1 Importance of Duckweeds in Basic Research and Their Industrial Applications

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Abstract

The Lemnaceae family, commonly called duckweeds, is 37 species of the smallest and simplest flowering plants found floating on nutrient-rich waters worldwide. Their small size and rapid clonal growth in aseptic conditions made them a stable and simple model for plant research especially from 1950 to 1990, when they were used to study plant physiology and biochemistry including auxin synthesis and sulfur metabolism. Duckweed research then saw a resurgence in 2008 when global fuel prices rose and the US Department of Energy funded the sequencing of the *Spirodela polyrhiza* genome. This launched not only the genomic investigations detailed in this book, but the regrowth of duckweed industrial applications. Thanks to their ability to quickly absorb nitrogen, phosphorous, and other nutrients while removing pathogens and growing at a rate of 13–38 dry tons/hectare year in water treatment lagoons, scientists are currently exploring ways that duckweed can convert agricultural and municipal wastewater into clean water and a high-protein animal feed. The potential of these plants for phytoremediation of heavy metals and organic compounds also allows the possibility to clean the wastewater from heavy industry while providing biofuels and even plastics. Finally, thanks to their superb nutritional profile *Wolffia* species grown in clean conditions promise to become one of the healthiest and most environmentally friendly vegetables. Given the importance of these incredible plants, it is no wonder researchers are investigating the genetic mechanisms that make it all possible.

This chapter was revised and significantly expanded upon, with the guidance of T. F., from the chapter "The Importance and Potential of Duckweeds as a Model and Crop Plant for Biomass-Based Applications and Beyond," in the Handbook on Environmental Materials Management, which X. H. C. and P. F. wrote for Springer Nature a year ago (Cao et al. 2018). We hope this chapter thoroughly explains non-genomic research and application topics, especially for those who are unfamiliar with the family.

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1.1 Introduction

Duckweed (known as monocotyledon family *Lemnaceae* or recently classified as subfamily *Lemnoideae* in the arum or aroid family *Araceae*) is a small group of aquatic plants with only five genera (*Spirodela*, *Landoltia*, *Lemna*, *Wolffia*, and *Wolffiella*) and 37 species (see Landolt 1986; Nauheimer et al. 2012; Sree et al. 2016). Except for *Wolffiella* (commonly named as bogmat) that is restricted to the Americas and Africa, species of other duckweed genera occur around the whole world. Although highly adaptable across a broad range of climates, most diverse species of duckweed appear in the subtropical or tropical zones. Duckweed species tend to be associated with nutrient-rich or eutrophic freshwater environments with quiet or slow-moving flow. However, they are extremely rare in deserts and are absent in the cold polar regions (Arctic and Antarctica).

Duckweed species are the smallest flowering plants with minute sizes from 0.5 mm to less than two cm (Landolt 1986). Species of duckweed can be easily distinguished morphologically from species of any other flowering plants, even closely related aquatic plants, due to their highly reduced body structure. The leaflike body of the duckweed species, sometimes called a frond or thallus, is a modified stem with only few cellular differentiations (Fig. 1.1). The growth of duckweed vegetatively occurs by budding within the pouches or cavities of the basal sections of the fronds. Each daughter frond emerging from the pouch of mother bud already contains two new generations of daughter fronds. Therefore, under optimal conditions, the growth rate of duckweed is nearly exponential. The frond number of fast-growing species (e.g., *Lemna aequinoctialis*, *Wolffiella hyalina*, and *Wolffia microscopica*) almost doubles within 24 h (Ziegler et al. 2015; Sree et al. 2015b), presenting the fastest growing flowering plants. With a miniaturized body plan and such rapid growth leading to maximum fitness, duckweed has arguably been interpreted as an example of the hypothetical Darwin–Wallace Demon for the lifetime reproductive success (Kutschera and Niklas 2015).

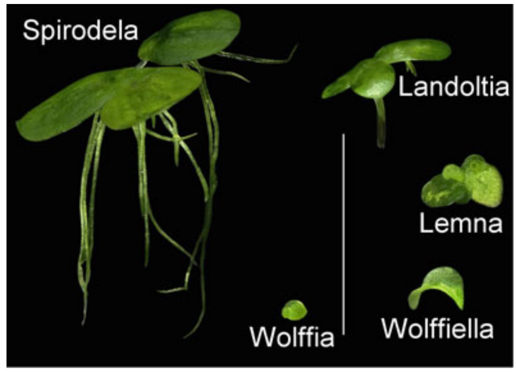

Fig. 1.1 Morphology of five representative species for duckweed genera. Spirodela: *Spirodela polyrhiza*; Landoltia: *Landoltia punctata*; Lemna: *Lemna minor*; Wolffiella: *Wolffiella lingulata*; Wolffia: *Wolffia arrhiza*. Bar: 1 cm

Only occasionally or very rarely, several species of duckweeds produce microscopic flowers in nature as well as under in vitro conditions (Fu et al. 2017; Schmitz and Kelm 2017; Sree et al. 2015a). In *Spirodela* and *Lemna* (belonging to the subfamily *Lemnoideae*), the flowering organs (1 membranous scale, 2 stamens, and 1 pistil) originate in the same pouches in which the daughter fronds are normally formed. In the subfamily *Wolffioideae* (consisting of *Wolffiella* and *Wolffia*), generative and vegetative reproductions are spatially separated occupying the floral cavity on the upper surface of the frond and the budding pouch, respectively.

Duckweed fronds are free floating on or near the surface of the water, often forming dense mats in suitable climatic and nutrient conditions. In unfavorable weather, such as drought or freezing winter seasons, in addition to flowering, several duckweed species are able to form special "resting fronds" (in the dormant phase) to persist until conditions return that can support growth. In place of a frond, the greater duckweed (*Spirodela polyrhiza*) produces a starch-rich tissue called a turion, which sinks to the bottom of the water. Turion production has been reported also for *Lemna turionifera*, *L. aequinoctialis*, *Wolffia brasiliensis*, *Wolffia borealis*, *Wolffia angusta*, *Wolffia australiana*, *Wolffia arrhiza*, *Wolffia columbiana*, and *Wolffia globosa*. These turions do not grow any further but can germinate and start a new life cycle from the bottom of the water body or mud when the water temperature reaches about 15 °C. In addition, resting fronds of the ivy duckweed (*Lemna trisulca*) and *Wolffiella gladiata* with reduced air spaces can accumulate starch and still rather slowly grow on the bottom of the water, forming new but similar fronds. However, the common duckweed (*Lemna minor*), gibbous duckweed (*Lemna gibba*), *Lemna perpusilla*, and some strains of *Lemna japonica* produce starch-rich fronds that do not sink to the bottom of the water but are just pressed down under the ice cover during freezing temperatures. Interestingly, formation of turions as a survival and adaptive capacity of *S. polyrhiza* strains collected from a wide geographical range seems to be genetically determined and highly influenced by the mean annual temperature of habitats (Kuehdorf et al. 2013). Furthermore, the family displays significant inter- and intraspecies differences of cell physiology (e.g., starch, protein, and oil contents) together with duckweed potential for industrial applications (Alvarado et al. 2008; Appenroth et al. 2017; Hou et al. 2007; Mkandawire and Dudel 2005; Tang et al. 2015; Yan et al. 2013; Zhang et al. 2009).

Due to their small and abbreviated structures, morphological and physiological classification of the 37 duckweed species (*Spirodela*: 2 species; *Landoltia*: 1; *Lemna*: 13; *Wolffiella*: 10; *Wolffia*: 11) can be challenging. In the past decade, for species assignment as well as resolving intraspecies differences, several attempts have been carried out to employ molecular genotyping techniques, including random amplified polymorphic DNA (RAPD; Martirosyan et al. 2008), inter-simple sequence repeats (ISSR; Fu et al. 2017; Xue et al. 2012), simple sequence repeats (SSR; Feng et al. 2017), amplified fragment length polymorphism (AFLP; Bog et al. 2010, 2013), and DNA barcoding using plastid sequences (Borisjuk et al. 2015; Wang et al. 2010) or nuclear ribosomal sequences (Tippery et al. 2015). Although DNA barcoding using two plastidic barcodes aids in identifying most duckweed species (at least 30 among 37 species) in a quite simple and straight forward manner, combination of different techniques or using additional barcodes may help to unambiguously and economically assign remaining duckweed species.

The Lemnaceae family was one of the earliest model plants due to their ease of aseptic cultivation in the laboratory and simple morphology. The second volume of Landolt and Kandeler's 1987 monographic study contains 360 pages dedicated to the physiological research of the family in particular and plants as a whole (Landolt and Kandeler 1987). The professors who organized the first duckweed conference summed up the duckweed research stating that duckweeds were the main model for plant biology from 1950 to 1990, when Arabidopsis and rice were used for their sexual reproduction and applicability to terrestrial crops (Zhao et al. 2012). In that time, investigations of duckweeds revealed the tryptophan-independent synthesis of auxin (Baldi et al. 1991), translational regulation in eukaryotes (Slovin and Tobin 1982), and seven of the first stable plant mutants (Posner 1962). Today, physiological studies continue largely in the fields of circadian rhythm research, xenobiotic plant–microbe interactions, and phytoremediation and toxicology. Starting in 2011, a biannual series of international duckweed conferences in research and applications has connected and helped expand this research community and increased public awareness and recognition of duckweed economic and environmental importance (Zhao et al. 2012; Lam et al. 2014; Appenroth et al. 2015). Together with the completion of the *Spirodela* genome in the year 2014 and rapid advances in sequencing technologies, this resurgence of interest has resulted in a proliferation of genome and transcriptome sequences for duckweed species and ecotypes discussed in the remainder of this book.

One of the largest fields of duckweed research is ecotoxicology, where the widely distributed *Lemna* species *minor* and *gibba* serve as model plants to determine the effect of a compound on an ecosystem. These growth tests have been standardized in the International Organization for Standardization's protocol ISO 20079 which handles environmental samples and the Organisation for Economic Co-operation and Development's assay OECD 221, which was developed for specific chemicals and compounds (ISO 2005; OECD 2006). Both are seven-day growth rate tests, which use different media, to measure the effective concentration of the substance, or EC_{50}, where the growth rate by frond count or frond area is half of the control. These tests date back to the 1970s and have surveyed the effects of heavy metals, pharmaceuticals, various pesticides and organic compounds, and even radioactivity on Lemna growth rate and health, helping us quickly asses and monitor environmental safety.

In order to perform major gene function studies, as well as to improve duckweed agronomic performance (Cao et al. 2016), it is required to establish an efficient system for genetic manipulation and transformation. Several stable transformation protocols for *Lemna* (Chhabra et al. 2011; Yamamoto et al. 2001), *Landoltia* (*Spirodela oligorrhiza*; Vunsh et al. 2007), and *Wolffia* (Boehm et al. 2001; Khvatkov et al. 2015) using either *Agrobacterium*-mediated or biolistic gene transfer together with a recent gene-silencing platform in *L. minor* (Cantó-Pastor et al. 2015) have been described, providing the means to further develop gene/genome-edited duckweed as a powerful biomanufacturing platform.

1.2 Current State of Duckweed-Based Applications

1.2.1 Historical

For centuries, people have seen the role duckweed can play in their food production. Perhaps by observing their livestock consume duckweed species, small-scale farmers in Southern Asia started feeding duckweed, often fresh as a portion of the diet, to their fish, ducks, chickens, pigs, and goats. In addition to animal feed, the people of Thailand, Laos, and Cambodia have consumed wild harvested and farmed *Wolffia*, mainly *globosa*, rinsed, and then incorporated into soups, salads, sauces, and a wide variety of foods (Bhanthumnavin and Mcgarry 1971). If the *Wolffia* is not cooked in with other ingredients, it is generally briefly boiled, thereby selecting a duckweed species without harmful calcium oxalate crystals and killing potential pathogens. Recently, farmer education programs in Guatemala, Indonesia, and across the globe have improved the use of duckweed to treat manure while using it as a fertilizer and expanded the practice within Asia and around the world, especially in Central America where a consortium of ~200 small-scale farmers grows duckweed and tilapia. It is estimated by the executive director of the International Lemna Association that the thousands of small-scale farmers collecting wild duckweed or growing it on site for human or animal consumption are currently a greater part of the duckweed applications by volume than the large-scale, higher tech companies.

1.2.2 Water Treatment

As global population rises, so does demand in clean water supply and wastewater treatment systems. While developed nations have often relied on a combination of aerobic bacteria degradation and chemical treatment in activated sludge systems, a variety of natural treatment systems have been growing in popularity for their often 50% lower capital and operating costs, ability to recapture nitrogen, phosphorous, and other valuable nutrients, and in some cases convert them into appropriate products. The main downsides of these natural treatment systems are their larger land requirements (up to 5 m^2/person), poorer performance at cold temperatures, and the requirement of knowledgeable and diligent staff to manage ecosystems through toxic wastewater streams, harsh weather, etc. All this indicates that natural treatment systems such as constructed wetlands are ideal in rural locations, especially of tropical countries, precisely where many of the 2.5 billion people without access to sanitary wastewater treatment live (Zhang et al. 2014b).

While a variety of plants have been used effectively in constructed wetlands, we will focus here on the 37 species of the Lemnaceae family for their global distribution, tolerance of ammonia, heavy metals, other stresses, high yield of biomass (especially at 20–30 °C), ease of harvest, high protein and starch content, and range of uses. As seen in Fig. 1.2, duckweed can treat agricultural, municipal, and even industrial wastewater streams into clean non-potable water, and a biomass that can be used for feed applications, or fuel if it was used to treat harmful industrial wastewater.

The classic example of a duckweed treatment system and feed application would be the Mirzapur Bangladesh hospital wastewater facility, which was designed by the PRISM group, monitored from 1989 to 1991, and thoroughly described in the book "Duckweed Aquaculture: A New Aquatic Farming System for Developing Countries" (Skillicorn et al. 1993). The book describes a pilot plant facility with clean effluent water of 1 mg/l BOD (biological oxygen demand) and 0.03 mg/l of both NH_3 and P, an annual duckweed dry yield of 13–38 metric tonnes/hectare year (t/ha yr), carp production of 10–15 t/ha yr, and positive economic analysis of duckweed, duckweed-fed carp, and duckweed-fed tilapia farming. As of 2015, the Mirzapur facility was still operational, profitably treating wastewater above the standards of any US city, while providing fresh, pathogen-free, sustainably farmed fish. Professor Zhao Hai's group from Chengdu Institute of Biology, Chinese Academy of Sciences, also has extensive records from their pilot plant at Dianchi Lake, in subtropical Yunnan, China (Fig. 1.2). In a year-long comparison between duckweed and water hyacinth, they found a higher total yield for water hyacinth (55 compared to 26.5 t/ha yr) and a higher nitrogen removal rate, partially due to denitrifying bacteria. However, they chose to focus on duckweed for its consistent year-round production, ~33% protein content, and biofuel potential as a low lignin, high starch ethanol feedstock (Zhao et al. 2014). In follow-up

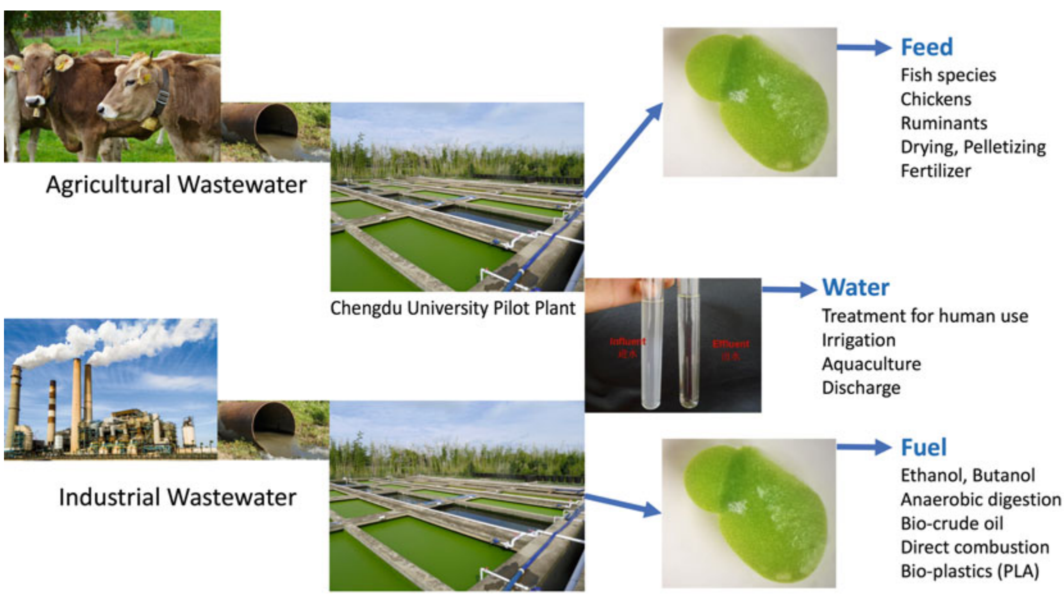

Fig. 1.2 Flowchart of duckweed wastewater treatment and biomass application. Farm and factory examples, and the pilot plant at Chengdu University. Their influent agricultural wastewater and effluent water in the two test tubes. Mother and daughter frond of *Lemna minor*. While duckweed can be grown on agricultural or industrial wastewater and used for feed or fuel, the applications of the biomass are determined by the input water source and local regulations. *Source* Hai Zhao, Chengdu University, China

experiments, they found they could increase duckweed starch content from 9.5 to 40% through 11 days of growth on clean water, and that a hydraulic residence time (HRT) of 6 days achieved their treatment standards and optimized the *Landoltia punctata* starch yield above maize and wheat to 13.9 t/ha yr. Considering that these are experimental water treatment plants, their duckweed yield is expected to rise with further optimization, or in more intensive cultivation. For their size, length of study, and abundance of publicized information, these two facilities stand as prime examples to study duckweed's water treatment capabilities, yield, and applications in practice.

If a wastewater stream comes from an industrial point source or a large municipality, it likely has persistent chemical compounds, such as textile dyes and metalworking fluids, or bioaccumulating heavy metals in it. There is a large body of academic evidence illustrating the potential of duckweed and other plants to treat wastewater from cities, tanneries, mines, metalworking shops, and textile mills by degrading compounds like pharmaceuticals and antibiotics, and accumulating phenols along with heavy metals (van der Spiegel et al. 2013). Rezania et al. reviewed the heavy metal absorption of 5 different plant species and described 19 studies evaluating *Lemna minor* and *gibba* as moderate or hyperaccumulators, often concentrating metals over 400-fold, depending on the metal and circumstance; even when used as a dried powder (Rezania et al. 2016). A table of 10 studies illustrated removal efficiencies of Cu, Cd, Pb, Zn, and 9 other metals, with the lowest being 29% and the majority being over 70%. In these cases, duckweed and its microbial communities can treat a variety of harmful wastewater streams and then be utilized outside of the food supply for biofuel applications to further concentrate the metals.

1.2.3 Bioenergy

While these applications have been researched academically, few have been practiced in large scale. The simplest bioenergy application would be direct combustion of dried duckweed, possibly as a drop-in fuel for a trash incinerator or coal-fired power plant. This would concentrate heavy metals in the smoke, which could be scrubbed, and ash for proper disposal, or even encapsulated reuse in concrete or gypsum as coal ash is in the USA. A second relatively simple option would be anaerobic digestion to produce methane. Conveniently, many municipal wastewater treatment plants already have anaerobic digesters to treat sludge, and the liquid digestate has been well studied as a fertilizer for duckweed ponds. A duckweed and pig manure mixture increased gas production 41% in comparison with pig manure, while the increased production from cow manure tapered after a 2% inclusion of duckweed (Cui and Cheng 2015).

Another possibility is pyrolysis of dried biomass or hydrothermal liquefaction (HTL) of wet biomass. Both processes are similar, yet we will focus on HTL since it conveniently avoids drying the ~90% water content duckweed biomass. In HTL, biomass and water processed at 200–400 °C and 50–200 times atmospheric pressure for 10–90 min to create aqueous solutes, H_2, CO_2, and CH_4 gasses, high molecular weight bio-char, and bio-crude oil with 95% of the energy content of diesel (Zhang et al. 2014a). A wide variety of feedstocks from algae to wood and to sewage sludge can be used, separately, or mixed, and each requires significant testing to optimize, which is likely why there are no large-scale HTL operations at the present day. The algae can be converted to crude oil with a 26–68% yield depending on the conditions, yet all the crude oil tends to have a high water content and require hydro-deoxygenation to dewater it thereby matching the stability and viscosity of petroleum crude oil. A wide range of molecules can be created and isolated so there is petrochemical potential as well. This option is interesting for its theoretical ability to match the wide variety of the crude oil applications in a carbon neutral manner and the ability to produce in hours what naturally takes ~150 million years.

The most versatile and best studied application of potentially harmful duckweed is fermentation of the starch, which can be accumulated at rates varying from 46% after 5 days to 31% after 10 days of nutrient starvation and fermented at 95% efficiency after enzymatic pre-treatment. These fermentation processes also create protein-rich distiller's grains, which can be used as an animal feed supplement if they are not concentrating heavy metals. As the first commercially viable example of ethanol fermentation, the Andrew Young Foundation conducted a private research trial using the ecosystem technology, produced by resource recovery experts Greenbelt Resources Corporation, which was presented in a feasibility study report conducted by an independent party Agregy and submitted to the USDA in 2017. With successful feasibility determined, the foundation created a corporation called Duckweed Days LLC, which partnered with Greenbelt Resources to conduct a pilot system development project in Paso Robles, California, USA, in 2018. Leveraging its farming and agricultural expertise as well as its engineering prowess, Greenbelt has developed a species agnostic prototype cultivation, harvesting and processing system. For the biorefining of the cultivated duckweed, Greenbelt's proprietary, partially AI-operated modular machinery uses membrane filtration to produce anhydrous bioethanol that can be sold as a fuel or solvent, plus chemically safe distillers' grains that can be used as animal feed or a nutritious protein concentrate.

Ethanol is not the only fermentation product, since *Clostridium acetobutylicum* bacteria can convert the sugars of 32% starch content duckweed into a mixture of 68% butanol, with acetone and ethanol coproducts (Cui and Cheng 2015). Ethanol can of course be blended into gasoline at rates up to 10% or 85% for certain flex-fuel vehicles, while significantly more expensive butanol behaves very similarly to gasoline. Finally, the Argentinian company MamaGrande experimented with fermentation as a means to generate lactic acid for polymerization into PLA. Polylactic acid, or PLA, is a renewable and degradable plastic produced by enzymatically digesting starch to glucose, fermenting the glucose to lactic acid, and then purifying and polymerizing it. At the moment, anaerobic digestion and ethanol fermentation appear to be the best studied options, while fermentation is the only biofuel in full-scale commercial application.

1.2.4 Animal Feed

Most agricultural wastewater and certain domestic wastewater streams will have undetectable or legally permissible levels of heavy metals, enabling a design where duckweed can recycle nutrients back into the food supply, provided it is monitored for heavy metals and other hazards, and legally approved. Agricultural wastewater, which can come from greenhouses, livestock barns, anaerobic digesters, or even food processing facilities, is often heavy metal "free" and therefore diluted down to 20–50 mg/l total nitrogen for optimal duckweed growth. Considering the pilot plant examples above, and publicly posted information from Paul Skillicorn's Agriquatics Blog, we see the following steps for domestic wastewater treatment (Fig. 1.3).

First, solids will be removed by screening and then primary settling lagoons or laminar flow systems and hydrocyclones, possibly for anaerobic digestion. Secondly, there may be a buffer lagoon or lagoons, which treat soaps and other chemicals that may harm duckweed or its downstream applications. Third will be the duckweed farm portion, where a diluted influent with NH_3 concentrations of 10–30 mg/l, BOD of 15–30 mg/l, and pH from 6.0 to 7.0 will fertilize rapidly growing high-protein duckweed biomass. Fourth, ponds with slower growing, high starch content duckweed can polish wastewater as the final cleaning step. Here, once nitrogen has been depleted heavy metals will be accumulated, with the majority of municipal effluents producing duckweed passing US food and feed safety standards. HRT can vary from 6 to 15 days depending on environment, degree of effluent recirculation, and treatment standards. For example, the Mirzapur duckweed ponds reduced NH_3 from 32 to 0.03 mg/l. This high HRT increases the footprint compared to a conventional system, while providing resilience against heavy rains or community crashes that

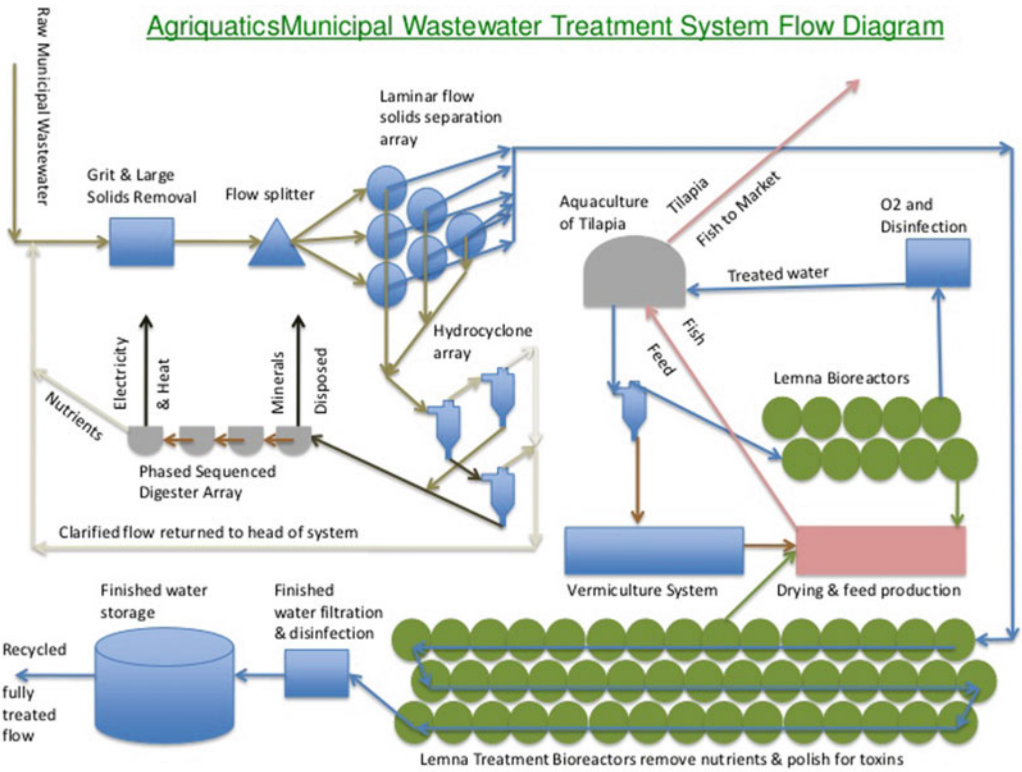

Fig. 1.3 Agriquatics wastewater treatment and aquaculture diagram for Olmito, Texas. Proposed blueprint for a municipal treatment facility designed by Agriquatics. The systems start with solids removal through laminar flow separators and hydrocyclones, and sends solids to an array of bacterial digesters, which act as an improved anaerobic digester similar to conventional methods. A series of duckweed ponds remove solutes, and their circular shape facilitates central harvesting. Water is then filtered and disinfected with conventional methods. Duckweed biomass can be tested, sterilized, and converted to Tilapia feed, while aquatic worms and duckweed purify water and provide food for the Tilapia. *Source* Paul Skillicorn, Lyndon Water Limited, UK, https://paulskillicorn.wordpress.com/about/

occasionally overwhelm smaller systems. Throughout this process, pathogens are largely killed off, evaporation is reduced 33%, mosquito populations are reduced, and odors are partially suppressed by the duckweed mat (Goopy and Murray 2003; van der Spiegel et al. 2013). Finally, polished water and duckweed biomass can be sterilized and utilized. In a budget estimate for a medium-sized treatment plant in Texas, USA, Agriquatics illustrated that their treatment system would have 52% of the capital and 66% of the total annual costs of a conventional oxidative ditch system. This budget completely excluded the proposed tilapia aquaculture system that had been proved profitable in Mirzapur. To make larger duckweed treatment systems, even more cost-effective Agriquatics has positioned them on the outskirts of cities to benefit from rising real estate value as the city grows, while providing greenspace and reducing pipe distance.

Since duckweeds have been a traditional feed for fish and poultry in South East Asia for centuries, they are now being quantitatively investigated in a variety of feed trials. In many cases, NH_3-tolerant *Lemna* and *Spirodela* species are used and harvested with dry weight protein contents of 20–30%. To minimize pathogen transfer, feed trials often use effluent from one species to grow duckweed, which is then fed to a different species. While ozone and microwave disinfection were used in the long-term commercial operation of Mirzapur, many feed trials have simply washed with water, or just harvested duckweed, and have no report of pathogens (Goopy and Murray 2003; Skillicorn et al. 1993). Surprisingly, several studies have found duckweed, including samples from hospital wastewater to be safe as chicken and fish feed with regard to *E. coli* and *Salmonella*, with no significant differences in the quantity of five different pathogens in chickens fed on duckweed compared to control, presumably due to the severe pathogen reduction seen in wastewater treated by duckweed and its associated microbial communities (Goopy and Murray 2003; van der Spiegel et al. 2013). The feed trials often use dried duckweed as a percentage of complete commercial feed or substitute it for a percentage of the soybean or fishmeal component, with duckweed performing very similarly to soy in the case of chickens, ducks, and fish, up to a point where it is suspected that oxalates or other anti-nutritives inhibit growth (Goopy and Murray 2003; Skillicorn et al. 1993). For tilapia, inclusion rates of 30% were found equivalent to control, and 30% replacement of fishmeal component was seen as the most cost effective (Goopy and Murray 2003). An ecosystem of 5 different carp species or the grass, catla, and mirror carp and tilapia species individually can be fed on a pure duckweed diet, with a carp yield of 10–15 t/ha yr (Skillicorn et al. 1993). Duckweed was found to be beneficial in replacing ~15% of the soybean meal in the feed for chicks or broilers, and 40% in the case of laying hens (Goopy and Murray 2003; Skillicorn et al. 1993). In some cases, pig saw decreased growth in response to small inclusion rates of duckweed, while the Mong Cai piglets of Vietnam had higher growth rates than their Large White counterparts due to higher nitrogen digestibility (Goopy and Murray 2003; Gwaze and Mwale 2015). Finally, ruminants have shown promising results with high nitrogen digestibility in merino sheep, and cattle consuming and effectively digesting up to 10% of their weight in dried duckweed per day (Goopy and Murray 2003). Taken together, these results show the potential of duckweed to reduce the environmental impact of livestock by recycling nitrogen phosphorous and other nutrients that currently cause eutrophication, while partially replacing human edible soy and non-sustainable fishmeal. Furthermore, recycling wastewater to grow animal feed has been shown in several economic analyses to raise farmer income, especially in developing countries.

Considering the economic and environmental benefits, and the success of duckweed as feed for a variety of livestock species, there will likely be a rapid expansion of the duckweed agricultural sector and its use as a sustainable animal feed. In the FAO's 2012 estimates, global demand for non-fish animal protein is expected to increase at 1.3% per year till 2050, with the largest growth of 4.2% in South Asia, with similar numbers in the 2030 projection (Alexandratos and Bruinsma 2012). Roughly, half of this increase is expected to be as poultry (OECD/FAO 2017). Additionally, the largest increase in animal protein supply will be aquaculture, which was ~17% of global fish supply in 1990, grew largely in Asia between 4 and 10% per year, and is forecasted to exceed the global catch in 2020 (OECD/FAO 2017). The livestock sector is, however, very feed, land, and water intensive, and all reports stress the need to reduce the environmental impact particularly through improving the feed supply. With their ability to treat agricultural wastewater on non-arable land and provide an affordable protein-rich feed, a greater number of farmers are turning to duckweeds as a cheap sustainable feed source. There are currently several commercial ventures and hundreds of thousands of small-scale farmers growing duckweed primarily in Asia and Central America feeding tilapia, ducks, chicken, and pigs. Since they are sustainably feeding the livestock species in the regions where the FAO expects the largest growth in the world, it is natural to expect this industry to grow. While working with farmer education programs in Guatemala and Indonesia, the ILA, International Lemna Association, has seen an increase in educational activities for small-scale farmers and 20% more businesses seeking to enter the industry for the past 7 years (Table 1.1, Director of the ILA).

Table 1.1 Summary of the duckweed applications in use or development and the major companies working on them

Application	Company (if blank academic)	Genera
Human food	Hinoman, Green Onyx, Parabel	Wolffia, Lemna
Protein isolate	Plantible, Parabel, CAIS	Lemna
Livestock	Many small-scale farmers	Lemna, Spirodela, others
Conversion chemicals	MamaGrande	Lemna
Wastewater treatment	MamaGrande, CAIS	Mixture
Space life support	Space Lab Technologies	Lemna, Wolffia
Isolation chemicals	CAIS	Mixture
Transformation		
Specialty (cosmetics, pets, tea)		
Biofuels or energy	Greenbelt Resources	

1.2.5 Human Nutrition

The high growth rate, protein content, and success in a variety of animal feed trials naturally beg the question of whether duckweeds could be a healthy and environmentally friendly food for humans? As previously stated, the *Wolffia* genus of the duckweed family has been traditional cuisine in Thailand, Burma, and Laos for centuries, since they lack the kidney stone forming calcium oxalate crystals found in the other genera. At the time of writing, there are three large companies producing *Wolffia* or *Lemna* for human consumption, namely Hinoman with greenhouse precision agriculture cultivation, Parabel with open pond *Lemna* cultivation and protein extraction, and Green Onyx, which has developed robotic farming systems that can dispense *Wolffia* on demand. Due to their successful scale-up since their founding in 2010, and abundant public information, we will focus on the Israeli company Hinoman here. They currently grow *Wolffia* (aka Mankai™) on formulated, clean water media in greenhouses with automated energy-efficient climate control and harvesting systems operated by their cultivation algorithm. Through this system, they are able to grow a pesticide- and herbicide-free vegetable year-round, with a fraction of the water used in cultivation of soy, spinach, or kale, (http://www.hinoman.biz/what-we-do/). Their product is stable with approximately 25% carbohydrate content, 45% protein content, and a complete and bioavailable amino acid profile such as egg or soy, with a higher PDCAAS than soy. They have currently conducted three publicly visible clinical trials demonstrating the protein and iron bioavailability, as well as the mitigating effect on Glycemic Index of their *Wolffia*, and posted multiple recipes for their product, which will soon be made available to consumers.

Furthermore, compared to kale *Wolffia* is more abundant in most minerals and vitamins A, B2, B12, and E, which survive the gentle drying process. An extensive academic investigation of the species *Wolffia microscopica* confirmed the high mineral content and that the protein (~25% of dry weight) exceeded WHO recommendations, while finding abundant antioxidants and a high omega-3 content (Ω6/Ω3 ratio is 0.61) for the relatively scarce lipids (Appenroth et al. 2017, 2018). Fresh, or dry powdered *Wolffia*, with a neutral taste, can be juiced, consumed fresh, or incorporated into breads, pastas, and sports nutrition products (Fig. 1.4). With supporting data from academic laboratories, records of historical consumption, and thorough testing of their product for harmful metals and oxalates, Hinoman and Green Onyx were able to achieve the generally recognized as safe (GRAS) status for the *Wolffia* species *arrhiza*

Fig. 1.4 *Wolffia* fortified breads. Hinoman has tested the addition of *Wolffia* to multiple food and beverage products. Note the retention of the chlorophyll pigments throughout the baking process, and unchanged texture and leavening of the bread. *Source* http://www.hinoman.biz

and *globosa* in the USA in 2015 and 2016, respectively. Now, with South East Asia, Israel, and the USA recognizing select *Wolffia* species and *Lemna minor* as human food the crop and its producers have significant potential to grow and provide abundant plant protein for minimal land, water, and energy inputs.

With their small size, growth rate, aquatic lifestyle, and high protein content, the duckweeds provide a promising new crop to grow and an assortment of cultivation and preparation processes for human consumption. Given the growing consumer demand for novel vegetables and healthy leafy greens, companies like Hinoman and Green Onyx grow these tiny nutritious vegetables in clean environments with robotic systems and plan to bring them into our grocery stores and homes both frozen and fresh. The global market for plant-based protein (57% of total global protein supply; Henchion et al. 2017) has been growing at 12.3% per year from 2013 to 2016, and is anticipated to grow 6.7% annually from 2018 to 2021, when it is anticipated to exceed 1 billion USD. Seeing this demand for protein, Plantible Foods is developing a gentle protein isolation process using *Lemna* in order to create a colorless, tasteless protein isolate with the physical properties of egg whites to create a vegan product that can finally match the textures of many beloved foods. Additionally, Parabel has chosen to sell its duckweed product as a high protein powder. Given the expansion of the plant protein market in both whole and extract formats, and their current progress, we expect these and other companies to increase in size, dramatically, providing a healthy and environmentally friendly alternative to less efficient protein sources.

As seen above, duckweed wastewater treatment performs well in tropical and subtropical environments, requires more land, yet less funding to operate, and even has the potential to generate revenue if duckweed biomass and clean effluent are well utilized. Agricultural wastewater can be converted into animal feed supplements, while industrial effluents can be treated to degrade or accumulate harmful chemicals and heavy metals while producing bioenergy, according to the laws of the land. The duckweed has proven to be a suitable food source for both humans and livestock, and will likely play an expanding role in meeting future food demands. There is plenty more to learn at the International Lemna Association and The Charms of Duckweed Web sites, and in the Duckweed Forum newsletter. Given the tremendous diversity of species, strains, environments, and applications, along with the relatively recent commercial interest, duckweed researchers are continuously rediscovering what is possible and practical.

1.3 Future Prospects in Duckweed-Based Applications

The field of duckweed applications has made tremendous progress recently. For centuries, it was harvested from wild ponds and used as a vegetable or animal feed in certain parts of the world, and largely in the twenty-first century humans have recognized the potential of these tiny overlooked plants and started applying them to wastewater treatment, and larger-scale animal feed and human nutrition operations. While certain applications are mature enough for large-scale deployment, those discussed below include important developing technologies. In terms of scale and possibility of duckweed applications, we believe in 2019 we are still looking at the tip of the iceberg.

Due to the success and low prices of other crops, many companies growing duckweed are focused on high-tech, high-value applications to avoid commodity markets. Similar to protein extracts, several high-value products, like sugars, antioxidants, and oils, are being extracted from duckweed biomass in academic and commercial research laboratories. Appenroth et al. conducted a thorough investigation of *W. microscopica* and found a complete plant protein, roughly 150 mg carotenoids and 22 mg of tocopherols/gram dry weight, and an oil profile of 61% polyunsaturated fatty acids with a high content omega-3 s and a phytosterol content minimum fivefold higher than common plant oils, presenting several healthy, high-value compounds that may be extracted (Appenroth et al. 2017). After or without extraction of certain compounds or protein, biomass can be converted to other products, for example MamaGrande's research in converting starch to sugar, and then polylactic acid valued at ~$2000 USD/ton. After enzymatically converting starch to sugar, the sugars can be fractionated and sold, or converted to levulinic, formic, or succinic acid (Liu et al. 2018). Pyrolysis and HTL discussed above can be used to create bio-char, gases, and a bio-crude oil. A subset of a single sample of duckweed derived bio-crude oil contained over 100 distinct compounds, mainly ketones, alcohols, fatty acids, and cyclic compounds (Duan et al. 2013). When considering the variables of biomass, solvents, temperature, pressure, and time HTL, pyrolysis can be adjusted to offer countless compounds that can be created and fractionated. Finally, there are a variety of other high-value application niches that duckweed can be used for including tea, cosmetics, pet food, and aquarium plants, which have been tested on small scale and may develop further. Major crops such as corn and soy have been used as feedstocks for hundreds of uses including food-thickening agents, cosmetics, construction adhesives, and ink. It is therefore reasonable to expect that as duckweed abundance grows there will be a greater number and variety of applications.

Another sector where duckweed species will likely play an expanding role is water reclamation and supply. In 2018, the Duckweed forum issue 22 described 23 companies in 9 countries, with 4 each working in water quality testing and water treatment (Shoham 2018). Provided the perpetual rise of water pollution and increased testing, and the roughly 50% lower capital and operating costs of duckweed (Skillicorn 2013) and constructed wetland (Zhang et al. 2014b) treatment systems compared to their bacterial counterparts, these industries are expected to grow, likely more so in developing countries. Sadly, 14 years of satellite observations reveal decreasing clean water availability across the world and in heavily populated areas like California, the Middle East, Northern India, and Northern China where groundwater is being depleted (Rodell et al. 2018). Many regions suffer clean water scarcity for at least 1 month of the year resulting in inadequate supply for people as well as agricultural losses. Duckweed treatment systems to reclaim water, as well as water efficient duckweed crops, with many other measures, might be utilized in these and other regions to increase supply. Similar to water reclamation, there is a lesser known need for phosphorous reclamation, since our current practice is to mine and refine phosphorous deposits, fertilize our crops, and then let the phosphorous run directly off of fields and into the ocean, or through our wastewater treatment systems into the ocean where it causes eutrophication damage like the Gulf of Mexico hypoxic zone. Economically mineable, organically available phosphorous is expected to be scarce by 2050 or 2100, and production might decline by 2030 raising its price possibly beyond the reach of poorer farmers (Childers et al. 2011). Fortunately, phosphorous can be recycled by better farming practices or by using more aquatic plants and other methods to recapture more than the current rate of 50% from human wastes. While phosphorous is a critical macronutrient and prime example, many other fertilizers have similar life cycles and would follow the phosphorous in any reduce, recapture, and reuse applications. Given the water and fertilizer scarcities this century will likely pose to billions of people, we sincerely hope that duckweed-based water treatment systems and many other water and nutrient reclamation technologies will be applied at larger scale to "close the loop" and avoid scarcity.

One of the earliest companies to work with duckweed, Biolex Therapeutics, saw the rapidly growing high protein biomass of *Lemna* as a great expression platform for transgenic proteins. They produced several complex antibodies, including one to target Leukemia, and trademarked the term Plantibodies™, yet sadly went out of business. Since their closure, there have been improvements in the transgenic expression within several duckweed species. There have even been academic papers reporting over 20 transgenic therapeutic proteins in duckweed reaching as high as 7% of total soluble protein (Balaji et al. 2016). Given the lower cost of production and lower risk of transmissible pathogens compared to mammalian cell lines, duckweed may once again provide genetically engineered proteins for medical or other applications.

Catalyst Agri-Innovations Society (CAIS) works with a number of diverse companies in several locations including an on-farm anaerobic digester with nutrient extraction and at a land-based fish farm. All of their work is on efficiency and resource recovery at the food/energy/water nexus in the overall agriculture domain. They currently work with several wastewater treatments like the Trident Processes system for separating manure solids, anaerobic digestion to extract energy, and duckweed or algae to remove solutes. Wastewater from multiple species is anaerobically digested to generate methane and energy, and the digestate moves on to enclosed stacked shelf growth chambers filled with duckweed. After doubling in under 48 h and cleaning the water, duckweed is fermented to separate protein from high-value simple sugars. Christopher Bush, Co-founder of CAIS, has worked with the XPRIZE Foundation, designing competitions including "Feeding the Next Billion." The team also works with the HeroX platform where a sponsor can publicly host a problem and cash prize for the solution, greatly increasing the number of scientists who can see and solve the problem and learn from the winning solution. This type of modern interdisciplinary research center, consulting firm, or incubator that relies on datasets from large sensor arrays and crowd sourcing looks to be increasing in popularity, and we look forward to the variety of applications that will be developed where duckweed will play a role as one of several options to reclaim resources or feed people and livestock more effectively.

Perhaps given their ability to clean wastewater while providing food and fresh air, duckweeds can be seen as not only a crop species, but a life support system. The current water recovery system on the International Space Station relies on complex chemical treatments and reagents while generating wastes, which has NASA interested in developing closed-loop life support systems for long-term missions. Many plants develop poorly in microgravity and produce inedible biomass, so non-gravitropic aquatic plants and specifically duckweeds have been studied for space flight in closed-loop systems, microgravity simulations, and space flights since 1966 (Landolt and Kandeler 1987; Gale et al. 1989; Bluem and Paris 2003). *Lemna aequinoctialis* was even found to have a 32% increase in growth rate in simulated microgravity (Yuan and Xu 2017). Therefore, Space Lab Technologies, LLC is currently collaborating with the University of Colorado at Boulder on a Phase 2 grant from NASA to develop the μG-LilyPond™ growth chamber as part of a life support system (Escobar and Escobar 2017). Part of their project is studying how bursts of high intensity light can stimulate production of carotenoids, vitamin E, and other nutritious secondary metabolites (Demmig-Adams and Adams 2002), and how these bursts within the light regimen can be optimized for energy use, plant yield, and nutritional content. Thanks to their high growth rate, ability to grow in shallow trays, preference for ammonia, and entirely edible nutritious biomass duckweed are currently the prime candidates for the system. Presently, it is designed to provide fresh food and oxygen, with the eventual goal of converting urine to clean water. Based on the previous literature, the goal is to create a 1 m^3 system capable of treating the wastewater and CO_2 of 4 crew members and providing an edible vegetable yield up to 250 g of dry weight per day (Gale et al. 1989; Landolt and Kandeler 1987). The μG-LilyPond™ system will need to overcome the unique challenges of space missions including size and weight restrictions, controlled growth and harvest in microgravity, water delivery via capillary action, sterility, minimal human maintenance, and rapid recovery from failures (Escobar and Escobar 2017). This intimate reliance on duckweed in a closed-loop system provides both a technical and a symbolic example of how humans and duckweed complement each other, and how we can use the smallest plants to solve the largest challenges.

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References

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. Food and Agriculture Organization of the United Nations (FAO), Rome

Alvarado S, Guédez M, Lué-Merú MP, Nelson G, Alvaro A, Jesús AC, Gyula Z (2008) Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*Eichhornia crassipes*) and lesser duckweed (*Lemna minor*). Bioresour Technol 99 (17):8436–8440

Appenroth KJ, Crawford DJ, Les DH (2015) After the genome sequencing of duckweed—how to proceed with research on the fastest growing angiosperm? Plant Biol (Stuttg) 17:1–4. https://doi.org/10.1111/Plb.12248

Appenroth K-J, Sree KS, Böhm V, Hammann S, Vetter W, Leiterer M, Jahreis G (2017) Nutritional value of duckweeds (*Lemnaceae*) as human food. Food Chem 217:266–273

Appenroth KJ, Sree KS, Bog M, Ecker J, Boehm V, Lorkowski S, Sommer K, Vetter W, Tolzin-Banasch K, Kirmse R (2018) Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human food. Frontiers in chemistry 6:483

Balaji P, Satheeshkumar PK, Venkataraman K, Vijayalakshmi MA (2016) Expression of anti-tumor necrosis factor alpha (TNFalpha) single-chain variable fragment (scFv) in *Spirodela punctata* plants transformed with Agrobacterium tumefaciens. Biotechnol Appl Biochem 63(3):354–361. https://doi.org/10.1002/bab.1373

Baldi BG, Maher BR, Slovin JP, Cohen JD (1991) Stable Isotope Labeling, in Vivo, of d- and l-Tryptophan Pools in *Lemna gibba* and the Low Incorporation of Label into Indole-3-Acetic Acid. Plant Physiol 95 (4):1203–1208. https://doi.org/10.1104/pp.95.4.1203

Bhanthumnavin K, Mcgarry MG (1971) *Wolffia* arrhiza as a possible source of inexpensive protein. Nature 232 (5311):495

Bluem V, Paris F (2003) Possible applications of aquatic bioregenerative life support modules for food production in a Martian base. Adv Space Res 31(1):77–86

Boehm R, Kruse C, Voeste D, Barth S, Schnabl H (2001) A transient transformation system for duckweed (*Wolffia columbiana*) using Agrobacterium-mediated gene transfer. J Appl Bot 75(3–4):107–111

Bog M, Baumbach H, Schween U, Hellwig F, Landolt E, Appenroth KJ (2010) Genetic structure of the genus *Lemna* L. (*Lemnaceae*) as revealed by amplified fragment length polymorphism. Planta 232 (3):609–619. https://doi.org/10.1007/s00425-010-1201-2

Bog M, Schneider P, Hellwig F, Sachse S, Kochieva EZ, Martyrosian E, Landolt E, Appenroth KJ (2013) Genetic characterization and barcoding of taxa in the genus *Wolffia* Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). Planta 237 (1):1–13. https://doi.org/10.1007/s00425-012-1777-9

Borisjuk N, Chu P, Gutierrez R, Zhang H, Acosta K, Friesen N, Sree KS, Garcia C, Appenroth KJ, Lam E (2015) Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. Plant Biol (Stuttg) 17:42–49. https://doi.org/10.1111/Plb.12229

Cantó-Pastor A, Mollá-Morales A, Ernst E, Dahl W, Zhai J, Yan Y, Meyers BC, Shanklin J, Martienssen R (2015) Efficient transformation and artificial miRNA gene silencing in *Lemna minor*. Plant Biol (Stuttg) 17:59–65. https://doi.org/10.1111/plb.12215

Cao HX, Wang W, Le HTT, Vu GTH (2016) The power of CRISPR-Cas9-induced genome editing to speed up plant breeding. Int J Genomics 2016:10. https://doi.org/10.1155/2016/5078796

Cao HX, Fourounjian P, Wang W (2018) The importance and potential of duckweeds as a model and crop plant for biomass-based applications and beyond. In:

Hussain CM (ed) Handbook of environmental materials management. Springer International Publishing, Cham, pp 1–16. https://doi.org/10.1007/978-3-319-58538-3_67-1

Chhabra G, Chaudhary D, Sainger M, Jaiwal PK (2011) Genetic transformation of Indian isolate of *Lemna minor* mediated by *Agrobacterium tumefaciens* and recovery of transgenic plants. Physiol Mol Biol Plants 17(2):129–136. https://doi.org/10.1007/s12298-011-0059-5

Childers DL, Corman J, Edwards M, Elser JJ (2011) Sustainability challenges of phosphorus and food: solutions from closing the human phosphorus cycle. Bioscience 61(2):117–124. https://doi.org/10.1525/bio.2011.61.2.6

Cui W, Cheng JJ (2015) Growing duckweed for biofuel production: a review. Plant Biol (Stuttg) 17:16–23. https://doi.org/10.1111/plb.12216

Demmig-Adams B, Adams WW 3rd (2002) Antioxidants in photosynthesis and human nutrition. Science 298 (5601):2149–2153. https://doi.org/10.1126/science.1078002

Duan P, Chang Z, Xu Y, Bai X, Wang F, Zhang L (2013) Hydrothermal processing of duckweed: effect of reaction conditions on product distribution and composition. Bioresour Technol 135:710–719. https://doi.org/10.1016/j.biortech.2012.08.106

Escobar CM, Escobar AC (2017, July) Duckweed: a tiny aquatic plant with enormous potential for bioregenerative life support systems. In: 47th International conference on environmental systems, Charleston, South Carolina, 16–20 July 2017. ICES, p 281

Feng B, Fang Y, Xu Z, Xiang C, Zhou C, Jiang F, Wang T, Zhao H (2017) Development of a new marker system for identification of *Spirodela polyrhiza* and *Landoltia punctata*. International Journal of Genomics 2017:8. https://doi.org/10.1155/2017/5196763

Fu L, Huang M, Han B, Sun X, Sree KS, Appenroth KJ, Zhang J (2017) Flower induction, microscope-aided cross-pollination, and seed production in the duckweed *Lemna gibba* with discovery of a male-sterile clone. Sci Rep 7(1):3047. https://doi.org/10.1038/s41598-017-03240-8

Gale J, Smernoff DT, Macler BA, MacElroy RD (1989) Carbon balance and productivity of *Lemna gibba*, a candidate plant for CELSS. Adv Space Res 9(8):43–52

Goopy J, Murray P (2003) A review on the role of duckweed in nutrient reclamation and as a source of animal feed. Asian-Australas J Anim Sci 16(2):297–305

Gwaze FR, Mwale M (2015) The prospect of Duckweed in pig nutrition: a review. J Agric Sci 7(11):189

Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6(7). https://doi.org/10.3390/foods6070053

Hou W, Chen X, Song G, Wang Q, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). Plant Physiol Biochem 45(1):62–69

International Organization of Standardization (2005) Water quality—Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*)—Duckweed growth inhibition test. ISO Geneva (CH), ISO guideline 20079

Khvatkov P, Chernobrovkina M, Okuneva A, Pushin A, Dolgov S (2015) Transformation of *Wolffia arrhiza* (L.) Horkel ex Wimm. Plant Cell Tissue Organ Cult (PCTOC) 123(2):299–307

Kuehdorf K, Jetschke G, Ballani L, Appenroth KJ (2013) The clonal dependence of turion formation in the duckweed *Spirodela polyrhiza*—an ecogeographical approach. Physiol Plant. https://doi.org/10.1111/ppl.12065

Kutschera U, Niklas KJ (2015) Darwin-Wallace Demons: survival of the fastest in populations of duckweeds and the evolutionary history of an enigmatic group of angiosperms. Plant Biol (Stuttg) 17:24–32. https://doi.org/10.1111/Plb.12171

Lam E, Appenroth KJ, Michael T, Mori K, Fakhoorian T (2014) Duckweed in bloom: the 2nd international conference on duckweed research and applications heralds the return of a plant model for plant biology. Plant Mol Biol 84 (6):737–742. https://doi.org/10.1007/s11103-013-0162-9

Landolt E (1986) The family of Lemnaceae—a monographic study, vol 1. Veroffentlichungen des Geobotanischen Institutes der Eidgenossischen Technischen Hochschule, Stiftung Rubel, Zurich

Landolt E, Kandeler R (1987) Biosystematic investigations in the family of duckweeds (*Lemnaceae*), Vol. 4: The family of Lemnaceae-a monographic study, Vol. 2 (phytochemistry, physiology, application, bibliography). Veroeffentlichungen des Geobotanischen Instituts der ETH, Stiftung Ruebel (Switzerland)

Liu C, Feng Q, Yang J, Qi X (2018) Catalytic production of levulinic acid and ethyl levulinate from uniconazole-induced duckweed (*Lemna minor*). Bioresour Technol 255:50–57. https://doi.org/10.1016/j.biortech.2018.01.087

Martirosyan EV, Ryzhova NN, Skryabin KG, Kochieva EZ (2008) RAPD analysis of genome polymorphism in the family Lemnaceae. Russ J Genet 44(3):360–364. https://doi.org/10.1134/s1022795408030198

Mkandawire M, Dudel EG (2005) Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. Sci Total Environ 336 (1):81–89

Nauheimer L, Metzler D, Renner SS (2012) Global history of the ancient monocot family Araceae inferred with models accounting for past continental positions and previous ranges based on fossils. New Phytol 195 (4):938–950. https://doi.org/10.1111/j.1469-8137.2012.04220.x

OECD/FAO (2017) OECD-FAO agricultural outlook 2017–2026. OECD Publishing, Paris. https://doi.org/10.1787/19991142

OECD (2006) Test No. 221: *Lemna* sp. growth inhibition test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264016194-en

Posner HB (1962) Characteristics of X-ray-induced aberrants of *Lemna perpusilla* 6746. Plant Cell Physiol 3(3):275–284

Rezania S, Taib SM, Din MFM, Dahalan FA, Kamyab H (2016) Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. J Hazard Mater 318:587–599

Rodell M, Famiglietti JS, Wiese DN, Reager JT, Beaudoing HK, Landerer FW, Lo MH (2018) Emerging trends in global freshwater availability. Nature 557 (7707):651–659. https://doi.org/10.1038/s41586-018-0123-1

Schmitz U, Kelm H (2017) First discovery of flowering *Wolffia arrhiza* in Central Europe. Aquat Bot 143:33–35

Shoham T (2018) On a mission to introduce duckweed as the 21st century cash crop: Current active industrial efforts. Duckweed Forum 6(3):92–97

Skillicorn P (2013) The agriquatics system vs activated sludge: a comparative analysis. https://paulskillicorn.wordpress.com/2013/04/17/the-agriquatics-system-vs-activated-sludge-a-comparative-analysis/. Accessed 9 Sept 2019

Skillicorn P, Spira W, Journey W (1993) Duckweed aquaculture: a new aquatic farming system for developing countries. World Bank

Slovin JP, Tobin EM (1982) Synthesis and turnover of the light-harvesting chlorophylla/b-protein in *Lemna gibba* grown with intermittent red light: possible translational control. Planta 154(5):465–472. https://doi.org/10.1007/BF01267815

van der Spiegel M, Noordam MY, van der Fels-Klerx HJ (2013) Safety of novel protein sources (Insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr Rev Food Sci F 12(6):662–678. https://doi.org/10.1111/1541-4337.12032

Sree KS, Bog M, Appenroth KJ (2016) Taxonomy of duckweeds (*Lemnaceae*), potential new crop plants. Emir J Food Agr 28(5):291–302. https://doi.org/10.9755/ejfa.2016-01-038

Sree KS, Maheshwari SC, Boka K, Khurana JP, Keresztes A, Appenroth KJ (2015a) The duckweed *Wolffia* microscopica: a unique aquatic monocot. Flora 210:31–39. https://doi.org/10.1016/j.flora.2014.10.006

Sree KS, Sudakaran S, Appenroth K-J (2015b) How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus *Wolffia* (Lemnaceae). Acta Physiol Plant 37(10):204

Tang J, Li Y, Ma J, Cheng J (2015) Survey of duckweed diversity in Lake Chao and total fatty acid, triacylglycerol, profiles of representative strains. Plant Biology 17(5):1066–1072

Tippery N, Les D, Crawford D (2015) Evaluation of phylogenetic relationships in Lemnaceae using nuclear ribosomal data. Plant Biology 17(s1):50–58

Vunsh R, Li J, Hanania U, Edelman M, Flaishman M, Perl A, Wisniewski JP, Freyssinet G (2007) High expression of transgene protein in Spirodela. Plant Cell Rep 26(9):1511–1519. https://doi.org/10.1007/s00299-007-0361-4

Wang W, Wu Y, Yan Y, Ermakova M, Kerstetter R, Messing J (2010) DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biol 10:205. https://doi.org/10.1186/1471-2229-10-205

Xue HL, Xiao Y, Jin YL, Li XB, Fang Y, Zhao H, Zhao Y, Guan JF (2012) Genetic diversity and geographic differentiation analysis of duckweed using inter-simple sequence repeat markers. Mol Biol Rep 39(1):547–554. https://doi.org/10.1007/s11033-011-0769-3

Yamamoto Y, Rajbhandari N, Lin X, Bergmann B, Nishimura Y, Stomp A-M (2001) Genetic transformation of duckweed *Lemna gibba* and *Lemna minor*. Vitro Cell Develop Biol-Plant 37(3):349–353. https://doi.org/10.1007/s11627-001-0062-6

Yan Y, Candreva J, Shi H, Ernst E, Martienssen R, Schwender J, Shanklin J (2013) Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species and cloning of a Δ6-desaturase responsible for the production of γ-linolenic and stearidonic acids in Lemna gibba. BMC Plant Biol 13(1):201

Yuan J, Xu K (2017) Effects of simulated microgravity on the performance of the duckweeds *Lemna* aequinoctialis and *Wolffia* globosa. Aquat Bot 137:65–71. https://doi.org/10.1016/j.aquabot.2016.11.010

Zhang C, Duan P, Xu Y, Wang B, Wang F, Zhang L (2014a) Catalytic upgrading of duckweed biocrude in subcritical water. Bioresour Technol 166:37–44. https://doi.org/10.1016/j.biortech.2014.05.022

Zhang DQ, Jinadasa K, Gersberg RM, Liu Y, Ng WJ, Tan SK (2014b) Application of constructed wetlands for wastewater treatment in developing countries–a review of recent developments (2000–2013). J Environ Manag 141:116–131

Zhang X, Zhao FJ, Huang Q, Williams PN, Sun GX, Zhu YG (2009) Arsenic uptake and speciation in the rootless duckweed *Wolffia* globosa. New Phytol 182 (2):421–428

Zhao Y, Fang Y, Jin Y, Huang J, Bao S, Fu T, He Z, Wang F, Zhao H (2014) Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: a pilot-scale comparison with water hyacinth. Bioresour Technol 163:82–91. https://doi.org/10.1016/j.biortech.2014.04.018

Zhao H, Appenroth K, Landesman L, Salmean AA, Lam E (2012) Duckweed rising at Chengdu: summary of the 1st international conference on Duckweed application and research. Plant Mol Biol 78 (6):627–632. https://doi.org/10.1007/s11103-012-9889-y

Ziegler P, Adelmann K, Zimmer S, Schmidt C, Appenroth KJ (2015) Relative in vitro growth rates of duckweeds (*Lemnaceae*)—the most rapidly growing higher plants. Plant Biol (Stuttg) 17:33–41. https://doi.org/10.1111/plb.12184