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DUCKWEED FORUM

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A small freshwater turtle emerging from an aquarium covered with duckweed

Cover page

A small freshwater turtle emerging from an aquarium covered with duckweed. From the Lam laboratory in New Jersey, USA. Photo by Eric Lam.

In this issue

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

Letter from the Editor:

January 29th, 2023

Dear *Duckweed Forum* readers,

Welcome to another issue of the community newsletter that serves up all things related to duckweed. As many parts of the world have begun to emerge from the restrictions imposed over the past 3 years of the COVID pandemic, I hope things are improving for everyone in our community.

In this issue, there are articles exploring the old and the new uses for duckweed in the society at-large. The description of ethnobotanical research by Fan et al. places the knowledge of duckweed in China back more than 2,000 years ago. As time passes, duckweeds had been used by Chinese farmers as a natural indicator to guide agricultural practices, while its uses in China extended from herbal remedies to art and culture that continues to this day. A companion article by our steering committee members Sowjanya and Klaus together with Marvin Edelman gave a fine historical account of the German polymath Hildegard von Bingen, who was a remarkably independent woman in the 12th Century. As a nun in the Benedictine cloisters, Hildegard pursued knowledge in science, music, philosophy, among other disciplines. She gained wide recognition in her days as a learned and wise person that was often sought out by others, especially other nonconformist women of her time. One of her specialities is in the herbal medicinal realm that was recorded in her book *Causa et Curae* on plants and medicine, which included descriptions of duckweeds as well as their use for medicinal purposes. These old recipes are still used to this date as seen by a search on the Internet. Remarkable! Together with the previously noted medicinal applications of duckweeds in ancient Chinese cultures and the Mayans in the Americas, this convergent discovery of duckweeds' efficacy for treating a wide variety of ailments is striking indeed. Armed with the power of modern-day analytical chemistry and more systematic studies, perhaps more wonders of duckweed as a novel source of beneficial compounds for human health will yet be discovered soon.

The lab of Marcel Jansen, who is also a member of our steering committee, addresses the more modern application of duckweeds in creating a sustainable Circular Economy. One approach is the coupling of duckweed farming to aquaculture of rainbow trout and perch, while another one integrates duckweed on a vertically integrated hydroponic system with LED lighting to grow on effluent from an anaerobic digester that processed waste-streams from a dairy industry. Both applications can have important impact to make our world and culture more waste-conscious, and duckweeds can play a key role in this equation to recover and valorize nutrients from organic waste-streams while minimizing the eutrophication of our lakes and streams. I look forward to seeing the realization of this potential of duckweed at large scales in the not-too-distant future.

With these positive thoughts, and on behalf of all the members in the International Steering Committee for Duckweed Research and Applications, I would like to wish everyone a Happy New Year of 2023. May we have peace on earth as well as more exciting discoveries and successes with duckweed to benefit our society everywhere.

Sincerely,

Eric Lam
Chair, ISCDRA

Duckweed research and applications on the Emerald Isle

Marcel AK Jansen, Simona Paolacci, Vlastimil Stejskal, Éamonn Walsh, Holger Kühnhold, Neil E Coughlan

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The world uses far more raw materials than can be sustainably replenished (Rees and Wackernagel, 2013), a concept elegantly expressed in "Earth overshoot day" (https://www.footprintnetwork.org/our-work/earth-overshoot-day/). As a consequence, planetary environmental boundaries are being crossed, endangering conditions upon which our society depends (Velenturf *et al.*, 1992). Furthermore, the bulk of these raw materials will, after use, be disposed of as waste, for example, within landfill sites. This exploitative (linear) use of raw resources cannot continue indefinitely as resources will be exhausted. This is where the concept of the "Circular Economy" comes in. The central tenet in the circular economy is to decouple the use of raw materials from consumption, while maintaining economic wealth and human well-being. The Ellen MacArthur Foundation defines the circular economy as: *"Looking beyond the current 'take, make and dispose' extractive industrial model, the circular economy is restorative and regenerative by design. Relying on system-wide innovation, it aims to redefine products and services to design waste out, while minimising negative impacts"* (https://www.ellenmacarthurfoundation.org/circular-economy). In practical terms this includes extending the life of products for as long as possible, through better design (value retention) and with built in options for repair and re-purposing (resource retention).

Notwithstanding attempts to minimise the loss of resources in a circular economy, waste is generated either during the production process and/or when products reach the end of their useful life. Here, another aspect of the circular economy kicks in, with waste being treated as a new resource that can be mined for value. Again, this may sound overly idealistic, but this has been done within the agricultural industry for generations; waste slurry produced by cattle and other animals is a resource, aka fertiliser. As such, waste has new value as a fertiliser, we refer to this as waste valorisation. The economic and environmental benefits of this simple, "old-fashioned" re-use of slurry should not be underestimated, as alternatives come at a considerable cost. For example, the production of synthetic ammonia fertiliser through the Haber-Bosch process is highly dependent on fossil fuel, and is responsible for 1.2% of global, anthropogenic CO_2 emissions (Smith *et al.*, 2020). Other fertiliser ingredients are obtained through unsustainable mining activities. In particular, phosphorus fertiliser is sourced from mineable rock phosphate deposits; deposits which are finite (Stamm *et al.*, 2022). Waste valorisation decreases demand for raw resources, but also minimises the (destructive) disposal of waste and therefore demand on land-fill sites, incinerators, and ultimately, a variety of negative impacts on the environment. Indeed, much of the world faces the peculiar dilemma of aquatic environments tending to become eutrophic due to poor management of plant nutrients, such as nitrogen and phosphorus, while simultaneously the cost of synthetic fertiliser is at record highs (which consequent impacts on food prices and affordability) (https://www.fao.org/3/cb9427en/cb9427en_special_feature_2.pdf). Therefore, any circular economy approach that can capture and valorise plant nutrients from agri-food industry waste streams will have both socio-economic and environmental benefits.

At University College Cork (UCC) in the south of Ireland, the group of Prof Marcel Jansen has been engaged in duckweed research since 2003. Initially, the focus was on the use of duckweed for toxicological testing (See Lahive *et al.*, 2015; Jagodzinski *et al.*, 2018; Mateos-Cárdenas *et al.*, 2019), but in the last decade the focus has moved to the use of Lemnaceae for wastewater valorisation. Two distinct approaches are being developed; 1) a low cost, outdoor approach focused on the integration of duckweed ponds and channels in aquaculture farms and 2) a high input, high-tech approach focused on the development of stacked duckweed bioreactors to valorise agri-food industrial waste under indoor conditions, irrespective of the seasons.

An Integrated, MultiTrophic Aquaculture farm at Mt Lucas, county Offaly, Ireland

In 2019, Bord Iascaigh Mhara (BIM), Ireland's Seafood Development Agency, developed an Integrated, MultiTrophic Aquaculture (IMTA) farm at Mt Lucas, under the leadership of Damien Toner. The main aim of the farm was to showcase a new type of sustainable, closed-loop, aquaculture, that integrates a duckweed-based water remediation system within a freshwater fish farm. The principle is that wastewater from fish ponds is utilised as input (i.e. fertilizer) for a primary producer species, aka duckweed. Water remediated in the duckweed system is then returned to the fish ponds. The concept comprises a circular economy whereby there is a balance between the nutrient extractive capability of the primary producer, and the waste production of the fish species. Furthermore, the duckweed biomass generated has economic value as a feed, fertiliser or protein source. The advantage of this system relates to environmental sustainability and economic stability (especially where the primary producer biomass presents economic value). Compared to traditional fish farms (freshwater taken upstream from a local river, and fouled water released downstream), the closed-loop design of the IMTA results in a dramatic reduction of water use, and minimised environmental impacts (Stejskal *et al.*, 2022).

Figure 1: Newly constructed duckweed channels at Mt Lucas fish farm, ready for inoculation with duckweed.

The Mt Lucas IMTA system was designed and constructed on cutaway peatland, i.e. land from which several meters of peat had previously been harvested. The remaining sub-soil is poorly drained, and therefore no pond-liners were required for the construction of a network of ponds and duckweed channels (Figure 1). The network consists of four ponds, each with two fish holding compartments and connected to a series of 16 duckweed channels (100 m long and 8 m wide). The total water surface of the duckweed channels is 10,910 m^2 (Stejskal *et al.*, 2022). Construction was completed in 2019. At that stage the duckweed group at UCC took over responsibility for monitoring and managing the duckweed-part of the IMTA farm.

This was a very exciting project for the UCC-group, as there was no experience in large-scale use of Lemnaceae for water remediation in Ireland. The general theory is that "*Lemnaceae species are tolerant of poor water quality, including highly eutrophic water*", but in practice our experience with a range of different wastewaters is that growth of duckweed on a particular wastewater cannot be automatically assumed. Fortunately, initial (*ex situ*) work by Dr Simona Paolacci showed that the effluent from fish ponds stocked with 30 tonnes rainbow trout (*Oncorhynchus mykiss*) and perch (*Perca fluviatilis*) is a suitable, although not optimal, growth medium for *Lemna minor* (Paolacci *et al.*, 2021). At this stage, populating the channels with duckweed started in earnest. The Irish strain *Lemna minor* "Blarney" (Strain 5500 in the Rutgers Duckweed Stock Cooperative database, collected at a mill-pond 5 miles east of Blarney, Cork county, Ireland) was extensively introduced, followed by a strain of *Lemna gibba* from north-western Ireland. Interestingly, over the past three years, we have noted an incremental increase in the proportion of *Lemna gibba* in the system, with *Lemna minor* "Blarney" becoming less prevalent. Clearly, this strain of *Lemna gibba* is more adapted to local conditions at the farm.

An obstacle to the establishment of a continuous duckweed mat was exposure to wind. As noted by Landolt (Landolt and Kandeler, 1987), wind can have a detrimental impact on Lemnaceae, and this is particularly the case on a wind-prone open system, located on a wind-prone island in the Atlantic. Simona, together with

Dr Vlastimil Stejskal developed and constructed a system of floaters and barriers to protect the duckweed mat, but in the long term, more permanent solutions will need to be implemented, perhaps reorientating the channels away from the most common wind direction. Nevertheless, from the summer of 2021 full duckweed cover of the channels was achieved, and it was estimated that the annual biomass production was around 30 tonnes dry matter (Figure 2). The good growth allowed us to assess the capacity for water remediation. Here, emphasis was very much on ammonia, as this is prevalent in fish effluents, while also being toxic to fish. So, adequate remediation was key for the success of the IMTA farm. To assess ammonia remediation, three independent approaches were taken. Firstly, the water quality was quantified pre- and post-duckweed channels, and this revealed that the duckweed mat in the 16 channels removed 0.78 tonnes Total Nitrogen (TN) per year. Secondly, based on the nitrogen percentage in harvested duckweed, it was assessed that 1.71 tonnes of TN is removed per year. Finally, based on laboratory measured nitrogen uptake rates it was estimated that 0.88 tonnes of TN can potentially be taken up each year (Paolacci *et al.*, 2022). These numbers are in reasonable agreement and show the efficiency of the system. However, more important than uptake of nitrogen by duckweed is the balance between the fish and extractive species contained within the IMTA system. Therefore, Vlastimil undertook a quantitative assessment of nitrogen excretion by fish. Based on the daily amount of fish feed applied, it was estimated that rainbow trout is able to retain just 35% of ingested nitrogen, giving excretion rates of 0.74 tonnes of nitrogen per year in the case of a stock of 30 tonnes of rainbow trout (the licence of the farm is for up to 32 tonnes of fish). Thus, the extractive capacity of the primary producer, and the waste production of the fish are in fair agreement. This is backed up by direct, bi-weekly, measurements of ammonia concentrations which were around 0.5 mg/L for much of the year, a concentration that is compatible with fish-culturing.

Figure 2. Summer of 2021, channels at Mt Lucas are fully covered by duckweed, ready for harvest.

The BIM-led IMTA farm at Mt Lucas has been a success. It has been demonstrated that duckweed can be cultured at a relatively large scale (1 hectare) in Ireland. The priority is now to develop a market for duckweed biomass in Ireland (Figure 3). Thus, a strong engagement with commercial companies interested in biomass for protein, biomass for feed, biomass for biofuel, biomass for soil improvement and biomass for bioactives is ongoing. The work at the Mt Lucas IMTA has already triggered further national and European funded research on the use of duckweed as a feed component in collaboration with a commercial feed company. It has also increased both academic and industrial interest in duckweed as a source of extractable protein. However, perhaps most importantly, the IMTA farm has drawn the attention of the general public and policymakers, as well as the wider agri-food industry to the opportunities of using duckweed as part of a green, sustainable, and circular agri-food industry in Ireland.

Figure 3. Summer of 2021, duckweed harvested at Mt Lucas for further trials. Harvests were partly automated (duckweed guzzler) and partly manual. This is an area where further technological development is required.

Valorisation of industrial agri-feed wastewaters

Unfortunately, the physicochemical composition of many waste streams is more complex than that of effluent from commercial fish ponds, and this complicates waste valorisation when using Lemnaceae. Nevertheless, there are substantial advantages in developing valorisation approaches for some of these complex waste streams, given the sheer volumes produced. One example relates to dairy processing waste. The dairy industry is a significant contributor to the global economy, with world milk production estimated to be 852 million tonnes in 2019. Between 0.2 to 11 litres of wastewater is produced per litre of milk processed, depending on the factory as well as the product made (i.e. cheese, yoghurt, milk powder). This is a massive wastewater volume that creates a substantial financial and technological challenge for the dairy processing industry, particularly as the wastewater is comprised of a complex mixture of organic compounds such as sugars (lactose), fat, and protein, as well as inorganics like ammonia, nitrate, phosphate, calcium, magnesium, potassium, iron, sodium and chloride. Dairy factories process this waste stream using wastewater treatment plants (WWTP) with the prime objective to clean the wastewater sufficiently to facilitate release on surface waters, in accordance with local emission limits. As part of wastewater treatment, nitrogen is typically lost to the atmosphere following nitrification-denitrification cycles, while phosphorus is commonly precipitated into a non-bioavailable precipitate through the addition of salts such as aluminum. Thus, two key ingredients of fertilisers, nitrogen and phosporus, are wasted, rather than being captured for re-use as part of a circular economy approach.

Dr Éamonn Walsh in the duckweed group at UCC explored the use of dairy processing waste as a growth medium for duckweed (Figure 4). Initial results were "dramatic"; strong microbial growth (lactose is a major component of dairy wastewater) resulted in the development of a thick bacterial layer, in which duckweed plants perished. Clearly, duckweed cultures cannot be used as a stand-alone system for remediation and valorisation of dairy processing waste. In response, a multi-component cascading system for valorisation of dairy wastewater was developed as part of the EPA-funded Newtrients-project. This system integrates microbial technologies of anaerobic digestion and aerobic dynamic feeding (ADF) with duckweed cultivation. Each step in the cascading system generates value-added output but also contributes to wastewater remediation. A UCC team led by Drs Niall O'Leary and David Wall developed bespoke anaerobic digestion reactors in which bacteria convert organic carbon into volatile fatty acids (VFAs) which are important chemical building blocks for a broad variety of applications. The ADF reactor within the cascade serves the circular economy through the production of biodegradable polymers derived from VFAs. The production of these polymers comprises a sustainable route for production of non-petrochemical bioplastics with lower environmental impact. The effluent of the microbial bioreactors is very low in organic compounds, but still contains much of its plant nutrient content. Éamonn was able to effectively grow duckweed on this wastewater, achieving good growth rates and nitrogen and phosphorus uptake activities (Walsh *et al.*, 2021).

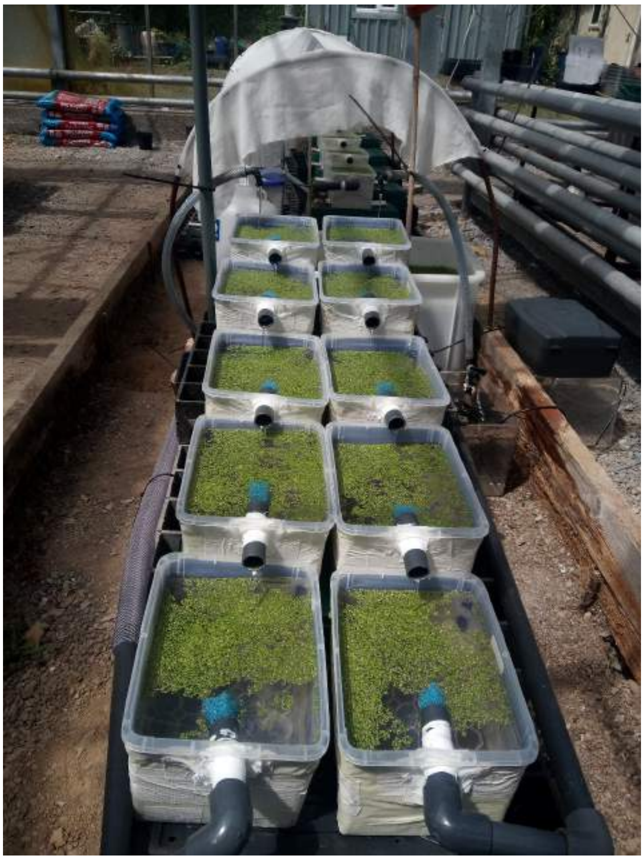

Figure 4. A simple cascading system at UCC, used for small scale experiments.

The integration of microbial and duckweed systems generates an effective platform to generate value from wastewater. However, unlike the IMTA farm at Mt Lucas, this was all done at a very small (< 1 litre), laboratory scale. While there is a lot of experience in upscaling of microbial reactors, much less has been published about duckweed bioreactors, particularly when these are expected to operate year-round. This is where

Dr Holger Kühnhold started to pioneer an indoor, stacked (multitiered) duckweed system. Initially, a "cheap and cheerful" stacked system was created. This was followed up by a very well-engineered recirculating, stacked system that was designed, built and trialled by Dr Neil Coughlan (Figure 5). The system consists of stacked stainless steel trays and a sump tank, operated with a combined capacity of 600 litres. Medium is pumped from the sump tank to the top tray, and then a gravity-fed flow drains tray 1 into tray 2, tray 2 into tray 3, and so on. Medium depth can be varied between 25 and 100 mm using flow regulators. Above each tray, a combination of red and blue LED strip lighting yields a maximum intensity of 150 µmol m^{-2} s^{-1}. This system facilitates effective duckweed growth, nitrogen and phosphorus uptake, and production of protein-rich biomass, thus generating a new feedstock. The system can operate 24 hours a day, seven days a week and year-round, irrespective of the seasons. This enables integration of the duckweed bioreactor in various industrial wastewater treatment plants, thus opening up opportunities for further applications in the agri-food industry.

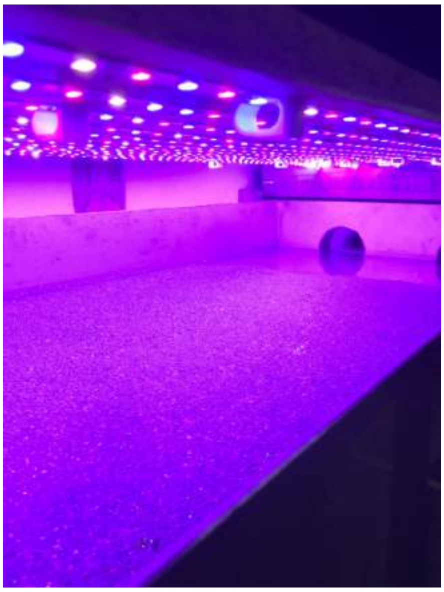
Figure 5. View of one of the tanks of a stacked duckweed incubator at UCC.

The Newtrients project has been very successful. One important outcome is the realisation that duckweed systems shouldn't be "go alone" systems but need to be seen as components of integrated wastewater valorisation systems. The combination of microbial and duckweed systems in the Newtrients project facilitated effective remediation of dairy processing wastewater, while generating value in the form of bioplastic precursors as well as protein-rich biomass. This opens a new perspective for a range of novel duckweed applications with other agri-food waste streams, and this is now facilitated by new funding. However, perhaps most importantly, the Newtrients acted as an exemplar of the technical feasibility of the circular economy, demonstrating a paradigm shift from simple wastewater treatment, to valorisation.

These Irish examples show that duckweed research is highly relevant for the development of a more circular economy. Thus, duckweed research and applications directly address the EU "Circular Economy Action Plan" (https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52020DC0098&from=EN) and are closely linked with UN Sustainable Development Goals (https://unece.org/trade/CircularEconomy). The story of the duckweed group at UCC shows the diversity of potential duckweed circular economy applications, ranging from simple, robust outdoor systems as have been traditionally used in South-East Asian countries for generations, to high-tech, integrated, indoor systems where duckweed species comprise a component of a more complex system. The example of effective remediation and valorisation of dairy processing wastewater shows that technologically the scope for duckweed systems is far larger than often assumed. Thus, the "return of the Lemnaceae" (Acosta *et al.*, 2021) is only just starting and exciting new opportunities are around the corner!

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Workshop Announcement

In transitioning to a circular economy, novel approaches are required for converting waste streams into novel resources. Duckweed (Lemnaceae spp.) is increasingly recognised as a promising tool for wastewater remediation and valorisation, and thus for enabling a more circular economy.

The duckweed group at University College Cork is pleased to present:

Duckweed Research and Applications for the Circular Economy in Ireland

A ONE-DAY, DISCUSSION-FOCUSED WORKSHOP

Friday June 9th, 2023
University College Cork, Ireland

The workshop will feature national and international expert speakers, creating a platform for knowledge exchange, learning from international best practice, interactive discussions and future collaboration. The emphasis of the workshop is on the use of duckweed species to *generate value from agri-food waste streams* and to *promote a more sustainable, circular economy.*

GROWTH - REMEDIATION – PROTEIN – AGRICULTURE – CIRCULAR ECONOMY

Confirmed speakers:

- Prof. Klaus Appenroth - Friedrich Schiller University Jena, Germany
- Dr Neil Coughlan – University College Cork, Ireland
- Dr Reindert Devlamynck – INAGRO, Belgium
- Dr Gruffydd Jones – Aberystwyth University – UK
- Dr Niall O'Leary – University College Cork, Ireland
- Dr Finn Petersen - Hochschule Osnabrück, Germany

This event is **FREE** but **places are limited.** Places are allocated on a first come first served basis. Please register in advance by emailing Sandra Jansen - a.jansen@ucc.ie

Further information: https://www.ucc.ie/en/plantstress/duckweed/
Or contact Prof. Marcel Jansen (m.jansen@ucc.ie)

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Department of Agriculture, Food and the Marine

ISCDRA
International Steering Committee on Duckweed Research and Applications

Commentary: Ethnobotanical importance of duckweeds in China

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1. Common weeds or useful plants?

Duckweeds are often regarded as common weeds, with little perceived value in economy or livelihood. They are the most widely distributed aquatic floating plants with miniature size and locally adapted populations found around the world. Taxonomically, these tiny aquatic plants consist of five genera and 36 currently recognized species in Lemnoideae (Araceae) according to APG IV (Angiosperm Phylogeny Group, 2016), but more recently has been proposed as a distinct family of Lemnaceae (Bog et al., 2019). In the folklore of China, duckweed or *Fu-ping* (浮萍) is a parataxon of tiny floating plants as a whole. In modern classification, it refers to plants in three genera including *Lemna*, *Spirodela* and *Wolffia* occurring in China. In particular two species, *Lemna minor* L. or *Spirodela polyrhiza* (L.) Schleid (Figure 1), are widely distributed and used in the country (Li, 1979). Plants in the subfamily Lemnoideae share the name of duckweed in their common names of Lesser Duckweed and Giant Duckweed, respectively (Ekperusi et al., 2019; Coughlan et al., 2022; Edelman et al., 2022).

Figure 1. Morphology and habitat of duckweed: (A) *Spirodela polyrhiza* (L.) Schleid; (B) *Lemna minor* L.

Morphologically, *L. minor* and *S. polyrhiza* can appear to be very similar at a first glance, but they have significant differences after careful inspection. *L. minor* is usually darker green on the top side (adaxial surface) with a single filiform root of 3-4 cm long hanging on the bottom side (abaxial surface), while *S. polyrhiza* is often purple on the back with 5-11 roots clustered around the center of the bottom side and are green and 3-5 cm long (Wożakowska-Natkaniec et al., 2015; Chen et al., 2016). These duckweeds grow in paddy fields, ponds, or other still-water areas, often mixing with other floating plants such as azolla or pistia to form floating communities that cover the water surface (Gallego et al., 2014; Huang et al., 2020; Acosta et al., 2021).

Currently, research on duckweeds is mainly focused on their ecological and energy values (Verma and Suthar, 2015; Chakrabarti et al., 2018; Kalderis et al., 2020; Appenroth et al., 2021; Chaudhary et al., 2021; Sauvage et al., 2021). Numerous studies have shown that *L. minor* has good absorption capacity for heavy metal pollutants such as cadmium, cobalt, vanadium, chromium, zinc, nickel, and lead. Other studies have reported resistance of *S. polyrhiza* to Mn at levels up to 70 mg/L, which can be of great significance for controlling heavy metal pollution in water sources (Kalderis, 2020; Farid et al., 2022). As an energy plant, duckweed has also attracted attention from researchers due to its high starch content under particular growth conditions. Pilot scale studies with duckweed grown on outdoor systems have reported its potential to generate starch-rich biomass that can be converted into bioethanol with 4-7 times more productivity than corn (Xu et al., 2011; Baliban et al., 2013). In contrast, little attention had been paid to the traditional uses of duckweeds, especially on its important role in Chinese history, traditional culture, or ethnic communities. In fact, duckweed has a long application history in China.

2. Records of duckweed in ancient Chinese literature

The ancient Chinese have categorically named plants floating on water as "*Ping*" (meaning duckweeds in English) for thousands of years, among which *L. minor* and *S. polyrhiza* received the most attention (Chen and Luo, 2005; Wang and Liu, 2016; Yang et al., 2021). Duckweeds are also known as *Zi Bei Fu Ping* 紫背浮萍, *Qing Bei Fu Ping* 青背浮萍, *Fu Ping Cao* 浮萍草, *Fu Ping* 浮萍, *Zi Ping* 紫萍, *Qing Ping* 青萍, *Shui Ping* 水萍 and *Ping* 萍（蘋）(Li, 1982; Zhao et al., 2020), which are known to have a variety of uses in China and occupies an important place in ancient Chinese literary works and medicinal texts (Table 1). However, most people including those in China, usually have less or incomplete knowledge and appreciation of duckweed.

Table 1. Plants named duckweeds in ancient Chinese literature

Family	Scientific name	Chinese name
Araceae	*Pistia stratiotes* L.	萍、大萍、大瓢
Lemnaceae (Lemnoideae)	*Lemna minor* L.	萍、青萍
	Spirodela polyrhiza (L.) Schleid.	萍、紫萍
(Wolffioideae)	*Wolffia arrhiza* (L.) Wimmer	萍、微萍、无根萍、萍沙
Hydrocharitaceae	*Hydrocharis dubia* (Bl.) Backer	萍、大萍、苹果萍水白、
	Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine	萍、圆心萍、肚兜萍
Marsileaceae	*Marsilea quadrifolia* L.	萍、蘋、田字草
Menyanthaceae	*Nymphoides peltata* (S. G. Gmelin) Kuntze	萍、荇菜
Nymphaeaceae	*Nuphar pumila* (Timm) de Candolle	萍、萍蓬草
Phyllanthaceae	*Phyllanthus fluitans* Benth.	萍、红浮萍、红毛丹
Salviniaceae	*Azolla pinnata* subsp. *asiatica* R. M. K. Saunders & K. Fowler	萍、红萍、满江红
	Salvinia natans (L.) All.	萍、槐叶萍、蜈蚣萍

The earliest records and descriptions of duckweeds from China can be found in *Literary Expositor* (爾雅; 476-221 BC). It was recorded in this book: "There are three species of duckweeds. The big one is called *Ping* 苹. The middle one is called *Xing* 荇. The small one is *Fu-ping* 浮萍. " This passage means that there are three kinds of plants called "duckweed", the big one called *Ping* is probably the *Nuphar pumila* (Timm) de Candolle, *Pistia stratiotes* L. or other plants belong to *Marsilea* L. The medium one could be *Nymphoides peltata* (S. G. Gmelin) Kuntze. The smallest variety, *Fuping*, could be *S. polyrhiza* or *L. minor* floating on the water. The duckweed used by herbalist is the one with smaller size (Deng, 2014).

Similarly, the *Tang Materia Medica* (唐本草; 659 AD) also pointed out: “There are three species of duckweeds. The big one is called *Ping*. There is *Xing* that is similar to *Ping* on the water, but the leaves are round. The smallest is the medicinal duckweed, which is very effective in treating fire sores (Su, 1985).”

Li Shizhen (李時珍), one of the great Chinese botanists from the time of the Ming Dynasty, described the form of duckweed in detail in *Compendium of Materia Medica* (本草纲目; 1552-1578 AD), and distinguished *S. polyrhiza* and *L. minor* for the first time (Figure 2). It says in this book, which was said to have taken 27 years to compile: “Duckweed is found everywhere, mostly in still water, and starts to grow in the spring. One leaf produced several leaves after a single night. There are whiskers under the leaves, which are their roots. There are two species of duckweeds. One is with green back and surface while the other with green surface and purple back. The one with purple back is called 紫萍 (*S. polyrhiza*), which is more suitable for medicinal purposes.” As for the collection and processing of duckweed, it is said in the book: “The duckweed with purple back is collected in July, picked up, cleaned, and dried using a bamboo screen. By placing a basin of water below the bamboo screen, reflected sun light can make it easier to dry (Li, 1982).”

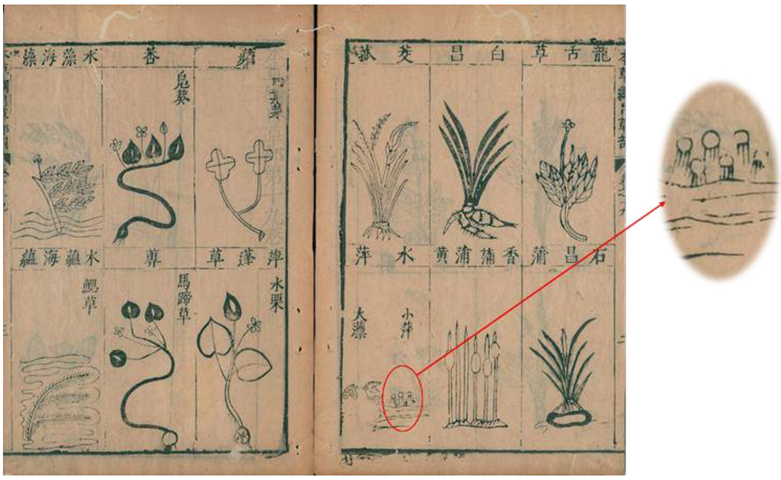

Figure 2. Morphological description of duckweed in Compendium of Materia Medica

3. Duckweeds used in ceremonies

Ceremony is a series of acts with specific symbolic significance and combined according to certain procedures and norms. It is an externalized, organized (even institutionalized) manifestation of concepts and emotions. In ancient civilization, the most mysterious rituals were various religious ceremonies, the main types of which were related to witchcraft, taboo, evasions, sacrifices, prayers, etc. (Esteban et al., 2021; Khazaie et al., 2021; Alabbad et al., 2022). Previous research shows that duckweed is an important material in performing the mantra for reducing fever and removing pox during the ritual of the Bacabs of the Mayan civilization. At the Sabbath dinner time in Judaism, duckweed plays a significant role in making oil lamp wick for lighting. It is prescribed in the Talmud that the festive Sabbath evening meal would be held in “well lighted quarters”. Indian Buddhism takes eating duckweed as a symbol of asceticism. Muslims will use duckweed in the water purification ceremony (Edelman et al., 2022). However, little is known about the application of duckweed in ancient Chinese rituals. We summarized use of duckweeds for ceremonies in Table 2.

Table 2. Duckweed used for ceremonies in China and other civilization

Religious rites	Ancient civilization	Appearing period	Role	Description
Talatuas	Chinese Confucianism	Western Zhou Dynasty 1046-771 BC	As food for ancestor worship	Ritual before marriage to inform ancestors that women have been successfully educated according to traditional etiquette and can marry and pray for protection by ancestors and gods
Ritual of the Bacabs	Mayan civilization	250–900 CE	Material for casting spells	Used in casting spell to cure fever and pox
The Sabbath	Judaism	around the year 200 CE	Materials for making oil lamp wick on Sabbath	The Sabbath is one of the main Jewish festivals. It is prescribed in the Talmud that the festive Sabbath evening meal be held in “well lighted quarters”.

Ritual of worshiping Shiva	Indian Hinduism	Medieval period	People passed their time with austerities and worshiping Shiva by eating duckweed	During austere penance in order to worship Lord Shiva, the sages perform several rituals, one of which is eating duckweed
Ritual Purification by Yemeni Muslims	Muslims	1837	Through water purification rituals, keeping water in the pool from changing its taste, color or smell for a long time.	the Yemenites claim, and perhaps believed, that the duckweed which covers the surface of stagnant waters, including their cisterns, can purify them (the waters), and they (the Yeminites) would not want to use standing water for purification where they would not see some (duckweed) floating.

In Chinese civilization, plants are one of the indispensable elements (Liu, 2014; Wang et al., 2022; Yang, 2022). They either serve as messengers to undertake the mission of dialogue with the gods, or as sacrifices to express the ancestors' reverence and reverence for the gods, or are endowed with specific meanings and beautiful wishes by their color, fragrance, taste, shape, and name (Xiao, 2014; Vaz et al., 2020). Duckweed is one of such plants, which was used as a messenger to help people communicate with their gods and ancestors in ancient China.

As an early example of ancient Chinese poetry, *the Book of Songs* collected poems from the early Western Zhou Dynasty to the middle of the Spring and Autumn Period (11th century BC to 6th century BC). Many ceremonial poems in *the Book of Songs* reflected people's usage of plants in rituals at that time (Jiang, 2004; Kong, 2008). Among them, the poem *Cai Ping* (采蘋) was about the preparation process of ceremony (Figure 3). This poem points out the role of duckweed as an offering. The original poem of *Cai Ping* is as follows:

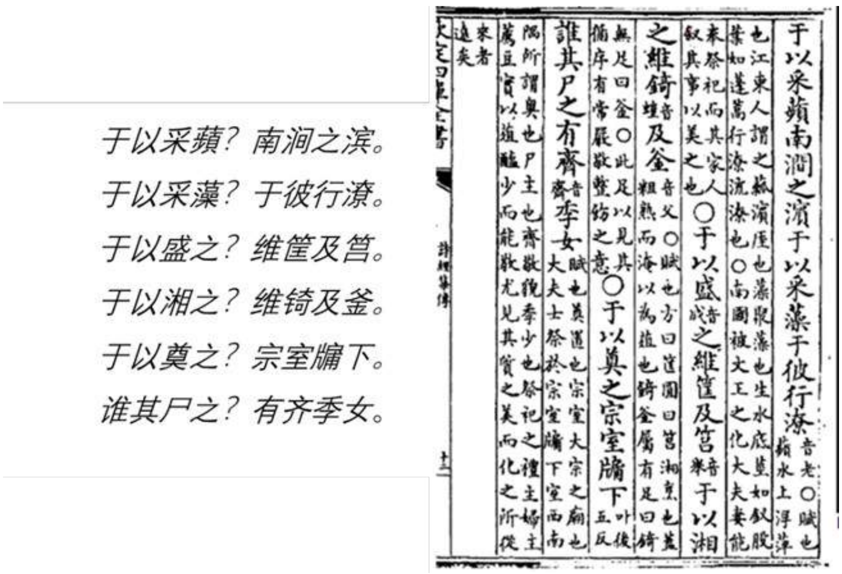

Figure 3. The poem Cai Ping 采蘋 recorded the ceremony

The general meaning of the poem is as follows: Where to collect the duckweeds (*Ping* 蘋)? It is near the stream in the south of the mountain. Where to collect the algae (*Zao* 藻)? Just where the water is shallow. With what to hold them in? Put them in the basket. What to cook them with? Use pots. Where to place the offerings? Under the windows of the shrine. Who will conduct the ritual? A respectful and devout woman to be married. The poem pointed out that the sacrificial objects prepared by people at that time were not things like bronze or gold, but a fresh plant, *Ping*, just picked from a mountain stream. The *Ping* referred to is likely the duckweed on the water (it is also believed that may be *Marsilea quadrifolia* L. – also called marsh clover), which is essential for communicating with the ancestors' souls and praying for blessings during the ceremonial process.

In addition to duckweed, there are many records of the frequent occurrence of various plants in ancient Chinese ritual activities, which reflects the simple natural view and primitive ecological view of ancient

Chinese ancestors, which is that all things have their unique spirit. It reflects the inseparable relationship between plants and human production, life, and culture, thus resulting in people's belief and worship of specific plants and plant communities (Hu and Zhang, 2017; Zeng and Qu, 2022).

4. Duckweeds as indicators of seasonal change

Before the advent of the technology for scientific monitoring of climatic patterns, the primary means for people to predict seasonal changes and weather was to observe the behavior pattern of animals and changes in plant growth (Beurs et al., 2004; Post and Inouye, 2008, 2008; Singh et al., 2013; Tao et al., 2022). With the knowledge of phenology, people can grasp the pattern of weather changes, learned how to arrange agricultural production activities according to environmental indicators, determine what crops are suitable for planting at different times, and how to optimize their work for maximum benefits (Xiao et al., 2015; Potgieter et al., 2021; Cann et al., 2022). The ancient Chinese have learned to integrate many phenological indicators to help them predict annual patterns of climatic conditions in order to plan their farming activities. Some of the common phenological phenomena used were behaviors of migratory birds and germination of particular plants (Veum et al., 2014; Zong et al., 2022). For example, according to *the Lü's Spring and Autumn Annals*, "*Acorus calamus, a kind of water grass, always germinates first every year, and thus indicates the time when farming activity should start* (Lü, 2011)".

Duckweed can always be seen in paddy fields, ponds, and other still water bodies in most areas. It was because of its wide distribution and frequent appearance in the vision of people engaged in agricultural production that early attention was paid to the relationship between basic biological characteristics of duckweed, such as its shape, species, growth environment and growth pattern, and associate them with seasonal changes in agricultural activities. As for the time when duckweed appeared every year, ancient Chinese linked it directly with the seasons and solar terms that agricultural production attached great importance to. As recorded in *Yue Ling* in *The Books of Rites*, "duckweed starting to germinate means it is the last month of spring." *Yue Ling* was an important work recording the phenology of the four seasons in ancient times, which provided experience accumulation and certain objective markers for summarizing the solar terms that are closely related to agricultural production (Hu and Zhang, 2017).

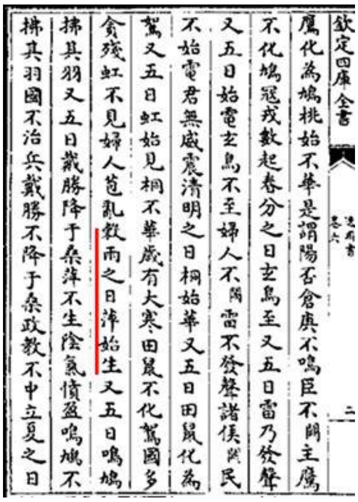
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Figure 4. Records of phenological function of duckweed in *Yi Zhou Shu*

Grain Rain (*Gu Yu* 穀雨 in Chinese), one of the 24 solar terms in Chinese tradition, literally means the rain that nourishes crop seeds. Grain rain is the last solar term in spring, which is significant for agricultural cultivation (Wang and Zhou, 2012; Xu, 2021). The *Yi Zhou Shu* says, "*On the day of the grain rain, the duckweed begins to grow* (Figure 4)." The emergence of duckweed thus reminds farmers that it is time for grain rain. In the season of grain rain, rice farmers in the south of the Yangtze River get busy raising rice seedlings and transplanting them to the paddy fields, while tea farmers are busy picking leaves and making tea. In addition to strengthening the management of wheat and garlic to prevent the occurrence of diseases and insect pests, the farmers in the north of the Yangtze River should also start planting sweet potatoes, peanuts, spring corn, cotton, and soybeans. It is also the season for livestock breeding and fish breeding (Huang, 1995).

The description in the *Yi Zhou Shu* is more direct than that in the *Yue Ling*. It directly relates the phenological phenomenon of duckweed growth to the important specific solar term, Grain Rain. It can be seen from the specific phenomenon of duckweed's renewal and growth in late spring that this aquatic plant has been well-known for its marker function to inform agricultural production. In this case, the emergence of duckweed in the Spring has its importance from the perspective of people's agricultural life.

5. Duckweeds appeared in literary and artistic works

The cultural function of duckweed mainly stems from the "imagery" it refers to. Imagery is used in both Chinese and Western literature, especially in ancient Chinese poetry. Ancient writers often described the unique images of natural landscapes, animals, and plants to convey a special emotion (Fridlund et al., 1992; Lang et al., 1998; Grol et al., 2017). As a kind of cultural thinking, imagery permeates ancient lyrical and narrative literature (Goetz et al., 1993; Mei, 2000; Zhai, 2015; Calafato and Simmonds, 2022). The use of imagery in Chinese literary works can be traced back to the West Zhou Dynasty (1046-771 BC). The ancestors of the West

Zhou Dynasty in China had an inherent worship and admiration for plants, which stemmed from the fear and mystery associated with nature. Thus, there are many imageries of plants in literary works from that time. It was common to use flowers as a metaphor for women, trees as a metaphor for men, and the fruit of plants as a metaphor for fertility and marriage (Kong, 2008; Qu, 2008; Cao, 2015).

The duckweeds, because of their floating growth habit (according to ancestors' cognition), drifting with the waves and moving with the wind, has become a representative imagery of living without a fixed place, being helpless and wandering without a base. There are many ancient poems in China that use duckweed as an image to express the situation of wandering and helplessness. An example is the poem "*Qu Fu Ci* 去妇词" written by Li Bai (701-762 AD), a famous poet of the Tang Dynasty (Editorial Department of Zhonghua Book Company, 1992). The extracts of the original poem that used duckweed as imagery are as follows：

君恩既断绝，相见何年月？

悔倾连理杯，虚作同心结。

女萝附青松，贵欲相依投。

浮萍失绿水，教作若为流。

This was a story of a woman's tragic situation after her husband abandoned her. The main idea of the original text above is: "When would we see each other again since your affection for me disappeared? I really regretted that I had married you and wanted to spend the rest of my life with you. I relied on you just like clinging to the green pine in my dreams, thinking that you were the one I can depend on. Like duckweed lost from the green water, how am I going to survive?" This poem written by Li Bai described the passive position of women in marriage and family during the ancient times, and the dilemma of being completely depended on and dominated by the husband. While condemning the husband who abandoned his wife, the poet also described the helplessness and worries of women about their future life. When a woman lost her husband, she was like a duckweed without water. The poem expressed the sympathy for the married women who were unable to live on their own and had the profound analysis of the marriage between men and women under the feudal social ethics (Wu, 2016).

Influenced by the culture of the Tang Dynasty (618-907 AD), duckweed was also often used as a symbol of transient emotion and life in Japanese poems in the Heian era (794-1185 AD) to convey a feeling of melancholy or sadness. A representative are the love poems of the outstanding Japanese poetess Ono Komachi (850 AD), which were included in Kokin waka shu (Shirane, 2012).

Nowadays, "Ping" has become a very common word in Chinese vocabulary system, and the phrases and allusions containing the word "*Ping*" are quite numerous. For example, *Ping Ji* 萍迹, *Ping Zong* 萍踪, *Ping Shui* 萍水, *Ping Ju* 萍聚, *Ping Shui Xiang Feng* 萍水相逢, and others. These words are familiar and frequently used. From the semantic content, almost all the words combined with "Ping" are related to the meanings of wandering and gathering and scattering (Xie, 2021). Therefore, in the Chinese cultural system, the meaning of "Ping" is not only the name of a kind of aquatic floating plants, but also contains the internal psychological and emotional sustenance that people have accumulated and formed over time. With the continuous development and evolution of literary creation, this emotional sustenance generates artistic styles and expression mechanisms suitable for different situations. A modern example of such usage in artistic work is a 2017 Chinese movie called Duckweed (乘风破浪), the Chinese title of which literally translates to "Ride the Winds, Break the Waves". While the storyline is one about the journey of a son's realization and appreciation of his father's turbulent life and struggles, the English translation of the title to Duckweed is clearly a reference to the constant challenges and resilience of everyday people in the face of events and situations that are seldom under their control. Although duckweeds may be small in comparison to wind and waves that constantly buffet them, they nevertheless can ride out these obstacles and flourish in their environment.

6. Duckweeds used as herbal medicines

Living conditions of our ancient ancestors were harsh in comparison to more modern civilizations. When people hunted animals or gathered plants from the wild for food, they inevitably ate some poisonous plants by mistake, which often led to vomiting, coma, and death. But sometimes these wild plants could also make their sick body feel better or even recover. Through repeated practice and experience summarization, people began to note that different plants can have diverse effects on the human body, and gradually began to accumulate knowledge about the use of medicinal plants. At that time, efficacy of medicinal plants was usually related to their morphology and growth environment (Smith-Hall et al., 2012; Alebie et al., 2017; Tang et al., 2021; Zhang et al., 2022). For example, as aquatic plants, duckweeds were generally believed to have the activity of cooling the human body and could thus help cure fever and inflammation.

The doctor/pharmacologist Pedianos Dioscorides (40-90 AD), who was born in Cilicia (today's Turkey) of the Roman Empire, recorded in his medical work "De Material Medica" that duckweed was a common water plant in still water and had the function of cooling, which was helpful to all phlegmon (diffuse inflammation), erisypelas (bacterial inflammation of the skin) and podagral (foot gout) (Staub et al., 2016; Edelman et al., 2022). Saint Hildegard von Bingen (1098-1179 AD) was a German theologian, scientist, physician, and philosopher in the Middle Ages. She also served as the dean of a Catholic convent. In her book Causae et Curae, she mentioned that *L. minor*, *Pyrethrum parthenium* (L.) Sm., *Salvia officinalis* L. and other plants could be used to make an ointment against colic. In addition, the book also records the role of duckweed in treating heart pain and rheumatism (Sakalauskaite-Juodeikiene et al., 2021; Edelman et al., 2022).

However, for the systematic records of duckweed medicinal use, we could find more clues from ancient Chinese books. Many Chinese classical medical works have recorded duckweed, an aquatic medicinal plant, including the detailed growth environment, plant morphology, methods of use, applicable diseases, etc. Our ethnobotanical survey also found that the traditional medicinal practice of using duckweed to treat diseases still exists in many ethnic communities of China to this day. There are 217 cases for use of duckweed recorded in ancient Chinese medicinal literature, including processing methods, usage methods and diseases that have been treated, through sorting out 8 ancient texts of classic Chinese medicine.

The ancient Chinese used a variety of methods to process medicinal materials. They would choose appropriate processing methods according to different indications of the patient, different aspects of the disease and the medicinal materials used. For duckweed, as shown in Figure 5, there are 12 processing methods mentioned in eight ancient Chinese medical texts. Among them, the method of making duckweed powder is the most common, which has been mentioned about 70 times. For example, according to the records in the *Prescriptions for Universal Relief* (普濟本事方), the duckweed that lives on the water is dried and made into powder (Xu, 2007). One tablespoon of duckweed powder is taken three times each day, which can cure dysuria (Zhu, 1959). The method of decoction (i.e. concentration by boiling or heating) has been applied 43 times. According to the *Medical Secrets from the Royal Library*, when there are abscesses on the body, take about 5 kg duckweed to decoct water and use the decocted water for bathing, which can be effective in half a day (Wang, 1993). Other processing methods include pelletizing, blending into paste, mashing into paste, pressing out juice, decocting into paste, incinerating, boiling with white liquor (baijiu), drying in the shade, boiling soup, and soaking in white liquor. It is worth mentioning that garlic puree, honey, raw eggs, raw oil, borneol – a terpene extracted from common valerian plant, lard, water, and vinegar should be added for blending with duckweed when making paste. Honey, batter, millet rice, human milk and milk should be added when making meatballs, which may play the role of adhesive.

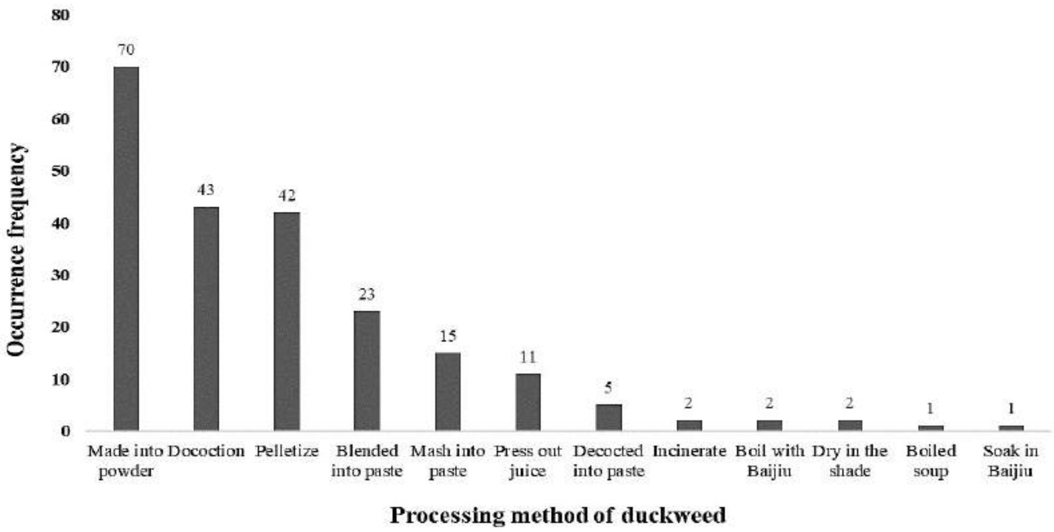

Figure 5. Processing methods of duckweeds in eight ancient Chinese medicinal books (*Baijiu* refers to liquor)

The methods for using duckweed in ancient medicinal books are divided into two categories: external use and internal use (Table 3). There are six methods for external use, which include applying or pasting to the affected part, soaking, and washing or bathing, burning smoke, facial dressings, washing the face, and blowing into the affected part. The method of applying or pasting the duckweed to the affected part is the most frequent among the eight ancient books, accounting for 24%. For example, *General Medical Collection of Royal Benevolence* records that a kind of prescription is called floating ointment. When making the floating ointment, dry the duckweed in the sun, weigh 250 g, pound it into powder, mix it with honey, and then put it into a porcelain box. Applying the floating ointment on the face before going to bed every night can make the face white and smooth (Zhao, 1992). The second is soaking and washing or bathing, accounting for 9% of the documented usage. It means using duckweed to boil water and take a bath to treat various skin diseases. The method of burning smoke by duckweed accounts for 5%. Burning smoke can drive away mosquitoes. The method of facial dressing, face washing, and blowing into the affected part are uncommon with 2%, 2% and 1% respectively.

Table 3. Statistics of duckweed usage types in eight ancient Chinese medicinal books.

Category	Subcategory	proportion
External	Apply or paste to the affected part	24.0%
	Immersion washing or bathing	9.0%
	Burn smoke	5.0%
	Facial dressing	2.0%
	Wash face	2.0%
	Blow into the affected part	1.0%
Take orally	Take orally directly	30.0%
	Take orally with warm Baijiu	10.0%
	Take orally after mixing with liquid	5.0%
	Contained in the mouth and swallow	4.0%
	Take orally with medicinal soup	3.0%
	Contained in the mouth and melted away	2.0%
	Take orally with rice soup	1.0%
	Take orally with honey water	1.0%
	Take orally with milk	0.5%
	Contained in the mouth and spit out	0.5%

There are ten ways to take duckweed orally as medicine. Taking duckweed directly is the most common method, accounting for 30% with lots of records in eight ancient books of Chinese medicine. The second is taking orally with warm white liquor, accounting for 10%. This could be due to the belief by the ancient Chinese that white liquor could stimulate the activity of duckweed, making it more effective. Take orally after mixing with liquid accounts for 5%, which include milk, white liquor, water, and goat liver juice. Holding in the mouth for some time and then swallow accounts for 4%. This method is mainly used to treat diseases in the mouth. Taking orally with medical soup accounts for 3%. The medicine soup mentioned in the eight ancient books for taking duckweed includes bog rush (*Juncus effusus* L.) decoction, thatch root (*Imperata cylindrica* (L.) Beauv.) decoction, *Acorus calamus* decoction, and mint (*Mentha canadensis* L.) decoction. Other methods, like holding in the mouth and melting away, taking orally with rice soup or honey water and so on, are only for specific diseases and not often mentioned (Wang, 1958; Zhu, 1959; Gu, 1963; Li, 1982; Su, 1982; Zhao, 1992; Wang, 1993; Su, 2011).

According to the statistics of the medicinal efficacy of duckweed in eight ancient books, 24 indications of duckweed can be summarized (Figure 6). In the order of the frequency mentioned, they are various skin diseases, body abscess, persistent thirst, ulceration of mouth and tongue caused by internal heat, urinary stammering, various poisoning, spots, pimples and dullness on the face, hair loss and not growing, paralysis, typhoid fever, mosquito infestation, various gynecological diseases, hands and feet cold or numbness, ocular disease, swelling, hematemesis, drunkenness, nosebleed, cholera and irritability, prolapse of anus, andrology, hypo-immunity, empyrosis, and traumatic injury. Various medicinal effects imply the importance of duckweed as a medicinal plant, and the high frequency of usage reveals that duckweeds may have great potential in treating various skin diseases, body abscesses, skin related issues on the face, hair loss and other disease manifestations related to human well-being.

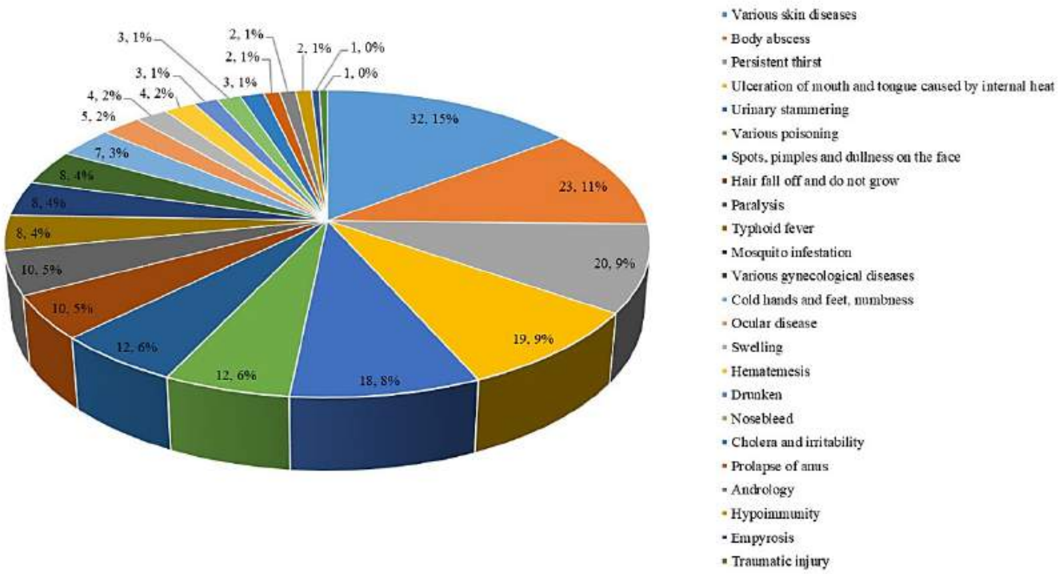

Figure 6. Statistics of duckweed medicinal efficacy in eight ancient Chinese medicinal books

7. Folk uses in traditional societies from diverse ethnic groups

The ethnobotanical field surveys are conducted in southern China to understand the traditional uses of duckweeds in local communities, particularly in remote rural areas (Figure 7). In seven provinces as marked on the map (Fig. 7), we have visited at least three sites since 2007. Yunnan and Guizhou provinces are the areas investigated most frequently.

Figure 7. Provinces investigated ethnobotanically: ①Yunnan; ②Guizhou; ③Guangxi; ④Hunan; ⑤Zhejiang; ⑥Sichuan; ⑦Hubei

Field investigations in southern China revealed two species, *L. minor* and *S. polyrhiza*, are widely distributed in aquatic systems with paddy rice fields as the most common habitats. In the investigation sites (Figure 8), for instance, local people have rich knowledge about duckweeds, including their ecological functions. The local people's familiarity and use of duckweeds have become an irreplaceable component in traditional agroecosystems and agricultural culture.

The Rice-Fish-Duck agroecosystem in Congjiang and Liping counties, Guizhou Province, has a long history practiced by Dong and other ethnic groups. The local people grow rice, fish, and duck at the same time in the paddies. It is a sustainable economic system combined within a virtuous eco-cycle in which, many traditional methods of farming and folk customs are harbored. Therefore, the Dong community's Rice-Fish-Duck agroecosystem was recognized as the Globally Important Agricultural Heritage Systems by FAO in 2011. Interestingly, the locals believe duckweeds are good for fish, duck, rice, soil, and water in the paddy fields. They feed the fish and duck with duckweed in the paddies, while collecting it from aquatic systems as pig forages. Research revealed that in the traditional rice cultivation system, auxin-producing bacteria isolated from both duckweed and rice has potential plant growth-promoting bacteria which may improve growth for both duckweed and rice in paddy fields (Huang et al., 2020). It is suggested that this agroecosystem may be amended as "Rice-Duckweed-Fish-Duck system".

Another example is the Honghe Hani terraced paddy rice fields in Yunnan, regarded as a wonder of terraced farming civilization created by the Hani people and other ethnic groups along with the local geographical climate (Yang et al., 2017; Luo et al., 2019; Li et al., 2021). It was successfully included in the Globally Important Agricultural Heritage Systems by FAO and the World Heritage List by UNESCO (Gu et al., 2012; Zhang et al., 2017; Luo et al., 2019). Hani people believe that duckweed, as a common species in paddy fields, has become an important component in their agroecosystems for its ecological services and other roles.

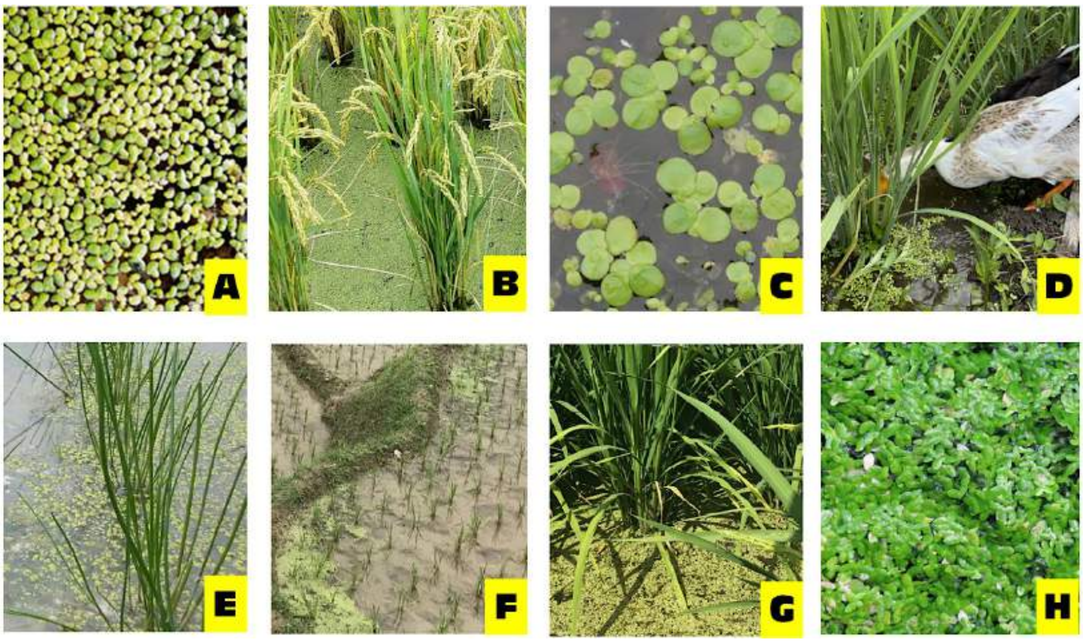

Figure 8. Duckweeds in different traditional agroecosystems of China. (A. *Spirodela polyrhiza* in Hani terraced paddy rice field, Jiayin, Honghe County, Yunnan; B. *Lemna minor* in paddy rice field, Pantiange, Weixi County, Yunnan; C. *Spirodela polyrhiza* in paddy rice field, Liping County, Guizhou; D. *Lemna aequinoctialis* in terraced paddy rice field, Gaozeng, Congjiang County, Guizhou; E. *Lemna minor* in *Eleocharis dulcis* field, Lipu County, Guangxi; F. *Lemna minor* in terraced paddy rice field, Longji, Longsheng County, Guangxi; G. *Lemna minor* in terraced paddy rice field, Ziquejie, Xinhua County, Hunan; H. *Lemna minor* in drainage, Zhuji City, Zhengjiang).

8. Medicinal practices of duckweeds in ethnic communities of China

According to our ethnobotanical surveys, many ethnic groups in the research sites still retain the traditional practice of using duckweed as medicine to protect their skin. As shown in Table 4, duckweed is used as a medicinal material by the De'ang, Yi, Shui, Miao, Dong, Mongolian and other ethnic groups to deal with various skin problems in their daily life, but the species of duckweed they use are different. For instance, the De'ang and Shui people only use *L. minor.* Dong people only use *S. polyrhiza.* The Yi, Miao and Mongolian used both species. While the species and names of duckweeds used by different ethnic groups are not the same, the methods used and the symptoms treated are roughly similar. The dried duckweed is ground into powder and applied externally to the affected area to treat skin related diseases such as urticaria, rubella, pruritus, edema, urosis, water fire scald, scabies, and ulcers. In addition, the Miao people also recorded that duckweed was burned and smoked to repel mosquitoes, which is consistent with the records in ancient books.

Table 4. Medicinal records of duckweeds used by ethnic groups

Scientific name	Local names	Ethnic group name	Used part	Method	Effect
L. minor	Langlang	De'ang	Whole grass	After drying, grind the powder and apply it to the affected area	Cure urticaria and edema
	Yiwei	Yi			Cure impenetrable rash, rubella pruritus, edema, urosis, water fire scald, scabies
	Bibiao	Shui			Cure traumatic injuries
	Duipenlue; Baolue; Pinao; Laizazhua	Miao			Cure rubella, heat toxin, bleeding, edema, impenetrable rash, and smoke after burning can repel mosquitoes
	n.a.	Mongolian			Indications: cold, impenetrable measles, rash, skin itching, urticaria, nephritis and edema, adverse urination, tinea, erysipelas, scald
S. polyrhiza	Yiwei	Yi			Cure impenetrable rash, rubella pruritus, edema, urosis, water fire scald, scabies
	Nai	Dong			Cure measles, lumbago, and edema
	Duipenlue; Baolue; Pinao; Laizazhua	Miao			Cure rubella, heat toxin, bleeding, edema, and impenetrable macula
	n.a.	Mongolian			Indications: cold, impenetrable measles, rash, skin itching, urticaria, nephritis and edema, adverse urination, tinea, erysipelas, scald

n.a.: none available

The De’ang, Yi, Shui, Miao, Dong and other ethnic groups lived most of the time in areas with hot and humid climates, and the sanitary conditions are often quite poor, which easily leads to skin itching, impenetrable rash, scabies, sores, and other diseases. They believe that, as an aquatic plant, duckweed had a cold nature, which has good efficacy for treating such diseases associated with inflammation. This view is consistent with the ancient records. However, with the improvement of sanitary conditions and living standards, the probability of such diseases gradually decreased, and people are more willing to use western medicine for treatment. Over time, the traditional medicinal knowledge relating to duckweed has only been preserved in the hands of a few herbalists, and most of these have limited knowledge to the fact that it is a good forage plant and can improve water quality if left in paddy fields. The traditional medicinal knowledge of duckweed is gradually being lost. Literature research and ethnobotanical investigations revealed the historical significance of duckweed in China. In ancient Chinese civilization, duckweed, as a ceremonial plant, was a bridge for people to communicate with gods. As a phenological plant, duckweed can guide people’s farming activities. As abstract imagery, duckweed can convey people's feelings and thus used for cultural and artistic purposes. What can contribute to human well-being is that duckweed, as a medicinal plant, may be able to treat many serious diseases that modern people are concerned about. In addition, duckweed also has important value as animal feed, for ecological services, and biological fertilizer applications. However, at present, the utilization rate of duckweed resources is still low, and there is relatively little awareness about its medicinal value in China. Results of ethnobotanical study has thus provided helpful information and directions for future development of duckweed in these areas.

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Historical account on St. Hildegard von Bingen

Inventor of Duckweed Elixir and polymath nun from medieval times

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St. Hildegard von Bingen from the 12th century (in Latin Hildegardis Bingensis; 1098-1179 CE) was a nun in the Benedictine cloister Disibodenberg in Rhineland-Palatinate, Germany and was later elected as Magistra (= Mother Superior). In 1150, she founded a new cloister, Rupertsberg, near Bingen at the river Rhine and became the abbess (Feuerein-Prasse et al., 2021). A decade and half later, she founded another Benedictine cloister in Eibingen (http://abtei-st-hildegard.de/english/). For a long time, Hildegard von Bingen was hardly known outside of Germany. Although regional calendars of the Roman Catholic church had listed her as a saint for centuries, she was officially promoted as a Saint and a Doctor of the Church in 2012 by Pope Benedict XVI (Joeckle, 2003, https://www.vatican.va/content/benedict-xvi/en/apost_letters/documents/hf_ben-xvi_apl_20121007_ildegarda-bingen.html).

Figure 1: Hildegard von Bingen in bronze. Author of “Causa et Curae” (Reasons and treatment of diseases), a medieval compilation of knowledge about plants, including the medicinal use of duckweeds. Sculpture by Karlheinz Oswald, 1998, in front of Eibingen Abbey St. Hildegard, Germany. picture credit: This file is licensed under the Creative Commons Attribution-Share Alike 3.0.

https://commons.wikimedia.org/wiki/File:Karlheinz_Oswald_Hildegard_von_Bingen,_Eibingen.JPG

Hildegard refused to be defined by the patriarchal hierarchy of the church and pushed the then established boundaries for women almost past their limits. For upper-class women, cloister convents fulfilled several basic needs, especially those of Benedictines as centres of knowledge and learning. They provided an outlet for nonconformist women, who did not wish to marry, e.g. because they saw in the convent a mode of life in which they could perform and perhaps distinguish themselves. The nunnery was a refuge for female intellectuals (Mark, 2019). Hildegard became an adviser to Popes, Cardinals and Statesmen.

The rich Benedictine cloisters in that time were famous for their huge libraries that provided Hildegard with an access to the compiled knowledge of her time. Moreover, as abbess, she stayed in contact with many scientific centres spread all over Europe. On these foundations, she developed into a polymath proficient in philosophy, musical composition, medieval literature, cosmology, theology – and biology, herbology, medicine and natural history. She is one of the best-known composers of sacred monophony. There are more surviving chants by Hildegard than by any other composer from the Middle Ages. Indeed her music had a vivid revival in the 19th century.

Hildegard is considered the founder of scientific natural history in Germany and popularized the pharmaceutical use of duckweeds. Knowledge of plants in medieval times was the domain of women, who were often treated with suspicion in the patriarchal society. Herbal plant experts were normally women who hardly had any education and described their knowledge in local languages, which was not considered worthy to be preserved. Hildegard, however, was highly educated and with her vast knowledge, she wrote two books (von Bingen, 1933, 1992, 2021) in Latin, the language of Science of that time: 1. "Physica", a nine volume book, describing the scientific and medicinal properties of plants together with information about animals and rocks. 2. "Causa et Curae" collected contemporary knowledge e.g. about plants, including duckweeds.

Her belief in the "greening of man," or *viriditas* as she called it, led her to trust that God had given herbs, spices, and foods to mankind in order to serve our bodies and keep us not only healthy but also full of joy and peace. Uehleke et al. (2012) investigated the herbal claims made by Hildegard using a statistical approach, focussing on 85 plants that are used today for medical purposes. There were 212 health claims described in Hildegard's books "*Physica"* (Physics) and "*Causae et Curae"* (Reasons and treatment of diseases) concerning this group of plants, 30 of which are correct according to contemporary standards. Randomly, the number would be 6 or 7. Hildegard's higher rate of correctness probably came from her responsibility for the cloister gardens in Disibodenberg and later on a larger scale in Rupertsberg and Eibingen. This way she collected practical knowledge of plants, beside the theoretical knowledge from the cloister library. Moreover, in all the cloisters, there were hospital wards where she had abundant opportunities to treat patients using herbal preparations. In total Hildegard described the properties and the medical uses of approximately 175 different herbs, among them, water lentils or duckweeds (Lemnaceae, now with 36 recognized species; Tippery et al., 2021).

Hildegard described several recipes that included duckweeds and also mentioned their usage. These preparations were used to treat respiratory and rheumatic problems, regulation of body temperature, and urinary ailments. Duckweeds with their high flavonoid content were included for their anti-inflammatory and diuretic properties to bring the body back to its natural balance (https://www.healthyhildegard.com/duckweed-elixir/). Duckweed was used as a component in preparations to treat chronic health conditions such as chest pain, constant colds, gastrointestinal deficiencies, and colorectal pain (Hertzka et al., 2020). Hildegard described these symptoms as a state of "vicht", or as we would say today, pre-cancerous. To prevent "vicht" Hildegard recommended a herbal mixture using duckweed, ginger (*Zingiber officinale* Roscoe), cinnamon (*Cinnamomum verum* J.Presl), sage (*Salvia officinalis* L.), fennel (*Foeniculum vulgare* (L.) Mill), tansy (*Tanacetum vulgaris* L.), white pepper (*Piper nigrum* L.), Blutwurz (*Potentilla erecta* L. (Raeusch.)), charlock mustard (*Sinapis arvensis* L.), and Lady's bedstraw (*Galium aparine* L. or *Galium verum* L.). It was called *Decoctum Lemnae* cp.("cp." most probably short for "compositum"). The preparations described in her scriptures are pretty complicated (Herzka et al., 2020, von Bingen, 2021). Here is an example: "… take a bit of ginger with more cinnamon and pulverize it. Take less sage than ginger, and more fennel than sage, and a little more tansy than sage, and crush them to a juice in a mortar, and strain it through a cloth. Then cook a bit of honey in wine, and add a little white pepper and put it in the powder and juice. Afterward, take duckweed, and twice as much blutwurz, and the same amount of mustard which grows in the field, and rub this to a juice in a mortar, and place it in a little bag, and pour the honeyed wine mixture over it, and make a clear drink." More details in tabular form are presented in Edelman et al. (2022).

Duckweed Elixir

Duckweed preparations, strictly following the advice of Hildegard, are now available as "Wasserlinsenelixier" (duckweed elixir) on a commercial basis under different brand names, e.g. "Bio Hildegard Wasserlinsen Elixier", "Vichtosan Wasserlinsenelxier", "Wasserlinsen Trank", "Wasserlinsenwein", "Wasserlinsenkraeutertrunk" or "Wasserlinsenkraeuter-Elxier". Hildegard was rediscovered by the green movement in many countries and her plant-based kitchen became very popular in the last decades all over the world (Fournier-Rosset, 2010). Plant-based products with duckweeds are well associated with St. Hildegard of Bingen.

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Student Spotlight: Cian Redmond

School of Biological, Earth and Environmental Sciences, University College Cork, Ireland. (Email: credmond@ucc.ie)

I am extremely lucky to have grown up in a small seaside town, that also happens to be only a 20 minute drive from the largest national park in Ireland – The Wicklow Mountains National Park. Living surrounded by these incredibly picturesque natural environments, it's no wonder I developed an interest in biology and the environment. Being witness to such a wide variety of ecosystems and habitats served only to motivate me to pursue a degree in Applied Plant Biology at the School of Biological, Earth and Environmental Sciences (BEES) in the University College Cork (UCC), Ireland.

This interest in the natural world coupled with my hobby of fish-keeping inevitably led me to a small, albeit fast growing, aquatic plant – duckweed. Within the aquarium hobby there are mixed opinions on duckweed, some see the extremely rapid growth as a nuisance and others see its benefit, I see the latter. I saw, first hand, the benefit of integrating duckweed as a natural biological filter, assisting in the removal of excess nutrients in my aquaria. The nitrogen cycle plays a crucial role in maintaining healthy aquaria. We rely on nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* to convert ammonia/ammonium to nitrite and finally to the least toxic form – nitrate. However, once the nitrogen is in the form of nitrate it begins to accumulate over time, eventually becoming toxic to aquatic life. The most cost-effective method of nitrate removal is regular water changes. However, when you have multiple tanks in operation, the volume of water required for these water changes increases. By simply incorporating duckweed into my aquaria, I found that I could decrease the frequency of water changes in lieu of a weekly duckweed harvest. The harvested duckweed nicely complemented garden waste in my compost heap – allowing me to recycle the nutrients that would otherwise have been lost to domestic wastewater treatment or quickly leached out from my garden soil.

Figure 1 Image of the indoor stacked duckweed bioreactors

A requirement in the final year of my degree in Applied Plant Biology was to undertake a research project. My interest in growing duckweed to remove nutrients from my aquaria wastewater aligned perfectly with the work of the Ireland-Wales funded Brainwaves project in UCC, spearheaded by Prof. Marcel A.K. Jansen. Over several months, I examined the effect of light intensity and photoperiod on both the growth of duckweed (*Lemna minor* 'Blarney') and its capacity for phytoremediation of phosphate and nitrogen. This research was carried out in three bespoke (i.e. custom-made) stacked indoor growth systems (Fig. 1). Each system had 500 L capacity with a total surface area of 3.96 m^2 for duckweed growth.

Figure 2 Image of the outdoor duckweed bioreactors

Upon completion of my degree, I returned to the Brainwaves project as a research assistant for the summer months. The focus of this work was on outdoor growth systems. Initially I assisted in the construction of 15 purpose-built bioreactor growth systems. Each bioreactor had a capacity of up to 1000 L. However, I operated them at a capacity of 500 L. Once the systems were operational, I began working on the cultivation of duckweed on an agricultural waste stream. The waste stream I used was a type of soiled water known colloquially as yard washings. Yard washings are generated on dairy farms when the milking parlour is hosed down after milking. In Ireland, it is usually stored separately from slurry and is regulated under different legislation. As it is more dilute than slurry it can be spread at a higher rate and is subject to less restrictions. While dilute when compared with slurry, it contains large amounts of nutrients – particularly nitrogen and phosphate.

Using the principles of circular economy, the aim was the valorisation of this waste stream. By taking this low value waste and using it as the growth media for duckweed it will potentially generate value from waste. The ideal product of this process is clean water that no longer poses an environmental threat, and a high protein biomass in the form of duckweed.
Having gained an insight into the world of research and developed valuable skills while working with the Brainwaves project, I was eager to further my studies or to continue working in this area. When an opportunity arose for a PhD position on another duckweed research project, led by Prof. Marcel A.K. Jansen, I eagerly applied.

I am now a few months into a three year PhD program in UCC, under the Duck-Feed Project funded by the Department of Agriculture, Food and the Marine in Ireland. This project focuses on the cultivation of duckweed on agricultural waste streams while maximising the protein content of duckweed. The end goal will be to develop the expertise to facilitate the roll-out of duckweed as a high protein agricultural crop in Ireland that can be incorporated into animal feed – reducing reliance on imported soymeal.

The first couple of months of my PhD research will focus on building upon the knowledge I have gained from my previous work with duckweed, as well as attempting to overcome some of the issues that negatively affected the growth and phytoremediation capacity of duckweed on yard washings. Some of the issues that I have encountered are related to the high levels of solids, the impact of microorganisms such as bacteria and algae, and the high variability of nutrient concentrations in agricultural waste streams between both farms and seasons.

I am looking forward to studying duckweed for the next three years, and to having the opportunity to discover something previously unknown. I am also looking forward to collaborating with other researchers in this field, and to be able to contribute knowledge to the ever-growing community of duckweed researchers – all of whom are hoping to solve some of the largest and most critical global issues we're facing, making use of the most humble family of plants – Lemnaceae.


Figure 3 Me, filtering yard washings in the lab

From the Database

Highlights

Flavone-associated resistance of two *Lemna* species to duckweed weevil attack

Lee, GS; Choi, H; Joo, Y; Kim, SG. Ecology and Evolution (2020) 12: e9459

Lemna perpusilla and *Lemna minor* are free-floating plants that often live in the same habitat. However, little is known about how they differ in response to herbivore attacks. In this study, we examined the species-specific resistance of two Lemna species to the duckweed weevil, *Tanysphyrus lemnae*. The female adults of *T. lemnae* preferred to lay eggs on *L. perpusilla* over *L. minor*. In addition, the larvae of *T. lemnae* performed better when fed on *L. perpusilla* than on *L. minor*. To understand the physiological basis of species-specific resistance in the two *Lemna* species, we measured the amounts of jasmonic acid (JA), phytosterols, and flavonoids. Attacks by duckweed weevils increased the levels of JA in the two *Lemna* species, but these levels did not differ significantly between the two species. Interestingly, the levels of flavones (isoorientin, vitexin, and isovitexin) in *L. minor* species were higher than those in *L. perpusilla*. The in vitro bioassay showed that three flavones significantly decreased the survival rate of duckweed weevil larvae. Although *L. perpusilla* was less resistant to duckweed weevil attack compared to *L. minor*, *L. perpusilla* grew faster than *L. minor* regardless of the duckweed weevil attack. These results suggest that these two Lemna species have different defense strategies against the duckweed weevil.

Alleviation of aqueous nitrogen loss from paddy fields by growth and decomposition of duckweed (*Lemna minor* L.) after fertilization

Wang, Y; Chen, X; Guo, B; Liu, C; Liu, J; Qiu, G; Fu, Q; Li, H. Chemosphere (2023) 311: 137073

Runoff loss of nitrogen from paddy fields has received increasing attention in recent years. Duckweed is an aquatic plant frequently found in paddy fields. In this study, the effects of duckweed (*Lemna minor* L.) in floodwater on aqueous nitrogen losses from paddy fields were systematically investigated. Results demonstrated that the growth of duckweed decreased total nitrogen concentrations in floodwater and nitrogen runoff loss from paddy fields by 16.7%-18.3% and 11.2%-13.6%, respectively. Moreover, compared with NO_3^-, NH_4^+ was preferentially removed by duckweed. ^{15}N isotope tracer experiments revealed that the growth and decomposition of duckweed acted as a "buffer" against the nitrogen variation in floodwater after fertilization. During the growth of duckweed, leaves were found to be the principal organ to assimilate NH_4^+ and release NO_3^- by using non-invasive micro-test technology. Duckweed degradation increased the content of hydrophobic acids and marine humic-like substances in floodwater, which promoted the migration of nitrogen from floodwater to soil. Redundancy analysis and structural equation models further illustrated that pH and temperature variation in floodwater caused by duckweed played a greater role in aqueous nitrogen loss reduction than the nitrogen accumulation in duckweed. This study suggested that the growth of duckweed in paddy fields was an effective supplementary method for controlling aqueous nitrogen loss during agricultural production.

Biochemistry

Exogenous melatonin enhances the growth and production of bioactive metabolites in *Lemna aequinoctialis* culture by modulating metabolic and lipidomic profiles

Baek, G; Lee, H; Ko, J; Choi, HK. BMC Plant Biology (2022) 22: 545

Lemna species are cosmopolitan floating plants that have great application potential in the food/feed, pharmaceutical, phytoremediation, biofuel, and bioplastic industries. In this study, the effects of exogenous melatonin (0.1, 1, and 10 μM) on the growth and production of various bioactive metabolites and intact lipid species were investigated in *Lemna aequinoctialis* culture. Melatonin treatment significantly enhanced the growth (total dry weight) of the *L. aequinoctialis* culture. Melatonin treatment also increased cellular production of metabolites including beta-alanine, ascorbic acid, aspartic acid, citric acid, chlorophyll, glutamic acid, phytosterols, serotonin, and sucrose, and intact lipid species; digalactosyldiacylglycerols, monogalactosyldiacylglycerols, phosphatidylinositols, and sulfoquinovosyldiacylglycerols. Among those metabolites, the productivity of campesterol (1.79 mg/L) and stigmasterol (10.9 mg/L) were the highest at day 28, when 10 μM melatonin was treated at day 7. These results suggest that melatonin treatment could be employed for enhanced production of biomass or various bioactive metabolites and intact lipid species in large-scale *L. aequinoctialis* cultivation as a resource for food, feed, and pharmaceutical industries.

Total active compounds and mineral contents in *Wolffia globosa*

Monthakantirat, O; Chulikhit, Y; Maneenet, J; Khamphukdee, C; Chotritthirong, Y; Limsakul, S; Punya, T; Turapra, B; Boonyarat, C; Daodee, S. Journal of Chemistry (2022) 2022: 9212872

Wolffia globosa, or watermeal, is an aquatic plant belonging to the Lemnaceae family that is consumed as food and sold in local markets of Thailand. The aim of this study was to quantify selected active compounds and minerals in *W. globosa* ethanolic extract and evaluate its antioxidant activity. Total phenolic, flavonoid, and anthocyanin contents were analyzed. High-performance liquid chromatography was used for the determination of beta-carotene, ferulic acid, luteolin-7-O-beta-D-glucoside, and kaempferol. Mineral contents (iron, potassium, calcium, magnesium, zinc, and sodium) were determined by atomic absorption spectroscopy. Antioxidative activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2 '-azobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays. The beta-carotene, ferulic acid, luteolin-7-O-beta-D-glucoside, and kaempferol contents of the extract were 2.52±0.10, 1.40±0.10, 2.42±0.50, and 1.57±0.14 mg/g extract, respectively. The highest mineral content in the *W. globosa* extract was magnesium. The wet extract of *W. globosa* showed higher amounts of all minerals than the dry extract. Freshly prepared and boiled *W. globosa* extracts showed radical scavenging activity at 1000 μg/ml with 75.77±0.93% and 67.10±0.20% inhibition of DPPH and 70.40±7.20% and 59.78±3.16% inhibition of ABTS, respectively. This plant is a promising novel source of natural phytochemical constituents and antioxidants and has potential for development as a plant-based nutraceutical product for the treatment of diseases caused by free radicals.

Biotechnology

Duckweed is a promising feedstock of biofuels: Advantages and approaches

Yang, G-L. International Journal of Molecular Sciences (2022) 23: DOI10.3390/ijms232315231

With the growing scarcity of traditional sources of energy and the accompanying acute environmental challenges, biofuels based on biomass are favored as the most promising alternative. As one of the core raw materials for biomass energy, research on its production methods and synthesis mechanisms is emerging. In recent years, duckweed has been used as a high-quality new biomass feedstock for its advantages, including fast biomass accumulation, high starch content, high biomass conversion efficiency, and sewage remediation. This study provides a systematic review of the growth characteristics, starch metabolism pathways, and methods to improve starch accumulation in the new energy plant, duckweed. The study also presents a prospect that might be used as a reference for the development of duckweed as a new energy-providing plant.

Re-circulating indoor vertical farm: Technicalities of an automated duckweed biomass production system and protein feed product quality evaluation

Petersen, F; Demann, J; von Salzen, J; Olfs, HW; Westendarp, H; Wolf, P; Appenroth, KJ; Ulbrich, A. Journal of Cleaner Prouction (2022) 380: 134894

Duckweeds are fast-growing and nutritious plants, which are gaining increased attention in different fields of application. Especially for animal nutrition, alternative protein sources are needed to substitute soybean meal. The current bottleneck is the standardized production of biomass, which yields stable quantities of a defined product quality. To solve this problem, an indoor vertical farm (IVF) for duckweed biomass production was developed. It consists of nine vertically stacked basins with a total production area of 25.5 m^2. The nutrient solution, a modified N-medium, re-circulated within the IVF with a maximum flow rate of 10 L min^{-1}. Nutrients were automatically added based on electrical conductivity. In contrast, ammonium was continuously supplied. A water temperature of 23°C and a light intensity of 105 µmol m^{-2} s^{-1} with a photoperiod of 12:12 h were applied. During a 40-day production phase, a total of 35.6 kg of fresh duckweed biomass (equals 2.1 kg of dried product) was harvested from the IVF. On average, 0.9 kg day^{-1} of fresh biomass was produced. The dried product contained 32% crude protein (CP) and high levels of proteinogenic amino acids (e.g. lysine: 5.42 g, threonine: 3.85 g and leucine: 7.59 g/100 g CP). Biomass of this quality could be used as a protein feed alternative to soybean meal. The described IVF represents a modular model system for duckweed biomass production in a controlled environment and further innovations and upscaling processes.

Graphene/hydroxyapatite nano-composite for enhancement of hydrogen productivity from delignified duckweed

Tawfik, A; Tan, XF; Elsamadony, M; Qyyum, MA; Azzam, AM; Mubashir, M; Ng, HS; Akhtar, MS; Khoo, KS. Fuel (2022) 330: 125537

The incredible growth of the duckweed (DW) in the water ecosystem causes severe environmental problems and reduces biodiversity. Harvesting and valorization of such plants represents a challenge due to its richness with organics. Hydrogen productivity (HP) from dark fermentation of delignified duckweed (DDW) is the main objective of current research. The anaerobe cultures were immobilized on the Graphene/hydroxyapatite nano-composite (nG/HAP) for enhancement of hydrogen yield (HY) and enzyme activities. The HP of 13.5±0.78 mL was obtained from the fermentation of native DW and significantly increased up to 208.9±12.6 mL for delignified one. This was linked to an increase of the hydrogenase enzyme (HE) activities from 0.02±0.0003 to 0.109±0.004 mg M.B reduced/min. The HP was further increased up to 387.1±13.6 mL for batches containing 80 mg/gVS of nG/HAP. nG/HAP inhibited the competitors and promoted the cooperators for the Firmicutes hydrogen producers.

Hydro-liquefaction of *Lemna minor* (Duckweed) with hydrogen-donor solvent at varying hydrogen pressures

Acharya, S; Kishore, N. International Journal of Sustainable Energy (2022) DOI10.1080/14786451.2022.2140157

The authors have investigated hydro-liquefaction of *Lemna minor* (duckweed) aquatic biomass and a hydrogen-donor solvent, methanol, at temperatures of 180°C, 200°C and 220°C and final reactor pressure values of 70, 80 and 90 bar. The rise in reactor pressure for all reaction temperatures led to an increase in biocrude yield with a maximum higher heating value of 22.19 MJ/kg, while the biochar also depicted its potential to be used in varied applications and as a bio-refinery feedstock. Fourier Transform Infrared Spectroscopy and Gas Chromatography Mass Spectrometry analysis of biocrude indicated the presence of esters and long chain alkanes, ethers, phenols, etc. The main finding of this work disclosed that fuel properties of the present biocrude (without any catalyst) were better than their non-catalytic and hydro-catalytic bio-oil counterparts obtained from similar biomass at a pyrolysis temperature of 550°C.

The response of duckweed *Lemna minor* to microplastics and its potential use as a bioindicator of microplastic pollution

Rozman, U; Kalcikova, G. Plants (2022) 11: 2953

Biomonitoring has become an indispensable tool for detecting various environmental pollutants, but microplastics have been greatly neglected in this context. They are currently monitored using multistep physico-chemical methods that are time-consuming and expensive, making the search for new monitoring options of great interest. In this context, the aim of this study was to investigate the possibility of using an aquatic macrophyte as a bioindicator of microplastic pollution in freshwaters. Therefore, the effects and adhesion of three types of microplastics (polyethylene microbeads, tire wear particles, and polyethylene terephthalate fibers) and two types of natural particles (wood dust and cellulose particles) to duckweed *Lemna minor* were investigated. The results showed that fibers and natural particles had no effect on the specific growth rate, chlorophyll a content, and root length of duckweed, while a significant reduction in the latter was observed when duckweed was exposed to microbeads and tire wear particles. The percentage of adhered particles was ten times higher for polyethylene microbeads than for other microplastics and natural particles, suggesting that the adhesion of polyethylene microbeads to duckweed is specific. Because the majority of microplastics in freshwaters are made of polyethylene, the use of duckweed for their biomonitoring could provide important information on microplastic pollution in freshwaters.

Ecology

Disentangling the mechanisms sustaining a stable state of submerged macrophyte dominance against free-floating competitors

Szabo, S; Koleszar, G; Zavanyi, G; Nagy, PT; Braun, M; Hilt, S. Frontiers in Plant Science (2022) 13: 963579

Free-floating and rootless submerged macrophytes are typical, mutually exclusive vegetation types that can alternatively dominate in stagnant and slow flowing inland water bodies. A dominance of free-floating plants has been associated with a lower number of aquatic ecosystem services and can be explained by shading of rootless submerged macrophytes. Vice versa, high pH and competition for several nutrients have been proposed to explain the dominance of rootless submerged macrophytes. Here, we performed co-culture experiments to disentangle the influence of limitation by different nutrients, by pH effects and by allelopathy in sustaining the dominance of rootless submerged macrophytes. Specifically, we compared the effects of nitrogen (N), phosphorus (P), iron (Fe) and manganese (Mn) deficiencies and an increased pH from 7 to 10 in reducing the growth of free-floating *Lemna gibba* by the rootless *Ceratophyllum demersum*. These macrophyte species are among the most common in highly eutrophic, temperate water bodies and known to mutually exclude each other. After co-culture experiments, additions of nutrients and pH neutralisation removed the growth inhibition of free-floating plants. Among the experimentally tested factors significantly inhibiting the growth of *L. gibba*, an increase in pH had the strongest effect, followed by depletion of P, N and Fe. Additional field monitoring data revealed that in water bodies dominated by *C. demersum*, orthophosphate concentrations were usually sufficient for optimal growth of free-floating plants. However, pH was high and dissolved inorganic N concentrations far below levels required for optimal growth. Low N concentrations and alkaline pH generated by dense *C. demersum* stands are thus key factors sustaining the stable dominance of rootless submerged vegetation against free-floating plants. Consequently, N loading from e.g. agricultural runoff, groundwater or stormwater is assumed to trigger regime shifts to a dominance of free-floating plants and associated losses in ecosystem services.

Pond greenhouse gas emissions controlled by duckweed coverage

Rabaey, J; Cotner, J. Frontiers in Environmental Science (2022) 10: 889289

Freshwaters are significant contributors of greenhouse gases to the atmosphere, including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Small waterbodies such as ponds are now recognized to have disproportionate greenhouse gas emissions relative to their size, but measured emissions from ponds have varied by several orders of magnitude. To assess drivers of variation in pond greenhouse gas dynamics, we measured concentrations and emissions of CO_2, CH_4, and N_2O across 26 ponds in Minnesota, United States, during the ice-free season. The studied ponds differed in land-use, from urban stormwater ponds to natural forested ponds. The ponds were all sources of greenhouse gases, driven by large CH_4 emissions (mean 704 [sd 840] mg CH_4-C m^{-2} d^{-1}). CO_2 fluxes were variable, but on average a sink (mean -25.9 [sd 862] mg CO_2-C m^{-2} d^{-1}), and N_2O emissions were generally low (mean 0.398 [sd 0.747] mg N_2O-N m^{-2} d^{-1}). Duckweed coverage on the water surfaces ranged from 0% to 100% coverage, and had the largest influence on water chemistry and greenhouse gas dynamics across the ponds. Duckweed covered ponds (ponds with greater than 85% coverage) had higher phosphorus levels and increased anoxia compared to ponds without duckweed (ponds with less than 12% coverage), leading to higher CH_4 concentrations and overall greenhouse gas emissions in the duckweed ponds. Duckweed ponds had a mean emission rate in CO_2 equivalents of 30.9 g C m^{-2} d^{-1} compared to 11.0 g C m^{-2} d^{-1} in non-duckweed ponds.

Feed & Food

Safety of *Lemna minor* and *Lemna gibba* whole plant material as a novel food pursuant to Regulation (EU) 2015/2283.

Turck, D; Bohn, T; Castenmiller, J; De Henauw, S; Hirsch-Ernst, KI; Maciuk, A; Mangelsdorf, I; McArdle, HJ; Naska, A; Pelaez, C; Pentieva, K; Siani, A; Thies, F; Tsabouri, S; Vinceti, M; Cubadda, F; Frenzel, T; Heinonen, M; Prieto Maradona, M; Marchelli, R; Neuhauser-Berthold, M; Poulsen, M; Schlatter, JR; van Loveren, H; Kouloura, E; Knutsen, HK. EFSA Journal. European Food Safety Authority (2022) 20: e07598

Following a request from the European Commission, the EFSA Panelon Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on *Lemna minor* and *Lemna gibba* whole plant material as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Lemna minor* and *L. gibba* are aquatic plants commonly named water lentils. The NF is produced by cultivation of *L. minor* and *L. gibba* plants, washing with water and heat treatment. The main constituents of the NF are water, protein and fibre. The Panel notes that the concentration of trace elements and contaminants in the NF is highly dependent on the conditions of cultivation of the plant and the fertiliser composition. The NF is intended to be used as a vegetable, similar to other leafy vegetables. The target population is the general population. The Panel considers that, with the exception of concerns related to the manganese intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The Panel considers that the risk that the consumption of the NF may trigger allergic reactions in humans is low. The Panel concludes that the NF, in consideration of its proposed uses and the concentration of manganese as compared to the normally present concentration of manganese in other leafy vegetables, may be of safety concern, therefore, the safety of the NF cannot currently be established.

Inclusion of duckweed (*Lemna minor*) in the diet improves flesh omega-3 long-chain polyunsaturated fatty acid profiles but not the growth of farmed Nile tilapia (*Oreochromis niloticus*)

Opiyo, MA; Muendo, P; Mbogo, K; Ngugi, CC; Charo-Karisa, H; Orina, P; Leschen, W; Glencross, BD; Tocher, DR. Animal Feed Science and Technology (2022) 292: 115442

A 12-week experiment was conducted to determine the effect of dietary duckweed (*Lemna minor*) on growth performance, body composition and fatty acid profiles of juvenile Nile tilapia (*Oreochromis niloticus*). Five isonitrogenous (30 % crude protein) diets were prepared with the inclusion of dry ground L. minor at 0 %, 5 %, 10 %, 15 % and 20 % of diet dry weight. Each diet was randomly allocated to triplicate tanks stocked with juvenile tilapia that had an initial mean weight of 2.00±0.01 g. The juvenile tilapia were reared in a flow-through system with each diet fed twice a day at a total ratio of 5 % body weight per day. While weight gain and specific growth rate (SGR) were generally higher, and food conversion ratio (FCR) generally lower in fish fed the control diet than fish fed *L. minor*, there were no significant differences in these performance parameters between tilapia fed *L. minor* at 15 % inclusion and fish fed the control diet. Survival ranged from 80 % to 96 % and was significantly higher in fish fed the control diet. Dietary *L. minor* significantly reduced whole-body total lipid contents and increased moisture contents. The dietary inclusion of *L. minor* significantly increased the proportions of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) in muscle, with eicosapentaenoic (EPA) and docosahex-aenoic (DHA) acids increasing by over 10-fold from 0.5 % each of total fatty acids, respectively, in fish fed the control diet to 5 % each in fish fed 20 % *L. minor*. Since the inclusion of *L. minor* increased the contents of 18:3n-3, but not EPA and DHA in the diets, this indicates that the increased proportions of EPA and DHA in the muscle of tilapia fed *L. minor* was due to endogenous biosynthesis of LC-PUFA with the conversion of dietary 18:3n-3 to EPA and DHA. Therefore, the present study indicated that dietary inclusion of 15-20 % *L. minor* in feeds for Nile tilapia can significantly improve the omega-3 fatty acid profile of muscle for human consumers, with only minor effects on growth and feed utilization. These findings demonstrated the potential for the utilization of *L. minor* as an ingredient to increase omega-3 LC-PUFA, EPA and DHA, contents of farmed tilapia while reducing the use of fish oil and fish meal in tilapia feeds.

Interaction with other organisms

The Effects of microbiota on the herbivory resistance of the Giant Duckweed are plant genotype-dependent

Schafer, M; Xu, S. Plants (2022) 23: DOI10.3390/plants11233317

In nature, all plants live with microbes, which can directly affect their host plants' physiology and metabolism, as well as their interacting partners, such as herbivores. However, to what extent the microbiota shapes the adaptive evolution to herbivory is unclear. To address this challenge, it is essential to quantify the intra-specific variations of microbiota effects on plant fitness. Here, we quantified the fitness effects of microbiota on the growth, tolerance, and resistance to herbivory among six genotypes of the giant duckweed, *Spirodela polyrhiza*. We found that the plant genotypes differed in their intrinsic growth rate and tolerance, but not in their resistance to a native herbivore, the great pond snail. Inoculation with microbiota associated with *S. polyrhiza* growing outdoors reduced the growth rate and tolerance in all genotypes. Additionally, the microbiota treatment altered the herbivory resistance in a genotype-specific manner. Together, these data show the potential of microbiota in shaping the adaptive evolution of plants.

Dynamic alteration of microbial communities of duckweeds from nature to nutrient-deficient condition

Bunyoo, C; Roongsattham, P; Khumwan, S; Phonmakham, J; Wonnapinij, P; Thamchaipenet, A. Plants (2022) 11: 2915

Duckweeds live with complex assemblages of microbes as holobionts that play an important role in duckweed growth and phytoremediation ability. In this study, the structure and diversity of duckweed-associated bacteria (DAB) among four duckweed subtypes under natural and nutrient-deficient conditions were investigated using V3-V4 16S rRNA amplicon sequencing. High throughput sequencing analysis indicated that phylum Proteobacteria was predominant in across duckweed samples. A total of 24 microbial genera were identified as a core microbiome that presented in high abundance with consistent proportions across all duckweed subtypes. The most abundant microbes belonged to the genus *Rhodobacter*, followed by other common DAB, including *Acinetobacter*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Pseudomonas*. After nutrient-deficient stress, diversity of microbial communities was significantly deceased. However, the relative abundance of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Pelomonas, Roseateles* and *Novosphingobium* were significantly enhanced in stressed duckweeds. Functional prediction of the metagenome data displayed the relative abundance of essential pathways involved in DAB colonization, such as bacterial motility and biofilm formation, as well as biodegradable ability, such as benzoate degradation and nitrogen metabolism, were significantly enriched under stress condition. The findings improve the understanding of the complexity of duckweed microbiomes and facilitate the establishment of a stable microbiome used for co-cultivation with duckweeds for enhancement of biomass and phytoremediation under environmental stress.

Molecular Biology

Chromosome numbers and genome sizes of all 36 duckweed species (Lemnaceae)

Hoang, PTN; Fuchs, J; Schubert, V; Tran, TBN; Schubert, I. Plants (2022) 11: 2674

Usually, chromosome sets (karyotypes) and genome sizes are rather stable for distinct species and therefore of diagnostic value for taxonomy. In combination with (cyto)genomics, both features provide essential clues for genome evolution and phylogenetic relationship studies within and between taxa above the species level. We present for the first time a survey on chromosome counts and genome size measurement for one or more accessions from all 36 duckweed species and discuss the evolutionary impact and peculiarities of both parameters in duckweeds.

Local endocytosis of sucrose transporter 2 in duckweed reveals the role of sucrose transporter 2 in guard cells

Liu, PH; Fang, Y; Tan, X; Hu, ZB; Jin, YL; Yi, ZL; He, KZ; Wei, CC; Chen, R; Zhao, H. Frontier in Plant Science (2022) 13: 996618

The local endocytosis of membrane proteins is critical for many physiological processes in plants, including the regulation of growth, development, nutrient absorption, and osmotic stress response. Much of our knowledge on the local endocytosis of plasma membrane (PM) protein only focuses on the polar growth of pollen tubes in plants and neuronal axon in animals. However, the role of local endocytosis of PM proteins in guard cells has not yet been researched. Here, we first cloned duckweed SUT2 (sucrose transporter 2) protein and then conducted subcellular and histological localization of the protein. Our results indicated that LpSUT2 (*Landoltia punctata* 0202 SUT2) is a PM protein highly expressed on guard cells. In vitro experiments on WT (wild type) lines treated with high sucrose concentration showed that the content of ROS (reactive oxygen species) in guard cells increased and stomatal conductance decreased. We observed the same results in the lines after overexpression of the LpSUT2 gene with newfound local endocytosis of LpSUT2. The local endocytosis mainly showed that LpSUT2 was uniformly distributed on the PM of guard cells in the early stage of development, and was only distributed in the endomembrane of guard cells in the mature stage. Therefore, we found the phenomenon of guard cell LpSUT2 local endocytosis through the changes of duckweed stomata and concluded that LpSUT2 local endocytosis might be dependent on ROS accumulation in the development of duckweed guard cells. This paper might provide future references for the genetic improvement and water-use efficiency in other crops.

Over-expression of phosphoserine aminotransferase-encoding gene (AtPSAT1) prompts starch accumulation in *L. turionifera* under nitrogen starvation

Wang, L; Li, SL; Sun, L; Tong, YN; Yang, L; Zhu, YR; Wang, Y. International Journal of Molecular Sciences (2022) 23: 11563

It has been demonstrated that the phosphorylation pathway of L-serine (Ser) biosynthesis (PPSB) is very important in plant growth and development, but whether and how PPSB affects nitrogen metabolism and starch accumulation has not been fully elucidated. In this study, we took the energy plant duckweed (strain *Lemna turionifera* 5511) as the research object and used a stable genetic transformation system to heterologously over-expressing Arabidopsis AtPSAT1 (the gene encoding phosphoserine aminotransferase, the second enzyme of PPSB). Our results showed that, under nitrogen starvation, the transgenic plants grew faster, with higher values of Fv/Fm, rETR, and Y(II), as well as fresh and dry weight, than the wild-type. More promisingly, the accumulation of starch was also found to be significantly improved when over-expressing AtPSAT1 in the transgenic plants. qRT-PCR analysis results showed that the expression of genes related to nitrogen assimilation, carbon metabolism, and starch biosynthesis was up-regulated, while the expression of starch degradation-related genes was down-regulated by AtPSAT1 over-expression. We propose that the increased starch accumulation caused by AtPSAT1 over-expression may result from both elevated photosynthetic capacity and nitrogen utilization efficiency. This research sheds new light on the mechanism underlying the ability of PPSB to coordinate nitrogen and carbon metabolism, and provides a feasible way to improve starch production, that is, through engineering PPSB in crops.

Genome-wide identification of nitrate transporter genes from *Spirodela polyrhiza* and characterization of SpNRT1.1 function in plant development

Lv, ML; Dong, TT; Wang, J; Zuo, KJ. Frontiers in Plant Science (2022) 13: 945470

Nitrate transporter (NRT) genes that participate in nitrate transport and distribution are indispensable for plant growth, development, and stress tolerance. *Spirodela polyrhiza* has the smallest genome among monocotyledon plants, and it has strong nitrate absorbance and phytoremediation abilities. However, the evolutionary history, expression patterns, and functions of the NRT gene family in *S. polyrhiza* are not well understood. Here, we identified 29 NRT members in the *S. polyrhiza* genome. Gene structure and phylogeny analyses showed that *S. polyrhiza* nitrate transporter (SpNRTs) genes were divided into eight clades without gene expansion compared with that in Arabidopsis. Transcriptomic analysis showed that SpNRT genes have spatiotemporal expression patterns and respond to abiotic stress. Functional analysis revealed that in *S.*

polyrhiza, SpNRT1.1 expression was strongly induced by treatment with nitrate and ammonium. Overexpression of SpNRT1.1 significantly repressed primary root length, and the number and total length of lateral roots. This was more pronounced in high ammonium concentration medium. Overexpressed SpNRT1.1 in Arabidopsis significantly improved biomass and delayed flowering time, indicating that the nitrate transport ability of SpNRT1.1 differs from AtNRT1.1. In conclusion, our results provide valuable information about the evolution of the NRT family in higher plants and the function of SpNRT1.1.

Physiology & Stress

DNA methylation in clonal duckweed (*Lemna minor* L.) lineages reflects current and historical environmental exposures

Van Antro, M; Prelovsek, S; Ivanovic, S; Gawehns, F; Wagemaker, NCAM; Mysara, M; Horemans, N; Vergeer, P; Verhoeven, KJF. Molecular Ecology (2022) DOI10.1111/mec.16757

Environmentally induced DNA methylation variants may mediate gene expression responses to environmental changes. If such induced variants are transgenerationally stable, there is potential for expression responses to persist over multiple generations. Our current knowledge in plants, however, is almost exclusively based on studies conducted in sexually reproducing species where the majority of DNA methylation changes are subject to resetting in germlines, limiting the potential for transgenerational epigenetics stress memory. Asexual reproduction circumvents germlines, and may therefore be more conducive to long-term inheritance of epigenetic marks. Taking advantage of the rapid clonal reproduction of the common duckweed *Lemna minor*, we hypothesize that long-term, transgenerational stress memory from exposure to high temperature can be detected in DNA methylation profiles. Using a reduced representation bisulphite sequencing approach (epiGBS), we show that temperature stress induces DNA hypermethylation at many CG and CHG cytosine contexts but not CHH. Additionally, differential methylation in CHG context that was observed was still detected in a subset of cytosines, even after 3-12 generations of culturing in a common environment. This demonstrates a memory effect of stress reflected in the methylome and that persists over multiple clonal generations. Structural annotation revealed that this memory effect in CHG methylation was enriched in transposable elements. The observed epigenetic stress memory is probably caused by stable transgenerational persistence of temperature-induced DNA methylation variants across clonal generations. To the extent that such epigenetic memory has functional consequences for gene expression and phenotypes, this result suggests potential for long-term modulation of stress responses in asexual plants.

Transcriptome analysis reveals genes expression pattern of *Spirodela polyrhiza* response to heat stress

Shang, S; Zhang, Z; Li, L; Chen, J; Zang, Y; Liu, X; Wang, J; Tang, X. International Journal of Biological Macromolecules (2022) DOI10.1016/j.ijbiomac.2022.11.139

With global warming, high-temperature stress has become an essential abiotic factor affecting plant growth and survival. However, little knowledge was available of the molecular mechanism that aquatic plants respond to this stress. In the present study, we explore the adaptation mechanism of *Spirodela polyrhiza*, a surface-water-grown duckweed species broadly distributed worldwide to high temperatures, and analyze its gene expression pattern of *S. polyrhiza* under heat stress. Three temperature stress treatments, including room temperature group (CK), middle high-temperature group (MTS), and high-temperature group (45°C, HTS) were set. The results showed that the contents of SOD first increased and then decreased, and those of MDA showed an upward trend under elevated high-temperature stress. According to the transcriptome data, 3145, 3487, and 3089 differently expressed genes (DEGs) were identified between MTS and CK, HTS and CK, and HTS and MTS, respectively. The transcription factors (TFs) analysis showed that 14 deferentially expressed TFs, including HSF, ERF, WRKY, and GRAS family, were responsive to heat stress, suggesting they might play vital roles in improving resistance to heat stress. In conclusion, *S. polyrhiza* could resist high temperatures by increasing SOD activity and MDA at the physiological level. Several transcription factors, energy accumulation processes, and cell membranes were involved in high-temperature stress at the molecular level. Our findings are helpful in better grasping the adaptation rules of some aquatic plants to high temperatures.

The importance of ecotype diversity on duckweed growth with and without salt stress

van Moorsel, SJ. Journal of Plant Ecology (2022) 15: 1065-1079

The pollution of freshwater ecosystems is threatening freshwater plant species diversity worldwide. Freshwater plants, such as the common duckweed (*Lemna minor*), are potentially sensitive to novel stressful environments. To test if ecotype diversity could increase resistance to stressful environments, I used seven *L. minor* populations and measured their growth rates with and without moderate salt stress across an ecotype diversity gradient. The *L. minor* populations were grown over 5 months in 92 experimental mesocosms, either in ecotype monocultures or in polyculture with either one or three conspecific ecotypes (23 unique compositions). After growing the duckweed in unperturbed conditions (phase 1), the cultures were subjected to moderate salt stress (50 mmol/L NaCl) for several weeks (phase 2). The experiment was conducted in the presence of the natural epimicrobial community associated with the different ecotypes. In phase 2, a subset of these algae added an unintentional second stressor to the experiment. The ecotypes differed in their growth rates, the fastest growing at twice the rate of others. The diversity context further shaped the ecotype growth rates. Ecotype polycultures showed higher abundances towards the end of the experiment, thus over time, as the environment deteriorated, ecotype diversity gained in importance. These findings show that within-species variation in growth rates can translate to a positive effect of ecotype diversity on population abundance. Exposure of *L. minor* to moderate salt levels did not significantly impact growth rates, although the effect may have been masked by reduced algal stress in the saline environments.

Phytoremediation

Potential of *Lemna minor* and *Eichhornia crassipes* for the phytoremediation of water contaminated with Nickel (II)

Moreno-Rubio, N; Ortega-Villamizar, D; Marimon-Bolivar, W; Bustillo-Lecompte, C; Tejeda-Benitez, LP. Environmental Monitoring and Assessment (2023) 195: 119

Phytoextraction of Nickel (II) in water by two types of aquatic macrophytes (*Lemna minor* and *Eichhornia crassipes*) was investigated using synthetic aqueous solutions of $NiSO_4$ at concentrations of 0.5, 1.5 and 2.5 mg/L. The toxic effects of nickel salt in plants were evaluated through the presence of necrosis and chlorosis. The bioconcentration factor, Nickel (II) removal efficiency and kinetics of removal were also calculated. Results of this study show bioconcentration factors higher than 1000, which categorize *L. minor* and *E. crassipes* as hyperaccumulators. Besides, *L. minor* presented a removal percentage higher than 68%, compared to *E. crassipes* that did not exceed 50% in any of the three concentrations studied. However, *E. crassipes* showed better resistance to the effects of nickel and obtained a greater removal capacity during the phytoremediation process that lasted for 10 days. In contrast, *L. minor* suffered necrosis and chlorosis in a concentration-dependent way. Consequently, both macrophytes are sustainable alternatives for nickel removal from contaminated water.

The impact of salt accumulation on the growth of duckweed in a continuous system for pig manure treatment

Lambert, M; Devlamynck, R; Fernandes de Souza, M; Leenknegt, J; Raes, K; Eeckhout, M; Meers, E. Plants (2022) 11: DOI10.3390/plants11233189

Duckweed (*Lemna*) is a possible solution for the treatment of aqueous waste streams and the simultaneous provision of protein-rich biomass. Nitrification-Denitrification effluent (NDNE) from pig manure treatment has been previously used as a growing medium for duckweed. This study investigated the use of a continuous duckweed cultivation system to treat NDNE as a stand-alone technology. For this purpose, a system with a continuous supply of waste streams from the pig manure treatment, continuous biomass production, and continuous discharge that meets the legal standards in Flanders (Belgium) was simulated for a 175-day growing season. In this simulation, salt accumulation was taken into account. To prevent accumulating salts from reaching a toxic concentration and consequently inhibiting growth, the cultivation system must be buffered, which can be achieved by altering the depth of the system. To determine the minimum depth of such a system, a tray experiment was set up. For that, salt accumulation data obtained from previous research were used for simulating systems with different pond depths. It was found that a depth of at least 1 m is needed to prevent a significant relative growth inhibition at the end of the growing season compared to the start. This implies a high water consumption (5-10 times more than maize). As a response, a second cultivation system was investigated for the use of more concentrated NDNE. For this purpose, salt tolerance experiments were conducted on synthetic and biological media. Surprisingly, it was observed that duckweed grows better on diluted NDNE (to 75% NDNE, or EC of 8 mS/cm) than on a synthetic medium (EC of 1.5 mS/cm), indicating the potential of such a system.

Cultivation of *Lemna minor* on industry-derived, anaerobically digested, dairy processing wastewater

O'Mahoney, R; Coughlan, NE; Walsh, E; Jansen, MAK. Plants (2022) 11: 3027

The growth and nutrient uptake capacity of a common duckweed (Lemnaceae) species, *Lemna minor* "Blarney", on dairy processing wastewater pre-treated by an anaerobic digester (AD-DPW) was explored. *L. minor* was cultivated in small stationary vessels in a controlled indoor environment, as well as in a semi-outdoor 35 L recirculatory system. The use of AD-DPW as a cultivation medium for *L. minor* offers a novel approach to dairy wastewater treatment, evolving from the current resource-intensive clean-up of wastewaters to duckweed-based valorisation, simultaneously generating valuable plant biomass and remediating the wastewater.

Integrated multitrophic aquaculture; Analysing contributions of different biological compartments to nutrient removal in a duckweed-based water remediation system

Paolacci, S; Stejskal, V; Toner, D; Jansen, MAK. Plants (2022) 11: 3103

Duckweed (Lemnaceae) can support the development of freshwater aquaculture if used as extractive species in Integrated MultiTrophic Aquaculture (IMTA) systems. These aquatic plants have the advantage of producing protein-rich biomass that has several potential uses. On the contrary, other biological compartments, such as microalgae and bacteria, present in the water and competing with duckweed for light and nutrients cannot be harvested easily from the water. Moreover, as phytoplankton cannot easily be harvested, nutrients are eventually re-released; hence, this compartment does not contribute to the overall water remediation process. In the present study, a mesocosm experiment was designed to quantify the portion of nutrients effectively removed by duckweed in a duckweed-based aquaculture wastewater remediation system. Three tanks were buried next to a pilot-scale IMTA system used for the production of rainbow trout and perch. The tanks received aquaculture effluents from the adjacent system, and 50% of their surface was covered by duckweed. Daily water analyses of samples at the inlet and outlet of the mesocosm allowed quantification of the amount of nutrients removed in total. The portion removed by duckweed was determined by examining the nutrient content in the initial and final biomass. The portion of nutrients removed by other compartments was similarly estimated. The results show that duckweed is responsible for the removal of 31% and 29% of N and P, respectively. Phytoplankton removed 33% and 38% of N and P, respectively, while the biofilm played no major role in nutrient removal. The remainder of the removed nutrients were probably assimilated by bacteria or sedimented. It is speculated that a higher initial duckweed density can limit phytoplankton growth and, therefore, increase the portion of nutrients removed by the duckweed compartment.

Removal of copper by *Azolla filiculoides* and *Lemna minor*: phytoremediation potential, adsorption kinetics and isotherms

Al-Baldawi, IA; Yasin, SR; Jasim, SS; Abdullah, SRS; Almansoory, AF; Ismail, N. Heliyon (2022) 8: 11456

Phytoremediation is an eco-friendly biotechnology with low costs. The removal of copper (Cu) from polluted water by the two floating plant species *Azolla filiculoides* and *Lemna minor* was observed and recorded. Plants were exposed to different Cu (II) concentration (0.25-1.00 mg/L) and sampling time (Days 0, 1, 2, 5 and 7). Both plants can remove Cu at 1.00 mg Cu/L water, with the highest removal rates of 100% for *A. filiculoides* and 74% for *L. minor* on the fifth day of exposure. At the end of the exposure period (Day 7), the growth of *A. filiculoides* exposed to 1.00 mg Cu/L was inhibited by Cu, but the structure of the inner cells of *A. filiculoides* was well organized as compared to the initial treatment period. Regarding *L. minor*, Cu at 1.00 mg/L negatively impacted both the growth and morphology (shrinking of its inner structure) of this plant. This is due to the higher accumulation of Cu in *L. minor* (2.86 mg/g) than in *A. filiculoides* (1.49 mg/g). Additionally, the rate of

Cu removal per dry mass of plant fitted a pseudo-second order model for both plants, whereas the adsorption equilibrium data fitted the Freundlich isotherm, indicating that Cu adsorption occurs in multiple layers. Based on the results, both species can be applied in the phytoremediation of Cu-polluted water.

Improvement of physicochemical parameters and removal of bacterial enteric pathogens in industrial wastewater by treatment with hybrid constructed wetlands

Hamoud, F; Bedouh, Y; Kahlouche, FZ; Filali, S; Merad, T. Chemistry and Ecology (2022) DOI10.1080/02757540.2022.2130272

The objective of this work was to evaluate the performance of a laboratory-scale Hybrid Constructed Wetland (HCW) planted with different macrophytes, at different hydraulic retention times (HRT), for physicochemical parameters improvement and bacterial enteric pathogens removal, in industrial wastewater. The results revealed that treatment efficiency of HCW planted with *Phragmites australis* and *Lemna minor* was kept on increasing with the increase in hydraulic retention time. Maximum efficiency of HCW was observed with a 12-day HRT, that is, 82.6, .90.9, 78.3, 92.1, 80.2, 93.5, 99.6, 99.3, and 100% reduction from the zero-time value for, TSS, BOD5, COD TKN, OP, TC, FC, MF, and FS, respectively. Interestingly, we found a strong negative relationship between pH and the majority of microbiological parameters which demonstrates its role in the phytoremediation process, especially in terms of mesophilic flora and faecal coliform removal.

Optimization of the elimination of antibiotics by *Lemna gibba* and *Azolla filiculoides* using response surface methodology (RSM)

Maldonado, I; Quispe, APV; Chacca, DM; Vilca, FZ. Frontiers in Environmental Science 10: 940971

Antibiotic residues have been found in environmental samples, such as water, soil, and even food, and usually come from wastewater, presenting environmental and human health risks. This study aimed to improve the elimination of the antibiotics tetracycline (Tet) and chloramphenicol (Chlor) by modifying three factors: contact time (3-7 days), plant biomass (10-14 g), and antibiotic concentration (5-15 mg/L Tet and 10-20 mg/L Chlor). An approach that optimizes time and resources, response surface methodology (RSM), was applied with a Box-Behnken design (BBD) to two plant species (*L. gibba* and *A. filiculoides*), i.e., one experimental design was used for each species. Antibiotic residues in water and plant samples were analyzed by liquid chromatography. The optimal conditions for Tet removal were 6.04 d, 11.4 g, and 13.4 mg/L with *Lemna* and 6.3 d, 11.9 g, and 14.7 mg/L with *Azolla*; the optimal conditions for Chlor removal were 7.8 d, 13.6 g, and 10.2 mg/L with *Lemna* and 4.6 d, 12.3 g, and 8.7 mg/L with *Azolla.* The results showed that the removal efficiency of antibiotics increased depending on the species used, reaching a maximum of up to 100%. Tet was better removed than Chlor, reaching maximum removal values of 100% and 84% with *Azolla* and *Lemna*, respectively. Chlor removal reached 70% and 64% with *Azolla* and *Lemna*, respectively. The mean bioconcentration factors (BCFs) of Tet were 2.9% in *Lemna* and 4.9% in *Azolla*, and the BCFs for Chlor were 38.1% in *Lemna* and 37.8% in *Azolla*. Thus, in general, better results were obtained with *Azolla.* In summary, the results demonstrate that this design and the selected plants contribute to the removal of antibiotics, presenting a sustainable and recommended alternative for the treatment of wastewater contaminated with antibiotic residues.

Phytotoxicity

Microplastics and co-pollutant with ciprofloxacin affect interactions between free-floating macrophytes

Mao, H; Yang, H; Xu, Z; Yang, Y; Zhang, X; Huang, F; Wei, L; Li, Z. Environmental Pollution (Barking, Essex) (2023) 316: 120546

Microplastic and antibiotic contamination are considered an increasing environmental problem in aquatic systems, while little is known about the impact of microplastics and co-pollutant with antibiotics on freshwater vascular plants, particularly the effects of interactions between macrophytes. Here, we performed a mesocosm experiment to evaluate the impact of polyethylene-microplastics and their co-pollutants with ciprofloxacin on the growth and physiological characteristics of *Spirodela polyrhiza* and *Lemna minor* and the interactions between these two macrophytes. Our results showed that microplastics alone cannot significantly influence fresh weight and specific leaf area of the two test free-floating macrophytes, but the effects on photosynthetic pigments, malondialdehyde, catalase and soluble sugar contents were species-specific. Ciprofloxacin can significant adverse effects on the growth and physiological traits of the two test macrophytes and microplastic mitigated the toxicity of ciprofloxacin on the two free-floating plants to a certain extent. In addition, our studies showed that microplastics and co-pollutants can influence relative yield and competitiveness of *S. polyrhiza* and *L. minor* by directly or indirectly influencing their physiology and growth. Therefore, our findings suggest that species-specific sensibility to microplastic and its co-pollutant among free-floating macrophytes may influence macrophyte population dynamics and thereby community structure and ecosystem functioning. And microplastics altered other contaminant behaviours and toxicity, and may directly or indirectly influence macrophytes interactions and community structure. The present study is the first experimental study exploring the effects of microplastics alone and with their co-pollutants on interactions between free-floating macrophytes.

Rare earth element scandium mitigates the chromium toxicity in *Lemna minor* by regulating photosynthetic performance, hormonal balance and antioxidant machinery

Alp, FN; Arikan, B; Ozfidan-Konakci, C; Ekim, R; Yildiztugay, E; Turan, M. Environmental Pollution (Barking, Essx) (2023) 316: 120636

Chromium (Cr) toxicity is a serious problem that threatens the health of living organisms and especially agricultural production. The presence of excess Cr leads to biomass loss by causing the imbalance of biochemical metabolism and inhibiting photosynthetic activity. A new critical approach to cope with Cr toxicity is the use of the rare earth elements (REEs) as an antioxidant defence system enhancer in plants. However, the effect of scandium (Sc), which is one of the REEs, is not clear enough in *Lemna minor* exposed to Cr toxicity. For this purpose, the photosynthetic and biochemical effects of scandium (50µM and 200µM Sc) treatments were investigated in *L. minor* under Cr stress (100µM, 200µM and 500µM Cr). Parameters related to photosynthesis (Fv/Fm, Fv/Fo) were suppressed under Cr stress. Stress altered antioxidant enzymes activities and hormone contents. Sc applications against stress increased the activities of superoxide dismutase (SOD), NADPH oxidase (NOX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and glutathione S-transferase (GST). In addition to the antioxidant system, the contents of indole-3-acetic acid (IAA), abscisic acid (ABA) and jasmonic acid (JA) were also rearranged. However, in all treatment groups, with the provision of ascorbate (AsA) regeneration and effective hormone signaling, reactive oxygen species (ROS) retention which result in high hydrogen peroxide (H_2O_2) content and lipid peroxidation (TBARS) were effectively removed. Sc promoted the maintenance of cellular redox state by regulating antioxidant pathways included in the AsA-GSH cycle. Our results showed that Sc has great potential to confer tolerance to duckweed by reducing Cr induced oxidative damage, protecting the biochemical reactions of photosynthesis, and improving hormone signaling.

Subcellular distribution, chemical forms of cadmium and rhizosphere microbial community in the process of cadmium hyperaccumulation in duckweed

Zheng, MM; Feng, D; Liu, H-J; Yang, G-L. The Science of the Total Environment (2023) 859: 160389.

Duckweed is a newly reported Cd hyperaccumulator that grow rapidly; however, little is known about its tolerance and detoxification mechanisms. In this study, we investigated the tissue, subcellular, and chemical form distribution of the Cd in duckweed and studied the influences of Cd on duckweed growth, ultrastructure, and rhizosphere microbial community. The results showed that Cd could negatively affect the growth of duckweed and shorten the root length. More Cd accumulated in the roots than in the leaves, and Cd was transferred from the roots to the leaves with time. During 12-24 h, Cd mainly existed in the cell wall fraction (2.05 %-95.52 %) and the organelle fraction (5.03 %-97.80 %), followed the soluble fraction (0.14 %-16.98 %). Over time, the proportion of Cd in the organelles increased (46.64 %-92.83 %), exceeding that in the cell wall (6.79 %-66.23 %), which indicated that duckweed detoxification mechanism may be related to the retention of cell wall and vacuole. The main chemical form of Cd was the NaCl-extracted state (30.15 %-88.66 %), which was integrated with pectate and protein. With increasing stress concentration and time, the proportion of the HCl-extracted state and HAc-extracted state increased, and they were low-toxic Cd oxalate and Cd phosphate, respectively. Cd damaged the ultrastructure of cells such as chloroplasts and mitochondria and inhibited the diversity of microbial communities in the duckweed rhizosphere; however, the dominant populations that could tolerate heavy metals increased. It was speculated that duckweed distributed Cd in a less toxic chemical form in a less active location, mainly through retention in the root cell wall and sequestration in the leaf vacuoles, and is dynamically adjusted. The rhizosphere microbial communities tolerate heavy metals may also be one of the mechanisms by which duckweed can tolerate Cd. This study revealed the mechanism of duckweed tolerance and detoxification of Cd at the molecular level and provides a theoretical basis for further development of duckweed.

Hormetic activation of nano-sized rare earth element terbium on growth, PSII photochemistry, antioxidant status and phytohormone regulation in *Lemna minor*

Alp, FN; Arikan, B; Ozfidan-Konakci, C; Gulenturk, C; Yildiztugay, E; Turan, M; Cavusoglu, H. Plant Physiology and Biochemistry (2022) 194: 361-373.

Soils contaminated with rare earth elements (REEs) can damage agriculture by causing physiological disorders in plants which are evaluated as the main connection of the human food chain. A biphasic dose response with excitatory responses to low concentrations and inhibitory/harmful responses to high concentrations has been defined as hormesis. However, not much is clear about the ecological effects and potential risks of REEs to plants. For this purpose, here we showed the impacts of different concentrations of nano terbium (Tb) applications (5-10-25-50-100-250-500 mgL^{-1}) on the accumulation of endogeneous certain ions and hormones, chlorophyll fluoresence, photochemical reaction capacity and antioxidant activity in duckweed (*Lemna minor*). Tb concentrations less than 100 mgL^{-1} increased the contents of nitrogen (N), phosphate (P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}) and iron (Fe^{2+}). Chlorophyll fluorescence (Fv/Fm and Fv/Fo) was suppressed under 250-500 mgL^{-1} Tb. In addition, Tb toxicity affected the trapped energy adversely by the active reaction center of photosystem II (PSII) and led to accumulation of inactive reaction centers, thus lowering the detected level of electron transport from photosystem II (PSII) to photosystem I (PSI). On the other hand, 5-100 mgL^{-1} Tb enhanced the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), NADPH oxidase (NOX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione S-transferase (GST). Tb (5-50 mgL^{-1}) supported the maintenance of cellular redox status by promoting antioxidant pathways involved in the ascorbate-glutathione (AsA-GSH) cycle. In addition to the antioxidant system, the contents of some hormones such as indole-3-acetic acid (IAA), gibberellic acid (GA), cytokinin (CK) and salicylic acid (SA) were also induced in the presence of 5-100 mgL^{-1} Tb. In addition, the levels of hydrogen peroxide (H_2O_2) and lipid peroxidation (TBARS) were controlled through ascorbate (AsA) regeneration and effective hormonal modulation in *L. minor*. However, this induction in the antioxidant system and phytohormone contents could not be resumed after applications higher than 250 mgL^{-1} Tb. TBARS and H_2O_2, which indicate the level of lipid peroxidation, increased. The results in this study showed that Tb at appropriate concentrations has great potential to confer tolerance of duckweed by supporting the antioxidant system, protecting the biochemical reactions of photosystems and improving hormonal regulation.

Screening of structural and functional alterations in duckweed (*Lemna minor*) induced by per- and polyfluoroalkyl substances (PFASs) with FTIR spectroscopy

Wu, Y-L; Xiong, Q; Wang, B; Liu, Y-S; Zhou, P-L; Hu, L-X; Liu, F; Ying, G-G. Environmental Pollution (Barking, Essex) (2022) 317: 120671

As a class of common emerging pollutants, per- and polyfluoroalkyl substances (PFASs) and their alternatives have been widely detected in various environmental matrices, exhibiting a great threat to the ecological environment and human health. Nevertheless, changes in biomolecular structure and function of duckweed caused by PFASs and their alternatives remain unknown thus far. Herein, the effects of four PFASs, including two common legacy PFASs (perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)) and two PFASs alternatives (perfluorobutane sulfonic acid (PFBS) and 1H,1H,2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)) on duckweed (*Lemna minor*) at biochemical level were investigated with Fourier transform infrared spectroscopy (FTIR). Although no obvious inhibitions were observed in the growth of *L. minor* with PFASs exposure at three levels of 1 μgL^{-1}, 100 μgL^{-1}, and 10 mgL^{-1}, significant structural and functional alterations were induced at the biochemical level. In response to PFASs exposure, lipid peroxidation, proteins aggregation and alpha-helix to beta-sheet transformation of the protein conformation, as well as changes of DNA conformations were detected. Moreover, alterations in lipid, protein, and DNA were proved to be concentration-related and compound-specific. Compared to the two legacy PFASs (PFOS and PFOA), alternative ones exhibited greater effects on the biological macromolecules of *L. minor*. The findings of this study firstly reveal structural and functional alterations in *L. minor* induced by PFASs exposure.

How aminomethylphosphonic acid (AMPA), the main glyphosate metabolite, interferes with chlorophyll biosynthesis?

Gomes, MP; Freitas, PL; Kitamura, RSA; Pereira, EG; Juneau, P. Environmental and Experimental Botany (2022) 203: 105039

Aminomethylphosphonic acid (AMPA) has been proposed to interfere with chlorophyll biosynthesis in photo-autotrophic organisms - which may explain its greater toxicity to plants in relation to animals. By using the aquatic macrophyte *Lemna minor*, we investigated the AMPA toxicity and its effects on chlorophyll biosynthesis. Environmentally relevant concentrations (35-50 μg l^{-1}) of AMPA reduced *L. minor* chlorophyll concentration with an EC10 and EC50 of 1.3 and 902.9 μg AMPA l^{-1} after seven days of exposure respectively but did not affect plant fresh weight/growth rate (EC10 and EC50 of 178.6/244.4 and 11,922.2/12,513.2 μg AMPA l^{-1}, respectively). AMPA decreased the activity of glycine decarboxylase (GDC), leading to glycine accumulation and glutamate (Glu) deprivation in plants. The lack of Glu prevents the synthesis of delta-aminolevulinic acid (ALA) in chloroplasts, interfering on chlorophyll synthesis. The negative effect of AMPA on chlorophyll concentration was reversible by supplementing growth media with Glu or ALA. The presence of AMPA in the aquatic ecosystems may contribute to their alteration by its negative effects on photosynthetic organisms through chlorophyll biosynthesis inhibition. Furthermore, we should stress out that AMPA could be toxic for animals and humans due to known toxicity of glycine induced by GDC inhibition.

Taxonomy & Geobotany

Target sequence data shed new light on the infrafamilial classification of Araceae

Haigh, AL; Gibernau, M; Maurin, O; Bailey, P; Carlsen, MM; Hay, A; Leempoel, K; McGinnie, C; Mayo, Simon; Wong, SY; Zuluaga, A; Zuntini, AR; Baker, WJ; Forest, F. American Journal of Botany (2022) DOI10.1002/ajb2.16117

Recent phylogenetic studies of the Araceae have confirmed the position of the duckweeds nested within the aroids, and the monophyly of a clade containing all the unisexual flowered aroids plus the bisexual-flowered *Calla palustris*. The main objective of this work was to better resolve the deep phylogenetic relationships among the main lineages within the family, particularly the relationships between the eight currently recognized subfamilies. We also aimed to confirm the phylogenetic position of the enigmatic genus *Calla* in relation to the long-debated evolutionary transition between bisexual and unisexual flowers in the family. Nuclear DNA sequence data were generated for 128 species across 111 genera (78%) of Araceae using target sequence capture and the Angiosperms353 universal probe set. The phylogenomic data confirmed the monophyly of the eight Araceae subfamilies but the phylogenetic position of subfamily *Lasioideae* remains uncertain. The genus *Calla* is included in subfamily *Aroideae* which has also been expanded to include *Zamioculcadoideae*. The tribe *Aglaonemateae* is newly defined to include the genera *Aglaonema* and *Boycea*. Our results strongly suggest that new research on African genera (*Callopsis, Nephthytis* and *Anubias*) and *Calla* will be important to understand the early evolution of the Aroideae. Also, of particular interest are the phylogenetic positions of the isolated genera *Montrichardia, Zantedeschia* and *Anchomanes*, which remain only moderately supported here.

Diversity and differentiation of duckweed species from Israel

Friedjung Yosef, A; Ghazaryan, L; Klamann, L; Kaufman, KS; Baubin, C; Poodiack, B; Ran, N; Gabay, T; Didi-Cohen, S; Bog, M; Khozin-Goldberg, I; Gillor, O. Plants (2022) 11: DOI10.3390/plants11233326

Duckweeds (Lemnaceae) are tiny plants that float on aquatic surfaces and are typically isolated from temperate and equatorial regions. Yet, duckweed diversity in Mediterranean and arid regions has been seldom explored. To address this gap in knowledge, we surveyed duckweed diversity in Israel, an ecological junction between Mediterranean and arid climates. We searched for duckweeds in the north and center of Israel on the surface of streams, ponds and waterholes. We collected and isolated 27 duckweeds and characterized their morphology, molecular barcodes (atpF-atpH and psbK-psbI) and biochemical features (protein content and fatty acids composition). Six species were identified- *Lemna minor*, *L. gibba* and *Wolffia arrhiza* dominated the duckweed populations, and together with past sightings, are suggested to be native to Israel. The fatty acid profiles and protein content further suggest that diverged functions have attributed to different haplotypes among the identified species. *Spirodela polyrhiza*, *W. globosa* and *L. minuta* were also identified but were rarer. *S. polyrhiza* was previously reported in our region, thus, its current low abundance should be revisited. However, *L. minuta* and *W. globosa* are native to America and Far East Asia, respectively, and are invasive in Europe. We hypothesize that they may be invasive species to our region as well, carried by migratory birds that disperse them through their migration routes. This study indicates that the duckweed population in Israel's aquatic environments consists of both native and transient species.

Strategies for intraspecific genotyping of duckweed: Comparison of five orthogonal methods applied to the Giant Duckweed *Spirodela polyrhiza*

Bog, M; Braglia, L; Morello, L; Melo, KIN; Schubert, I; Shchepin, ON; Sree, KS; Xu, SQ; Lam, E; Appenroth, KJ.
Plants (2022) 11: 3033

The predominantly vegetative propagating duckweeds are of growing commercial interest. Since clonal accessions within a respective species can vary considerably with respect to their physiological as well as biochemical traits, it is critical to be able to track the clones of species of interest after their characterization. Here, we compared the efficacy of five different genotyping methods for *Spirodela polyrhiza*, a species with very low intraspecific sequence variations, including polymorphic NB-ARC-related loci, tubulin-gene-based polymorphism (TBP), simple sequence repeat variations (SSR), multiplexed ISSR genotyping by sequencing (MIG-seq), and low-coverage, reduced-representation genome sequencing (GBS). Four of the five approaches could distinguish 20 to 22 genotypes out of the 23 investigated clones, while TBP resolved just seven genotypes. The choice for a particular method for intraspecific genotyping can depend on the research question and the project budget, while the combination of orthogonal methods may increase the confidence and resolution for the results obtained.

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). 3,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

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

Title:

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

Authors:

- All authors are responsible for the content of the manuscript.
- Provide the complete names of all authors.
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Image resolution and submission:

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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