southeastern Asia (e.g., in Thailand), the USA, the EU and the Israeli markets have granted regulatory approvals for the commercial sale of duckweeds-based products for human consumption as follow:

- Fresh *Wolffia globosa* and *Wolffia arrhiza* species in USA, EU and Israel.
- The Mankai strain (*Wolffia globosa*) and its related processed products in USA and Israel
- The Lentein powder (*Lemna* & *Wolffia*) in USA

We are hoping to see further regulatory approvals for additional duckweed species and associated products based on the pending applications and future new ones.

Ancient names for duckweeds

Marvin Edelman[1], Klaus-J Appenroth[2], K Sowjanya Sree[3] and Tokitaka Oyama[4]

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We recently published an article entitled "Ethnobotanical History: Duckweeds in Different Civilizations" (Edelman et al., 2022; https://www.mdpi.com/2223-7747/11/16/2124/htm), which documents duckweed uses in ancient/medieval times. There is a detailing of the exact references and their contextual background in ancient texts from Chinese, Christian, Greek, Hebrew, Hindu, Japanese, Mayan, Roman and Yemeni cultures and civilizations. The entries ranged from botanical descriptions, religious usage, medicinal recipes, cultural significances, and poetic lores (co-authors in the picture). Of particular note is the apparent convergent medicinal uses described by ancient Mayan and Chinese texts.

The authors (from left to right): Klaus-J. Appenroth, K. Sowjanya Sree, Marvin Edelman and Tokitaka Oyama

Recently, there were discussions about the very name 'DUCK-WEED' in several of the international platforms. Concerns were raised about the very name implying an animal feed (Duck) and / or an unwanted plant (weed), in view of its use as human food. This article presents several terms for duckweed in different ancient cultures widely distributed across continents, as shown in the table below. We look forward to see how well the application specialists may be able to make use of this historical information, and we should be glad to see some of these names reappearing in modern societies on the supermarket shelves.

Local names of Duckweed in antiquity through the Middle Ages (Edelman et al., 2022).

Culture	Period of history	Local name	Literal meaning
Chinese	Han Dynasty c.200 CE	*Shui Ping* *Shui Hua*	duckweed water flower
	Ming Dynasty c.1500 CE	*Fu Ping*	duckweed
Christian	Hildegard von Bingen c.1150 CE	*Lemna*	duckweed
Greek	Theophrastus c.330 BCE	*Lemna*	water plant
Hebrew	Book of Psalms c.1000 BCE	*Yawvein* *Tachlav*	duckweed duckweed
	Talmud (Mishna) c.200 CE	*Yaroka*	greenery on water surface
Hindu	Kurma purana c.700 CE	*Shaivaal*	weed on water
Japanese	Ono no Komachi c.850 CE	*Ukikusa* *Ne-nashi-k(g)usa*	floating weeds weeds without a root
Maya	Ritual of the Bicabs c.200 CE	*Ixim ha*	maize-water
Roman	Dioscorides c.70 CE	*Lens*	lens-shaped
Yemini	Zaydism c.1000 CE	*Simsim*	Sesame-seed shaped

Cost efficient nutrient solutions from commercially available fertilizers

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A variety of different nutrient solutions is described in the literature, such as the Murashige-Skoog medium, the Schenk-Hildebrandt medium, the Hoagland's medium, or the N medium (Appenroth 2015). In all these cases, they are based on expensive *pro analysi* (p.a.) chemicals with high purity, as they are usually used for small-scale laboratory purposes.

When duckweed is cultivated on a larger scale, much greater quantities of chemicals are needed for nutrient solution or stock solution preparation. Depending on the amount of chemicals needed, this can become very expensive, especially on an agricultural scale.

Therefore, a less expensive way of preparing the stock solutions for a modified N medium is described in the literature (Petersen et al. 2021) Exchanging p.a. chemicals with commercially available fertilizers can reduce the costs for duckweed growers.

At the University of Applied Sciences Osnabrück, Germany, a modified N medium with a nitrate-N to ammonium-N ratio of 75 % to 25 % is applied for duckweed cultivation (Table 1). The corresponding six stock solutions (stock solution 2 is not used for this ratio) are mainly prepared with commercially available fertilizers. The cost for one liter of stock solution is currently about 1.55 €. For nutrient solution preparation this stock solution will be diluted at least 1:200, giving a final cost of 0.009 € per liter, including the cost for local tap water.

Marked in red is the nitrate-N to ammonium-N ratio which resulted in the highest RGR (0.22 d^{-1}), crude protein content (43.9 %) and relative protein yield (45 g $week^{-1}$ m^{-2}) for the species *Wolffiella hyalina* (clone 9525). For cultivation, a water temperature of 20°C, a light intensity of 350 µmol m^{-2} s^{-1} and a photoperiod of 8:16 hours light to dark cycle was applied (Petersen et al. 2021).

Table 1: Formulation of seven stock solutions (g L^{-1}) for five different nitrate-N to ammonium-N ratios ([100-0], [75-25], [50-50], [25-75], and [0-100]), based on the N-medium. Marked in red color is the nitrate-N to ammonium-N ratio which resulted in highest RGR, crude protein content and relative protein yield (Petersen et al. 2021).

Stock solution	Product name	Producer	[100-0] (g L^{-1})	[75-25] (g L^{-1})	[50-50] (g L^{-1})	[25-75] (g L^{-1})	[0-100] (g L^{-1})
1	Calcinit	Yara	47.2	35.4	23.6	11.8	0
1	Krista K Plus	Yara	161.8	121.3	80.9	40.4	0
2	NH_4Cl	Merck	0	0	26.7	53.5	80.2
3	OCI Granular 2	OCI NV	0	33	33	33	33
4	KCl	AppliChem	0	29.8	59.6	89.5	119.3
4	$CaCl_2 * 2 H_20$	Merck	0	7.4	14.7	22.1	29.4
5	Krista MKP	Yara	27.2	27.2	27.2	27.2	27.2
6	Epso Combitop	K+S	49.3	49.3	49.3	49.3	49.3
6	Borax	Horticoop	0.06	0.06	0.06	0.06	0.06
6	Mangaan	Horticoop	0.44	0.44	0.44	0.44	0.44
6	$MoNa_2O_4 * 2 H_20$	AppliChem	0.02	0.02	0.02	0.02	0.02
7	Ferty 72	Planta	2.2	2.2	2.2	2.2	2.2

Such a described medium was also successfully used for other scientific experiments (Petersen et al. 2022).

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Petersen, Finn; Demann, Johannes; Restemeyer, Dina; Olfs, Hans-Werner; Westendarp, Heiner; Appenroth, Klaus-Juergen; Ulbrich, Andreas (2022): Influence of Light Intensity and Spectrum on Duckweed Growth and Proteins in a Small-Scale, Re-Circulating Indoor Vertical Farm. In: *Plants* 11 (8), S. 1010. DOI: 10.3390/plants11081010.

Duckweed helps fire brigades to extinguish fire

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Late spring to summer are the months for a green mat of duckweed in some of the village ponds and canals in Germany. This summer, a friend sent to us a photo from a pond in a village close to Jena, Großlohma, part of the city Blankenhain in Thuringia (Fig. 1), Germany. The photo of the pond was not unusual, and also not that the duckweed were afloat. What took us to surprise was a label hung on the fence around pond (Fig. 2). This label read in German: "Wasserlinsen abschoepfen verboten!!!". It is translated as "Skimming of duckweed forbidden!!!" Such ponds in Germany are used as static fire reservoir in case of emergency for centuries.

Label attached of the fence of a village pond. The text read: "Skimming of duckweed prohibited!!!"

This text surprised us as in many villages in this area farmers harvest duckweed to feed their poultry. A phone call with the Mayor, Mr. Gerold Bloethner, brought in remarkable information. He explained that this pond is used as a static water tank for fire emergency cases, following the German laws. Talking further, he said that in the earlier times, large amounts of filamentous green algae grew in this pond. And, that when the fire brigade tried to suck this pond water, the algae would quickly block the exhaust suction device. And then they realized that when duckweed grows on the water surface, algal growth goes down strongly. Continuing further he said, moreover, duckweed do not block the suction device but flow through it and the fire brigade does its job."

From a scientific point of view this effect is easy to explain. When duckweed form a mat on the surface of water, they absorb almost completely all the photosynthetically active radiation, and their fast growth ensures that the mat is thick enough, when not disturbed, to block maximal amount of light from passing through to the lower depths of the water body. Thus, reducing the growth of sub-surface life forms, like the submerged algal species as in this case.

Visiting this village pond was an amazing duckweed excursion, together with the member of the Green Party and the ex-Mayor of the City Jena, Frank Schenker and his wife Jutta Schenker (Fig. 3). Jutta Schenker commented: "I have seen this village and the scenic pond for the first time. I am very much surprised that by the rule to not to skim duckweed from the pond, the layer became so dense that no light penetrates for the algae. Fascinating!" And Frank Schenker, also a member of the city parliament in Jena, said: "Duckweed in a water pond used for fire emergency service, to prevent algal growth is an unusual application – but evidently effective. As a member of the Green Party I would like to suggest to the farmer coops in our area to use such spontaneous growth for animal feed. The present debate in agriculture would suggest this."

The authors can only add that we identified the duckweeds species in this pond as *Lemna minor* and the alga as *Cladophora sp*.

In front of the fire pond in Grosslohma (from left to right): K. Sowjanya Sree, Jutta Schenker, Klaus J. Appenroth, Frank Schenker.

Final Announcement

for Duckweed Workshop at PAG 30

Final Announcement: *Important Dates for attendees*

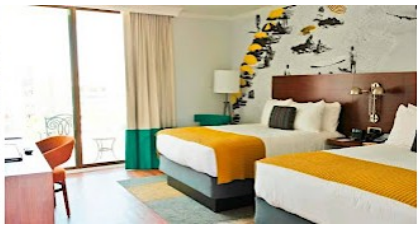
Duckweed Research and Applications Workshop

in the

Plant and Animal Genome (PAG 30) Conference 2023

January 13-18, 2023

at the fully renovated Town & Country Conference Center in San Diego, CA, USA



Organizers Eric Lam (Rutgers U.) and Todd Michael (Salk Institute) are organizing the Duckweed Research and Applications Workshop to take place on the afternoon of January 13th during PAG 30 in San Diego (January 13-18, 2022) and would encourage everyone in the community to attend. We expect to have an excellent lineup of exciting speakers with new results from diverse studies with duckweed.

Please register at this link: https://pag.confex.com/pag/30/gateway.cgi? or at the link from the homepage if you are a new user: https://pag.confex.com/pag/30/registration/index.cgi?username=119237&password=951188

Please also note these deadlines:

Deadline for poster abstract submission: November 4th, 2022
Deadline for posting speakers by organizers: November 18, 2022
Deadline for workshop abstract submission: December 9th, 2022

Confirmed Speakers and Topics:

Arturo Mari-Ordonez: "*Transposons as drivers of epigenetic landscapes in plants, lessons from duckweeds*"
Shogo Ito: "*Analysis of genes expressed during the flowering and the dormancy induction in duckweeds*"
Autar Mattoo: "*Polyamine biosynthesis and catabolism pathways in the duckweed S. polyrhiza*"
Ryan Sartor: "*Setting Up a program for trait improvement of Lemna gibba through selective breeding*"
Alex Harkess: "Automated phenotyping of duckweed"
Leo Orozco: "*Gene network regulation of carbon and nitrogen metabolism by the environment and plant- microbe interaction*"
Kenneth Acosta: "*Microbial ecology of Lemna minor*"

My duckweed research experience and new research focus areas: Yubin Ma

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Duckweed has been considered as a valuable feedstock for bioethanol production due to its high biomass and starch production. My research between 2013 and 2019 at Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, focused on duckweed resource collection, duckweed stain selection and heavy-ion irradiation mutagenesis, genetic transformation and genome editing, starch accumulation molecular mechanism as well as large-scale duckweed cultivation system development. 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 and *Landoltia punctata* 6001 from Chongqing city were obtained with the highest starch accumulation and Cd tolerance, respectively [1, 2]. On the other hand, heavy-ion irradiation mutagenesis was applied in duckweed breeding [3]. As to influence factor in duckweed for starch accumulation, we had investigated the daily variations in starch content, and the effects of different culture media and salt concentrations on *L. aequinoctialis* 6000 starch accumulation [1]. 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) as well as light intensities (20, 50, 80, 110, 200 and 400 mol m μ^{-2} s^{-1}) [4]. Moreover, comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol was done in my group [5]. Nitrogen starvation could induce starch accumulation in duckweed. To further investigate the starch accumulation in *L. aequinoctialis* 6000, transcriptome was analyzed after cultivated under nitrogen starvation for 0, 3 and 7 days. In addition, comparative transcriptome of *L. punctata* 6001 after cadmium treatment for 0, 1, 3, and 6 days was also analyzed [6]. To understand the function of genes and traits in duckweeds, we developed efficient genetic transformation and CRISPR/Cas9 mediated genome editing in *L. aequinoctialis* [7]. To promote duckweed commercialization, space-saving cultivation model was designed. Further, we established a 1500 m^2 duckweed large-scale multilayer cultivation system using wastewater from raising cows [8]. The duckweed was cultivated in photovoltaic greenhouse to keep the system working throughout the whole year. Moreover, duckweed starch automatic isolation system was developed in my group.

I am honored to be a member in 3rd and 4th International Steering Committee on Duckweed Research and Applications. During service for the duckweed community, I attended meetings and gave advice for preparing the *Duckweed Forum*. I also tried my best to promote the development of duckweed industry in China.

At 2019, I move to Ocean University of China with my research focus on *Urechis unicinctus* development biology and stress biology, as well as genetic breeding. The Echiura worm *U. unicinctus* is a benthic organism inhabiting U-shaped burrows in the intertidal zone in China, Korea, and Japan, and is a delicious seafood with high nutritional value. The intertidal zone is a peculiar and dynamic coastal environment between land and ocean, and is one of the most productive coastal ecosystems with estuaries. As an ecotone, it represents a particular environment characterized by changing environmental factors like temperature, salinity, oxygen concentration, sulfide, etc. For *U. unicinctus* stress biology, I mainly focus on the molecular mechanisms for sulfide and temperature adaptation. On the other hand, the worm has a typical free-swimming larva beginning with the early trochophore stage and the planktonic larva settles to the bottom during the segmentation larva stage, and then burrows into the sediment and metamorphoses to the benthic worm. For *U. unicinctus* development biology, I currently focus on the molecular mechanism for different phototaxis in *U. unicinctus* planktonic larva and benthic worm. In China, the natural resources of *U. unicinctus* have declined sharply

because of overfishing. *U. unicinctus* has become an emerging aquaculture species in China. At present, *U. unicinctus* breeding technology is still nascent. In my group, I also work on *U. unicinctus* artificial breeding as well as genetic breeding to develop improved varieties for future *U. unicinctus* aquaculture. At present, I have sequenced *U. unicinctus* genome and published a few papers in this field [9-13].

Because duckweeds can be rich in starch, protein and flavonoids, I also think this plant family could be considered as an alternative feed for *U. unicinctus* aquaculture. In future studies, I will try it for *U. unicinctus* cultivation. So, I will likely be back into duckweed-related research in the near future.

Urechis unicinctus, about 30 g weight and 12 cm long.

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Student Spotlight: Johannes Demann

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I grew up on a pig farm close in the 'Emsland', a rural area in Germany. Therefore, I had a childhood in nature with thriving plants and animals. After high school, I did a practical education as a farmer for two years, learning how to grow barley, corn, wheat and canola, and to care for animals. Because I wanted to know more about agriculture, I started studying in Osnabrück and focused on the scientific bases in plant and animal sciences for the practical observations I had made before. I have studied a variety of subjects; especially animal physiology and animal nutrition caught my interest. With my Bachelor's thesis, I got the chance to work on the areas of circular economy and nutrient efficiency in animal nutrition. I understood that livestock transforms non-edible biomass into nutritious food and is a valuable part of the circular economy. During my Master's studium and the final thesis, I got in touch with feed evaluation, nutrition physiology and novel protein feeds. In particular, I had a few talks and essays about the production, the nutritional value of duckweed, and their integration into local nutrient cycles.

After my Master's degree, Professor Westendarp, who also supervised my Bachelor's and Master's theses, offered me a position as a Ph.D. student in cooperation with the University of Rostock. The corresponding project has interdisciplinary goals and is focused on the development of standardized production of duckweed as feed for broiler chickens and the nutritional evaluation of duckweed as protein feed for broiler chickens. Figure 1 shows the indoor vertical farm in which the duckweed biomass for subsequent experiments has been produced. This has been presented earlier in Duckweed Forum Issue #33.

From an agricultural perspective, intensive livestock farming characterizes northwestern Germany, which leads to imports of protein feeds such as soybean meal and excess nutrients, mainly in the form of manure. A manure-based duckweed production can reduce nutrient surpluses and soybean imports. Large-scale duckweed production (especially in an indoor vertical farm) is still in its infancy and knowledge about its nutritional properties is limited. This is also due to the high variability in plant composition caused by different cultivation conditions. However, I consider the variable composition of duckweed biomass a strength, as growing conditions can be targeted to a specific use, in my case, feeding broiler chickens. Moreover, certain species can be selected based on their amino acid profile, which is characteristic for them (Appenroth et al. 2017).

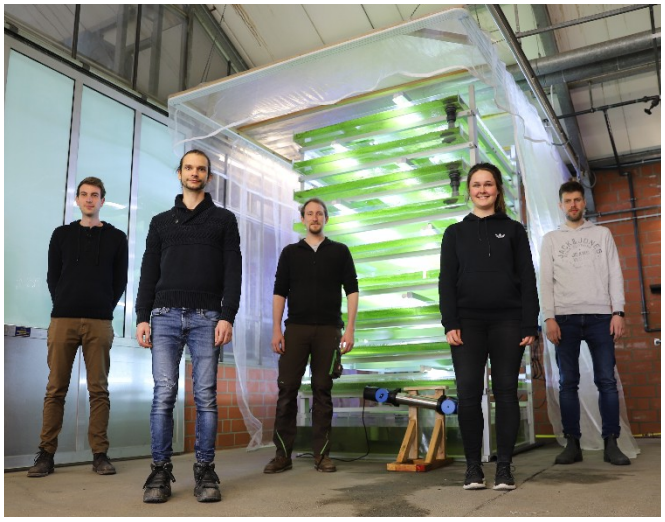

Figure 1: The student team in front of the duckweed indoor vertical farm. From left to right: Finn Petersen, Tim Dargatz, Jannis von Salzen, Dina Restemeyer and me

Analytical parameters in duckweed biomass can provide an indication of nutritional quality. With these parameters, duckweed production can be optimized to yield nutritious duckweed biomass. Key parameters are particularly protein and amino acid content when duckweed is used as a protein source. Moreover, there are limiting substances, such as anti-nutritive factors (ANF) and plant fibers, whose content must be minimized in an optimization process for the intended application. Both ANF and plant fibers reduce protein value as feed, as they can limit the absorption of valuable substances during digestion. The term ANF describes an extensive group of plant compounds that reduce feed digestibility due to their chemical properties or feed intake due to their unfavorable taste. Such ANFs, previously detected in duckweed, are tannins (Negesse et al. 2009).

Besides chemical analysis, animal trials are needed to evaluate the nutritional value of duckweed biomass. The main parameters are digestibility and energy content, which are hardly available for duckweed. Digestibility describes the proportion of nutrients that is absorbed in the digestive system and are also available for metabolism. The main nutrients are amino acids and phosphorus. Energy content is called "metabolizable energy" for broiler chickens and describes the amount of chemical feed energy that is not excreted but available for metabolism. Both parameters are highly relevant for today's feed production because animals have to be sufficiently supplied. The results are an absolute precondition for the practical application of duckweed in the feed of broiler chickens. As cultivation conditions and plant composition strongly influence these parameters, product standardization has to be improved.

In the future, interdisciplinary approaches are needed to produce duckweed biomass for animal nutrition. For example, new findings in the fields of duckweed production or breeding can be applied to yield a very specific product for specific livestock. Thus, duckweed can be a solution to close nutrient cycles, improve countries' self-sufficiency in protein, and use nutrients as well as land more effectively. I consider duckweed a valuable component of sustainable feed and food production.

Figure 2: Part of the student team at the animal research site. From right to left: Lukas Eiligmann and me

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From the Database

Highlights

Characterization of defense responses against bacterial pathogens in duckweeds lacking EDS1

Baggs, EL; Tiersma, MB; Abramson, BW; Michael, TP; Krasileva, KV. New Phytologist (2022) DOI10.1111/nph.18453

ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) mediates the induction of defense responses against pathogens in most angiosperms. However, it has recently been shown that a few species have lost EDS1. It is unknown how defense against disease unfolds and evolves in the absence of EDS1. We utilize duckweeds; a collection of aquatic species that lack EDS1, to investigate this question. We established duckweed-Pseudomonas pathosystems and used growth curves and microscopy to characterize pathogen-induced responses. Through comparative genomics and transcriptomics, we show that the copy number of infection-associated genes and the infection-induced transcriptional responses of duckweeds differ from other model species. Pathogen defense in duckweeds has evolved along different trajectories than in other plants, including genomic and transcriptional reprogramming. Specifically, the miAMP1 domain-containing proteins, which are absent in *Arabidopsis*, showed pathogen responsive upregulation in duckweeds. Despite such divergence between *Arabidopsis* and duckweed species, we found conservation of upregulation of certain genes and the role of hormones in response to disease. Our work highlights the importance of expanding the pool of model species to study defense responses that have evolved in the plant kingdom independent of EDS1.

Engineering Triacylglycerol Accumulation in Duckweed (*Lemna japonica*)

Liang, Y; Yu, X-H; Anaokar, S; Shi, H; Dahl, WB; Cai, Y; Luo, G; Chai, J; Cai, Y; Molla-Morales, A; Altpeter, F; Ernst, E; Schwender, J; Martienssen, RA; Shanklin, J. Plant Biotechnology Journal (2022) DOI10.1111/pbi.13943

Duckweeds are amongst the fastest growing of higher plants, making them attractive high-biomass targets for biofuel feedstock production. Their fronds have high rates of fatty acid synthesis to meet the demand for new membranes, but triacylglycerols (TAG) only accumulate to very low levels. Here we report the engineering of *Lemna japonica* for the synthesis and accumulation of TAG in its fronds. This was achieved by expression of an estradiol-inducible cyan fluorescent protein-Arabidopsis WRINKLED1 fusion protein (CFP-AtWRI1), strong constitutive expression of a mouse diacylglycerol:acyl-CoA acyltransferase2 (MmDGAT), and a sesame oleosin variant (SiOLE(*)). Individual expression of each gene increased TAG accumulation by 1- to 7-fold relative to controls, while expression of pairs of these genes increased TAG by 7- to 45-fold. In uninduced transgenics containing all three genes, TAG accumulation increased by 45-fold to 3.6% of dry weight (DW) without severely impacting growth, and by 108-fold to 8.7% of DW after incubation on medium containing 100 µM estradiol for four days. TAG accumulation was accompanied by an increase in total fatty acids of up to 3-fold to approximately 15% of DW. Lipid droplets from fronds of all transgenic lines were visible by confocal microscopy of BODIPY-stained fronds. At a conservative 12 tonnes (dry matter) per acre and 10% (DW) TAG, duckweed could produce 350 gallons of oil/acre/year, approximately 7-fold the yield of soybean, and similar to that of oil palm. These findings provide the foundation for optimizing TAG accumulation in duckweed and present a new opportunity for producing biofuels and lipidic bioproducts.

Biotechnology

Co-pyrolysis of paper mill sludge and textile dyeing sludge with high calorific value solid waste: Pyrolysis kinetics, products distribution, and pollutants transformation

Feng, SH; Zhang, GX; Yuan, DK; Li, YC; Zhou, YF; Lin, FW. Fuel (2022) 329: 125433

Paper mill sludge (PMS) and printing and textile dyeing sludge (TDS) are two typical industrial sludge with high calcium content, high ash content and low calorific value. To attain effective removal of industrial solid waste, in this study, PMS and TDS are first pyrolyzed independently to study their thermochemical behavior. Results show that 600°C is the optimal pyrolysis temperature for both PMS and TDS from the perspective of organics degradation. However, the yield and quality of pyrolysis oil and gas are still at low level due to the characteristics of high ash content and low organic content, which is not suitable for fuel recovery. Two kinds of organic solid wastes with high calorific value, duckweed (DW) and waste tire (WT), are co-pyrolyzed with PMS and TDS, which can effectively reduce activation energy and increase the yield of liquid and gas products. The most prominent is P1D1 pyrolysis gas, the production of CH_4 and C-2-C-3 increased from 10.80 L/kg and 3.89 L/kg to 20.16 L/kg and 15.58 L/kg respectively, and the high calorific value also increased to 230.93 kJ/kg. The quality of pyrolysis liquid has been improved significantly as the declined N and O-heteroatom content (especially for P1D1 and T1W1) due to the inhibition effect of co-pyrolysis on the N/O migration into pyrolytic liquid. The solid residue from the co-pyrolysis of PMS and DW contains rich N-functional groups and can promote the conversion of pyridinic-N and pyrrolic-N to quaternary-N. Compared with independent pyrolysis, co-pyrolysis can promote the degradation of harmful organic matter in sludge. The addition of DW and WT co-pyrolysis reduced the solid residue yield of PMS by 19.9 wt% and 14.9 wt% (13.4 wt% and 10.5 wt% for TDS). This work provides inspiration for further understanding the safe disposal of PMS and TDS by pyrolysis.

Ecology

Ultraviolet B modulates gamma radiation-induced stress responses in *Lemna minor* at multiple levels of biological organisation

Xie, L; Song, Y; Petersen, K; Solhaug, KA; Lind, OC; Brede, DA; Salbu, B; Tollefsen, KE. Science of the Total Environment (2022) 846: 157457

Elevated levels of ionizing and non-ionizing radiation may co-occur and pose cumulative hazards to biota. However, the combined effects and underlying toxicity mechanisms of different types of radiation in aquatic plants remain poorly understood. The present study aims to demonstrate how different combined toxicity prediction approaches can collectively characterise how chronic (7 days) exposure to ultraviolet B (UVB) radiation (0.5 W m^{-2}) modulates gamma (gamma) radiation (14.9, 19.5, 43.6 mGy h^{-1}) induced stress responses in the macrophyte *Lemna minor*. A suite of bioassays was applied to quantify stress responses at multiple levels of biological organisation. The combined effects (no-enhancement, additivity, synergism, antagonism) were determined by two-way analysis of variance (2 W-ANOVA) and a modified Independent Action (IA) model. The toxicological responses and the potential causality between stressors were further visualised by a network of toxicity pathways. The results showed that gamma-radiation or UVB alone induced oxidative stress and programmed cell death (PCD) as well as impaired oxidative phosphorylation (OXPHOS) and photosystem II (PSII) activity in *L. minor*. Gamma-radiation also activated antioxidant responses, DNA damage repair and chlorophyll metabolism, and inhibited growth at higher dose rates (≥ 20 mGy h^{-1}). When co-exposed, UVB predominantly caused non-interaction (no-enhancement or additive) effects on gamma-radiation-induced antioxidant gene expression, energy quenching in PSII and growth for all dose rates, whereas antagonistic effects were observed for lipid peroxidation, OXPHOS, PCD, oxidative stress, chlorophyll metabolism and genes involved in DNA damage responses. Synergistic effects were observed for changes in photochemical

quenching and non-photochemical quenching, and up-regulation of antioxidant enzyme genes (GST) at one or more dose rates, while synergistic reproductive inhibition occurred at all three gamma-radiation dose rates. The present study provides mechanistic knowledge, quantitative understanding and novel analytical strategies to decipher combined effects across levels of biological organisation, which should facilitate future cumulative hazard assessments of multiple stressors.

Effects of wildfire ash on the growth and biochemical profiles of the aquatic macrophyte *Lemna minor*

Mesquita, AF; Abrantes, N; Campos, I; Nunes, C; Coimbra, MA; Goncalves, FJM; Marques, JC; Goncalves, AMM. Aquatic Toxicology (2022) 250: 106245

Wildfires are a social and environmental concern to the world due to their many adverse effects, including risk to the public health and security, economic damages in prevention and fight, ecosystems pollution, land usage sustainability, and biodiversity. In the Mediterranean region, these events have increased in the last years. Although several studies evaluated the impacts of the wildfires on the structure and function of the ecosystems and their communities, there is a lack of information at the biochemical level beyond the toxicological effects to the organisms. So, aiming to evaluate the potential toxic and biochemical effects of pine and eucalypt ash from high and low severity burned areas in the aquatic environments, *L. minor* growth, fatty acid and carbohydrate profiles were studied. Data showed that the wildfires ash from high severity burned areas are more toxic, with a higher growth inhibition than when exposed to ash from low severity burned areas. Considering the ash from low severity burned areas, eucalypt ash revealed to be more noxious to the macrophyte than pine ash. Furthermore, it was observed a decrease in the diversity and abundance of fatty acids content, comparing with the control. An opposite trend was observed in carbohydrates which increased with the organisms' exposure to almost all ash types, except in case of the organisms exposed to eucalypt ash from high severity burned areas, where carbohydrate content decreased.

Monitoring duckweeds (*Lemna minor*) in small rivers using sentinel-2 satellite imagery: Application of vegetation and water indices to the Lis river (Portugal)

Gerardo, R; de Lima, IP. Water (2022) 14: 2284

Duckweed species, particularly *Lemna minor*, are widely found in freshwaters all over the world. This macrophyte provides multiple ecosystems' functions and services, but its excessive proliferation can have negative environmental impacts (including ecological and socio-economic impacts). This work explores the use of remote sensing tools for mapping the dynamics of *L. minor* in open watercourses, which could contribute to identifying suitable monitoring programs and integrated management practices. The study focuses on a selected section of the Lis River (Portugal), a small river that is often affected by water pollution. The study approach uses spatiotemporal multispectral data from the Sentinel-2 satellite and from 2021 and investigates the potential of remote sensing-based vegetation and water indices (Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI), Normalized Difference Aquatic Vegetation Index (NDAVI), Green Red Vegetation Index (GRVI), Normalized Difference Water Index (NDWI)) for detecting duckweeds' infestation and its severity. The NDAVI was identified as the vegetation index (VI) that better depicted the presence of duckweeds in the surface of the water course; however, results obtained for the other VIs are also encouraging, with NDVI showing a response that is very similar to NDAVI. Results are promising regarding the ability of remote sensing products to provide insight into the behavior of *L. minor* and to identify problematic sections along small watercourses.

Occurrence and accumulation of pharmaceutical products in water and biota of urban lowland rivers

Mastrangelo, MM; Valdes, ME; Eissa, B; Ossana, NA; Barcelo, D; Sabater, S; Rodriguez-Mozaz, S; Giorgi, AND. Science of the Total Environment (2022) 828: 154303

We evaluated the distribution of eleven groups of pharmaceutically active compounds (PhACs) in surface waters and biota of different trophic levels, in five sites of two lowland urban rivers in Argentine. Twenty-nine out of 39 PhACs and two metabolites were detected in at least one water sample (2-9622 ng/L), eleven detected in biofilms (1-179 ng/g d. w) and eight in the macrophyte *Lemna gibba* (4-112 ng/g d.w). The two more polluted sites had a similar distribution of the main groups of compounds. In surface waters, the largest concentrations were for the analgesic acetaminophen (9622 ng/L), the antibiotic sulfamethoxazole (326 ng/L), the antihypertensive valsartan (963 ng/L), the beta-blocking agent atenolol (427 ng/L), the diuretic hydrochlorothiazide (445 ng/L) and the psychiatric drug carbamazepine (99 ng/L). The antibiotic ciprofloxacin exhibited the highest concentration in the biofilm (179 ng/g d.w) and in the macrophyte *L. gibba* (112 ng/g d.w). Several compounds were detected in the water but not in the biota (eg., codeine and bezafibrate), and others (e.g., azithromycin and citalopram) were found in the biota but not in the surface water. Significant bioaccumulation factors (>1000 L/Isg d.w.) were obtained for venlafaxine and ciprofloxacin in biofilm. Our results show that PhACs may accumulate in several biological compartments. Within an environmental compartment, similar PhACs profile and concentrations were found in different sites receiving urban pollution. Among different compartments, biofilms may be the most suitable biota matrix to monitor the immediate reception of PhACs in the biota. Our results indicate that the presence of PhACs in urban rivers and their accumulation in the biota could be incorporated as symptoms of the urban stream syndrome.

Feed & Food

Assessment of selenium and zinc enriched sludge and duckweed as slow-release micronutrient biofertilizers for *Phaseolus vulgaris* growth

Li, Jun; Otero-Gonzalez, L; Lens, PNL; Ferrer, I; Du Laing, G. Journal of Environmental Management (2022) 324: 116397

Selenium (Se) and zinc (Zn) are essential micronutrients that are often lacking in the diet of humans and animals. Application of mineral Se and Zn fertilizers into soils may lead to a waste of Se and Zn due to the fast leaching and low utilization by plants. Slow-release Se and Zn biofertilizer may therefore be beneficial. This study aims to assess the potential of SeZn-enriched duckweed and sludge produced from wastewater as slow-release Se and Zn biofertilizers. Pot experiments with green beans (*Phaseolus vulgaris*) and sampling of Rhizon soil pore water were conducted to evaluate the bioavailability of Se and Zn in sandy and loamy soils mixed with SeZn-enriched duckweed and sludge. Both the Se and Zn concentrations in the soil pore water increased upon amending the two biomaterials. The concentration of Se released from SeZn-enriched duckweed rapidly decreased in the first 21 days and slowly declined afterwards, while it remained stable during the entire experiment upon application of SeZn-enriched sludge. The Zn content in the soil pore water gradually increased over time. The application of SeZn-enriched duckweed and sludge significantly increased the Se concentrations in plant tissues, in particular in the form of organic Se-methionine in seeds, without a negative impact on plant growth when an appropriate dose was applied (1mg Se/kg soil). While, it did not increase Zn concentrations in plant seeds. The results indicate that the SeZn-enriched duckweed and sludge could be only used as organic Se biofertilizers for Se-deficient soils. Particularly, the SeZn-enriched sludge dominated with elemental nano-Se was an effective Se source and slow-release Se biofertilizer. These results could offer a theoretical reference to choose an alternative to chemical Se fertilizers for biofortification, avoiding the problem of Se losses by leaching from mineral Se fertilizers while recovering resources from wastewater. This could contribute to the driver for a future circular economy.

The effect of high-polyphenol Mediterranean diet on visceral adiposity: the DIRECT PLUS randomized controlled trial

Zelicha, H; Kloting, N; Kaplan, A. et al. BMC Medicine (2022) 20: 327

Mediterranean (MED) diet is a rich source of polyphenols, which benefit adiposity by several mechanisms. We explored the effect of the green-MED diet, twice fortified in dietary polyphenols and lower in red/processed meat, on visceral adipose tissue (VAT). In the 18-month Dietary Intervention Randomized Controlled Trial Polyphenols Unprocessed (DIRECT-PLUS) weight-loss trial, 294 participants were randomized to (A) healthy dietary guidelines (HDG), (B) MED, or (C) green-MED diets, all combined with physical activity. Both isocaloric MED groups consumed 28 g/day of walnuts (+ 440 mg/day polyphenols). The green-MED group further consumed green tea (3-4 cups/day) and *Wolffia globosa* (duckweed strain) plant green shake (100 g frozen cubes/day) (+ 800mg/day polyphenols) and reduced red meat intake. We used magnetic resonance imaging (MRI) to quantify the abdominal adipose tissues. Participants (age = 51 years; 88% men; body mass index = 31.2 kg/m^2; 29% VAT) had an 89.8% retention rate and 79.3% completed eligible MRIs. While both MED diets reached similar moderate weight (MED: - 2.7%, green-MED: - 3.9%) and waist circumference (MED: - 4.7%, green-MED: - 5.7%) loss, the green-MED dieters doubled the VAT loss (HDG: - 4.2%, MED: - 6.0%, green-MED: - 14.1%; $p < 0.05$, independent of age, sex, waist circumference, or weight loss). Higher dietary consumption of green tea, walnuts, and *W. globosa*; lower red meat intake; higher total plasma polyphenols (mainly hippuric acid), and elevated urine urolithin A polyphenol were significantly related to greater VAT loss ($p < 0.05$, multivariate models). A green-MED diet, enriched with plant-based polyphenols and lower in red/processed meat, may be a potent intervention to promote visceral adiposity regression.

A novel multitrophic concept for the cultivation of fish and duckweed: A technical note

Stejskal, V; Paolacci, S; Toner, D; Jansen, MAK. Journal of Cleaner Production (2022) 366: 132881

The authors report on the design and operation of an advanced, innovative and environmentally friendly freshwater integrated multitrophic aquaculture (IMTA) system. The novel system facilitates fish production while controlling water quality using duckweed treatment units. The basic design consists of ponds for farming of Eurasian perch and rainbow trout, treatment areas for the culture of duckweed (*Lemna minor* and *Lemna gibba*), as well as connecting channels. An important aspect of the farm is its close association with a renewable energy source. The system is driven by electric energy produced by a wind turbine and uses unused cutaway peatland left after peat harvesting was completed. Water analysis shows that a combination of fish and duckweed farming can maintain a favourable water quality. Moreover, production of both fish and plant biomass are substantial, with fish production of 0.49 ± 0.20 kg fish m^{-2} year-1, water use efficiency of 1.21 ±0.42 kg fish m^{-3} year^{-1} and electricity use of 16.1 ±11.2 kWh kg^{-1} year^{-1}. Additionally, the system produced in excess of 30 tonne (dry weight) protein-rich duckweed biomass per year, which is a suitable feedstock for a variety of farmed animals.

History

Ethnobotanical History: Duckweeds in Different Civilizations

Edelman, M; Appenroth, KJ; Sree, KS; Oyama, T. Plants (2022) 11: 2124

This presentation examines the history of duckweeds in Chinese, Christian, Greek, Hebrew, Hindu, Japanese, Maya, Muslim, and Roman cultures and details the usage of these diminutive freshwater plants from ancient times through the Middle Ages. We find that duckweeds were widely distributed geographically already in antiquity and were integrated in classical cultures in the Americas, Europe, the Near East, and the Far East 2000 years ago. In ancient medicinal sources, duckweeds are encountered in procedures, concoctions, and incantations involving the reduction of high fever. In this regard, we discuss a potential case of ethnobotanical convergence between the Chinese Han and Classical Maya cultures. Duckweeds played a part in several ancient rituals. In one, the unsuitability of its roots to serve as a wick for Sabbath oil lamps. In another reference to its early use as human food during penitence. In a third, a prominent ingredient in a medicinal incantation, and in a fourth, as a crucial element in ritual body purifications. Unexpectedly, it emerged that in

several ancient cultures, the floating duckweed plant featured prominently in the vernacular and religious poetry of the day.

Interaction with other organisms

Long-term effects of duckweed cover on the performance and microbial community of a pilot-scale waste stabilization pond

Zhao, YG; Tu, Q; Yang, YT; Shu, XD; Ma, W; Fang, Y; Li, B; Huang, J; Zhao, H; Duan, CQ. Journal of Cleaner Production (2022) 371: 133531

Duckweed-based ponds (DP), as a modified type of waste stabilization ponds (WSP) are receiving growing attention due to the sustainable production of high-quality duckweed biomass. However, the comprehensive effects of duckweed cover on nutrient removal, greenhouse gas emission and microbial community of the WSP have rarely been studied. In this study, two pilot-scale WSP (12 m^2) with (DP) and without (OP) a duckweed mat on the surface were compared over 1 year to determine the effects. The results showed that, compared to OP, DP had higher TP, but lower TN and NH_4^+-N removal efficiencies, and lower pH, dissolved oxygen (DO) and sedimentation rate of settling detritus. For both nitrogen and phosphorus removal, the dominant pathway was duckweed uptake (66.95% and 93.18%, respectively) in DP; however, it became other pathways (mainly nitrification/denitrification, 82.27%) and sedimentation (87.65%), respectively, in OP. Meanwhile, DP had lower N_2O, but higher CH_4 emission fluxes than OP, resulting in comparable global warming potential between OP and DP (approximately 2800 g $CO_2/m^2/yr$). Microbial community analysis indicated that the greater nitrogen removal and N_2O emission in OP could be attributed to higher abundance of ammonia-oxidizing bacteria in the water and denitrifiers in the sediment, supported by higher DO and settling organic carbon, respectively. Meanwhile, the greater CH_4 emission in DP could be attributed to higher abundance of methanogens and syntrophic methanogenic bacteria in the sediment. Thus, despite favourable effects on nutrient recovery and phosphorus removal mainly attributed to duckweed uptake, duckweed cover had unfavourable effects on nitrogen removal and CH_4 emission reduction of the WSP mainly attributed to microbial taxa which were influenced by environmental conditions (such as DO and settling detritus) in the WSP. These findings help to clarify the influencing mechanism of duckweed cover, providing overall insight into the function and application of the DP.

Influence of cadmium and microplastics on physiological responses, ultrastructure and rhizosphere microbial community of duckweed

Yang, GL; Zheng, MM; Liao, HM; Tan, AJ; Feng, D; Lv, SM. Ecotoxicology and Environmental Safety (2022) 243: 114011

The combined contamination of heavy metals and microplastics is widespread in freshwater environments. However, there are few researches on their combined effects on aquatic plants. In this study, the effects of single and combined stress of 0.01 mg L^{-1} cadmium (Cd), 50 mg L^{-1} polyethylene and 50 mg L^{-1} polypropylene for 15 days on the physiological response, ultrastructure and rhizosphere microbial community of duckweed were investigated. The results showed that Cd and microplastics single or combined stress inhibited the growth of duckweed, shortened the root length and decreased the chlorophyll content. Compared with single Cd treatments, the combination of microplastics and Cd increased duckweed growth rate and increased superoxide dismutase activity and malondialdehyde content and reduced chloroplast structural damage, indicating that the combined stress could reduce the toxicity of heavy metals to duckweed. Through the study of rhizosphere microbial diversity, 1381 Operational Taxonomic Unit (OTUs) were identified and rich microbial communities were detected in the duckweed rhizosphere. Among them, the main microbial communities were Proteobacteria, Bacteroidetes, and Cyanobacteria. Compared with Cd single stress, the ACE and chao index of rhizosphere microbial community increased under combined stress, indicating that the diversity and abundance of microbial communities were improved after combined stress treatment. Our study revealed the

effects of heavy metals and microplastics on aquatic plants, providing a theoretical basis for duckweed applications in complex water pollution.

Harnessing plant-microbiome interactions for bioremediation across a freshwater urbanization gradient

O'Brien, AM; Yu, ZH; Pencer, C; Frederickson, ME; LeFevre, GH; Passeport, E. Water Research (2022) 223: 118926

Urbanization impacts land, air, and water, creating environmental gradients between cities and rural areas. Urban stormwater delivers myriad co-occurring, understudied, and mostly unregulated contaminants to aquatic ecosystems, causing a pollution gradient. Recipient ecosystems host interacting species that can affect each other's' growth and responses to these contaminants. For example, plants and their microbiomes often reciprocally increase growth and contaminant tolerance. Here, we identified ecological variables affecting contaminant fate across an urban-rural gradient using 50 sources of the aquatic plant *Lemna minor* (duckweed) and associated microbes, and two co-occurring winter contaminants of temperate cities, benzotriazole and salt. We conducted experiments totalling >2,500 independent host-microbe-contaminant microcosms. Benzotriazole and salt negatively affected duckweed growth, but not microbial growth, and duckweeds maintained faster growth with their local, rather than disrupted, microbiota. Benzotriazole transformation products of plant, microbial, and phototransformation pathways were linked to duckweed and microbial growth, and were affected by salt co-contamination, microbiome disruption, and source sites of duckweeds and microbes. Duckweeds from urban sites grew faster and enhanced phytotransformation, but supported less total transformation of benzotriazole. Increasing microbial community diversity correlated with greater removal of benzotriazole, but taxonomic groups may explain shifts across transformation pathways: the genus *Aeromonas* was linked to increasing phototransformation. Because benzotriazole toxicity could depend on amount and type of in situ transformation, this variation across duckweeds and microbes could be harnessed for better management of urban stormwater. Broadly, our results demonstrate that plant-microbiome interactions harbour manipulable variation for bioremediation applications.

A metabolomic approach to assess the toxicity of the olive tree endophyte *Bacillus* sp. PTA13 lipopeptides to the aquatic macrophyte *Lemna minor* L.

Papadopoulou, EA; Giaki, K; Angelis, A; Skaltsounis, AL; Aliferis, KA. Toxics (2022) 10: 494

Pesticides represent a major human input into the ecosystem, posing a serious risk to non-target organisms. Therefore, there is pressure toward the reduction in their use and the discovery of alternative sources of bioactivity. Endophytic microorganisms represent a source of bioactivity, whose potential for plant protection has been recently established. In this context, an olive tree endophytic Bacillus sp. was isolated, exhibiting superior antifungal activity, mainly attributed to its major surfactin, iturin, and fengycin and the minor gageotetrin and bacilotetrin groups of lipopeptides (LP). Based on the potential of LP and the lack of information on their toxicity to aquatic organisms, we have investigated the toxicity of an LP extract to the model macrophyte *Lemna minor* L. The extract exhibited low phytotoxicity (EC50 = 419 μg mL^{-1}), and for the investigation of its effect on the plant, GC/EI/MS metabolomics was applied following exposure to sub-lethal doses (EC25 and EC50). Results revealed a general disturbance of plants' biosynthetic capacity in response to LP treatments, with substantial effect on the amino acid pool and the defense mechanism regulated by jasmonate. There are no previous reports on the phytotoxicity of LP to *L. minor*, with evidence supporting their improved toxicological profile and potential in plant protection.

Isolation of aquatic plant growth-promoting bacteria for the floating plant duckweed (*Lemna minor*)

Makino, A; Nakai, R; Yoneda, Y; Toyama, T; Tanaka, Y; Meng, XY; Mori, K; Ike, M; Morikawa, M; Kamagata, Y; Tamaki, H. Microorganisms (2022) 10: 1564

Plant growth-promoting bacteria (PGPB) can exert beneficial growth effects on their host plants. Little is known about the phylogeny and growth-promoting mechanisms of PGPB associated with aquatic plants, although those of terrestrial PGPB have been well-studied. Here, we report four novel aquatic PGPB strains, MRB1-4 (NITE P-01645-P-01648), for duckweed *Lemna minor* from our rhizobacterial collection isolated from *Lythrum anceps*. The number of *L. minor* fronds during 14 days co-culture with the strains MRB1-4 increased by 2.1-3.8-fold, compared with an uninoculated control; the plant biomass and chlorophyll content in co-cultures also increased. Moreover, all strains possessed an indole-3-acetic acid production trait in common with a plant growth-promoting trait of terrestrial PGPB. Phylogenetic analysis showed that three strains, MRB-1, -3, and -4, were affiliated with known proteobacterial genera (*Bradyrhizobium* and *Pelomonas*); this report is the first to describe a plant-growth promoting activity of *Pelomonas* members. The *gammaproteobacterial* strain MRB2 was suggested to be phylogenetically novel at the genus level. Under microscopic observation, the *Pelomonas* strain MRB3 was epiphytic and adhered to both the root surfaces and fronds of duckweed. The duckweed PGPB obtained here could serve as a new model for understanding unforeseen mechanisms behind aquatic plant-microbe interactions.

Antimicrobial and functional properties of duckweed (*Wolffia globosa*) protein and peptide extracts prepared by ultrasound-assisted extraction

Duangjarus, N; Chaiworapuek, W; Rachtanapun, C; Ritthiruangdej, P; Charoensiddhi, S. Foods (2022) 11: 2348

Wolffia globosa is an interesting alternative plant-based protein source containing up to 40% protein dry weight. Dried duckweed protein extract (PE) was obtained using ultrasound-assisted extraction (UAE) before isoelectric precipitation (pH 3.5) to yield protein concentrate (PC) and protein solution (PS). The PC was hydrolyzed using Alcalase enzyme to obtain protein concentrate hydrolysate (PCH). Among all fractions, PCH exhibited antimicrobial properties by decreasing populations of *Vibrio parahaemolyticus* and *Candida albicans* at 0.43 ± 1.31 log reduction (66.21%) and 3.70 ± 0.11 log reduction (99.98%), respectively. The PE and PS also showed high solubilities at pH 8 of 90.49% and 86.84%, respectively. The PE demonstrated the highest emulsifying capacity (EC) (71.29%) at pH 4, while the highest emulsifying stability (ES) (similar to 98%) was obtained from the PE and PS at pH 6 and pH 2, respectively. The major molecular weights (Mw) of the PE, PC, PCH and PS were observed at 25, 45, 63 and 100 kDa, with a decrease in the Mw of the PCH (<5 kDa). The PCH contained the highest total amino acids, with aspartic acid and glutamic acid being the major components. The results revealed the antimicrobial and functional properties of duckweed protein and hydrolysate for the first time and showed their potential for further development as functional food ingredients.

Molecular Biology

Genome-wide analysis of the growth-regulating factor (GRF) family in aquatic plants and their roles in the ABA-induced turion formation of *Spirodela polyrhiza*

Li, GJ; Chen, Y; Zhao, XY; Yang, JJ; Wang, XY; Li, XZ; Hu, SQ; Hou, HW. International Journal of Molecular Sciences (2022) 23: 10485

Growth-regulating factors (GRFs) are plant-specific transcription factors that play essential roles in regulating plant growth and stress response. The GRF gene families have been described in several terrestrial plants, but a comprehensive analysis of these genes in diverse aquatic species has not been reported yet. In this study, we identified 130 GRF genes in 13 aquatic plants, including floating plants (*Azolla filiculoides, Wolffia australiana, Lemna minuta, Spirodela intermedia*, and *Spirodela polyrhiza*), floating-leaved plants (*Nymphaea colorata* and *Luryale ferox*), submersed plants (*Zostera marina, Ceratophyllum demersum, Aldrovanda*

vesiculosa, and *Utricularia gibba*), an emergent plant (*Nelumbo nucifera*), and an amphibious plant (*Cladopus chinensis*). The gene structures, motifs, and cis-acting regulatory elements of these genes were analyzed. Phylogenetic analysis divided these GRFs into five clusters, and ABRE cis-elements were highly enriched in the promoter region of the GRFs in floating plants. We found that abscisic acid (ABA) is efficient at inducing the turion of *Spirodela polyrhiza* (giant duckweed), accompanied by the fluctuated expression of SpGRF genes in their fronds. Our results provide information about the GRF gene family in aquatic species and lay the foundation for future studies on the functions of these genes.

Genome-wide characterization of aquaporins in *Spirodela polyrhiza* and expression of aquaporin gene family under high-temperature stress

Shang, S; Zhang, ZW; Liu, XX; Liu, LX; Tang, XX. Nordic Journal of Botany (2022) 03567

As one of the most conserved classes of gene families, aquaporins (AQP) genes play an essential role in water transport and stress resistance in organisms. However, little information was available for their evolutionary and functional roles in aquatic plant species. In the present study, gene sequences, gene structure, phylogeny, conserved motif and promoter of the AQP genes in *Spirodela polyrhiza* were investigated to explore the mechanisms of osmoregulation underwater. A total of 24 putative AQP genes divided into four clades representing four distinct AQPs subfamilies were identified. The gene structure and intron numbers of the AQP genes were conserved among subfamilies. The Froger's residues, conserved Ar/R selectivity filters and conserved NPA domain showed high differences among different subfamilies. Moreover, the promoter profiling of SpAQP genes for conserved cis-acting regulatory elements revealed enrichment of cis-elements involved in light response and stress responsiveness, reflecting their varying gene expression pattern. Our results demonstrated that the AQP genes in the *S. polyrhiza* were similar to those in the *Zostera marina* and might have experienced adaptive evolution to water. Transcriptome results showed that the SpTIP2-3, SpPIP2-2, SpPIP1-3, SpTIP4-3 and SpNIP5-2 were expressed significantly among different treatments indicating they might play important roles under temperature stress. These data offer valuable sight into understanding the adaptation to the water of the *S. polyrhiza*. They may further inform our understanding of the evolution of water tolerance in *S. polyrhiza*.

Morphology

Automated imaging of duckweed growth and development

Cox, KL; Manchego, J; Meyers, BC; Czymmek, KJ; Harkess, A. Plant Direct (2022) 6: 439

Duckweeds are the smallest angiosperms, possessing a simple body architecture and highest rates of biomass accumulation. They can grow near-exponentially via clonal propagation. Understanding their reproductive biology, growth, and development is essential to unlock their potential for phytoremediation, carbon capture, and nutrition. However, there is a lack of non-laborious and convenient methods for spatially and temporally imaging an array of duckweed plants and growth conditions in the same experiment. We developed an automated microscopy approach to record time-lapse images of duckweed plants growing in 12-well cell culture plates. As a proof-of-concept experiment, we grew duckweed on semi-solid media with and without sucrose and monitored its effect on their growth over 3 days. Using the PlantCV toolkit, we quantified the thallus area of individual plantlets over time, and showed that *L. minor* grown on sucrose had an average growth rate four times higher than without sucrose. This method will serve as a blueprint to perform automated high-throughput growth assays for studying the development patterns of duckweeds from different species, genotypes, and conditions.

Physiology & Stress

Mechanism of calcium signal response to cadmium stress in duckweed

Ren, QT; Xu, ZY; Xue, Y; Yang, R; Ma, X; Sun, JG; Wang, J; Lin, S; Wang, WQ; Yang, L; Sun, ZP. Plant Signaling & Behavior (2022) 17: 2119340

Cadmium (Cd) causes serious damage to plants. Although calcium (Ca) signal has been found to respond to certain stress, the localization of Ca and molecular mechanisms underlying Ca signal in plants during Cd stress are largely unknown. In this study, Ca^{2+}-sensing fluorescent reporter (GCaMP3) transgenic duckweed showed the Ca^{2+} signal response in *Lemna turionifera* 5511 (duckweed) during Cd stress. Subsequently, the subcellular localization of Ca^{2+} has been studied during Cd stress by transmission electron microscopy, showing the accumulation of Ca^{2+} in vacuoles. Also, Ca^{2+} flow during Cd stress has been measured. At the same time, the effects of exogenous glutamic acid (Glu) and γ-aminobutyric (GABA) on duckweed can better clarify the signal operation mechanism of plants to Cd stress. The molecular mechanism of Ca^{2+} signal responded during Cd stress showed that Cd treatment promotes the positive response of Ca signaling channels in plant cells, and thus affects the intracellular Ca content. These novel signal studies provided an important Ca^{2+} signal molecular mechanism during Cd stress.

Acute and multigenerational effects of petroleum- and cellulose-based microfibers on growth and photosynthetic capacity of *Lemna minor*

Cui, RX; Kwak, JI; An, YJ. Marine Pollution Bulletin (2022) 182: 113953

Comparative toxicological assessment studies on the effects of petroleum-and cellulose-based microfibers on aquatic plants are limited. Therefore, we evaluated the acute and 10-generational toxicological effects of two types of petroleum-and cellulose-based microfibers on the duckweed *Lemna minor*. Plant growth and photosynthesis parameters were monitored as bioindicators. The multigenerational test revealed the following ranking of the microfibers according to the severity of their effects on *L. minor*: polypropylene > lyocell > viscose > polyethylene terephthalate. The acute tests revealed a significant increase in the energy required to initiate photosynthesis, although the growth of *L. minor* was not adversely affected by any microfiber. Both petroleum -and cellulose-based microfibers induced adverse effects on the growth and photosynthesis of *L. minor* in multigenerational tests. The results of the generational tests contribute to the understanding of the long-term adverse effects of microfibers on aquatic plants.

Assessment of the effects of chitosan, chitooligosaccharides and their derivatives on *Lemna minor*

Boros, BV; Dascalu, D; Ostafe, V; Isvoran, A. Molecules (2022) 27: 6123

Chitosan, chitooligosaccharides and their derivatives' production and use in many fields may result in their release to the environment, possibly affecting aquatic organisms. Both an experimental and a computational approach were considered for evaluating the effects of these compounds on *Lemna minor*. Based on the determined EC50 values against *L. minor*, only D-glucosamine hydrochloride (EC50 = 11.55 mg/L) was considered as "slightly toxic" for aquatic environments, while all the other investigated compounds, having EC50 > 100 mg/L, were considered as "practically non-toxic". The results obtained in the experimental approach were in good agreement with the predictions obtained using the admetSAR2.0 computational tool, revealing that the investigated compounds were not considered toxic for crustacean, fish and *Tetrahymena pyriformis* aquatic microorganisms. The ADMETLab2.0 computational tool predicted the values of IGC(50) for *T. pyriformis* and the LC50 for fathead minnow and *Daphnia magna*, with the lowest values of these parameters being revealed by totally acetylated chitooligosaccharides in correlation with their lowest solubility. The effects of the chitooligosaccharides and chitosan on *L. minor* decreased with increased molecular weight, increased with the degree of deacetylation and were reliant on acetylation patterns. Furthermore, the solubility mainly influenced the effects on the aqueous environment, with a higher solubility conducted to lower toxicity.

Effects of descendent phenotypic diversity mediated by ancestor environmental variation on population productivity of a clonal plant

Jin, Y; Chen, JS; Luo, FL; Huang, L; Lei, NF; Yu, FH. Diversity (2022) 14: 616

Phenotypic variation of individuals within populations can be influenced by not only genetic diversity and environmental variation experienced by these individuals but also environmental variation experienced by their parents. Although many studies have tested impacts of phenotypic diversity caused by genotypic or species diversity on productivity, no study has assessed the effects of phenotypic diversity induced by parental environmental variation on productivity. To address this novel question, we conducted two experiments with the widespread, fast-growing, clonal, floating plant *Spirodela polyrhiza*. We first grew mother (ancestor) ramets of *S. polyrhiza* under different environmental conditions to obtain descendent ramets with different phenotypes. Then, these ramets were used to construct descendent populations with different levels of phenotypic diversity caused by ancestor environmental variation and examined the effect of phenotypic diversity on population productivity. Environmental variation (changes in nutrient availability, plant density and light intensity) had significant effects on descendent populations of *S. polyrhiza*. However, descendent phenotypic diversity induced by ancestor environmental variation had no significant effect on total biomass or number of ramets of the descendent populations and such an effect did not depend on the nutrient availability that the descendent populations experienced. Although our results failed to support the idea that phenotypic diversity induced by ancestor environment variation can influence descendent population productivity, we propose that this novel idea should be tested with more species in different ecosystems.

Phytoremediation

Mechanisms, toxicity and optimal conditions - research on the removal of benzotriazoles from water using *Wolffia arrhiza*

Polinska, W; Piotrowska-Niczyporuk, A; Karpinska, J; Struk-Sokolowska, J; Kotowska, U. The Science of the Total Environment (2022) 847: 157571

In the presented work, phytoremediation with the use of floating plant *Wolffia arrhiza* (L.) Horkel ex Wimm. was proposed as a method of removing the selected benzotriazoles (BTRs): 1H-benzotriazole (1H-BTR), 4-methyl-1H-benzotriazole (4M-BTR), 5-methyl-1H-benzotriazole (5M-BTR) and 5-chlorobenzotriazole (5Cl-BTR) from water. The efficiency of phytoremediation depends on three factors: daily time of exposure to light, pH of the model solution, and the amount of plants. Using a design of experiment (DoE) methods the following optimal values were selected: plant amount 1.8 g, light exposure 13 h and pH 7 per 100 mL of the model solution. It was found that the loss of BTRs in optimal conditions ranged from 92 to 100 % except for 4M-BTR, for which only 23 % of removal was achieved after 14 days of cultivation of *W. arrhiza*. The half-life values for studied compounds ranged from 0.98 days for 5Cl-BTR to 36.19 for 4M-BTR. The observed rapid vanishing of 5M-BTR is supposed by the simultaneous transformation of 5M-BTR into 4M-BTR. The detailed study of BTRs degradation pointed that the plant uptake is mainly responsible for the benzotriazoles concentration decrease. Toxicity tests showed that the tested organic compounds induce oxidative stress in *W. arrhiza*, which manifested among others, in reduced levels of chlorophyll in cultures with benzotriazoles compared to control.

Lemnaceae clones collected from a small geographic region display diverse traits relevant for the remediation of wastewater

Walsh, E; Cialis, E; Dillane, E; Jansen, MAK. Environmental Technology and Innovation (2022) 28: 102599

The Lemnaceae (duckweed) are a family of aquatic plants which can be used as part of a circular economy approach to remediate and valorise wastewater. The suitability of duckweeds relates to their tolerance of wastewater conditions, fast growth rates and valuable biomass. Duckweed species and clones display a wide range of remediation abilities and nutritional composition. In order to find duckweed species and clones best

suited for remediation and valorisation of dairy processing wastewater, plant samples were collected from a number of locations around southern Ireland. These species and clones were cultured on a synthetic dairy processing wastewater while growth, TN/TP removal and protein content were assessed. Synthetic dairy wastewater was found to be suitable for remediation by different species and clones. However, significant differences were found in relative growth rate (RGR), TN removal rate and protein content. As per PCA, it was found that species and clones with higher RGR tended to have a lower protein content. Furthermore, nutrient removal rate was not strongly associated with growth and protein content. The results demonstrate that remediation is not positively correlated with valorisation across duckweed species and clones. Thus, clonal selection for duckweed reactors will need to consider the prime objective of remediation i.e. biomass production or wastewater remediation.

Flow rate and water depth alters biomass production and phytoremediation capacity of *Lemna minor*

Coughlan, NE; Walsh, E; Ahern, R; Burnell, G; O'Mahoney, R; Kuehnhold, H; Jansen, MAK. Plants (2022) 11: 2170

DOI10.3390/plants11162170

Given its high biomass production, phytoremediation capacity and suitability as a feedstock for animal and human nutrition, duckweeds are valuable multipurpose plants that can underpin circular economy applications. In recent years, the use of duckweeds to mitigate environmental pollution and valorise wastewaters through the removal of excess nitrogen and phosphate from wastewaters has gained considerable scientific attention. However, quantitative data on optimisation of duckweed performance in phytoremediation systems remain scant. In particular, a mechanistical understanding of how physical flows affect duckweed growth and remediation capacity within vertical indoor multi-tiered bioreactors is unknown. Here, effects of flow rate (0.5, 1.5 or 3.0 L min^{-1}) and medium depth (25 mm or 50 mm) on *Lemna minor* biomass production and phytoremediation capacity were investigated. Results show that flow rates and water depths significantly affect both parameters. *L. minor* grew best at 1.5 L min^{-1} maintained at 50 mm, corresponding to a flow velocity of 0.0012 m s^{-1}. The data are interpreted to mean that flow velocities should be low enough not to physically disturb duckweed but still allow for adequate nutrient mixing. The data presented will considerably advance the optimisation of large-scale indoor (multi-tiered, stacked), as well as outdoor (pond, lagoon, canal), duckweed-based remediation of high nutrient wastewaters.

Performance assessment of local aquatic macrophytes for domestic wastewater treatment in Nigerian communities: A review

Justin, LD; Olukanni, DO; Babaremu, KO. Heliyon (2022) 8: 10093

The concept of treating wastewater before disposal is a global necessity. Recent mechanisms of doing this include the use of Constructed Wetland Systems (CWS). This technique is believed to be cost-effective and simpler compared to conventional methods. The application of this system is primarily dependent on the use of plants through the phytoremediation process. There is evidence of the potential of some locally found Nigerian aquatic plants such as water lettuce, water hyacinth and duckweed to be applicable for this purpose. However, there is little information on their performance level in remediating domestic wastewater. Thus, this review paper assessed the performance of these local macrophytes for domestic wastewater treatment and the potential of contributing the same in Nigerian communities. This was done by reviewing recent literature on the role of water lettuce, water hyacinth and duckweed, their occurrence and their efficiency in minimising different wastewater contaminants. Contaminant indicators such as total solids, electrical conductivity (EC), BOD, COD, dissolved oxygen, total phosphorous, total nitrogen, and heavy metals have been reduced using these macrophytes. The review indicates that the selected macrophytes do not only have the potential for wastewater purification but high efficiencies in doing so when applied appropriately in the Nigerian communities.

Lemna trisulca L.: A novel phytoremediator for the removal of zinc oxide nanoparticles (ZnO NP) from aqueous media

Ergonul, MB; Nassouhi, D; Celik, M; Dilbaz, D; Sazli, D; Atasagun, S. Environmental Science and Pollution Research (2022) DOI10.1007/s11356-022-22112-x

Several aquatic plant species have been proposed for phytoremediation of waters polluted with heavy metals and pesticides. According to the limited information available, aquatic macrophytes also have a promising potential to remove NPs from aqueous media. Although there is considerable information on the remediation potential of *Lemna* spp., the capacity of *Lemna trisulca* seems to be neglected, particularly for nanoparticle removal. Therefore, in the current study, we aimed to investigate the moval efficiency of *L. trisulca* exposed to 3 different ZnO NP concentrations (2.5, 5, and 10 ppm) for 1, 4, and 7 days in Hoagland solutions and the removal percentage were measured on each duration and compared among groups. The accumulated zinc levels were measured in whole plant material and bioconcentration factors were calculated for each group. In addition, the effect of ZnO NPs on the photosynthetic activity of the plant was evaluated via analyzing the photosynthetic pigment (chlorophyll a and b) concentration. The removal percentage ranged between 9.3 and 72.9% and showed a gradual increase in all experimental groups based both on dose and test duration. The statistical comparisons of the removal percentage among the groups with or without the plant indicate *that L. trisulca* had a significant effect on removal rates particularly between 1st and 4th days of exposure, however, did not show any progress at 7th days. The only significant difference for chl-a and chl-b levels was observed in 10 ppm ZnO NP-exposed plants at 7th days.

Phytotoxicity

Differential phytotoxic effect of silver nitrate ($AgNO_3$) and bifunctionalized silver nanoparticles (AgNPs-Cit-L-Cys) on *Lemna* plants (duckweeds)

Iannelli, MA; Bellini, A; Venditti, I; Casentini, B; Battocchio, C; Scalici, M; Ceschin, S. Aquatic Toxicology (2022) 250: 106260

Duckweeds are aquatic plants often used in phytotoxic studies for their small size, simple structure, rapid growth, high sensitivity to pollutants and facility of maintaining under laboratory conditions. In this paper, induced phytotoxic effects were investigated in *Lemna minor* and *Lemna minuta* after exposition to silver nitrate ($AgNO_3$) and silver nanoparticles stabilized with sodium citrate and L-Cysteine (AgNPs-Cit-L-Cys) at different concen-trations (0, 20 and 50 mg/L) and times (7 and 14 days). *Lemna* species responses were evaluated analyzing plant growth (mat thickness, fresh and dry biomass, relative growth rate - RGR) and physiological parameters (chlorophyll - Chl, malondialdehyde - MDA, ascorbate peroxidase - APX and catalase - CAT). Ag content was measured in the fronds of the two *Lemna* species by inductively coupled plasma optical emission spectrometry. $AgNO_3$ and AgNPs-Cit-L-CYs produced phytotoxic effects on both duckweed species (plant growth and Chl reduction, MDA increase) that enhanced in response to increasing concentrations and exposure times. AgNPs-Cit-L-Cys caused much less alteration in the plants compared to $AgNO_3$ suggesting that the presence of bifunc-tionalized AgNPs-Cit-L-Cys have a reduced phytotoxic effect as compared to Ag^+ released in water. Based on the physiological performance, *L. minuta* plants showed a large growth reduction and higher levels of chlorosis and stress in respect to *L. minor* plants, probably due to greater Ag^+ ions accumulation in the fronds. Albeit with some differences, both *Lemna* species were able to uptake Ag^+ ions from the aqueous medium, especially over a period of 14 days, and could be considered adapt as phytoremediation agents for decontaminating silver ion-polluted water.

Duckweed: a potential phytosensor for heavy metals

Sharma, R; Lenaghan, SC. Plant Cell Reports (2022) DOI10.1007/s00299-022-02913-7

Globally, heavy metal (HM) contamination is one of the primary causes of environmental pollution leading to decreased quality of life for those affected. In particular, HM contamination in groundwater poses a serious risk to human health and the potential for destabilization of aquatic ecosystems. At present, strategies to remove HM contamination from wastewater are inefficient, costly, laborious, and often the removal poses as much risk to the environment as the initial contamination. Phytoremediation, plant-based removal of contaminants from soil or water, has long been viewed as an economical and sustainable solution to remove toxic metals from the environment. However, to date, phytoremediation has demonstrated limited successes despite a large volume of literature supporting its potential. A key aspect for achieving robust and meaningful phytoremediation is the selection of a plant species that is well suited to the task. For the removal of pollutants from wastewater, hydrophytes, like duckweed, exhibit significant potential due to their rapid growth on nutrient-rich water, ease of collection, and ability to survive in various ecosystems. As a model for ecotoxicity studies, duckweed is an ideal candidate, as it is easy to cultivate under controlled and even sterile conditions, and the rapid growth enables multi-generational studies. Similarly, recent advances in the genetic engineering and genome-editing of duckweed will enable the transition from fundamental ecotoxicity studies to engineered solutions for phytoremediation of HMs. This review will provide insight into the suitability of duckweeds for phytoremediation of HMs and strategies for engineering next-generation duckweed to provide real-world environmental solutions.

The first plastic produced, but the latest studied in microplastics research: The assessment of leaching, ecotoxicity and bioadhesion of Bakelite microplastics

Klun, B; Rozman, U; Ogrizek, M; Kalcikova, G. Environmental Pollution (2022) 307: 119454

Bakelite, the first synthetic plastic, is a rather unexplored material in the field of ecotoxicology, despite its long production and use. The aim of this study was to investigate the ecotoxicity of Bakelite microplastics (before and after leaching) and its leachates on four aquatic organisms: the crustacean *Daphnia magna*, the plant *Lemna minor*, the bacterium *Allivibrio fischeri* and the alga *Pseudokirchneriella subcapitata*. Bakelite microplastics before and after leaching and leachates affected all organisms, but to varying degrees. Leachates showed increased ecotoxicity to *Daphnia magna*, while *Pseudokirchneriella subcapitata* was more affected by particles. For *Lemna minor* and *Allivibrio fischeri*, the effects of particles before leaching and leachate were comparable, while the negative effect of particles after leaching was minimal or not present. All leachates were analysed, and phenol and phenol like compounds were the predominant organics found. In addition, bioadhesion of Bakelite microplastics to the surface of *Daphnia magna* and *Lemna minor* was confirmed, but the particles were mainly weakly adhered. Results of this study suggest that, in addition to the recently studied microplastics from consumer products (e.g. from polyethylene and polystyrene), microplastics from industrial plastics such as Bakelite may be of increasing concern, primarily due to leaching of toxic chemicals.

Short-term test for toxicogenomic analysis of ecotoxic modes of action in *Lemna minor*

Loll, A; Reinwald, H; Ayobahan, SU; Goeckener, B; Salinas, G; Schaefers, C; Schlich, K; Hamscher, G; Eilebrecht, S. Environmental Science & Technology (2022) DOI10.1021/acs.est.2c01777

In the environmental risk assessment of substances, toxicity to aquatic plants is evaluated using, among other methods, the 7-day *Lemna* sp. growth inhibition test following the OECD TG 221. So far, the test is not applicable for short-term screening of toxicity, nor does it allow evaluation of toxic modes of action (MoA). The latter is also complicated by the lack of knowledge of gene functions in the test species. Using ecotoxicogenomics, we developed a time-shortened 3-day assay in *Lemna minor* which allows discrimination of ecotoxic MoA. By examining the changes in gene expression induced by low effect concentrations of the

pharmaceutical atorvastatin and the herbicide bentazon at the transcriptome and proteome levels, we were able to identify candidate biomarkers for the respective MoA. We developed a homology-based functional annotation pipeline for the reference genome of *L. minor*, which allowed overrepresentation analysis of the gene ontologies affected by both test compounds. Genes affected by atorvastatin mainly influenced lipid synthesis and metabolism, whereas the bentazon-responsive genes were mainly involved in light response. Our approach is therefore less time-consuming but sensitive and allows assessment of MoA in *L. minor*. Using this shortened assay, investigation of expression changes of the identified candidate biomarkers may allow the development of MoA-specific screening approaches in the future.

New insight into the effect of riluzole on cadmium tolerance and accumulation in duckweed (*Lemna turionifera*)

Yang, L; Ren, QT; Ma, X; Wang, MW; Sun, JE; Wang, S; Wu, XY; Chen, X; Wang, CX; Li, QQ; Sun, JS. Ecotoxicology and Environmental Safety (2022) 241: 113783

Cadmium (Cd) damages plant photosynthesis, affects roots and leaves growth, and triggers molecular responses. Riluzole (RIL), which protected neuronal damage via inhibiting excess Glu release in animals, has been found to improve Cd tolerance in duckweed in this study. Firstly, RIL treatment alleviated leaf chlorosis by protecting chlorophyll and decreased root abscission under Cd stress. Secondly, RIL declines Cd accumulation by alleviating excess Glu release during Cd shock. RIL mitigate Glu outburst in duckweed during Cd stress by a decline in Glu in roots. The Cd^{2+} influx was repressed by RIL addition with Cd shock. Finally, differentially expressed genes (DEGs) of duckweed under Cd stress with RIL have been investigated. 2141 genes were substantially up -regulated and 3282 genes were substantially down-regulated with RIL addition. RIL down-regulates the genes related to the Glu synthesis, and genes related to DNA repair have been up-regulated with RIL treatment under Cd stress. These results provide new insights into the possibility of RIL to reduce Cd accumulation and increase Cd tolerance in duckweed, and lay the foundation for decreasing Cd accumulation in crops.

Effect of 17 beta-Estradiol on growth and biosynthesis of microalgae *Scenedesmus quadricauda* (CPCC-158) and duckweed *Lemna minor* (CPCC-490) grown in three different media

Kozlova, TA; Levin, DB. Plants (2022) 11: 1669

As fish farm wastewaters have detectable levels of fish hormones, such as 17 beta-estradiol (E2), an understanding of the influence of fish steroids on algal (*Scenedesmus quadricauda*) and duckweed (*Lemna minor*) physiology is relevant to the potential use of fishery wastewaters for microalgae and plant biomass production. The study was conducted using three types of media: Bold Basal Medium (BBM), natural fishery wastewater (FWW), and reconstituted fishery wastewater (RFWW) with the nutrient composition adjusted to mimic FWW. During the experiment, the media were aerated and changes in the pH and conductivity of the water were closely monitored. E2 promoted the growth of *S. quadricauda* and *L. minor*, with significant accumulation of high-value biomolecules at very low steroid concentrations. However, clear differences in growth performance were observed in both test cultures, *S. quadricauda* and *L. minor*, grown in different media, and the most effective hormone concentrations were evidently different for the algae and the plant.

Instructions to Contributors for the Duckweed Forum

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

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

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

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

Title:

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

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It is extremely important that figures be prepared with the proper resolution for publication in order to avoid inaccurate presentation of the data. The minimum acceptable resolution for all figures is 300 dpi. Excessive file compression can distort images, so files should be carefully checked after compression. Note that figures that contain both line art (such as graphs) and RGB/grayscale areas (such as photographs) are best prepared as EPS (vector) files with embedded TIFF images for the RGB/grayscale portions. The resolution of those embedded TIFF images should be at least 300 dpi. Original images should be submitted as a separate file to the text file. It would be helpful to insert the intended into the Word file as well, if desired, to indicate the location for it. The legend to the image/figure should be added at the end of the text file and labeled as "Legend to Figures".

Links for Further Reading

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

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

http://thecharmsofduckweed.org Comprehensive site on all things duckweed-related, By Dr. John Cross, maintained by Paul Fourounjian.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

Community Resources - Updated Table for Duckweed Collections in the Community

For information related to the location, collection size and contact email for duckweed collections in our community, please access the website of the RDSC (Rutgers Duckweed Stock Cooperative) under the heading "List of Worldwide Duckweed Collections". This Table will be updated as new entries for duckweed collections are being supplied to members of the International Steering Committee for Duckweed Research and Applications (ISCDRA). We also plan to publish the updated table in the first issue of each Duckweed Forum newsletter volume starting in 2021.

Note to the Reader

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Please let us know via email to the Chair of ISCDRA, Prof. Eric Lam: ericL89@hotmail.com