Picturesque Dal Lake in Kashmir, India: a living repository of duckweeds
Picturesque Dal Lake in Kashmir, India: a living repository of duckweeds

The floating aquatic ecosystem of the picturesque Dal Lake (Kashmir, India) is a living repository of Duckweed Flora. The lake is situated at an altitude of ~1,584 m above m.s.l. and covers an area of 11.5 km². With the region exposed to severe winters, duckweed thrive in this lake from Spring to Autumn (between March and October) and are usually found in the lakeside shallow waters together with other aquatic macrophytes (Picture credit: Mr. S. Khursheed Qadri, former Director General, Archaeology, Museums & Archives, Jammu and Kashmir, India).

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Science meets art: *Wolffia globosa* (Roxb.) Hartog & Plas

*Wolffia globosa* is commonly found in the tropical and subtropical regions of Asian as well as some African countries. Moreover, it is the species that is almost exclusively used traditionally for human nutrition in several Asian countries. The various clones originating from different geographical areas possess very large differences in physiological parameters like growths rates, with doubling time ranging from 4.5 days to 1.2 days. The fronds are ellipsoid in shape and are without any prominent papulae on the upper surface. With approximately 1300 Mbp/1C its genome belongs to the three largest duckweed genomes. This might be the reason why its genome has not yet been sequenced. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.
Letter from the Editor:

Dear readers,

On behalf of the members of the International Steering Committee for Duckweed Research and Applications (ISCDRA), I send you our greetings and hope you are doing fine under this complicated time. As the COVID-19 pandemic continues to rage on in various parts of our planet since January, the future remains uncertain for humanity as to the timetable for an effective strategy to control its spread and damage while restarting the global economy in a prudent and sustainable fashion. As we are seeing, one of the most challenging issues for society in dealing with an urgent crisis such as this pandemic is the proper messaging of the evolving data, their interpretation, and swift implementation of a science-based solution. The role for a cohort of trusted experts to direct a balanced response to this type of crisis is critical and its importance cannot be overstated. Since the virus does not recognize any man-made boundaries, an internationally coordinated effort is also crucial for the long-term success of any mitigation strategy. The efficient and effective sharing of information in a globalized community thus has an important role to play in this public health crisis.

In its own way, I believe the Duckweed Forum could also serve as an important tool to support our growing community and rise to the challenges facing our efforts to bring the great potential of this family of aquatic plants to benefit humanity. In this issue, Autar Mattoo from the USDA lab in Beltsville (USA), wrote a comprehensive and fond description of his personal history in working with duckweed several decades ago. His work with Marvin Edelman and other colleagues made seminal discoveries on the key protein in Photosystem II that plays a crucial function in oxygenic photosynthesis, using Landoltia punctata as the model system. We are all looking forward to the fruits of his renewed research interest in examining nutritional aspects of duckweed that may also be associated with the phytohormone ethylene. The following article is the second contribution by Melanie Binggeli (ETH, Zurich) that describes her work as a Pioneer Fellow supported by the ETH. Together with her collaborator, Cyrill Hess, they have worked for a year in learning the various facets of building a startup business venture from a research platform. Her article should be of special interest to students and researchers alike who may be contemplating of becoming an entrepreneur in the duckweed space that is rapidly expanding. As their 1-year fellowship is ending this August, we wish Melanie and Cyrill well in their next adventures and hope to see the fruits of their labor in the marketplace one day. An Opinion article on the curious challenge posed by Wolffia brasiliensis for its taxonomic identification relative to other duckweed species is from Klaus Appenroth and colleagues, while the Student Spotlight section presents Yan Chen from Wuhan, China, who works on heavy metal interaction with Spirodela polyrhiza. Lastly, the issue is completed by the Database section with duckweed-related publications that appeared in the past 3 months.

As always, the ISCDRA and I hope you will enjoy this issue of the DF. I especially like to acknowledge all the ad hoc contributors for their time and effort to make our DF truly a community platform for sharing. We look forward to receiving comments and contributions from you in the near future.

Sincerely,

Eric Lam
Chair, ISCDRA
Historical Account: Duckweeds, Photosynthesis & Ethylene - Tryst with Destiny

Autar K. Mattoo

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Aquatic higher plants have been used as biological/biochemical models since a long time to address important biological questions in addition to some of them becoming a medium for purifying wastewater (Oron et al., 1985; Edelman et al., 2018). One such aquatic plant groups is called duckweed, which are classified within the family of Lemnaceae. In recent years research using duckweed has intensified largely because of their value as nutritious food for human consumption. My first exposure to the floating duckweeds was as a boy living in my grandfather’s house at Rainawari (Srinagar, Kashmir, India) close to the backwaters of the Nageen/Dal Lake. This waterway was full of beautiful ducks and floating duckweeds (Fig. 1a). Sitting on the riverbank steps of our house, we would enjoy the ripples and breezy waves of waterway mingled with all sorts of aquatic plants. I had just learned in my primary school about seed germination and the fact that plants have life. One late afternoon, sitting next to my young cousin, I saw him disturb the aquatic flora of duckweeds and recall telling him that ‘plants too were living organisms’! Nonetheless, my serious tryst with duckweed species *Spirodela oligorrhiza* (now classified as *Landoltia punctata*) (Fig. 1b) as a model plant to study photosynthesis happened some 26 years later after I had worked with rats (Master’s), plants/fruits (Ph.D.) and microorganisms (Baroda University), and completed my postdoctoral/visiting fellowships (Adelaide University – back to rat biochemistry; and USDA, Beltsville – fruits, plant hormones, and fungal biology), and returned to Baroda (1977). In early 1979, I resigned from my faculty position at Baroda University and accepted a DAAD Fellowship to work at the Weizmann Institute of Science in the laboratory of Prof. Marvin Edelman.

**Fig. 1a**: Duckweeds plus other aquatic flora in the Dal Lake, Kashmir, India (Picture credit: Mr. S. Khursheed Qadri, former Director General, Archaeology, Museums & Archives, Jammu and Kashmir, India); **b**: *Spirodela oligorrhiza* (*Landoltia punctata*) (Picture credit: Prof. Marvin Edelman, Weizmann Institute of Science, Israel).
The challenge

In the 1970’s, the Edelman laboratory was utilizing *Landoltia punctata* as a model plant system to study the biology of a rapidly synthesized thylakoid protein named 32 kDa (Edelman and Reisfeld, 1978; Edelman and Reisfeld, 1980). The 32 kDa membrane protein was known to be synthesized in the chloroplast at levels equivalent to that of ribulose-1,5-bisphosphate carboxylase (rubisco) but due to its rapid turnover it did not accumulate as much (its turnover rate was 50 to 60 times more rapid than rubisco or the apoprotein of the chlorophyll a/b light-harvesting complex). The function of the 32 kDa protein had eluded discovery. It did not appear to be involved in thylakoid biogenesis nor rate limiting for CO$_2$ fixation (Weinbaum et al., 1979). Upon joining the Edelman Laboratory I was given the task (challenge) to determine the function of the 32 kDa protein!

Chance favored the prepared mind & Landoltia made it happen

Sharing thoughts while socializing with eminent scholars can lead to welcoming pastures. I had just about settled down and was quickly getting oriented to photosynthesis and chloroplast literature when one evening I was invited to a birthday celebration party of a Volcani Center senior scientist. It offered a great opportunity to meet distinguished scientists including the eminent photosynthesis scholar Mordehay Avron who knew my USA mentor Morris Lieberman. Mordehay inquired about my research in the Edelman lab and upon my explaining about the unknown function of the 32 kDa protein he suggested that I should read a hypothesis paper (Renger, 1976) for the ‘existence of a ’proteinaceous shield’ covering the primary electron acceptor of photosystem II (PS II) and acting as a regulator of electron flow between PS II and photosystem I (PS I) in the chloroplast’.

As soon as the partying was done I went straight to the Weizmann Institute library and read Renger’s hypothesis paper several times while highlighting passages that I surmised were important to address the question I was asked to study and solve. To move rapidly I convinced one of Edelman’s masters students, Hedda Hoffman-Falk, to join me in pursuing the question after she too read the article and felt I may be up to something important. Incidentally, Marvin Edelman was away holding the fort at Army Reserve duty and I did not want to bother him since we had not yet performed the critical experiments.

Duckweeds, *Landoltia punctata* in particular, have the advantage of simple growth conditions, relatively short doubling time, aseptic cultivation, easy uptake of solutes including radiolabeled molecules, amenable to dissection, easy handling, and well suited for studying plant biology. Renger’s postulate was that such a proteinaceous shield (32 kDa?) regulates PS II and inhibits the effect of the herbicide diuron in plants (Renger, 1976). Since the questions we were asking were related to chloroplast biology and photosynthesis, quick uptake of radiolabeled substrates by a model organism was of great advantage. As this story moves forward it will become apparent that the selection of the duckweed *Landoltia* to study the function and life story of the 32 kDa protein was fortuitous and a great asset indeed.

The 32 kDa protein, better known now as D1, is a PSII reaction center protein

Quickly, we first ascertained that partial proteolytic digestion patterns of the 32 kDa protein from maize, peas, and Chlamydomonas were similar in *Landoltia punctata* (Hoffman-Falk et al., 1982; Mattoo et al., 1982), therefore the latter likely has a very similar structure to those in the other photosynthetic organisms and duckweed could be a good choice. We determined many features of the 32 kDa protein including its localization near the outer surface of the thylakoid membrane, that its light-dependent trypsinization induces diuron-insensitive PSII electron transport, and electron transfer in 32 kDa-depleted thylakoids is specifically inhibited at the reducing side of PSII and
between the two photosystems (Fig. 2a, b). These findings with *Landoltia* 32 kDa protein were consistent with Renger's postulate of the presence of a proteinaceous shield that regulates PSII electron flow (Mattoo et al., 1981). It was a major discovery whose follow up kept the Edelman (Israel) - Mattoo (USA) team competitive and busy for a number of decades thereafter. Continuing to utilize *Landoltia punctata*, we demonstrated that photosynthetic electron transport is coupled to *in vivo* degradation of the ‘rapidly metabolized 32 kDa chloroplast membrane protein’ (Mattoo et al., 1984), which is copiously synthesized and degraded in mature chloroplasts and became known later on as the D1 protein. Other features unraveled include (a) that light intensity regulates its rates of synthesis and degradation, (b) that it is unstable in the light but stable in the dark, (c) ATP is critical for its light-driven synthesis but not for its degradation, (d) inhibition of PSII electron transport by herbicides diuron (aka DCMU) and atrazine blocked 32 kDa / D1 protein degradation. Thus, both anabolism and catabolism of the 32 kDa / D1 protein were found to be photoregulated, with its degradation coupled to electron transport but not phosphorylation (Mattoo et al., 1984).

![Fig. 2a. SDS-PAGE fractionation of 35Smet-labeled thylakoid proteins after trypsinization in the absence or presence of diuron. Shown are D1 (32 kDa) and LH2 (26 kDa) proteins. The different digestion pattern for D1 in the absence and presence of diuron are apparent. Diuron induces a conformational change in D1 retarding its trypsin digestibility.](image)

### Rapid metabolism of the 32 kDa/D1 PSII protein

*Landoltia punctata* as our model system enabled understanding the *in vivo* regulation of the 32 kDa/D1 protein at a faster pace than utilizing etiolated and green tissues from non-aquatic plants because of low uptake of radiolabel and their more complex metabolism in the latter. Our further experiments were slated for unraveling regulation of the 32 kDa/D1 protein metabolism vis a vis photosynthetic electron transport (Fig. 3). We had discovered that light-mediated regulation of the 32 kDa/D1 protein by photophosphorylation also controlled its degradation and involved photosynthetic electron flow. This led to demonstrating photo-regulation of both the anabolic and catabolic...
processes of the D1 protein. While we were engaged in this research, an outstanding discovery unraveled the molecular structure of the photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis* to a resolution of 3 Å (Deisenhofer et al., 1985). The resolved structure showed a central part consisting of two subunits, L and M, each spanning the membrane five times and sharing a special pair of chlorophyll molecules. Further, the authors had noted that L and M sequences were homologous to the D1 and D2 proteins of PSII, and went on to propose that the D1 and D2 proteins form the core of the PS II reaction center (Deisenhofer et al., 1985). This team included Hartmut Michel, Johann Deisenhofer, and Robert Huber whose pioneering work earned them a Nobel Prize in Chemistry (for more details see Edelman and Mattoo, 2006).

Getting back to the bench, we demonstrated that the 33.5 kDa precursor of the 32 kDa/D1 protein and its processing occur in the unstacked stromal lamellae before the mature 32 kDa/D1 protein translocates within the thylakoids to topologically distinct, stacked granal lamellae (Fig. 3; Mattoo and Edelman, 1987). Once processed, the mature 32 kDa/D1 protein was found to undergo posttranslational palmitoylation which was mainly confined to the protein assembled in the granal lamellae where the PSII centers are concentrated (Fig. 3). The aquatic duckweed model (e.g., *Landoltia punctata*) is not only amenable for quick uptake of nutrients from a water body or the exogenously supplied radiolabeled compounds, the clear resolution of the fractionated samples on gels was found to be remarkable, apparent in the data shown in Fig. 3 that demonstrate intramembrane translocation and posttranslational palmitoylation of the 32 kDa/D1 protein. The membrane associated events in the life history of the D1 protein are summarized in Fig. 4 (after Mattoo and Edelman, 1987).

Meanwhile, Edelman's postdocs Bruce Greenberg and Victor Gaba were exploring the contribution of photosensitizers to 32 kDa/D1 protein degradation. These studies implicated chlorophyll in the visible and far red, and the plastoquinone anion radical in the UV region. The degradation of the 32 kDa/D1 protein in sunlight was attributed to UV-B irradiance, which suggested that UV-activated D1 turnover may have ecological implications (Greenberg et al., 1989). In later studies by Marcel Jansen and colleagues in the lab, it became apparent that *Landoltia* houses protection mechanisms against UV-B irradiance (Jansen et al., 1996). Three mechanisms were unearthed: (1) protection by UV-B screening pigments; (2) elevated oxygen-radical detoxifying system; (3) a mechanism other than screening pigments or increased radical scavenging capacity.
Landoltia (Spirodela) delivered another new discovery – dual location of the 32 kDa/D1 protein

My postdoc Frank Callahan enjoyed working with Landoltia fronds. He developed a collaboration with my colleague Bill Wergin, an electron microscopy specialist, to unravel the ultrastructure and cytochemistry of *Landoltia punctata* chloroplast fractions (Fig. 5; Callahan et al., 1989). These studies revealed interesting aspects of the duckweed thylakoid network, including clear separation of granal and stromal lamellae, identification of PSII/PSI/ATPase proteins and their specific association with distinct lamella (Fig. 6; Callahan et al., 1989). The dual location of PSII proteins between stromal and granal lamellae was highlighted for the first time (Callahan et al., 1989). These data also highlighted the strength of using Landoltia as a model system for cytochemistry and ultrastructure studies.

Once a week my lab group would meet at Hard Times café for a beer, such interactive happy hours I had picked up at the University of Adelaide and later also helped initiate when I joined the Weizmann Institute at Rehovot. On one such an evening in early 1990 I received a call late at night from Frank telling me that he had forgotten he was running an SDS-PAGE gel in the lab and had gone home. I advised him to go back to the lab and retrieve the gel and follow up the next steps. As a serious researcher, he followed the advice and retrieved the gel, carried out the remaining protocol, and went home. Amazingly, next day when that particular SDS page gel was analyzed, Frank was surprised to find an additional slower moving band than the parent D1 in grana-localized reaction center (Callahan et al., 1990), which turned out to be a variant of the D1 protein. Tedd Elich, another postdoctoral fellow in my lab, followed this work and identified the modified form as phosphorylated D1. The *in vivo* phosphorylation was found to occur on a threonine residue(s) localized within 1 kDa from the N terminus of mature D1. The steady-state level of phosphorylated D1 *in vivo* was found to vary with light intensity, reaching up to 20% of the total D1 (Elich et al., 1992).

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**Fig. 5.** Ultrastructural and cytochemical characteristics of *Landoltia punctata* chloroplast fractions.

**Fig. 6.** Immunological identification/localization of major thylakoid proteins in *Landoltia punctata* whole thylakoids (T), granal (G), and stromal (S) lamellae. Blots were immunodecorated with antibodies specific for individual proteins (see Callahan et al., 1989).
Another visiting scientist, Sudhir Sopory from India, and other lab members studied the effects of free radical scavengers, propylgallate and uric acid, on D1 degradation. They found that both these free radical scavengers inhibited 32 kDa/D1 degradation without affecting the linear electron flow (Sopory et al., 1990). Thus, free-radical damage was implicated in D1 degradation, suggesting oxygen environment as a causative factor for destabilizing the protein. Meanwhile, postdoc Maria Ghirardi joined hands with Sudhir Sopory and another visiting scientist from India, Sudha Mahajan, to address the question whether only D1 or the whole PSII reaction center cycles between grana and stromal lamellae, since a fully assembled stromal lamellae-localized PSII reaction center had not yet been isolated nor identified. Their joint efforts and working round the clock led to the isolation and characterization of a PSII reaction center complex from the stromal lamellae (Ghirardi et al., 1993). This newly identified stromal lamellae PSII was enriched in both D1 and its sister protein D2 at the same level as in the grana but contained only half of the cytochrome b$_{559}$ levels found in the grana-localized complex.

**Photosystem II core protein dephosphorylation is light-regulated in vivo**

As is apparent from the above, our aim was basically linked to demonstrating the D1 life story in vivo, since in vitro findings need to be finally demonstrated in a living tissue/cell/organism. In this regard, as also mentioned above, our approach was eased by utilizing Landoltia as a model. Tedd Elich, having identified and characterized the in vivo D1 phosphorylation, addressed the next question regarding the light regulation of PSII core phosphoproteins. Earlier, the in vitro studies had indicated that dephosphorylation was insensitive to light or redox control (Bennett, 1980; Michel et al., 1987). Tedd’s experiments demonstrated that in fact the D1, D2 and CP43 PSII core proteins undergo light-stimulated but linear electron-transport-independent dephosphorylation in vivo. Among other characteristics, the in vivo D1 dephosphorylation was found dependent upon light intensity and occurred throughout the visible light spectrum. We proposed that PSI excitation is involved in regulating dephosphorylation of PSII core proteins in vivo (Elich et al., 1993). Later, we found that the compound propylgallate inhibits LHCII phosphorylation in vivo but had little effect on PSII core protein phosphorylation. Using this inhibitor, LHCII dephosphorylation but not PSII core protein dephosphorylation was found to be insensitive to light in vivo. These data indicated that multiple phosphatases are involved in thylakoid protein dephosphorylation in vivo (Elich et al. 1997). Thus, both D1 phosphorylation and dephosphorylation were found to be light dependent; however, their role(s) in the life of D1 or photosynthesis remain undetermined.

**D1 phosphorylation is regulated by an endogenous diurnal rhythm but likely not linked to its degradation**

While our discussions on the possible physiological function of D1 phosphorylation continued, postdocs Mark Swegle and Isabelle Booij-James embarked on experiments to find what regulates D1 phosphorylation. We synthesized synthetic D1 peptides and raised antibodies that selectively detected phosphorylated and unphosphorylated forms of D1. Landoltia plants were entrained to the natural light/dark diurnal cycle in a greenhouse. Thus, we were able to measure the ratio of phosphorylated D1 to total D1 protein (D1-P index: [D1-P]/[D1] + [D1-P]). We demonstrated that Landoltia plants exhibit diurnal oscillation in the D1-P index, which was paralleled by de novo D1 phosphorylation in vivo. Interestingly, when the plants were shifted from a light/dark cycle to continuous light conditions, the D1-P index rhythm was maintained for several cycles (Booij-James et al., 2002). The diurnal regulation of D1 phosphorylation added another novel dimension to the light dependent D1 protein turnover and D1 chloroplast biology.

We surmised that the circadian oscillations in D1 phosphorylation could be part of a regulatory system controlling the operation of the photosynthetic apparatus, as well as a signal to alter D1 protein metabolism. It had been suggested that light cycle-dependent damage to proteins may get
corrected by new synthesis early during the light period of the following day (Riesselmann and Piechulla, 1992). In such a scenario, an endogenous diurnal rhythm could enhance synthesis of thylakoid membrane protein complexes early in the morning to allow optimal photosynthesis thereafter. Diurnal regulation of PSII D1 metabolism was not unexpected since phototrophs are known to be subjected to a daily light/dark cycle. However, such a diurnal rhythm-mediated regulation of D1 in cyanobacteria was not necessarily important since oxygenic bacterial phototrophs possess multiple copies of the psbA D1 gene (Bustos et al., 1990; Clarke et al., 1993; Kulkarni and Golden, 1994; Golden, 1995; Chen et al., 1999), with specific D1 isoform synthesis adapted to either lower or higher light intensity. We hypothesized that reversible D1 phosphorylation (and likely other phosphorylated PSII proteins) may have evolved in higher plants as a more energy-efficient substrate for diurnal regulation of PSII core metabolism. Later, we showed that inhibition of \textit{in vivo} D1 phosphorylation in \textit{Landoltia} plants by nitric oxide (NO) donors did not lead to inhibition of D1 degradation, suggesting that the two processes are not tightly linked (Booij-James et al., 2009).

**D1 degradation is a low-fluence event in the intact plant**

Marcel Jansen and colleagues in Edelman's lab explored D1 as a sensitive \textit{in vivo} probe for UV-B damage. The D1 and its sister protein, D2, form the PSII reaction center core. Both the D1 and D2 proteins were shown to be stable in the dark but not in light. The kinetics for D2 degradation mirrored those for D1, with D2 protein's half-life ~five times longer than the D1 in visible light but being specifically accelerated by UV-B radiation to almost equal that of the D1 degradation rate (Jansen et al., 1996b). As a result, at high but physiological light intensities and in environmentally relevant mixtures of both radiations the PSII reaction center core becomes prone to degradation (Jansen et al., 1996a, b, c). Jansen followed this up with a study of the light saturation kinetics of D1/D2 degradation. At a fluence as low as 5 \( \mu \text{mol.m}^{-2}.\text{s}^{-1} \) > 25% of the total degradation response of the D1 protein occurs, while > 90% of the degradation potential was attained at ~750 \( \mu \text{mol.m}^{-2}.\text{s}^{-1} \), intensities that are below saturation for photosynthesis (Jansen et al., 1999). Thus, in intact plants, D1 degradation increases with photon flux in a complex, multiphasic manner. Four phases were uncovered over the fluence range from 0-1600 \( \mu \text{mol.m}^{-2}.\text{s}^{-1} \). It was concluded that the mechanistic details of low light D1-D2 degradation are crucial in determining their relation to the photochemistry of PSII. The D1/D2 heterodimers undergo a number of modifications during their life cycle. For example, differentially phosphorylated PSII populations were isolated and, interestingly, these were found to differ in their sensitivity to strong light (Giardi, 1993). In recent studies, other aspects of D1 function have been revealed including its moonlighting role in rewiring specific metabolic pathways possibly involving retrograde signaling, in addition to its pivotal function in photosynthesis (Giardi et al., 2013; Antonacci et al. 2018).

**Landoltia and ethylene**

My doctoral thesis was on the biochemistry of ripening in mango and, among many findings, the involvement of ethylene in mango ripening (Mattoo and Modi, 1969) and the discovery of the citrate cleavage enzyme in mango fruit (Mattoo and Modi, 1970) were highlights. My sabbatical with Morris Lieberman at the USDA's Beltsville Agricultural Research Center, in 1977/1978, brought me close to Israeli scientist Edo Chalutz from the Volcani Center, who joined the Lieberman lab during the same time period, and we together worked on various aspects of ethylene, publishing six manuscripts. During my DAAD fellowship in Edelman's lab in Israel, I was itching to also interact with the Volcani Center's Postharvest Biology Laboratory group, which, at the time, was located contiguous to the Weizmann Campus. Edelman was supportive and the result was a number of ethylene-related papers with Volcani Center friends, which, in turn, generated collaborations with researchers at the Horticulture Department of the Hebrew University of Jerusalem, conveniently located across the...
street from the Weizmann Institute. Back in the USA, while working on the D1 protein in Landoltia, I wondered if I could employ this plant to answer the question, “do aquatic plants like Landoltia follow the same ethylene biosynthesis pathway as terrestrial crop plants”. We soon found that Landoltia plants produce very little (0.05 ppm) ethylene when cultivated axenically in standard Hutner’s medium. To augment this miniscule amount of ethylene production, we added 0.02 mM Cu(II) to the medium before transferring Landoltia cultures. This treatment resulted in a 15 to 30-fold increase of ethylene production, mostly in 10 to 20 day old cultures exposed to light intensity > ~45 µmol.m⁻².s⁻¹ and in a medium containing > 50% D₂O (Fig. 7a). Singlet oxygen quenchers and lipid peroxidation inhibitors (e.g., propylgallate, selomethionine) inhibited the Cu(II)-stimulated ethylene production but neither AVG (an inhibitor of 1-aminocyclopropane-1-carboxylate synthase) nor the cytoplasmic protein synthesis inhibitor cycloheximide affected the induction of ethylene production by Cu(II) (Fig. 7b). The increase in ethylene production in the presence of Cu(II) was associated with intracellular membrane and organelle damage observed using electron microscopy. We thus suggested that Cu(II)-induced ethylene in Landoltia was likely due to cellular membrane damage mediated by singlet oxygen and free radicals (Mattoo et al., 1986).

To further differentiate between the aquatic Landoltia and terrestrial plants, in the next study we induced ethylene production by cupric ions in leaf discs from tobacco (Nicotiana tabacum L. cv Xanthi) and whole fronds of Landoltia. Ethylene was induced at higher levels in younger tissues of both systems, which was inhibited by the lipid peroxidation/singlet oxygen inhibitor (DABCO) but not by cycloheximide, and was stabilized by D₂O in the presence of light. Also apparent was the stimulation of copper-induced ethylene production by selomethionine in tobacco leaf discs in contrast to it being inhibitory in Landoltia. Importantly, Landoltia was able to utilize both methionine or linoleic acid as substrates to produce ethylene to similar extents in the presence of cupric ions; however, in contrast, tobacco leaf discs were more efficient in converting methionine than linoleic acid to ethylene (Fig. 8). Thus, both these plants produced ethylene in response to cupric ions albeit via likely different routes (Mattoo et al., 1992).
Questions remaining

In 2019, I had the delight of being at the Weizmann Institute of Science to attend the 5th International Duckweed conference in Rehovot, Israel and help celebrate the 80th birthday of my mentor, Marvin Edelman. As was anticipated, many colleagues were also present for the double occasion and it was one of the great homecoming experiences that will remain etched in my memory. One afternoon, Marcel Jansen asked me why I had not followed up the ethylene story on Landoltia, as much more had been unearthed in the ethylene field since 1992. Likewise, Edelman was encouraging me to get back to research with duckweeds, one reason being our common interest in 'nutrition', since my lab developed a tomato model to delve into increasing nutritional quality of crop plants (Mehta et al., 2002; Mattoo et al., 2007; Anwar et al., 2019). I had no clear answer for Jansen, however I gave it serious thought. Suffice it to say that currently my lab has re-initiated work using duckweed as a model system in addition to tomato. I thank Marcel Jansen for the discussions and Marvin Edelman for sending me the duckweed strains. Hopefully sooner than later I look forward to re-contributing to the duckweed literature. The odyssey that you have just read is a good start!

Acknowledgments

I wish to thank Marvin Edelman for his valuable comments and suggestions that improved this manuscript and for continued consul and friendship. I acknowledge all the collaborators listed in this story whose contributions enabled it to happen. Along the way of this adventure many contributed by discussions and contributed to other aspects of D1 research. I am thankful to all of them: James D. Anderson, Adi Avni, Alessandra Cona, Bharat Chattoo, Robert Fluhr, Maria Teresa Giardi, Giuseppina Rea, Dina Heller, Karl Jakob, Michael Koblizek, Chiara Leonardi, Alexander Raskind, Judy St. John. I thank Mr. S. Khursheed Qadri for the picture of aquatic plants in Dal Lake (Kashmir, India).
References


Commentary: A year as pioneer fellows- Building a bridge from research towards application

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In 2019, Cyrill Hess (environmental scientist) and I (agricultural scientist) won a Pioneer Fellowship together at the Swiss Federal Institute of Technology (ETH). This is a grant that allows graduates from ETH Zurich to transform their research into potential business applications. We started this year with a focus on a growing system for the duckweed species Wolffia arrhiza.

We began in September 2019 by using lean innovation principles and created about ten different business canvases (such as the "lean canvas" by Ash Maurya, a very useful tool which we’ve learned to appreciate during this year) on how duckweed might be used in our society. We built up a network of stakeholders and collected information from the market to understand which business canvases may make sense. We went through the canvases in several iterations, filling and adapting them based on the feedback we received from our network and the market. Some canvases we got rid of quickly, because we saw in experiments that we cannot produce the feature we were looking for (such as changing the color or influence the taste of Wolffia with natural colorants or ingredients), or because we saw that there are already existing products on the market which provide the same function whilst being much cheaper (such as dried and pelleted animal feed). Some canvases we couldn’t find valid faults with and we still believe they have potential for the future, such as to interrupt the market of micro-greens, to provide a high-quality duckweed-based protein-rich powder or to use duckweed as an ingredient to fortify products such as pasta. There are additional ideas that we agree not to share yet and are thus still kept confidential, some of which we were working on with other research groups.

Based on the scenarios and canvases, we identified several potential B2B (business-to-business) customers and distilled their real needs related to using duckweed with them. We also tackled questions such as why our potential end customers would spend money on buying duckweed at all. In order to better understand customer perception of duckweed, we worked on food sensory surveys together with a team. It's currently unclear whether this data can be published. If so, you may read more about it in a future publication. We also built up a network of interested buyers in the food
industry and stakeholders in the area of agriculture and food innovation. Combining all these resources, we worked on several roadmaps and financial plans in order to estimate the cost and time-frame that would be needed to reach specific milestones.

To improve our understanding of startups, such as startup law, venture capital financing and economics, we had several brilliant trainings at the ieLab (the innovation and entrepreneurship laboratory) at ETH, which was the organizer of our Pioneer Fellowship program. Further we also read some books, my favorite that I’d recommend everyone interested in startup business funding and wants to learn about venture capital is a book called “Venture Deals” written by Brad Feld.

Another chapter of our work was to create a roadmap for tackling the Novel Food Admission of Wolffia derived products in the European Union. The Novel Food Admission rule for European Union states that all food which has not been eaten in the EU in substantial quantities before 15th of May 1997 is subject to thorough testing, before a single gram of it can be sold as food on the European market. The research and preparation required for the admission process of a novel food in the EU typically cost millions of dollars and many years of time. As an example, the process for Stevia (the sugar replacing plant) to be granted the Novel Food Admission in the EU took about two decades. In contrast to a patent, where the owner has 20 years of time to recover the costs, novel foods are only protected for 5 years for the applicant before other competitors can enter the market, which makes it really tough for startups in Europe who want to introduce novel foods.

Talking about this, intellectual property was another point that had to be tackled as well. There are several ways to protect intellectual property, ranging from patents, copyrights, to trade secrets. A good IP strategy is important and combines several of these elements, however it needs to be considered too that "being right" is not necessary "getting right" in front of the law. For a startup with IP, it’s a big challenge to keep track if others on the globe may be infringing on its IP rights, and if so, there may be insufficient resources to sue that person or company, especially if the company is bigger and has more means to process in front of the court. On the other hand, many investors require patents because that gives them more perceived security for their investment. Thus, the whole IP subject is another sensitive topic that requires time and expertise to handle properly, and should not be underestimated.

In parallel to this, we optimized the technology of the duckweed growing system and analyzed several Wolffia arrhiza strains for selected parameters. We took several new prototypes into operation and measured the effects on duckweed growth by a number of influence factors. We also worked quite intensely on tackling food safety and contaminations, as it is tricky to grow a freshwater plant with lots of nutrients in an open system under optimal light conditions, enhancing not only the growth of duckweed, but also of many other organisms that thrive under these same conditions. I contributed an earlier article dedicated to organic contaminations in duckweed growth systems to summarize these learnings, please refer to the last edition of this newsletter to find it.

Our pioneer fellowship will end in August 2020. Stay tuned to the Duckweed Forum or reach out to us if you want to hear more about our experience.

Last but not least, we want to thank the international duckweed community which we enjoyed to meet with for the first time at the conference in Rehovot during the beginning of our fellowship in September 2019. Many of you inspired us, gave us great advice and even became friends along the way. Thank you for building up and being such an amazing, warm and inspirational community. We hope our ways will cross again in the future!
At the onset of this millennium, with the publication of Les et al. (2002), molecular tools started to revolutionize the field of duckweed taxonomy. To date, for the genera Spirodela and Landoltia the delineation of species is clear while for the genus Lemna, only delineation of the two species Lemna minor and Lemna japonica needs further clarification (Bog et al., 2019), which is currently in progress. In contrast, more significant challenges exist for the genera Wolffia and Wolffella (Bog et al., 2013, 2018). A particularly enigmatic issue is the taxonomic position for the species Wolffia brasiliensis. In the early study of Les et al. (2002), on the basis of several plastidic markers this species was positioned either unresolved between the clades of Wolffia and Wolffella or positioned within the genus Wolffella. Wang et al. (2010) also presented results with several plastidic markers and Wolffia brasiliensis was also found to be closer to Wolffella than to Wolffia in this study, in spite of the fact that morphological markers clearly point this species as belonging to the genus Wolffia (Bog et al., 2020).

Recently, Park et al. (2020a, b) reported the complete plastidic sequences for several duckweed species. Using these information, they showed that W. globosa and W. australiana can be clearly separated from Wolffella lingulata. However, W. brasiliensis was found to be closer to Wolffella lingulata than to W. australiana and therefore resolving the genus Wolffia as non-monophyletic using the whole plastidic genome— in strong contrast to the morphology. Park et al. (2020a) wrote: “The node posterior between W. brasiliensis and Wolffella lingulata was as low as 0.5. Suggesting the classification of W. brasiliensis is still ambiguous even with whole chloroplast genome information”. Evidently, investigating plastidic DNA sequence could not solve this taxonomic problem.

Tippery et al. (2015) investigated not only plastidic markers (matK/ trnK + rbcL + rpl16) but also for the first time nuclear marker (ETS + 18S + ITS-1 + 5.8S + ITS-2 + 26S) in connection with duckweed taxonomy. Whereas plastidic markers showed the aforementioned position of W. brasiliensis, i.e.,
closer to *Wolffiella* than to *Wolffia*, results were not revealing using nuclear markers. Here, the position of *W. brasiliensis* was, additionally to *W. borealis* and *W. microscopica*, unresolved between the two genera. Further, *W. australiana* was placed as a sister group to all other species of *Wolffia* and *Wolffiella*.

In conclusion, based on all these results discussed here, the taxonomic position of *W. brasiliensis* remains unresolved between *Wolffia* and *Wolffiella* genera and its assignment still ambiguous. It is likely that this conflict between results from genotyping markers and morphological criteria can only be resolved by whole genome sequencing of multiple species in the *Wolffia* and *Wolffiella* genera.

**References**


Student Spotlight: Yan Chen

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I was interested in all kinds of plants when I was a child, for their beautiful appearance. Later, this growing interest made me decide to study plant sciences for my postgraduation. During the Summer Camp-2017 at the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, I was fortunate to meet my PhD supervisor, Dr. Hongwei Hou and to be admitted as a bachelor-straight-to-doctorate student at Professor Hou's lab. Prof. Hou leads a research group of aquatic plant physiology, which is focused on aquatic macrophyte physiology. One of his study areas is duckweed research and application. It was in the summer camp where I had first learned about this amazing tiny plant, duckweed. Duckweeds have small size, simple structure, rapid growth, high protein content, and strong ability for enrichment of heavy metals and many other amazing features, which inspired me to study these plants. Therefore, I chose duckweeds as my research subject at the postgraduate stage.

The afore-mentioned characteristics of duckweeds make them potential subjects of study in the field of plant morphology, development and evolution, as well as in biotechnological applications. On the one hand, duckweeds can contain high protein and can be a rich source of starch, which made them attractive as a potential bioreactor for antibody and alcohol production. On the other, duckweed-based wastewater treatment has been proven to be a feasible and inexpensive solution to remove nitrogen, phosphorus and heavy metals from eutrophic water bodies. In addition, duckweeds are regarded as a good raw material for biotechnological studies, because it has the characteristics of high biomass accumulation rate, short life cycle and easy collection. With heightened awareness of environmental protection, people pay more and more attention to ecological problems such as water eutrophication, atmospheric pollution, soil pollution, and soil erosion. There are several studies on heavy metal enrichment in water plants while it is urgent to clean the toxic substances in water, such as heavy metals. Metal ions are distributed in all components of aquatic ecosystem after entering, which has a strong toxic effect on the ecological environment and organisms including aquatic animals, aquatic plants, and even humans, when the content of metal ions exceeds a certain range.

Therefore, it is necessary to study the mechanism of heavy metal enrichment in plants. In order to understand the mechanism of heavy metal removal by plants, we have studied the absorption and transport of metal ions in Spirodela polyrhiza. Wang et al. (2014) reported the first draft sequence for the genome of S. polyrhiza (Clone 7498), which laid the foundation for studying these mechanisms by molecular biological approaches. It also provides an essential tool for using genetic engineering to develop S. polyrhiza as a platform to remediate aquatic ecosystem. It is reported that duckweeds can absorb heavy metals from the environment mainly through chelation and entrapment, microbial sequestration, physical and chemical volatilization, adsorption and sedimentation.
My work is to study the mechanism of heavy metal enrichment in duckweeds. We hope to improve water quality by using duckweed with strong enrichment capability to absorb heavy metals. In order to achieve this goal, I have cloned some genes of \textit{S. polyrhiza} which are associated with heavy metal enrichment, as revealed by bioinformatics analysis, including \textit{SpNramps} (\textsc{NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN}), \textit{SpGSH} (\textsc{GLUTAMATE-CYSTEINE LIGASE}) and \textit{SpPCS} (\textsc{PHYTOCHELATIN SYNTHASE}). To better understand the regulation of these genes, cloning of their promoters and their functional validation in yeast and Arabidopsis model systems were also performed.

Based on the result obtained from heterologous expression in yeast, I have found that \textit{SpNramps}, \textit{SpGSH} and \textit{SpPCS} genes are able to transport metal ions and \textit{SpGSH} and \textit{SpPCS} gene have great ability for cadmium transport. It was also found that the promoter of these genes attached to \textsc{GUS} was mainly expressed in the root and the reproductive pouch. Apart from my focus on the mechanism of \textit{SpNRAMP}s, \textit{SpGSH} and \textit{SpPCS} genes involved in metal transport in duckweeds, I also try to study the effects of the three detoxification methods viz., "isolation", "antioxidation" and "chelation". Our experiments attempt to analyze the mechanism of duckweed tolerance to heavy metals and then identify the optimal lines that can tolerate high levels of metal absorption for water restoration and the sensitive strains for monitoring water quality, as well as to discover the reasons for the tolerance and hypersensitivity of transporters in higher plants.

Cadmium, arsenic and other heavy metals are highly biotoxic in the environment, some of these not only affect the normal physiological metabolism and growth of plants, but also can enter the food chain, thus endangering the health of animals and humans. At present, most of the information about the harmful effects of heavy metals on plants focus on the external manifestations, ultrastructure, peroxide stress, antioxidant enzyme system, photosynthetic system, yield and quality of crop products and effects on the root system of terrestrial plants. However, the molecular mechanisms and pathways of tolerance and toxicological effects of heavy metals on aquatic plants remain to be clarified. Therefore, my research will use the \textit{SpNramps}, \textit{SpGSH} and \textit{SpPCS} genes to study heavy metal enrichment mechanisms in \textit{S. polyrhiza}. In addition to measuring the physiological and biochemical indices of the plants, we will also study the oxidative stress induced by cadmium and other heavy metals more comprehensively by fluorescence quantitative analysis, Western blot, RT-PCR and other methods, and try to understand the response mechanism of \textit{S. polyrhiza} to heavy metal stresses. To some extent, it is expected to provide a theoretical basis for the study of heavy metal enrichment in aquatic plants. Further, we want to develop \textit{S. polyrhiza} as a monitor for heavy metal pollution in the aquatic ecosystem, and to identify a strong enrichment plant variety with high tolerance to remediate wastewater polluted with heavy metals.
Aquatic plants, *Landoltia punctata*, and *Azolla filiculoides* as bioconverters of wastewater to biofuel

Miranda, AF; Kumar, NR; Spangenberg, G; Subudhi, S; Lal, B; Mouradov, A (2020) Plants 9: DOI:10.3390/plants9040437

The aquatic plants, *Azolla filiculoides*, and *Landoltia punctata*, were used as complementing phytoremediators of wastewater containing high levels of phosphate, which simulates the effluents from textile, dyeing, and laundry detergent industries. Their complementarities are based on differences in capacities to uptake nitrogen and phosphate components from wastewater. Sequential treatment by *L. punctata* followed by *A. filiculoides* led to complete removal of $\text{NH}_4^+$, $\text{NO}_3^-$, and up to 93% reduction of $\text{PO}_4^{3-}$. In experiments where *L. punctata* treatment was followed by fresh *L. punctata*, $\text{PO}_4^{3-}$ concentration was reduced by 65%. The toxicity of wastewater assessed by shrimps, *Paratya australiensis*, showed a four-fold reduction of their mortality (LC$_{50}$ value) after treatment. Collected dry biomass was used as an alternative carbon source for heterotrophic marine protists, thraustochytrids, which produced up to 35% dry weight of lipids rich in palmitic acid (50% of total fatty acids), the key fatty acid for biodiesel production. The fermentation of treated *L. punctata* biomass by *Enterobacter cloacae* yielded up to 2.14 mol H$_2$/mole of reduced sugar, which is comparable with leading terrestrial feedstocks. *A. filiculoides* and *L. punctata* can be used as a new generation of feedstock, which can treat different types of wastewater and represent renewable and sustainable feedstock for bioenergy production.

Biochemistry

*Lemna minor* studies under various storage periods using extended-polarity extraction and metabolite non-target screening analysis

Wahman, R; Graßmann, J; Sauvetre, A; Schröder, P; Letzel, T (2020) Journal of Pharmaceutical and Biomedical Analysis 188:113362

Plant metabolomic studies cover a broad band of compounds, including various functional groups with different polarities and other physicochemical properties. For this reason, specific optimized methods are needed in order to enable efficient and non-destructive extraction of molecules over a large range of LogD values. This study presents a simple and efficient extraction procedure for *Lemna minor* samples demonstrating polarity extension of the molecular range. The *Lemna* samples chosen were kept under the following storage conditions: 1) fresh, 2) stored for a few days at -80 °C, and 3) stored for 6 months at -80 °C. The samples were extracted using five specifically chosen solvents: 100 % ethanol, 100 % methanol (MeOH), acidic 90 % MeOH (MeOH-water-formic acid (FAC) (90:9:5:0.5, v/v/v/v), MeOH-water (50:50, v/v), and 100 % water. The final extraction procedure was conducted subject to three solvent conditions, and the subsequent polarity-extended analysis was applied for *Lemna minor* samples using RPLC-HILIC-ESI-TOF-MS. The extraction yield is in descending order (acidic 90 % MeOH), 50 % MeOH, 100 % water and 100 % MeOH. The results displayed significant molecular differences, both in the extracts investigated and in the fresh *Lemna* samples, compared to stored samples, in terms of the extraction yield and reducing contents as well.
as the number of features. The storage of *Lemna minor* resulted in changes to the fingerprint of its metabolites as the reducing contents increased. The comparisons enable a direct view of molecule characterizations, in terms of their polarity, molecular mass, and signal intensity. This parametric information would appear ideal for further statistical data analysis. Consequently, the extraction procedure and the analysis/data evaluation are highly suitable for the so-called extended-polarity non-target screening procedure.

**Biotechnology**

**Duckweed derived nitrogen self-doped porous carbon materials as cost-effective electrocatalysts for oxygen reduction reaction in microbial fuel cells**


Cost-effective metal-free electrocatalysts for oxygen reduction reaction were incredible significance of improvement about microbial fuel cells. In this research, a novel nitrogen self-doped porous carbon material is effectively inferred with KOH activation from a natural and renewable biomass, duckweed. Self-doped nitrogen in carbon matrix of nitrogen-doped porous carbon at 800 degrees C provides abundant active sites for oxygen reduction and improves the oxygen reduction kinetics significantly. Moreover, the porous structure of nitrogen-doped porous carbon at 800° C encourages the transition of electrolyte and oxygen molecules throughout the oxygen reduction reaction. Oxygen on the three-phase boundary is reduced to water according to a four-electron pathway on nitrogen-doped porous carbon electrocatalyst. The single-chamber microbial fuel cell with nitrogen-doped porous carbon as electrocatalyst achieves comparable power density (625.9 mW m⁻²) and better stability compared to the commercial Pt/C electrocatalyst. This simple and low-cost approach provides a straightforward strategy to prepare excellent nitrogen-doped electrocatalyst derived from natural and renewable biomass directly as a promising alternate to precious platinum-based catalysts in microbial fuel cells.

**Hydrothermal carbonization and pellet production from *Egeria densa* and *Lemna minor***

Alvarez, X; Cancela, A; Freitas, V; Valero, E; Sanchez, A; Acuna-Alonso, C (2020) Plants 9: DOI:10.3390/plants9040425

Biofuels are seen as a potential option for mitigating the effects of fossil fuel use. On the other hand, nutrient pollution is accelerating eutrophication rates in rivers, lakes, and coastal waters. Harvesting aquatic plants to produce biofuels could mitigate this problem, though it is important to attack the problem at source, mainly as regards the contribution of nutrients. For the first time, solid biofuels were obtained in the forms of carbon and pellets from the aquatic plants *Egeria densa*, which is classed as an invasive plant under the Spanish Catalogue of Exotic Invasive Species, and *Lemna minor*, both of which can be found in the Umia River in north-west Spain. The essential oils and macro- and microelements present in both these plants were also extracted and analyzed. The higher heating values (HHVs) of the carbon products obtained ranged from 14.28 to 17.25 MJ/kg. The ash content ranged from 22.69% to 49.57%. The maximum yield obtained for biochar for *Egeria densa* at 200 °C was 66.89%. Temperature significantly affects solid hydrochar yield. The HHVs of the pellets obtained ranged from 11.38 to 13.49 MJ/kg. The use of these species to obtain biofuels through hydrothermal carbonization (HTC) and pellets is a novel and effective approach that will...
facilitate the removal of nutrients that cause eutrophication in the Umia River. The elements extracted show that harvesting these plants will help to remove excessive nutrients from the ecosystem.

**Vermicomposting of duckweed (Spirodela polyrhiza) by employing Eisenia fetida: Changes in nutrient contents, microbial enzyme activities and earthworm biodynamics**

Gusain, R; Suthar, S (2020) Bioresource Technology 311:123585

This study investigated the vermicomposting of duckweed (DW) mixed with cow dung in 25 (T25), 50 (T50), 75 (T75), 100% (T100) ratio using *Eisenia fetida* under a 35 d trial. Decrease in pH, organic carbon (33.54-38.25%), C/N ratio (43.6-56.6%), but increase in total N (18.2-42.4%), Paval (137-187%), and TK (7.76-79.4%) was recorded. Macro-elements (Mg, Fe, Zn, Mn, and Cu) also showed a many-fold increase in vermicomposts. T50 and T75 showed the highest mineralization rates. Activities of enzymes (proteases; dehydrogenases; beta-galactosidase; acid phosphatase; and alkali phosphatases) and soil respiration rate was also higher in DW-rich waste mixtures. Seed bioassay test indicates the high agronomic application of DW-based vermicomposts. High earthworm biomass (975-1395mg) and fecundity rate (1.53-4.07 cocoons worm⁻¹) was recorded in all vermi-setups suggesting the suitability of DW as a substrate for *E. fetida* culture.

**Comparison of protein extraction methods for 2DE-based proteomic analysis of duckweed Spirodela polyrhiza, a small aquatic model plant**

Wang, YB; Wang, XY; Yang, RX; Niu, LJ; Wang, W (2020) Aquatic Botany 163: 103216

Duckweed, a small floating aquatic plant in the family Lemnaceae, has considerable potential for agricultural and environmental applications. Proteomic analysis will facilitate the study of plant biology and plant-environment interactions. A reliable and reproducible protein extraction method is critical for successful proteomic analysis. To date, there have been no evaluation studies of extraction methods for duckweed proteomics. In this study, we developed a trichloroacetic acid (TCA)/acetone/TCA precipitation method (TAT) for *Spirodela polyrhiza* and compared it with three extraction methods, primarily by two-dimensional gel electrophoresis (2DE). These methods were based on TCA/acetone precipitation, phenol extraction or their combination to concentrate proteins and remove interfering substances. As a result, these methods produced significant differences in protein yield, and each revealed a unique set of proteins. In general, TAT produced better 2DE protein patterns with high reproducibility regarding the number, coverage and abundance of protein spots. Our results provide useful information for selecting suitable extraction methods for proteomic analysis of duckweed plants.

**Ecology**

**Growth and morphological responses of duckweed to clonal fragmentation, nutrient availability, and population density**

Zhang, LM; Jin, Y; Yao, SM; Lei, NF; Chen, JS; Zhang, Q; Yu, FH (2020) Frontiers in Plant Science 11: 618

Connected ramets of aquatic clonal plants are susceptible to fragmentation by disturbance. Such clonal fragmentation may interact with nutrient availability and individual density to affect growth and morphology of aquatic clonal plants. We grew the widespread floating clonal plant *Spirodela*
polyrhiza (duckweed) under three levels of population density (low, medium, or high), two levels of nutrient availability (low or high), and two levels of clonal fragmentation (with or without). Clonal fragmentation and high nutrients increased biomass and ramet number, but decreased frond width, frond length, and specific frond area of S. polyrhiza. Increasing population density decreased growth (biomass and ramet number) and frond and root size, and increased frond thickness of individual ramets of S. polyrhiza. The negative effect of population density on growth of S. polyrhiza was greater under high than under low nutrient availability. Furthermore, the negative effect of population density on total mass and frond mass of S. polyrhiza was greater with fragmentation than without. These results suggest that clonal fragmentation, nutrient availability and population density can interact to affect growth and morphology of clonal floating plants. Competition for nutrients and space, rather than light, may be the mechanisms underlying reduced growth of clonal floating plants. As clonal fragmentation can increase biomass and ramet production of S. polyrhiza, disturbance that potentially causes clonal fragmentation cannot be recommended as a measure to limit the spread of clonal floating plants.

Interaction with other organisms

Community dynamics of duckweed-associated bacteria upon inoculation of plant growth-promoting bacteria

Ishizawa, H; Kuroda, M; Inoue, D; Morikawa, M; Ike, M (2020) FEMS Microbiology Ecology DOI:10.1093/femsec/fiaa101

Plant growth-promoting bacteria (PGPB) have recently been demonstrated as a promising agent to improve wastewater treatment and biomass production efficiency of duckweed hydrocultures. For their reliable use in aqueous environments, this study analyzed the plant colonization dynamics of PGPB and its ecological consequence on the entire duckweed-associated bacterial communities. A PGPB strain, Aquitalea magnusonii H3, was inoculated to duckweed at different cell densities or timings in the presence of three environmental bacterial communities. The results showed that strain H3 improved duckweed growth by 11.7-32.1% in five out of nine experiments. Quantitative-PCR and amplicon sequencing analyses showed that strain H3 successfully colonized duckweed after 1 and 3 d of inoculations in all cultivation tests. However, it significantly decreased in numbers after 7 d, and similar bacterial communities were observed on duckweed regardless of H3 inoculation. Predicted metagenome analysis suggested that genes related to bacterial chemotactic motility and surface attachment system are consistently enriched through community assembly on duckweed. Taken together, strain H3 dominantly colonized duckweed for a short period and improved duckweed growth. However, the inoculation of the PGPB did not have a lasting impact due to the strong resilience of natural duckweed microbiome.

Phenol removal capacity of the common duckweed (Lemna minor L.) and six phenol-resistant bacterial strains from its rhizosphere: In vitro evaluation at high phenol concentrations

Radulovic, O; Stankovic, S; Uzelac, B; Tadic, V; Trifunovic-Momcilov, M; Lozo, J; Markovic, M (2020) Plants 9: DOI:10.3390/plants9050599

The main topic of this study is the bioremediation potential of the common duckweed, Lemna minor L., and selected rhizospheric bacterial strains in removing phenol from aqueous environments at extremely high initial phenol concentrations. To that end, fluorescence microscopy, MIC tests, biofilm formation, the phenol removal test (4-AAP method), the Salkowski essay, and studies of
multiplication rates of sterile and inoculated duckweed in MS medium with phenol (200, 500, 750, and 1000 mg L\(^{-1}\)) were conducted. Out of seven bacterial strains, six were identified as epiphytes or endophytes that efficiently removed phenol. The phenol removal experiment showed that the bacteria/duckweed system was more efficient during the first 24 h compared to the sterile duckweed control group. At the end of this experiment, almost 90% of the initial phenol concentration was removed by both groups, respectively. The bacteria stimulated the duckweed multiplication even at a high bacterial population density (>10\(^{8}\) CFU mL\(^{-1}\)) over a prolonged period of time (14 days). All bacterial strains were sensitive to all the applied antibiotics and formed biofilms in vitro. The dual bacteria/duckweed system, especially the one containing strain 43-\textit{Hafnia paralvei} C32-106/3, Accession No. MF526939, had a number of characteristics that are advantageous in bioremediation, such as high phenol removal efficiency, biofilm formation, safety (antibiotic sensitivity), and stimulation of duckweed multiplication.

**Experimental evidence of the consumption of the invasive alien duckweed \textit{Lemna minuta} by herbivorous larvae of the moth \textit{Cataclysta lemnata} in Italy**

Mariani, F; Di Giulio, A; Fattorini, S; Ceschin, S (2020) Aquatic Botany 161: 103172

Alien plant invasion is a serious threat for biodiversity conservation. Most theories on the mechanisms regulating the expansion of an alien species, agree that herbivory is one of the main factors affecting the success or failure of these species. One worrying example in Europe is the American duckweed \textit{Lemna minuta} that, since its arrival in the 1940s, has become wide spread throughout many countries. This study focused on determining and quantifying the herbivorous nature of the larvae of the moth \textit{Cataclysta lemnata} (native to Europe) with regards to \textit{L. minuta}. On the premise that, if a native herbivore feeds on an alien plant species, it could help to contain its expansion. We tested the effectiveness of larvae at three different instars in consuming \textit{L. minuta} under laboratory conditions. Laval preference for the alien \textit{L. minuta} and the native \textit{L. minor} was determined by quantifying the removal of monolayer mats of these two duckweeds. Firstly, we found that \textit{C. lemnata} larvae was able to use \textit{L. minuta} as atrophic resource and also to build protective cases. Moreover, they feed effectively, and seemingly without preference, on both the native and the alien species, contrary to the Enemy Release Hypothesis, which assumes that native consumers are better adapted to consume native species than alien ones. In addition, \textit{C. lemnata} late-instar larvae were more efficient in \textit{Lemna} consumption. This study suggests that where \textit{C. lemnata} is a native herbivore the spread of \textit{L. minuta} could be effectively contained.

**Removal of indicator and pathogenic bacteria by \textit{Lemna minuta} Kunth in an aquaponics recirculation system**

Velichkova, K; Sirakov, I; Dinev, T (2020) Fresenius Environmental Bulletin 29: 2222-2227

The use of eco-technologies, such as duckweed insetting, for wastewater treatment is becoming popular because of its affordability and efficiency of pathogen removal. The purpose of the current research was to study the influence of \textit{Lemna minuta} as a single plant in aquaponic recirculation system on the indicator microorganisms and pathogens in water. The aquaponic recirculation system consisted of 10 fish cultivation tanks and 4 plant tanks. The cleaning block of the system consisted of one mechanical filter (settling tank) and moving bed biofilter. Weekly samples of control water, fish tanks, and tanks with \textit{L. minuta} were taken in four replicates. For the quantitative detection of some sanitary indicator microorganisms (coliforms, \textit{Enterobacteriaceae}), pathogens (\textit{Salmonella} spp.) and total count in the treated water, medium plates (Compact Dry EC; Compact
Dry ETB; Compact Dry SL and Compact Dry TC, R-Biopharm AG, Germany) coated with dry culture medium were used.

**Molecular Biology**

Comparative and phylogenetic analysis of the complete chloroplast genome of *Wolffia brasiliensis* (duckweed) in Araceae

Park, JH; Park, H; Jeon, HH; Woo, DU; Lee, Y; Kang, YJ (2020) Mitochondrial DNA Part B-Resources 5: 1767-1768

*Wolffia brasiliensis*, a species of duckweed, is an aquatic plant belonging to the family Araceae. In this study, the complete chloroplast genome of *W. brasiliensis* was assembled from the whole genome Illumina sequencing data. The chloroplast genome is 168,514 bp in length, which contained one large single copy (LSC; 90,790 bp) and one small single copy (SSC; 13,930 bp) separated by two inverted repeat (IR) regions of 31,897 bp. It encodes 4 rRNA, 29 tRNA, and 78 protein-coding genes, with 20 double copies. Total GC content is 36.24%. Bayesian phylogenetic analysis indicated that *W. brasiliensis* occupied the ambiguous phylogenetic position between Wolffielia and Wolffia.

Characterization of the complete chloroplast genome sequence of *Wolffia globosa* (Lemnoideae) and its phylogenetic relationships to other Araceae family

Park, H; Park, JH; Jeon, HH; Woo, DU; Lee, Y; Kang, YJ (2020) Mitochondrial DNA Part B-Resources 5: 1905-1907

*Wolffia globosa* is the smallest angiosperm in the world and can be found in Asia and parts of America. Also, it is commonly used as food in Southeast Asia. In this study, the complete chloroplast genome of the *Wolffia globosa* was assembled from the whole genome Illumina sequencing data. The assembled genome size is 169,405 bp in length, which composed of a large single copy region (LSC) of 92,171 bp, a small single copy (SSC) regions of 13,570 bp and separated by a pair of inverted repeat (IR) regions of 31,810 bp each. It encodes a total of 113 genes, including 78 protein-coding genes, 31 tRNA genes, and 4 rRNA genes. There are 22 duplicated genes in the predicted gene catalog. The overall GC content is 35.9% while the GC content of the LSC, SSC, and IR regions are 33.8%, 31.1%, and 40.0%, separately. Based on Bayesian phylogenetic analysis, it represents that *Wolffia globosa* was closely related to *Wolffia australiana*.

Duckweed Forum: *Wolffia angusta* is the smallest angiosperm on Earth, known till date

**Physiology & Stress**

Biosynthesis of the starch is improved by the supplement of nickel (Ni\(^{2+}\)) in duckweed (*Landoltia punctata*)

Shao, J; Liu, ZB; Ding, YQ; Wang, JM; Li, XF; Yang, Y (2020) Journal of Plant Research DOI: 10.1007/s10265-020-01204-0

Duckweed is a kind of floating aquatic plant and increasing its starch production is favorable for bioenergy. In this study, we found that starch biosynthesis was greatly promoted by the supplement of nickel ion (Ni\(^{2+}\)) through the comparison of other different ions. The starch content in duckweed
was increased by nearly eightfold when duckweed was treated with 20 µM Ni²⁺. The analysis of paraffin sections visually found that starch granules were more complete and dark blue in Ni²⁺ treated duckweed than the control. Quantitative real-time PCR demonstrated that the expressions of starch synthesis-related enzymes were up-regulated in Ni²⁺ treated duckweed. Further analysis revealed that the accumulation of Ni²⁺ in duckweed effectively increased the activity of urease, which compensated for the deficiency of certain decrease in biomass and accelerated biosynthesis of the starch. Thus, our results represent another strategy to improve starch production of duckweed.

Exogenous jasmonic acid enhances oxidative protection of Lemna valdiviana subjected to arsenic
Coelho, DG; de Andrade, HM; Marinato, CS; Araujo, SC; de Matos, LP; da Silva, VM; de Oliveira, JA (2020) Acta Physiologica Plantarum 42: 97

Oxidative damage is one of the most harmful effects arising from arsenic (As) toxicity in plants. Herein, the role of exogenous jasmonic acid (JA) in the modulation of As-induced oxidative stress in Lemna valdiviana was investigated. Plants were grown for 24 h in Clark’s nutritive solution containing As (4.0 mg L⁻¹) or As + JA (50, 100, 250 and 500 µM). Chlorophyll a and b content decreased under As stress, either in isolation or associated with JA. The decreased chlorophyll a/b ratio in As-exposed plants was recovered by JA treatment at 100 µM. The carotenoid content was higher in plants exposed to As compared to controls and lower when it was associated with JA. Arsenic triggered the accumulation of O₂⁻ and H₂O₂, in addition to severely increasing lipid peroxidation. Application of JA in As-grown plants resulted in lower O₂⁻ content and lipid peroxidation than in those grown under As alone, as a result of enhanced SOD activity. On the other hand, H₂O₂ accumulation was increased by JA in As-stressed plants, associated with higher CAT, POX and GPX activity. The As content and bioaccumulation factor (BF) were improved by application of JA in the nutritive solution at 250 and 500 µM. Our findings indicate that JA modulates the pigment balance, thereby fine-tuning energy dissipation as well as alleviating As-induced oxidative damage in L. valdiviana through modulation of ROS homeostasis and improvement of the antioxidant enzymatic system, allowing increased accumulation of As without showing major damage.

Alteration of metabolic profiles in Lemna paucicostata culture and enhanced production of GABA and ferulic acid by ethephon treatment
Kim, E; Kim, M; Choi, HK (2020) PLOS ONE 15: e0231652

Lemna species have been used in the food, feed, and pharmaceutical industries, as they are inexpensive sources of proteins, starches, and fatty acids. In this study, we treated L. paucicostata with different concentrations (0.05, 0.1, 0.2, 0.5, or 1 mM) of ethephon. The total dry weight decreased in all ethephon-treated groups compared to the control group. We also investigated the alteration of metabolic profiles induced by ethephon treatment by using gas chromatography-mass spectrometry. This analysis identified 48 metabolites, and the relative levels of most of alcohols, amino acids, fatty acids, and phenols increased by the ethephon treatment, whereas levels of organic acids and sugars decreased. Among these, the highest production of γ-aminobutyric acid (GABA, 5.041±1.373 mg/L) and ferulic acid (0.640±0.071 mg/L) was observed in the 0.5 mM and the 0.2 mM ethephon treatment groups, respectively. These results could be useful for large-scale culture of L. paucicostata with enhanced GABA and ferulic acid content for utilization in the food, feed, cosmetic, and pharmaceutical industries.

Duckweed Forum: The correct name is Lemna aequinoctialis
**Diallyl phthalate-triggered oxidative stress in Spirodela polyrhiza L. Schleiden: physiological effects and role of antioxidant defence system**


The present study was designed to investigate the manifestations of diallyl phthalate (DAP)-induced oxidative stress in *Spirodela polyrhiza*. Plants were exposed to varying concentrations of DAP, viz. 10, 20, 40, 80, 100, 200, 400 mg L\(^{-1}\) for 7 days under in vitro conditions. Biochemical analysis after harvesting revealed various phytotoxic effects of DAP on *S. polyrhiza* which was quite evident from a significant decline in fresh weight and dry-to-fresh weight ratio with progressively increasing concentrations of DAP. Plants accumulated a significant amount of DAP (92.00 mg/kg FW) at 80 mg L\(^{-1}\) concentration which led to alterations in photosynthetic pigments (chl a, chl b, total chlorophyll), increase in carotenoid and anthocyanin pigment, increase in lipid peroxidation, decrease in protein and carbohydrate content. Results also revealed significant effects of DAP on increased proline, phenols and electrolyte leakage. In response to this and to confer DAP tolerance in *S. polyrhiza*, activities of antioxidant enzymes (SOD, APX, CAT, POD and GR) elevated with proceeding concentrations. Moreover, toxicological implications in plants were confirmed by observing scanning electron micrographs and confocal micrographs of frond and root, respectively, implying oxidative damage faced by plant under DAP exposure. Considering all these results into account, it appeared that there are alarming consequences of DAP toxicity to *S. polyrhiza* and this plant exhibited better phthalate tolerance ability attributed to its efficient antioxidant machinery which may play a cardinal role in combating diallyl phthalate-mediated stress.

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**Abscisic acid-enhanced starch accumulation of bioenergy crop duckweed (Spirodela polyrrhiza)**

Wang, XZ; Cui, WH; Hu, WW; Feng, CP (2020) RSC Advances 10: 10394-10401

To meet the increasing energy consumption around the world and fight global climate change, there is an urgent need to explore renewable energy crops to replace the traditional energy sources. Duckweed (*Spirodela polyrrhiza*) is widely distributed in the world and has high starch and low lignin contents, which is perhaps an ideal feedstock for bioenergy production. To investigate the effects of abscisic acid (ABA) on duckweed biomass and starch accumulation, *Spirodela polyrrhiza* was cultivated at different ABA concentrations. The results showed that the highest starch content in duckweed (21.8% dry weight) was achieved in 1.0 x 10\(^{-2}\) mg L\(^{-1}\) ABA medium, 70.3% higher than that of the control medium without ABA. The number of starch granules in 1.0 x 10\(^{-2}\) mg L\(^{-1}\) ABA medium was far more than that in the control medium. The highest adenosine diphosphate (ADP)-glucose pyrophosphorylase (AGPase) activity was observed in the 1.0 x 10\(^{-2}\) mg L\(^{-1}\) ABA medium, which was caused by the up-regulation expression of ADP-glucose pyrophosphorylase 2 (APL2). Further investigations on cell ultra-structures and stomatal property of the duckweed indicated that ABA increased the number and size of starch granules and stomatal size in duckweed cells. These enhancements lead to a greatly improved energy flow in the aquatic plant from photosynthesis to carbon storage, making duckweed a potential renewable bioenergy crop.
**Phytoremediation**

**Assessment of the potential of duckweed (Lemna minor L.) in treating lead-contaminated water through phytoremediation in stationary and recirculated set-ups**

Ubuza, LJA; Padero, PCS; Nacalaban, CMN; Tolentino, JT; Alcoran, DC; Tolentino, JC; Ido, AL; Mabayo, VIF; Arazo, RO (2020) Environmental Engineering Research 25: 977-982.

The problems of heavy metal contamination in water have become alarming and necessitate efficient remediation. However, conventional water and wastewater treatment techniques are considered costly, and some are even not environment-friendly. These problems trigger the idea of utilizing plants in the treatment process of metal-contaminated water. The current work investigated the potential of duckweed (Lemna minor L.) in treating lead-contaminated water through phytoremediation. The duckweed was used as bioaccumulator of lead (Pb) in the prepared stationary and recirculated set-ups at 3, 6, and 9 d. The physicochemical characteristics such as pH, BOD5, DO, turbidity, and temperature of the influent and effluent were compared. The highest bioaccumulation of 62.8% was achieved at 3 d in the recirculated set-up. The result of the analysis showed that duckweed has the potential in phytoremediation considering better quality effluent. The concentration of Pb in the effluent of 0.93 mg/L in the recirculated set-up with duckweed in 3 d was much lower compared to the initial concentration in the influent at 2.5 mg/L. This study demonstrated that duckweed could be a suitable plant for Pb removal from water with big implications in remediating heavy metal-contaminated water from various industries.

**Intraspecific variations in cadmium tolerance and phytoaccumulation in giant duckweed (Spirodela polyrhiza)**

Chen, Daoqian; Zhang, Hao; Wang, Qiongli; Shao, Min; Li, Xinyu; Chen, Dongmei; Zeng, Rensen; Song, Yuanyuan (2020) Journal of Hazardous Materials 395:122672

Duckweeds are widely recognized for the heavy metal phytoremediation. However, the intraspecific variations in biological responses of duckweeds to heavy metal remain largely unknown. Here, the toxicity and phytoaccumulation of cadmium (Cd) were synchronously evaluated in 30 accessions of giant duckweed (Spirodela polyrhiza) collected from different provenances in Southern China. Exposure to 1 µM Cd decreased relative growth rates of dry weight, fronds number and fronds area, as well as photosynthetic pigment contents, while it increased H$_2$O$_2$ accumulation, lipid peroxidation and activities of anti-oxidant enzymes in the majority of accessions. Cd treatment led to remarkable Cd accumulation but little changes in the starch content in giant duckweed. The biological responses to Cd varied among the accessions. Further correlation analysis indicated that growth traits and Cd concentration were positively correlated with Cd accumulation, while the contents of chlorophyll, H$_2$O$_2$, and MDA were negatively associated with Cd accumulation. Our results proved the great intraspecific variation in Cd tolerance of giant duckweed, suggesting a valuable natural resource for Cd phytoremediation. Moreover, different mechanisms may be exploited by S. polyrhiza for phytoaccumulation, but growth maintenance, Cd uptake and antioxidative enzyme-independent ROS-scavenging under Cd exposure are the common mechanisms contributing to Cd accumulation ability.
Removal of eight perfluoroalkyl acids from aqueous solutions by aeration and duckweed

Zhang, WL; Liang, YN (2020) Science of the Total Environment 724: 138357

Poly- and perfluoroalkyl substances (PFAS) are surfactants. Leveraging their surface active feature, this work investigated using aeration to remove perfluoroalkyl acids (PFAAs) from aqueous solutions. Eight PFAAs were spiked to either deionized water or Hoagland solution at three pHs. After 7 h of aeration, removals of perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorobutanesulfonic acid (PFBS), and perfluorohexanoic acid (PFHxA) were marginal and much lower than those of perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), perfluoroctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS). In deionized water, close to 80% of PFOA and PFOS at 200 ppb were removed when the pH was 2.3. The Hoagland solution at pH 2.3 and 5.0 benefited removal of long-chain PFAS at 2 ppb, but not at 200 ppb. With duckweed growing on the Hoagland solution surface, >95% of PFHpA, PFHxS, PFOA, and PFOS at 200 ppb were removed after 2 weeks. Aeration enhanced duckweed uptake of PFHxS, PFOA, and PFOS at 2 ppb significantly. Specific to PFOS, duckweed accumulated 14.4% of this compound initially spiked at 2 ppb in 2 weeks. These results demonstrated that aeration plus duckweed could be a viable and scalable remediation solution for surface water contaminated by PFAS.

Nutrient removal by duckweed from anaerobically treated swine wastewater in lab-scale stabilization ponds in Vietnam

Dinh, TTU; Soda, S; Nguyen, TAH; Nakajima, J; Cao, TH (2020) Science of the Total Environment 722: 137854

In Vietnam, swine wastewater is generally treated using anaerobic processes. Nevertheless, the level of pollutants in effluent after anaerobic treatment remains very high, thereby necessitating further treatment. This research was conducted to assess the applicability of duckweed (Lemna minor) for purifying wastewater collected from a household swine wastewater treatment system in Hanoi, Vietnam. After the anaerobically treated wastewater was diluted 10-fold, it was fed continuously to lab-scale stabilization ponds with and without planted duckweed at a hydraulic retention time of 5 days under ambient conditions. The chemical oxygen demand (COD), total nitrogen (T-N), and total phosphorus (T-P) concentrations in the influent were, respectively, 260-290 mg/L, 24-28 mg/L, and 1.4-1.8 mg/L. The COD, T-N, T-P removals in the pond with duckweed (74%, 84%, and 84%) were much higher than in the pond without duckweed (71%, 55%, and 58%). The duckweed greatly enhanced the first-order removal rates by 1.4, 2.0, and 3.2 times, respectively, for COD, T-N, and T-P in the ponds. Although the primary purification mechanisms in the ponds were sedimentation and adsorption, the duckweed grown with the relative growth rate of 0.07-0.16 d⁻¹ showed nutrient uptake activity from the wastewater. Biofilms formed on the duckweed roots apparently promoted COD removal and degradation of organic nitrogen into ammonia. Stabilization ponds planted with duckweed are anticipated for use as co-beneficial systems for wastewater treatment and biomass production.

Mechanistic and economic assessment of polyester wastewater treatment via baffled duckweed pond

Osama, R; Awad, HM; Ibrahim, MG; Tawfik, A (2020) Journal of Water Process Engineering 35: 101179
Lemna gibba (L. gibba) was successfully used for phytoremediation of wastewater rich 1,4-dioxane resulting biomass with high protein and carbon content. However, the efficiency of L. gibba was strongly dependent on the reactor configuration i.e. classical and baffled duckweed pond (DWP&BDWP) system. The BDWP configuration provided a removal efficiency of 69.3±18.6% for 1,4-dioxane resulting a residual value of 50.2±29.7 mg/L in the treated effluent. The classical DWP module provided a lower removal efficiency of 20.8±11.9% and a higher residual value of 1,4-dioxane (115.6±30.7 mg/L) in the treated effluent. 1,4-dioxane uptake using L. gibba was occurred after decomposing into metabolite products in terms of glycolic and oxalic acids. Afterwards, these acids further degraded into phosphoglycerate which easily absorbed by the plants and stored as amino acids in the tissues. Therefore, alanine, aspartic acid, glycine, threonine and serine were boosted by values of 47.3, 32.5, 26.5, 26.1 and 20%, respectively. The removal mechanism of 1,4-dioxane via duckweed pond was mainly due to uptake followed by photo-degradation process. Economic analysis is clearly emphasized on the applicability of the proposed configuration, which achieved net profit and payback period of 159.4 $/year and 37.7 years, respectively.

Nutrient uptakes and biochemical composition of Lemna minor in brackish water

Nesan, D; Selvabala, K; Chieh, DCJ (2020) Aquaculture Research DOI: 10.1111/are.14693

The freshwater plant species Lemna minor suffers significantly in nutrient uptake and biomass accumulation performance when grown in saline effluents, such as those produced in brackish aquaculture operations. To determine the exact impact of salinity to these plant traits, this study measured the nutrient uptakes and biochemical composition of L. minor grown in synthetic aquaculture medium of increasing salinity levels. The overall trend for biomass growth showed that higher salinity levels resulted in lower growth with a mass gain of about 13% in 12.5 ppt medium compared with 96% in the control. However, the NO$_3^-$ uptake appeared to be unaffected by differences in salinity. NH$_4^+$ uptake was significantly affected only at salinity concentrations of 10 ppt and 12.5 ppt. The relationship between PO$_4^{3-}$ uptake and salinity was less clear, as PO$_4^{3-}$ levels appeared to decrease linearly for all test groups and were shown to be statistically insignificant. At the end of the experiment period, the control medium showed the lowest measured COD levels, 17 mg/L while the 12.5 ppt solution has the highest COD level, 61 mg/L. Protein content showed a decline with increasing salinity of growth medium, while carbohydrate content was shown to be increasing. These preliminary data identify the general relationship between salinity and the measured criteria of L. minor and will subsequently serve as the basis for further remediation studies and the development of salinity mitigation methods.

Assessing and modelling the efficacy of Lemna paucicostata for the phytoremediation of petroleum hydrocarbons in crude oil-contaminated wetlands

Ekperusi, AO; Nwachukwu, EO; Sikoki, FD (2020) Scientific Reports 10:8489

The potentials of the invasive duckweed species, Lemna paucicostata (accepted name: Lemna aequinoctialis, DF) to remove pollutants from aquatic environment was tested in a constructed wetland as an ecological based system for the phytoremediation of petroleum hydrocarbons in crude oil-contaminated waters within 120 days. Total petroleum hydrocarbons in wetlands and tissues of duckweed were analyzed using gas chromatography with flame ionization detector following established methods while the experimental data were subjected to the first-order kinetic rate model to understand the remediation rate of duckweed in wetlands. L. paucicostata effected a significant (F=253.405, P<0.05) removal of hydrocarbons from wetlands reaching 97.91% after 120
days. Assessment on the transport and fate of hydrocarbons in duckweed indicated that \textit{L. paucicostata} bioaccumulated less than 1% and significantly biodegraded 97.74% of hydrocarbons in wetlands at the end of the study. The experimental data reasonably fitted ($r^2=0.938$) into the first-order kinetic rate model. From the result of the study, it is reasonable to infer that \textit{L. paucicostata} is an effective aquatic macrophyte for the removal of petroleum hydrocarbons in moderately polluted waters.

**Phytoremediation of nutrients from water by aquatic floating duckweed (\textit{Lemna minor}) in rearing of African cichlid (\textit{Labidochromis lividus}) fingerlings**

Sarkheil, M; Safari, O (2020) Environmental Technology and Innovation 18: 100747

Water treatment, reuse, and reducing the nutrients loading to the aquatic environments are key ways to achieve sustainable aquaculture. The usage of aquatic plants is an effective and environment-friendly method for water treatment. This study was conducted to investigate the nutrient removal efficiency of aquatic plant \textit{Lemna minor} by static test and flow test using a water recirculation system for rearing of African cichlid (\textit{Labidochromis lividus}) fingerlings during 7 and 30 days, respectively. The growth performance of fish and water quality parameters were compared between the \textit{L. minor} and control groups in triplicate. The results of static test showed that \textit{L. minor} removed the total nitrogen ammonia (TAN) and total phosphorus (TP) by 43.7% and 52.38% after 48 h and 7 days, respectively. The results of flow test revealed that the survival rate (%) and growth performance including final weight, final length, weight gain, specific growth rate (SGR%), body weight increase (BWI%) and daily growth index (DGI) of fish cultured in a water recirculation system containing \textit{L. minor} as a biofilter were significantly higher than the control (P<0.05). The utilization of \textit{L. minor} decreased the concentrations of TAN, TP, electrical conductivity (EC) and total suspended solids (TSS) by 41%, 37.80%, 2.60% and 81.11% compared to the control after 30 days of cultivation period. The nitrate (NO$_3^-$) concentration increased to the maximum level on day 20 and then it decreased significantly on day 30 in the \textit{L. minor} treatment (P<0.05). These findings indicated that the usage of aquatic plant, \textit{L. minor} could be considered as an effective biological method for water treatment in aquaculture.

**Phytotoxicity**

**Growth and essential carotenoid micronutrients in \textit{Lemna gibba} as a function of growth light intensity**

Stewart, JJ; Adams, WW; Escobar, CM; Lopez-Pozo, M; Demmig-Adams, B (2020) Frontiers in Plant Science 11: 480

Duckweed is a promising food crop with multiple benefits for space applications. Fresh duckweed could deliver synergistically acting essential antioxidant nutrients to a crew - but only if growth conditions provide the plant with the right cues to trigger antioxidant formation. We grew \textit{Lemna gibba} under continuous growth light ranging from low to very high intensities (photosynthetic photon flux densities = PPFDs) in order to investigate the effect on plant growth, photosynthesis, and level of carotenoid antioxidants that are essential human micronutrients. \textit{Lemna gibba} achieved remarkably high growth rates under modest growth PPFD by virtue of superior light absorption resulting from minimal self-shading and high chlorophyll levels. Conversely, \textit{L. gibba}'s growth rate remained high even under very high growth PPFDs. This notable ability of \textit{L. gibba} to avoid inactivation of photosynthesis and diminished growth under very high growth PPFDs resulted from a combination
of downregulation of chlorophyll synthesis and increased biochemical photoprotection that limited a build-up of excessive excitation energy. This biochemical photoprotection included accumulation of zeaxanthin (an essential human micronutrient) and high levels of zeaxanthin-catalyzed thermal energy dissipation of excess excitation. Compared to the light levels needed to saturate *L. gibba* photosynthesis and growth, higher light levels were thus required for strong induction of the essential antioxidant zeaxanthin. These results indicate a need for design of light protocols that achieve simultaneous optimization of plant yield, nutritional quality, and light-use efficiency to circumvent the fact that the light requirement to saturate plant growth is lower than that for production of high zeaxanthin levels. How this trade-off between light-use efficiency of growth and nutritional quality might be minimized or circumvented to co-optimize all desired features is discussed.

**Exposure of *Lemna minor* L. to gentian violet or Congo red is associated with changes in the biosynthesis pathway of biogenic amines**

Adomas, B; Sikorski, L; Bes, A; Warminski, K (2020) Chemosphere 254:126752

In the literature, there is a lack of data on the effect of gentian violet (GV) and congo red (CR) dyes on the biosynthesis pathway of biogenic amines (BAs) in *Lemna minor* L. (common duckweed). This plant species is an important link in the food chain. Both dyes inhibited growth, biomass yield and the biosynthesis of chlorophyll a in common duckweed. The predicted toxic units demonstrated that GV had a more toxic effect on the growth rate and biomass yield of common duckweed than CR. Decarboxylase activity in the biosynthesis of BAs in common duckweed is also a useful indicator for evaluating the toxicity of both dyes. Gentian violet also exerted more phytotoxic effects on the analyzed biochemical features of common duckweed because it changed the putrescine (Put) biosynthesis pathway, increased tyramine content 1.6 fold, inhibited the activity of S-adenosylmethionine decarboxylase by 40% and the activity of ornithine decarboxylase (ODC) by 80%. Tyrosine decarboxylase (TDC) was most active in plants exposed to the highest concentration of GV. Similarly, to control plants, in common duckweed exposed to CR, Put was synthesized from ornithine; however, spermidine content was 86% higher, Put content was 51% lower, and ODC activity was 86% lower.

**Surface modification induced cuprous oxide nanoparticle toxicity to duckweed at sub-toxic metal concentrations**

Rippner, DA; Lien, J; Balla, H; Guo, T; Green, PG; Young, TM; Parikh, SJ (2020) Science of the Total Environment 722: 137607

Nanoparticle capping agents are critical for controlling the growth, oxidation state, and final particle size during aqueous synthesis. However, despite the known phytotoxicity of cetyltrimethylammonium bromide (CTAB) to plants, it is used to synthesize metal oxide nanoparticles of uniform size and with mesoporous structure. Among the few studies that have investigated how CTAB influences nanoparticle toxicity, CTAB has never been identified as the primary cause of nanoparticle toxicity in environmental systems; rather nanoparticle surface charge or morphology was identified as the driver of toxicity in environmentally relevant systems. In the current study, CTAB release from CTAB surface modified Cu$_2$O nanoparticles (SM-Cu$_2$O NPs) inhibited duckweed (*Landoltia punctata*) growth, even when administered at subtoxic Cu concentrations. Organic ligands, such as humic acid (HA) and ethylenediaminetetraacetic acid (EDTA), lessened growth inhibition associated with exposure to SM-Cu$_2$O NPs, likely through electrostatic and hydrophobic interactions with CTAB. Such results highlight the need for a more
holistic approach to nanoparticle surface modification and improved communication between toxicologists and synthetic chemists to develop green alternatives for nanoparticle synthesis.

Single and combined effects of the drugs salicylic acid and acetazolamide: adverse changes in physiological parameters of the freshwater macrophyte, *Lemna gibba*

Daniel, D; de Alkimin, GD; Nunes, B (2020) Environmental Toxicology and Pharmacology Pages:103431, DOI:10.1016/j.etap.2020.103431

Pharmaceutical drugs are among the most used chemicals, for human and veterinary medicines, aquaculture and agriculture. Pharmaceuticals are biologically active molecules, having also environmental persistence, thereby exerting biological effects on non-target species. Among the most used pharmaceuticals, one may find salicylic acid (SA), a non-steroid anti-inflammatory drugs (NSAIDs), and acetazolamide (ACZ), a diuretic drug that acts by inhibiting the activity of carbonic anhydrase (CA). In this work, single and combined effects of SA and ACZ were assessed in the aquatic macrophyte *Lemna gibba* L., focusing on physiological parameters, namely photosynthetic pigments, (chlorophyll a, b and total (Chl a, b and TChl) as well as carotenoids (Car)). In addition, chemical biomarkers namely glutathione S-transferases (GSTs), catalase (CAT) and CA. The highest concentrations of ACZ, caused a decrease in the contents of all chlorophylls; this effect was however reverted by SA exposure. Both ACZ and SA levels caused a decrease in CA activity. Nevertheless, when in combination, this inhibition was not observed in plants exposed to the lowest concentration of these drugs. In conclusion, both pharmaceuticals have the capacity to cause alterations in *L. gibba* enzymatic activity and photosynthetic pigments content. Additionally, SA seems to exert a protective effect on this species against deleterious effects caused by ACZ.

Phytotoxicity of Class B aqueous firefighting formulations, Tridol S 3 and 6% to *Lemna minor*

Logeshwaran, P; Sivaram, AK; Yadav, M; Chadalavada, S; Naidu, R; Megharaj, M (2020) Environmental Technology and Innovation 18: 100688

Phytotoxicity of Class B aqueous firefighting concentrates, Tridol-S 3%, and Tridol-S 6% to *Lemna minor* were studied using the parameters such as the frond number, biomass production in terms of dry weight, chlorophyll content and proline accumulation. Decrease in fresh weight, dry weight, and chlorophyll pigments; increase in proline content suggested that both the firefighting concentrates are potentially toxic to *L. minor*. Relative growth rate (RGR) also showed a similar pattern of toxicity with the corresponding increase in test concentrations of both the compounds. The EC$_{50}$ values show Tridol-S 3% was more toxic than Tridol-S 6% in terms of frond number and dry weight. From our findings, it is clear that *L. minor* is highly sensitive to the exposure of firefighting foams, and is suitable for its use as an indicator organism for assessing the aquatic toxicity of aqueous firefighting foams. This study clearly suggests that the migration of Tridol AFFF into aquatic environments is likely to have detrimental effects on the aquatic flora. To the best of our knowledge, this study constitutes the first report on the phytotoxicity of firefighting concentrates, Tridol-S 3% and Tridol-S 6% to *Lemna minor* L.

Comparative ecotoxicity of single and binary mixtures exposures of cadmium and zinc on growth and biomarkers of *Lemna gibba*

Martinez, S; Saenz, ME; Alberdi, JL; Di Marzio, WD (2020) Ecotoxicology DOI: 10.1007/s10646-020-02213-4
In the present study, single and mixture effects of cadmium (Cd) and zinc (Zn) on Lemna gibba were analyzed and compared using growth parameters, based on frond number and fresh weight, and biochemical parameters, such as pigment, protein content and activity of antioxidant enzymes. Plants were exposed for 7 days to these metals in nutrient solution. Single and mixture exposures affected plant growth and the biomarkers of the antioxidant response. Considering the growth parameters, Cd was found to be much more toxic than Zn. IC<sub>50</sub>-7d, based on growth rate calculated on frond number, were 17.8 and 76.73 mg/L, and on fresh weight were 1.08 and 76.93 mg/L, for Cd and Zn respectively. For Cd, LOEC values were obtained at 2.06 and 1.03 mg/L, for frond number and fresh weight respectively, while for Zn, at 20.1 and 74.6 mg/L. A high toxicity effect, considering the same response variables, was observed in plants exposed to the mixtures. Three fixed ratios, based on toxic units (TU) were assayed, ratio 1: 2/3 Cd-1/3 Zn, ratio 2: 1/2 Cd-1/2 Zn and ratio 3: 1/3 Cd-2/3 Zn. Ratio 3 (where Zn was added in higher proportion) was the less toxic. All concentrations of Ratio 1 and 2 significantly inhibited plant growth, showing a 100% inhibition of growth rate at the highest concentrations when based on frond number. Catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APOX; EC 1.11.1.11) and guaiacol peroxidase (GPOX; EC 1.11.1.7) activities in single metals assays were higher than controls. In mixture tests, the activity of APOX and GPOX was significantly stimulated in plants exposed to all evaluated combinations, while CAT was mainly stimulated in Ratio 3. It was observed that the activity of the enzymes was increased in the mixtures compared with similar concentrations evaluated individually. APOX activity was observed to fit the CA model and following a concentration-response pattern. The response of this antioxidant enzyme could serve as a sensitive stressor biomarker for Cd-Zn interactions. Frond number in Cd-Zn mixtures was not well predicted from dissolved metal concentration in solution using concentration addition (CA) as reference model, as results showed that toxicity was more than additive, with an average of sigma TU = 0.75. This synergistic effect was observed up to 50 mg Zn/L in the mixture, but when it was present in higher concentrations a less than additive effect was observed, indicating a protective effect of Zn. A synergistic and dose-ratio deviations from CA model were also observed.

Effects of silver(I) toxicity on microstructure, biochemical activities, and genic material of Lemna minor L. with special reference to application of bioindicator

Li, HB; Mo, F; Li, YH; Wang, MS; Li, Z; Hu, HY; Deng, WH; Zhang, R (2020) Environmental Science and Pollution Research DOI: 10.1007/s11356-020-08844-8

In this research, several biochemical variations in plant of Lemna minor L. were investigated to reflect Ag<sup>+</sup> toxicity. Lemna minor L. changed colorless AgNO<sub>3</sub> to colloidal brown at doses equal to and greater than 1 mg L<sup>-1</sup>. Optical and fluorescence microscopy revealed the presence of bright spots in roots of tested plant related to Ag/Ag<sub>2</sub>O-NPs. Photosynthetic pigment contents of Lemna minor L. declined upon exposure to Ag<sup>+</sup> with an evidently higher decrease in chlorophyll a than in chlorophyll b. Similarly, Ag<sup>+</sup> treatment caused an evident reduction in the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). The reduction in antioxidant activity was significantly higher in POD than in SOD and CAT. Ag<sup>+</sup> treatment resulted in a significant increment in the level of malondialdehyde (MDA) content as the judging criteria of cellular injury which showed sign of dose-related. The alterations occurred in RAPD profiles of treated samples following Ag<sup>+</sup> toxicity containing loss of normal bands, appearance of new bands, and variation in band intensities compared with the normal plants. In addition, morphological character and biomass of Lemna minor L. subjected to increasing Ag<sup>+</sup> concentrations were evaluated to reveal Ag<sup>+</sup> toxicity. Our study demonstrated that Lemna minor L. have a high sensitivity to indicate fluctuation of water quality. It would be beneficial that modulating the genotype of Lemna minor L. to bear high proportion of contaminates.
Taxonomy

A taxonomic revision of *Lemna* sect. *Uninerves* (Lemnaceae)

Bog, M; Sree, KS; Fuchs, J; Hoang, PTN; Schubert, I; Kuever, J; Rabenstein, A; Paolacci, S; Jansen, MAK; Appenroth, KJ (2020) Taxon 69: 56-66

*Lemna* sect. *Uninerves* Hegelm. consists of three species, *Lemna minuta* Kunth (synonym *L. minuscula*), *L. valdiviana* Phil. and *L. yungensis* Landolt. *Lemna yungensis* was discovered growing on rocks in the Yungas in Bolivia by E. Landolt and was described just 20 years ago. In the original description, Landolt reported that this species is closely related to *L. valdiviana* and that it is difficult to distinguish the three species on a morphological basis. Therefore, the taxonomic position and status of *L. yungensis* remained controversial. Here, we carried out a detailed taxonomic study, integrating approaches that include quantitative morphometry, metabolomic profiling by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) as well as molecular genetic analysis using amplified fragment length polymorphism (AFLP), and barcoding of plastidic sequences. We also investigated genome sizes of clones of the three species. Whereas *L. minuta* can easily be differentiated from *L. valdiviana* and *L. yungensis*, it was not possible to distinguish *L. valdiviana* from *L. yungensis* with any of the methods used. These data imply that *L. yungensis* is identical to *L. valdiviana*. Thus, the name *L. yungensis* should be synonymised with the name *L. valdiviana*, since this is the older name.

Genomics- Edited Book

The Duckweed Genomes. Compendium of Plant Genomes

Editors: Cao, Xuan Hieu, Fourounjian, Paul, Wang, Wenqin (Eds.)

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Instructions to Contributors for the Duckweed Forum

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

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

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

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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.

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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.

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• Number all pages, including those with figures on the bottom and center of each page.

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• 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:
  
  o 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

  o be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

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• Provide the complete names of all authors.

• Identify which author will receive correspondence regarding the contribution.

• Provide the corresponding author’s name, telephone number, and current e-mail address.

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

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Links for Further Reading

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

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

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

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

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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