Unravelling the impact of light, temperature and nutrient dynamics on duckweed growth: A meta-analysis study

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Abstract

Nature-based solutions have been proven in recent decades as a reliable and cost-effective technology for the treatment of wastewater. They are widely used in several countries, mainly as secondary or tertiary biological treatment. Such systems rely on the ability of photosynthetic organisms to assimilate and remove, to a certain extent, nutrients valuable for their own growth. Different plant species have been studied for this purpose, but particular attention has been given to duckweeds, the smallest flowering plant in the world. These plants have been proven to be highly efficient for wastewater treatment given their rapid growth, natural abundance among macrophytes, and the quality of the biomass produced. However, despite being considered a seemingly simple technology, the performance of treatment systems based on duckweed is dependent on environmental and operational conditions not very well understood. While there have been many studies on growth of duckweed for wastewater treatment, the difference in species, systems, variables, scales and reporting units make it very difficult to draw comparisons across studies. This study employs a systematic review approach to conduct a meta-analysis of the effect of temperature, light, and nutrient availability on duckweed growth by means of standardized IQ-scores. The analysis of the results considered the duckweed species being used and the interaction between these parameters. The results suggest that daily light integral (DLI) is a useful parameter to assess the overall effect of light (photoperiod and intensity) on duckweed growth and that the effect of nitrogen and phosphorus supply should consider the nitrogen species available for plant growth and its ratio to phosphorus concentrations. By establishing the optimal range of culture conditions for duckweed , this study provides important insights for optimizing wastewater treatment systems that rely on duckweed for nutrient control and recovery, which is primarily mediated by duckweed growth.

Keywords: Duckweed, Light intensity, Nutrient control, Temperature, Wastewater, meta-analysis

Abbreviations: NBS, Nature-based solutions; WWT, Wastewater treatment; RGR, Relative growth rate; TP, total phosphorus; TN, Total nitrogen; DLI, Daily light integral

Highlights

- Comparing duckweed growth studies in varying experimental conditions is challenging.
- A meta-analysis with standardised scores can overcome this limitation.
- Different genera have different temperature optima.
- The daily light integral is a useful parameter for assessing the impact of light on duckweed growth.

- There is no clearly defined preference for N source.
- The ratio of N:P has important effects on growth rates.

1. INTRODUCTION

Nature-based solutions (NBS) which harness the growth of photosynthetic organisms in wastewater are being thoroughly investigated as a cost-effective method for decentralized wastewater treatment. Among the NBS, treatment systems based on aquatic plants – e.g., macrophytes – have been widely used to remove pollutants from water, as a tool for proper wastewater management and disposal. For more than forty years, these systems have been implemented in Europe and North America for nutrient control and recovery from wastewater at low loading rates – i.e., wastewater treatment units for polishing final effluents (Brix, 1994; Donde et al., 2018). Today, macrophytes are increasingly being used worldwide to treat different types of effluent, including municipal and industrial wastewaters, acid mine drainage, agricultural and livestock wastes, and leachate from landfills, among others. In rural areas and developing countries, macrophyte-based systems play a vital role in the treatment of municipal wastewater from small and decentralised systems, where energy intensive treatment units are not suitable due to technical or economic constrains (Upadhyay et al., 2016).

Aquatic macrophytes act as a biological filter, taking up nutrients from polluted waters to support biomass production, while fixing atmospheric carbon dioxide. The great diversity of macrophytes has resulted in a wide variety of systems being used for wastewater treatment, ranging from systems using large aquatic plants like water hyacinth, to very small plants like duckweed. The latter have proven to be efficient in removing nutrients, organic matter, and toxic substances from water. The success of such treatment systems is based on their adaptability and fast-growing capacity. Furthermore, wastewater treatment systems using duckweeds have proven their ability to perform well in both urban and rural settings, and strong environmental credentials due to their low energy consumption and operational costs (Brix, 1994).

Duckweeds have been tested for a wide range of wastewater treatment conditions (El-Shafai et al., 2004; Hassan & Edwards, 1992). These plants grow very rapidly and remove nutrients at a higher rate than other aquatic macrophytes (Oron et al., 1988). Under optimal growth conditions, including nutrient bioavailability, light intensity and water temperature, they can double their weight every 2 or 3 days (Rusoff et al., 1980). This reproduction rate is greater than that of any other higher plant, resulting in the formation of dense mantles over the surface of water bodies, especially when the concentrations of nitrogen and phosphorous in the water column correspond to mesotrophic/eutrophic environments (Portielje & Roijackers, 1995).

Despite the multiple benefits of duckweed-based systems for wastewater treatment, some limitations associated with their engineering design and operation persist. For instance, the efficiency of treatment processes is seasonal, in response to changing environmental conditions and free surface area available to support biomass growth and photosynthesis. These conditions have a direct effect on the ability of duckweed to take up and metabolise nutrients, which ultimately affect the quality of the final effluent. For this reason, it is necessary to firstly appraise the performance of duckweed-based systems for wastewater treatment under a range of culture conditions, typical to the corresponding application (i.e., nutrient loading rates, flow rates, retention times, climate conditions, etc.,)

As other photosynthetic organisms, duckweeds require a supply of macronutrients (carbon, nitrogen and phosphorus) and trace nutrients to grow. These nutrients are all present in wastewaters, either in mineral or organic form, hence the potential for using wastewater as a medium to support duckweed biomass growth. Apart from the concentration of nutrients in the growth medium (i.e., wastewaters), culture conditions such as temperature, pH, initial mantle density, surface area availability, photoperiod and light intensity, have a significant influence on duckweed growth and nutrient uptake.

Moreover, to successfully improve the quality of wastewater effluents, we need to be aware that not all duckweed species are equally effective at taking up nutrients and hence, biomass productivity and composition vary. For that reason, process performance is highly dependent on duckweed strains which may be well or poorly adapted to specific operation and/or environmental conditions (Bergmann et al., 2000; Cheng & Stomp, 2009). In this sense, appropriate selection of duckweed strains to work with must be undertaken.

Overall, reported outcomes on how environmental and operational conditions impact growth and nutrient uptake by duckweeds are highly variable in published literature. Therefore, it is very difficult to extract meaningful comparisons for such diverse studies which use different duckweed species, different growth media or effluents, different culture setups and controlled or naturally varying photoperiod and temperature. To try to synthesise this information and draw meaningful conclusions a systematic review was undertaken, and a meta-analysis applied to the data from the retrieved publications. This approach has its origins in medical studies where there are often small sample sizes and confounding variables, but the methodology is much more generally applicable (Page et al., 2021). By drawing on many studies, patterns or trends emerge which are not visible in individual studies.

This meta-analysis study focuses on establishing the influence that temperature, light and Nitrogen and Phosphorus have on duckweed biomass growth and nutrient uptake, considering tested natural and engineered environments, that will support the importance of selecting suitable duckweed isolates and species for process development studies and engineering applications. By comparing different outcomes under a standardized methodology, it is possible to plan and design more reliable, robust, and resilient duckweed-based systems for wastewater treatment and nutrient recovery. The goal is to offer an integrated analysis of the dynamics involved in nutrient reclamation and biomass production by duckweeds.

2. METHODS

2.1. Literature search

The data for the present meta-analysis study was put together from three different peer-reviewed literature databases (PubMed, Web of Science and Scopus) following the Prisma guidelines (http://www.prisma-statement.org/) (Figure 1). All scientific articles published prior to June 2021 were retrieved using the advanced search tool from each database. Different keywords and synonyms were grouped into five topics to be searched using the following Boolean operation: TITLE-ABSTRACT-KEYWORDS - (growth OR composition) AND (duckweed) AND (nutrient OR reclamation).

2.2. Inclusion and exclusion criteria

Article titles and abstracts were manually screened to exclude studies not related to the topic. Only studies in wastewater treatment and nutrient recovery using different species of duckweed were included in the analysis. In a further step, relevant articles were examined to determine fit to the eligibility criteria of this review.

The exclusion criteria included the following:

1. Toxicological studies using duckweeds: Studies assessing the potential of plants for emerging contaminants remediation or the ecotoxicological effect of pollutants on duckweed growth. These studies were excluded as the use of standard culture conditions for the cultivation of duckweeds was limited to the control experiment.
2. Review papers: Publications collecting and reviewing data from other authors already included within the database or papers presenting the state of art of duckweeds in wastewater treatment.
3. Not enough data of interest: Papers in which either the relative growth rate of plants or the data/plots required for its calculation is not presented.
4. Different research question: Scientific reports whose objective was other than assessing the effect of temperature, light, and nutrient availability on the growth of duckweeds.
5. Non-retrieved papers: Papers that cannot be found using selected databases or without any response from contacted authors.
6. Language: Papers published in a language other than English or without any English translation available.

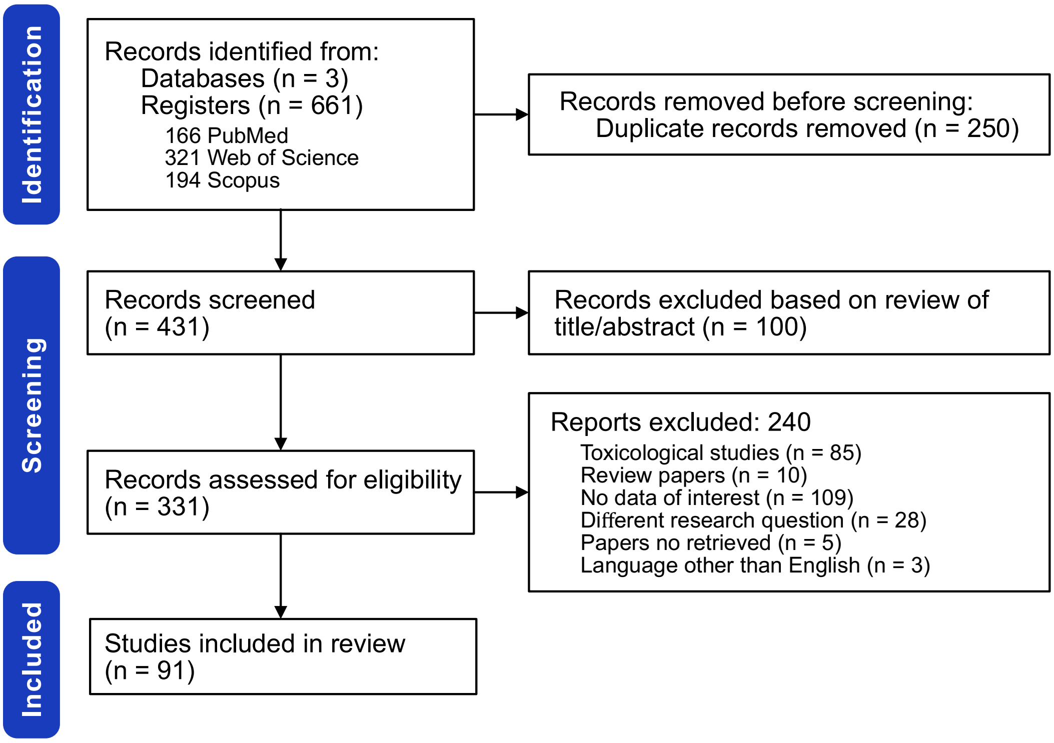

Figure 1. Identification of studies via databases and registers. *This PRISMA flow diagram (Moher et al., 2010) shows the literature search results, highlighting the main exclusion criteria used in the screening stage, of peer-reviewed papers published in English prior June 2021.*

2.3. Data extraction

All data retrieved from the studies included in the review are available in Supplementary Material S1. From each study, the following data was extracted: (1) authors and year of publication, (2) test species, (3) culture conditions, (4) culture media characteristics, and (5) observed response in the treatments. A summary of extracted variables and their respective units is presented in Table 1.

Whenever provided, data on the characteristics of duckweed studied, such as genus, species, collection reference number and country of origin, were included. Culture conditions tested in each of the studies were collected and classified either as environmental or simulated culture conditions. When provided, the volume and total surface area of cultivation, initial stocking density, temperature, photoperiod, and light intensity were noted as in the original publication. In some cases, surface area was calculated upon the dimensions of the containers in which the experiments were done. The initial stocking density, or mat density, was calculated as the amount plant material, in fresh or dry basis, per unit of surface area at the beginning of the experiment. Where experiments were conducted under ambient/outdoor culture conditions, and data on temperature, photoperiod and light intensity were not reported, these data were retrieved from the Photovoltaic Geographical Information System from the European Commission (https://re.jrc.ec.europa.eu/pvg_tools/en/) for the location. Normal direct irradiance values were converted to Photosynthetically Active Radiation (PAR) using a conversion factor of 4.6 (Langhans & Tibbitts, 1997).

Table 1. *Variables and reported units extracted from independent experiments in reviewed reports.*

No. Reports: 91 **No. Experiments:** 220 **No. Datapoints:** 920

Duckweed	**Culture conditions**		**Culture media**		**Responses**	
Genera	Real / Simulated	-	Real / Synthetic	-	RGR	(d^{-1})
Species	Stocking density	(mg m^{-2})	Nitrogen source	-	BC	(% dw)
Clone	Coverage	(%)	Total N	(mg N L^{-1})	EC	(% dw)
Origin	Temperature	(°C)	Ammonium	(mg NH_4-N L^{-1})	N removal rate	(mg N L^{-1} d^{-1})
	Photoperiod	(Light hours)	Nitrate	(mg NO_3-N L^{-1})	P removal rate	(mg P L^{-1} d^{-1})
	Light intensity	(µmol m^{-2} s^{-1})	Total P	(mg P L^{-1})		
			Orthophosphate	(mg PO_4-P L^{-1})		

RGR = Relative growth rate, BC = Biochemical composition (protein, lipid, starch), EC = Elemental composition (C, H, O, N)

In addition to this, some characteristics of the culture media in which plants were grown were recorded. Medium was classified as synthetic or real, based on the methods described by the authors. Total Nitrogen (TN) was noted along with the initial concentration of both ammonium and nitrate in the media, all expressed as mg N L^{-1}. Total Phosphorus (TP) and phosphate concentrations are reported as mg P L^{-1}.

Finally, duckweed growth parameters like biomass productivity, relative growth rate (RGR) and doubling time were taken out from the screened literature. When data was not provided, RGR was calculated as $RGR = Ln\ (X_f / X_i) / t$, with X_f and X_i either the dry biomass, wet biomass, number of fronds or total fronds area at the end and start of the experiment respectively, and t the cultivation time in days. In cases where biomass growth was presented in time course plots, the corresponding RGR was calculated by fitting growth curves data to the differential form of the equation $dX/dt = RGR \times t$.

When possible, data were extracted from tables and text of the publication; however, when results were presented only on graphs, they were retrieved by reversing data visualizations using the software WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/). To facilitate the analysis, all data collected for each variable were converted to the same units (as per Table 1) using relevant conversion factors.

2.4. Data analysis

Data obtained from the literature search was catalogued and curated using Microsoft Excel software; data analysis and visualisation was conducted using *R* software. Statistical analysis of RGR values included one-way ANOVA with Tukey's multiple comparisons test. Statistical significance criterium was defined as p value < 0.05. *Z*-scores were used to standardize the size effect of culture conditions on response variables, to a same scale, to make them comparable. For each independent experiment, *Z*-values for any response variable were obtained as $Z = (x - \mu)/\sigma$, where x corresponds to the value of the response variable at any given culture condition within the experiment; μ is the mean response, and σ the standard deviation. A further transformation was performed on the data to avoid negative values at the time of obtaining regression curves. For this, Z-values were adjusted to have a mean of 100 and a standard deviation of 15. The new values, so called IQ-scores, were calculated as $IQ = Z * 15 + 100$.

3. RESULTS AND DISCUSSION

3.1. Study sample and experimental design characteristics

This review identified 661 studies that met the inclusion criteria, see Figure 1 for the PRISMA flow diagram summarizing the study selection process. Out of these studies, 91 provided sufficient information to be included in the final quantitative analysis.

Lemna seems to be the most studied duckweed genus as 62% of the studies included in the review had this genus as a study subject (Figure 2A). *L. minor* and *L. gibba* were the species for which most experimental data was available, from 39 and 23 publications respectively. The fact that *L. minor*, also known as common duckweed, is the most widespread duckweed species and broadly used in toxicity testing (Moody & Miller, 2005; OECD, 2002) makes it the most extensively studied of all duckweed species (Ceschin et al., 2016; Wang, 1986). Of the 14 species discovered for this genus, data were available for 8 of them. The next most studied duckweed genera were *Spirodela* and *Landoltia*, of which the same number of studies (16) was available for the species *S. polyrrhiza* and *L. punctata*. Finally, *Wolffia* and *Wolffiela* are the least studied duckweed genera. Recognised as rootless duckweed, both genera contribute only 11% of the papers selected for analysis. Being studied in 6 of the selected publications, *W. arrhiza* is the species with the highest representation of this group. Overall, our database has a good representation of the different duckweed species (19 out of 36 species discovered so far) for each of the genera and, the variability of climates from which each species is representative (61% from temperate climate locations – including China, 24% from tropical climate regions, and 15% from subtropical climate areas).

In terms of culture conditions, data related to temperature, photoperiod and light intensity was collected, and classified according to the degree of control over the experiments (Figure 2B). As per the density plots, 50% of the selected experiments were carried out at temperatures between 23 and 26°C, with a median value of 24°C, under controlled cultured conditions. The temperature range increased when cultures were carried out under ambient/outdoor conditions (from 19 to 27°C) due to seasonality in temperate climate regions. In the latter case, the distribution of data is multimodal, with peaks at 20, 26 and 39°C.

Regarding photoperiod, under controlled culture conditions, the preference was to carry out trials under simulated long daylight conditions (16h of light for 50% of the data), while under ambient conditions, most of the studies were carried out under natural light, with photoperiods varying between 8.5 and 14 hours of light per day, with a median of 12h of light per day. Perhaps the biggest difference between data collected at ambient and controlled growing conditions concerns light intensity. At the former condition, several authors used sunlight as a source of energy radiation. Light intensity values were normally distributed, with most of the tests carried out between 270 and 450 µmol m^{-2} s^{-1}, consistent with average values for solar PAR radiation in countries with climates ranging from temperate to subtropical (*Global Solar Atlas*, n.d.; Wang et al., 2013). In contrast, data from experiments performed in controlled environments present a right-skewed distribution, and only 25% of the data exceeds 150 µmol m^{-2} s^{-1}.

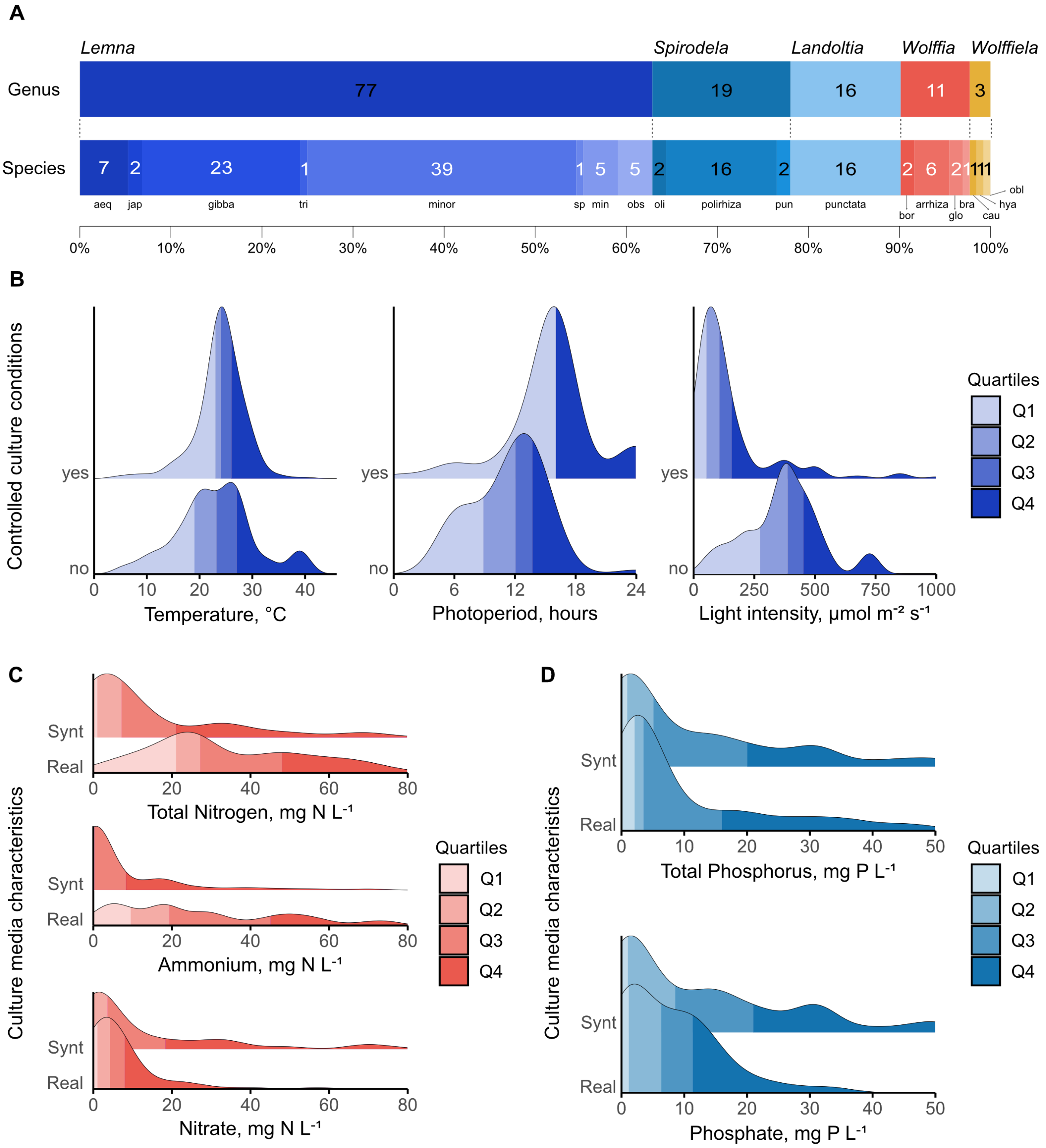

Figure 2. ***Summary of selected dataset descriptors grouped by variable category.*** *(A) Number of experiments per duckweed Genus and species included in the review, (B) Density plots showing the data distribution of environmental factors studied across different studies performed under controlled and uncontrolled culture conditions, (C) Density plots showing the data distribution for Total Nitrogen and nitrogen species concentration from papers using real and synthetic wastewater as culture media, (D) Density plots showing the data distribution for Total Phosphorus and Phosphate concentration from papers using real and synthetic wastewater as culture media.*

When considering the characteristics of the culture medium, whether synthetic or real wastewater, there are differences in the composition of the different phosphorus and nitrogen species. As far as nitrogen is concerned, there are two main species that contribute to the total nitrogen content of the culture medium, nitrate and ammonium. Figure 2C shows the number of publications studying the effect of either nitrogen species or total nitrogen on duckweed growth. In works using urban wastewater, the major input of total nitrogen comes from ammonium resulting from the decomposition of organically bound nitrogen. In this sense, the plots show how the number of studies on ammonium significantly influences the results that can be found when total nitrogen is the variable of study. The nitrogen concentration ranges used in the studies on ammonium and total nitrogen removal/uptake were distinct from each other. Half of the research on ammonium utilized concentrations ranging from 11 to 51 mg N L^{-1}. Meanwhile, around 50% of the studies on total nitrogen employed concentrations between 25 to 70 mg N L^{-1}. These ranges are similar to what is typically observed in urban wastewater (Ma et al., 2016; Metcalf et al., 2004). Higher ammonia concentrations tested by authors correspond to the use of wastewater from sources other than urban areas. In contrast, studies on ammonium carried out with synthetic media barely exceeded 8 mg L^{-1} (just 25% of the data).

In general, experiments carried out using nitrate as the sole nitrogen source in both synthetic and real wastewater showed that the concentrations of nitrate were usually lower than the total nitrogen concentrations tested in other studies. Whereas nitrate concentration varied between 0 – 8 mg N L^{-1} in 75% of cases, higher concentrations were used only in synthetic media, where the nitrogen source was adjusted in such a way as to match the total nitrogen values normally found in wastewater (25% of the test run in synthetic media had a nitrate concentration over 18 mg N L^{-1}).

Finally, data distribution regarding total phosphorus (TP) and phosphate (PO_4-P) concentration in synthetic and real wastewater is reported in (Figure 2D). For both types of culture media (real and synthetic media), there is a correlation between the phosphate concentration and the total phosphorus values tested. Regardless of the type of culture media, half of the experiments tested phosphorus concentrations below 6 mg P L^{-1}, which was reported either as phosphate or total phosphorus. Moreover, authors using synthetic media in their studies tended to cover a wider range of phosphate concentration to assess scenarios mimicking repleted and depleted nutrient conditions.

3.2. Effect of environmental factors on plant growth

In recent years an increasing number of researchers have focused on understanding how global climate is changing and the corresponding impacts on life on earth. It is undeniable that any change in environmental conditions has direct repercussions on living organisms, consequently influencing their metabolism (e.g., growth rates) and performance in engineering applications. For biomass growth, duckweeds use light and nutrients to carry out photosynthesis. While growing, these aquatic plants also produce and accumulate metabolic products, of which relative amounts in biomass depend upon the specific species studied, and environmental conditions tested (i.e., Light intensity and photoperiod, temperature, and availability of nutrients). To have a comprehensive understanding on the effect of these parameters on the growth of duckweeds, it is necessary to critically assess the existing literature.

3.2.1. Temperature

Temperature is probably the most important environmental factor regulating duckweed growth, composition, and nutrient uptake. As for other aquatic organisms, water temperature controls the rate at which biochemical reactions take place, including duckweed's photosynthesis, metabolism and catabolism. These plants can grow in a broad range of temperatures, subject to species and isolate, acclimation, and seasonal ambient conditions. The relationship between temperature and duckweed growth can be described by the Arrhenius equations, previously used in kinetic models for other photosynthetic organisms (Feng et al., 1990; Goldman & Carpenter, 1974). It is assumed that duckweed growth rate continuously increases with temperature increments up to a point in which growth rate decreases, i.e., optimal temperature. Therefore, to have a more accurate representation of the relationship between growth rate and temperature data, the thermal performance model curve (TPC), described by the Hinshelwood equation was employed (Hinshelwood, 1947). The Hinshelwood thermal model assumes that the rate of biomass growth is proportional to the overall enzyme activity and the kinetic growth rate constant. It also assumes that changes in the kinetic growth rate constant as a function of temperature can be described by the Arrhenius equation. The model predicts a unimodal relationship between biomass growth rate and temperature, with an optimal temperature at which the rate is at its maximum.

Based on the data obtained from published literature (Figure 3), the tested ambient air temperatures ranged from 5 to 40°C, within which the actual relative growth rate (RGR) varied between 0.0 and 0.41 d^{-1}; the highest RGR value correspond to *Lemna minor* cultured in synthetic media under controlled culture conditions (Lasfar et al., 2007). In general, it is found that temperature affects duckweed biomass growth in similar ways in the different studies analysed. The growth rate increases as the temperature rises from 5°C and reaches a maximum at around 25ºC. Above this temperature, plants become stressed and reduce their growth rate. This behaviour follows well known fundamental principles of plant growth and experimental results from other species of aquatic plants and microalgae used for wastewater treatment (Carr et al., 1997; Ras et al., 2013). Outside the optimal temperature range (20-30°C), or even at extreme temperature values, the plants do not grow as fast or simply die. This fact explains why *L. minor* and *L. minuta* do not survive over winter in uncontrolled outdoor experiments (Paolacci et al., 2018). Duckweeds sense environmental conditions and when these are not favourable, most of them can enter a dormant state by turion formation (Appenroth, 2002; Kuehdorf & Appenroth, 2012). This ability allows these plants to survive in environments with seasonal climatic variability.

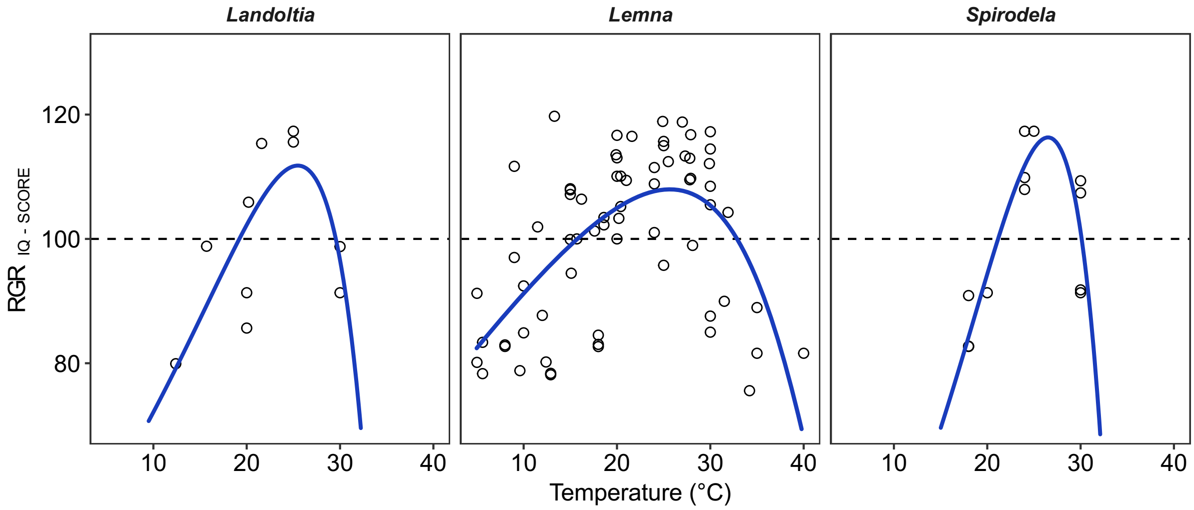

Figure 3. Effect of temperature on the relative growth rate (RGR) of different duckweed genera. *Thermal performance curves for Landoltia, Lemna and Spirodela were fitted to datasets from 3, 15 and 4 independent experiments respectively, using the Hinshelwood model. In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed lines represent the $RGR_{IQ\text{-}score}$ baseline (= 100). Coefficients and standard errors for the fitted curves are given in Supporting Information, Table S2.*

When analysing the variation of RGR IQ-scores with respect to temperature, a region is revealed, above the baseline (IQ-score = 100), where plants growth is better than the average from all the experiments included in the review (average growth rate = 0.119 d^{-1}). Based on the thermal performance curves resulting from the datasets for different genera of duckweed (Figure 3), we found that the temperature range within which the area of optimal growth is contained varied between genera. *Lemna* species can cope better with extreme temperatures and exhibit a good growth performance in a wide range of temperatures (11.4 to 38.1°C) while the range of temperatures for optimal growth was narrower for *Landoltia* and *Spirodela* species (18.1 to 32.3°C, and 19.0 to 29.2°C, respectively). In the case of *Lemna*, plants that are grown outside the optimal temperature range end up having a RGR 55% lower than the average RGR above the IQ-scores baseline. These results highlight the importance of choosing the most suitable duckweed species according to local temperature conditions.

The temperature range for RGR IQ-scores higher than 100% is of great importance for the development of duckweed-based processes for wastewater treatment. If temperature alone is considered, a treatment system operated at ambient conditions will be reliable if the selected duckweed species perform well within the local temperature variations. Thus, the implementation of duckweed-based systems for wastewater treatment in regions with tropical or subtropical climates is favoured due to narrow temperature variations from optimal duckweed growth conditions throughout the year. In cool/cold temperature regions actions need to be taken to engineered wastewater treatment systems to avoid temperature falling below the optimal range.

3.2.2. Light

Light is an essential factor for plant growth as it is the energy source for photosynthesis, which enables plants to fix atmospheric inorganic carbon and turn it into organic compounds. When referring to light during the cultivation of duckweeds, three factors must be considered: light intensity, light/dark cycles or photoperiod, and light spectral composition. All factors affect duckweed biomass growth through their impact on photosynthesis. In terms of light intensity, it has been found that the growth rate of aquatic plants and microalgae increases with increasing light intensity, up to a maximum RGR value when light saturation conditions are reached (Madsen & Sand-Jensen, 1994; Sorokin & Krauss, 1958). Further light intensity increments above this point reduce plant growth rates and may even inhibit photosynthesis (photo-inhibition). However, results may vary depending on the species and isolates studied, as well as on photoadaptation processes that improve the photosynthetic efficiency of the organisms. This includes changes in chlorophyll content and ratios, number of chloroplasts and respiration patterns (Lichtenthaler et al., 1981).

Although light intensity plays a fundamental role in photosynthesis, the time during which the radiation is incident on the plants must also be considered. At low light intensities the RGR of duckweeds increases with longer day conditions, but at high light intensities longer photoperiods negatively impact plants growth rate (Lasfar et al., 2007; Yin et al., 2015). In this sense, it is necessary to consider the total amount of radiation that reaches the plants while they are exposed to the light (e.g., daily light integral – DLI) to avoid photosystem inhibition and damage, so that photosynthesis can continue (Sundby et al., 1993).

Light intensity

The relative growth rate of different duckweed species increases with increasing light intensity, reaching maximum biomass growth at around 200 μmol m^{-2} s^{-1} (Figure 4); further increases in light intensity do not significantly affect plant growth (even up to 800 μmol m^{-2} s^{-1}, not shown in the figure). The change in IQ-scores of the selected duckweed species with respect to light intensity was fitted to a Monod-like model widely used for microalgae (Béchet et al., 2013). From the results, it can be established that overall, for all duckweed species, light saturation is reached at around 100 μmol m^{-2} s^{-1}. One exception is *L. aequinoctialis* grown in continuous light, which is saturated by light at an intensity of 50 μmol m^{-2} s^{-1}. Furthermore, within the range of reported light intensities (0 – 800 μmol m^{-2} s^{-1}), no evidence of photoinhibition can be seen. Similar results were reported by Wedge et. al (1982), who found that, depending on the temperature, light saturation in *Lemna minor* plants occurs between 300 – 600 μmol m^{-2} s^{-1} and that there is no photoinhibition unless the light intensity is greater than 1200 μmol m^{-2} s^{-1}.

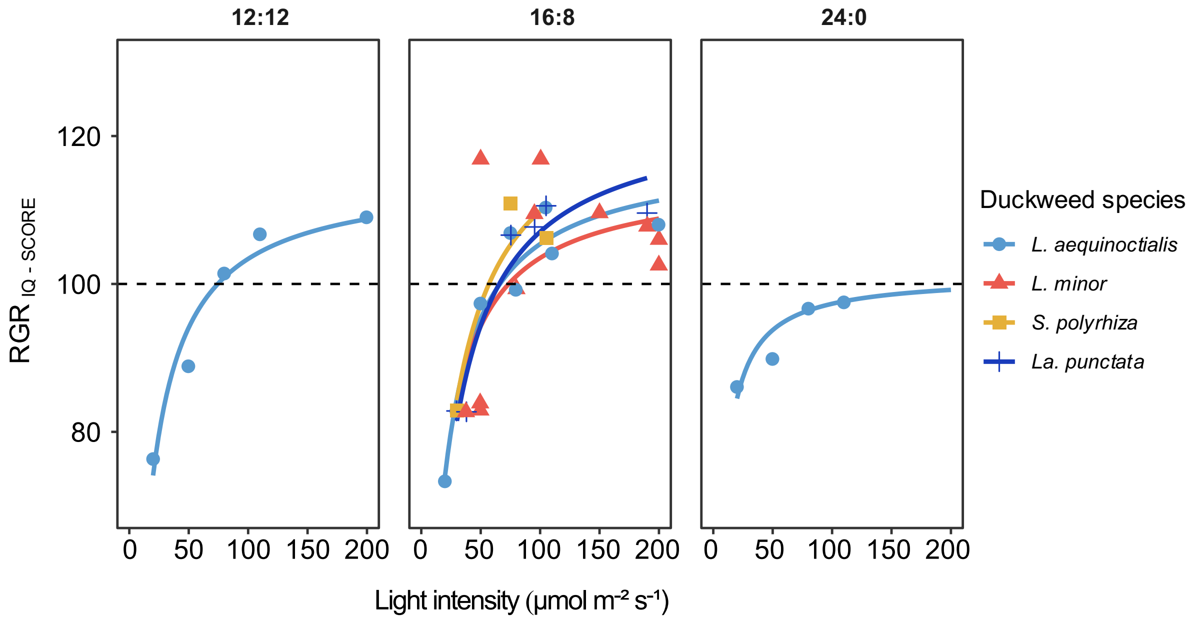

Figure 4. Effect of light intensity on the relative growth rate (RGR) of duckweeds cultivated under different photoperiods (light hours: dark hours). *Curves represent the general trend of the data for different duckweed species upon parametric fitting of datasets to Monod-like equations. The numbers above the boxes represent the number of hours light: dark per day. In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the* $RGR_{IQ\text{-}score}$ *baseline (= 100). Data for L. aequinoctialis, L. minor, S. polyrrhiza and La. Punctata was retrieved from 4, 4, 1 and 2 independent experiments respectively.*

Although the overall effect of light intensity on the RGR of the plants is the same (direct increment until saturation), the magnitude of the effect varies according to the photoperiod and duckweed species in question. On the one hand, the positive effect of increasing light intensity on RGR is compromised as the length of light hours increases. In the case of *L. aequinoctialis*, there is no significant effect of increasing the photoperiod from 12 to 16 hours, but an additional increase of 8 hours reduces the RGR by 24% (Yin et al., 2015). On the other hand, when grown at the same day length (16 h) and below light saturation condition, the RGR of different duckweeds species improves differently for each unit by which the light intensity is increased. As an example, an increment of 50 µmol m^{-2} s^{-1} improves the RGR of *L. minor*, *L. aequinoctialis*, *La. punctata* and *S. polyrrhiza* by 23.1, 31.9, 31.2 and 33.3% respectively (Y. Li et al., 2016; Walsh et al., 2021; Z. Zhao et al., 2014).

Photoperiod

When it comes to photoperiod, two different trends are discernible when analysing the growth of *L. minor* under constant light intensity (Figure 5A). When the light intensity at which duckweeds grow is higher than 300 µmol m^{-2} s^{-1}, the RGR increases from 0.01 to 0.43 day^{-1} when the light exposure is increased from 0 to 12 hours a day. Longer photoperiods reduce the rate at which the plants grow. In this case there is a region above the IQ-scores baseline, between 7 and 18 hours of day length, in which the RGR of duckweeds is greater than the average RGR of all the retrieved data. This range can be defined as the optimal photoperiod range for duckweed growth. At low light intensities (e.g., 156 μmol m^{-2} s^{-1}) the effect of photoperiod on the RGR of *L. minor* is the same as at high intensities, however, the optimal range in which plants can grow is extended by 7 hours. In this case, we found that the effect of photoperiod on the RGR was the same despite the difference in culture temperature between the experiments (Lasfar et al., 2007; Paterson et al., 2020).

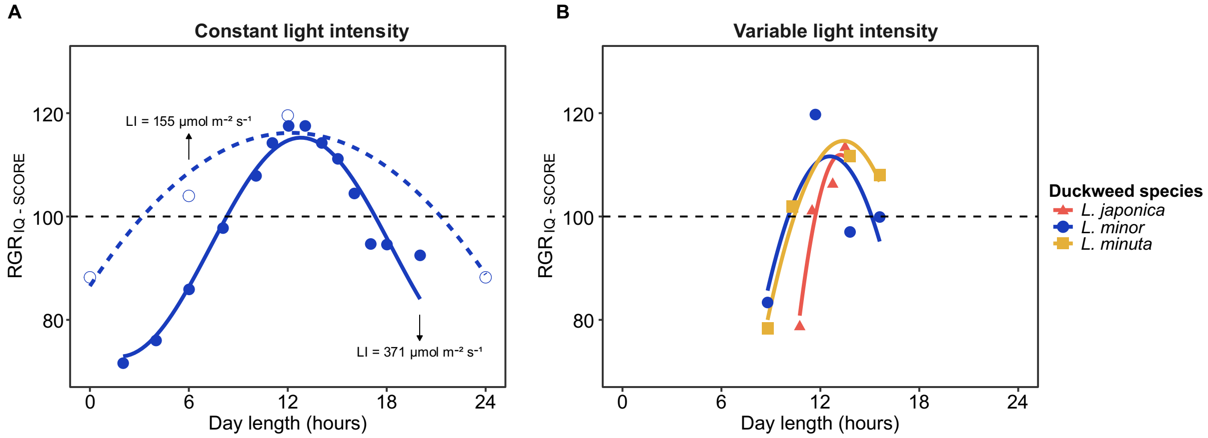

Figure 5. Effect of photoperiod on the relative growth rate (RGR) of duckweeds under natural and controlled culture conditions. *(A) Duckweed cultivated in controlled environments at two different constant light intensities; (B) Duckweed cultivated in real environments with varying photoperiod and light intensities (ranging between 100 – 400 μmol m^{-2} s^{-1}). Curves represent the general trend of the data upon non-parametric fitting of datasets. In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the RGR $_{IQ\text{-}score}$ baseline (= 100). Data for L. minor, L. japonica and L. minuta was retrieved from 3, 1 and 1 independent experiments respectively.*

A particular case is that of *L. aequinoctialis*, whose RGR increases with longer day length, reaching a maximum under continuous light independently of the light intensity (ranging from 20 to 400 μmol m^{-2} s^{-1}, data not shown) (Yin et al., 2015). The difference between both Lemna species highlights the importance of species selection in the design of wastewater treatment systems. *L. minor* copes better with daylength changes in open air treatment systems, while *L. aequinoctialis* can be used in engineered indoors systems with a continuous supply of light. When analysing the effect of the photoperiod on the RGR of plants that were grown outdoors under variable light intensity conditions (Figure 5 B), it is observed that the data follows similar trends to those of plants cultivated under constant light intensities, but in a narrower range. For light intensities varying between 100 and 300 μmol m^{-2} s^{-1} the optimal photoperiod range for three different duckweed species is reduced to 7 hours only, between 9 and 15h of day length on average.

Although the results do not establish a direct relationship between photoperiod and other duckweed genera and species (not enough data from data collection process), they do lead to the conclusion that photoperiod and light intensity should be considered together when analysing the effect of light on plant growth.

Combined effect of light intensity and photoperiod – The daily light integral concept

By integrating the light intensity at which the plants are grown together with the time at which they are exposed to light, it is possible to analyse the combined effect of those two variables on the relative growth rate of duckweeds (Figure 6). This combined variable is named as daily light integral (DLI), which describes the number of photosynthetically active radiation (PAR) measured as photons (individual particles of light in the 400-700 nm range) that are delivered to a specific area over a period of time (mol m^{-2}). When the two effects of light intensity and exposure are integrated into the DLI variable, it was found that a biomass growth rate increases with DLI until reaching a maximum at at 15 mol m^{-2}, corresponding to the light saturation value. Below this value, plant growth declines rapidly because they do not receive enough energy to efficiently carry out photosynthesis and therefore there is no cell reproduction. On the other hand, when a DLI of 24 mol m^{-2} is reached, the effect of photosystem inhibition becomes significant and the RGR falls below the IQ-scores baseline.

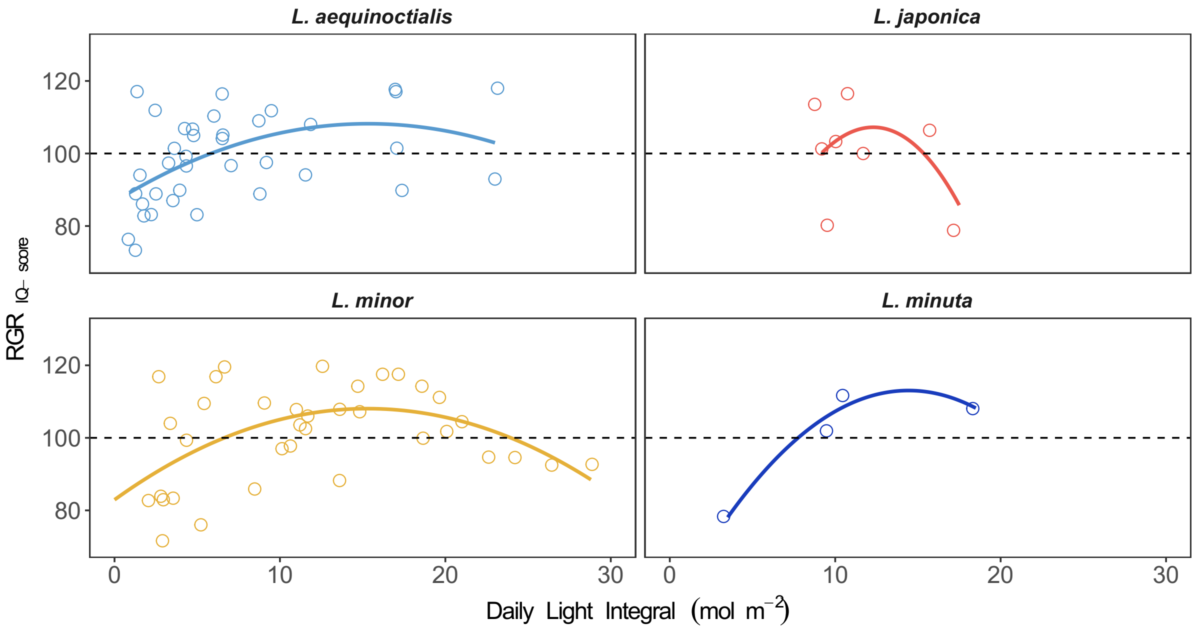

Figure 6. Daily light integral (DLI) as a parameter to assess the effect of light on the relative growth rate (RGR) of Lemna species. *Curves represent the general trend of the data upon non-parametric fitting of datasets. In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the RGR IQ-score baseline (= 100). Data for L. aequinoctialis, L. japonica, L. minor and L. minuta was retrieved from 10, 2, 7 and 1 independent experiments respectively.*

In this regard, further studies testing the turnover of D1 protein of photosystem II in varying DLI values need to be addressed to confirm the extent to which the damage in the photosystem affect the RGR in duckweeds (Aro et al., 1993). In the past, it has been proven that DLI not only affects plant growth but many other plant traits (Poorter et al., 2019). In general, it was found in the literature that plant growth is limited below a DLI of 5 mol m^{-2}, whereas saturation of most traits occurs beyond 20 mol m^{-2}. The fact that the reported data fell within this range supports the idea that there is little difference in plasticity with respect to DLI between different plant species.

The analysis of the DLI as a control variable, suggest that the effect of light on RGR of duckweed is independent of the duckweed species (different *Lemna* species in this case) being tested. The finding is useful for the design of engineered treatment systems based on duckweed biomass and using either natural or artificial light. In the former case, the DLI supports the potential use of solar energy as a source of radiation, thus reducing energy costs and dependence on fossil fuels. Furthermore, DLI monitoring would allow prediction of biomass growth in environments where the intensity and amount of light is not constant during the system operation period, making it possible to get more reliable systems for engineering applications (e.g., wastewater treatment). Moreover, the analysis of the data reveals that after exceeding a threshold value in DLI (7.5 mol m^{-2}), there is no major gain in terms of RGR so that energy savings can be considered during the design of the treatment process. For instance, by doubling the DLI from 7.5 to 15 mol m^{-2}, the energy cost doubles in a system of constant area while the RGR of the plants improves by only 5.8%.

The spectral composition of light, or the specific wavelengths of light that are present, is another parameter that can affect the growth of duckweed. Different pigments in the plant absorb different wavelengths of light, which can stimulate or inhibit growth. Studies have concluded that red and blue light are the most effective in promoting growth and increasing biomass production. Duckweed grown in either blue or red light resulted in 10% and 31% increase in dry weight, respectively, in comparison to cultures under cool white light (Q. Li et al., 2022). Moreover, the combination of red and blue light at different ratios does not significantly impact duckweed growth but influences the accumulation of starch (Q. Li et al., 2022; Petersen et al., 2022). These findings have important implications for optimizing duckweed cultivation for applications such as wastewater treatment with resource recovery (i.e., production of starch-rich duckweed biomass for animal feed).

3.3. Effect of nutrient supply on duckweed growth

In addition to light, plants need the right combination of nutrients to live, grow and reproduce. Both excess and deficiency of nutrients can cause problems to plant growth. Among the elements that plants need in relatively high amounts, macronutrients like phosphorus and nitrogen are of most interest due to their low bioavailability in aquatic environments (Vitousek et al., 2010). Although both nutrients are abundant in agricultural runoff and wastewater discharges, their presence in aquatic ecosystems is undesirable due to the potential development of anoxia and eutrophication in surface waters. As we well know, nitrogen and phosphorus fertilizers are usually added to soils to ensure that plants have adequate access to these essential nutrients. In plants, both nutrients are present either as ionic species or as constituents of biomolecules of great importance for the plant (Maathuis, 2009). Like terrestrial plants, duckweeds can acquire significant amounts of inorganic nutrients through their root system (Cedergreen & Vindbadk, 2002; Ying Fang et al., 2007). However, due to their aquatic nature and the fact that the fronds float directly on the surface of the water, nutrient absorption is mostly carried out from the underside of the frond (Ice & Couch, 1987; Oron, 1994). The extent to which duckweeds growth is affected by nitrogen and phosphorus supply is reviewed in the context of their use for wastewater treatment.

3.3.1. Nitrogen

Nitrogen (N) is involved in the synthesis of amino acids (the building blocks of proteins), chlorophyll and nucleic acids (DNA, RNA). It promotes the photosynthetic capacity and the growth of plant tissue, making it an important performance factor (Barker & Bryson, 2006; Novoa & Loomis, 1981). N is present in wastewater in mineral ($N\text{-}NO_3$ and $N\text{-}NH_4$) or organic forms, but it is mainly absorbed by duckweeds in mineral form like ammonium and /or nitrate (Ding et al., 2018; Joy, 1969). Both nitrogen deficiency and excess affect plant growth, but the extent to which it is affected depends on the species of nitrogen used for cultivation.

Nitrogen species

The forms of nitrogen in wastewater vary depending on the type of wastewater, pH and temperature (Caicedo et al., 2000). As a result of different biological and chemical processes, the main nitrogen compounds in wastewater are ammonium, nitrate and nitrite. Among them, ammonium is the main chemical specie, as it originates from the decomposition of organic matter. However, significant amounts of nitrate can be found in wastewaters and runoff resulting from industrial or farming activities requiring significant amounts of nitrate-based chemicals or fertilisers.

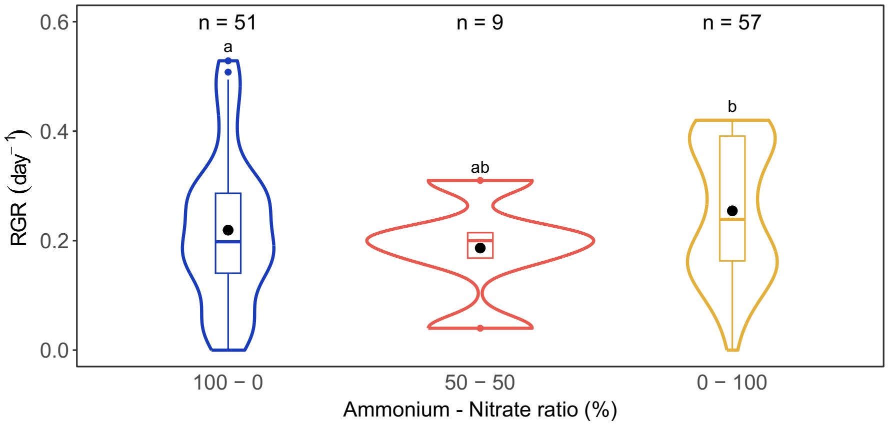

Figure 7. Differences in the relative growth rate (RGR) of duckweeds grown at different ammonium to nitrate ratios. *Violin plots represent the distribution of datapoints, and box plots represent the median, the 25th and 75th percentiles, minimum, maximum and outlying points. Black points mark the average RGR value for each* NH_4 *-* NO_3 *ratio. Only data for different species of the genus Lemna are presented. The number of observations per group (n) is presented on top of each plot. Lower case letters represent statistical significance (*$p < 0.5$*).*

In the *Lemnaceae* family, the preference for ammonium over nitrate is still a subject of discussion. Most of authors have stated that ammonium is adopted as the first source of nitrogen by duckweed, because it is important for the synthesis of amino acids and proteins, and there is an associated saving of energy for the assimilation process (Oron, 1994; Porath & Pollock, 1982). However, ammonium assimilation is temperature sensitive and occurs only at pH values between 6 and 8 (Caicedo et al., 2000). It has also been pointed out that ammonium is a limited source of nitrogen due to its toxicity to plants (Joy, 1969). When both nitrogen sources are available in the medium, the plant prefers to absorb ammonium, but can take nitrate when it is the only nitrogen source (Ying Fang et al., 2007). When a wider range of pH values is considered, some duckweeds species have shown predilection for nitrate over ammonium while the absorption of other macronutrients (P) was enhanced (Paterson et al., 2020).

The possibility of using both ionic species as a source of nitrogen to grow duckweeds is reflected in the number of publications studying the effect of different ammonium to nitrate ratios on the RGR of plants. In the case of Lemna species, when considering the sole effect of the nitrogen source on duckweeds RGR, it was found that there is no statistically significant difference ($p > 0.05$) between mean RGR values when using ammonia, nitrate or both nitrogen species in the culture medium (Figure 7). The differences between the culture conditions employed in studies considered in the analysis reveal that the preferred nitrogen source for each duckweed species is species-dependent and may be determined by the acclimatisation of plants to the growing conditions, the nitrogen concentration and the N:P ratio.

Nitrogen concentration

Total nitrogen concentration in domestic wastewater varies between 20 and 80 mg N L^{-1}. Ammonia is the major contributor to total nitrogen (~ 60%), followed organic nitrogen and nitrate (Henze et al., 2002). In some cases, total nitrogen concentration can be as high as 200 mg N L^{-1}, especially in wastewater from industries like aquaculture, and run-off water from agriculture (Korner et al., 2003). If duckweeds are intended to remediate wastewater from aquaculture, it is necessary not only to understand the effect of the nitrogen species present in the effluent of this industry, but also the effect of the concentration of nitrogen on the potential of these plants to grow.

Studies have shown that duckweeds have a wide range of tolerance to nitrogen concentrations, and the optimum concentration may vary depending on the species, growing conditions and nitrogen source (Figure 8). In general, when nitrate is the only nitrogen source, duckweed growth is supported at moderate concentrations (between 2-70 mg N L^{-1}, Figure 8-A). In presence of ammonium duckweed growth is supported at lower concentrations (between 5-15 mg N L^{-1}, Figure 8-B). However, higher nitrogen concentrations beyond RGR maxima are detrimental to duckweed growth in both cases. In addition to the above, when comparing the kinetic curves obtained with respect to N concentrations and the $RGR_{IQ\text{-}score}$ baseline, the RGR of the *Lemna* species is higher than the average RGR value in cultures grown with nitrate (2 – 195 mg N L^{-1}) than those grown in ammonium (5 – 60 mg N L^{-1}).

The fact that the RGR response curves to different nitrogen concentrations follow the same trend for nitrate and ammonium suggests that duckweed does not have a particular preference for a specific nitrogen source, since, under certain conditions, both nitrogen sources benefit plant growth. What the results suggest is that to some extent ammonium has greater inhibitory effects on the growth of *L. gibba* and *L. minor* than nitrate. This can be explained due to potential ammonium toxicity.

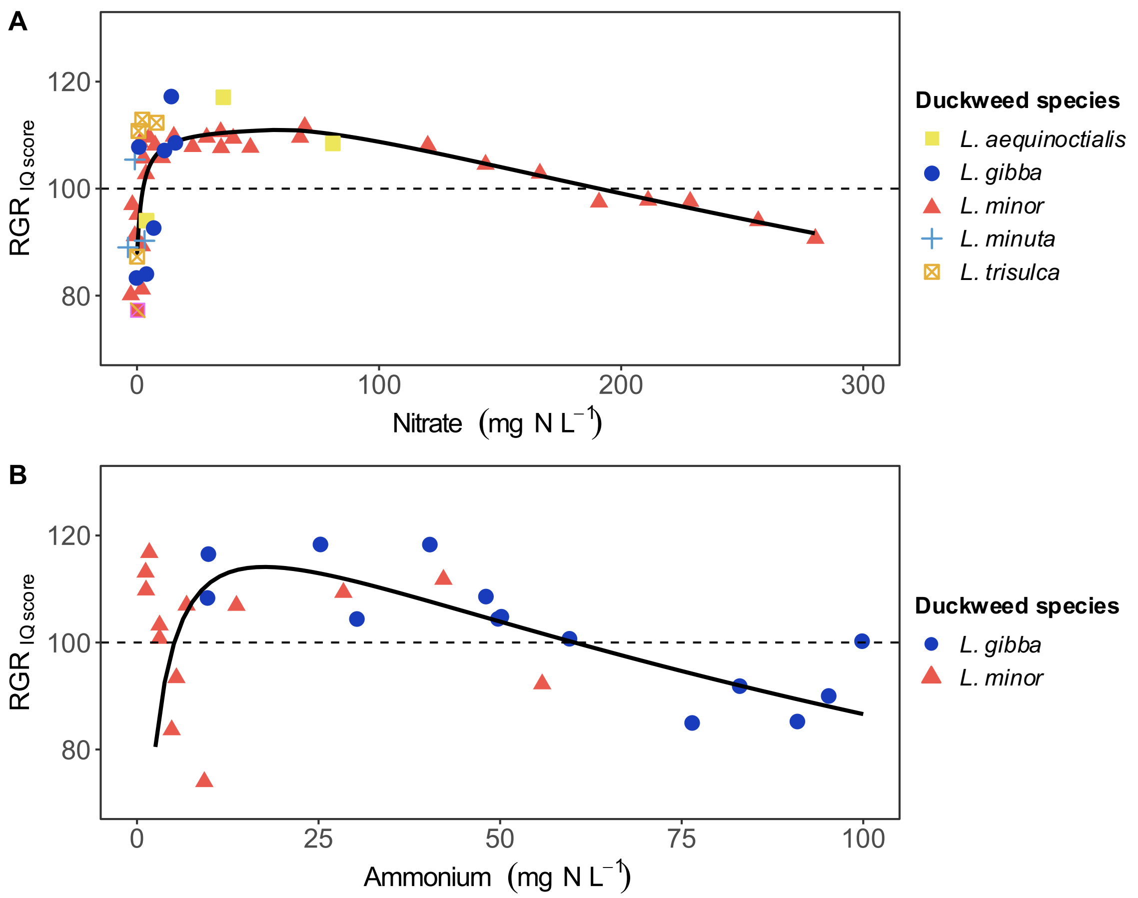

Figure 8. Effect of the supply of different nitrogen species on the relative growth rate (RGR) of duckweeds. *(A) duckweeds grown in media with nitrate as only source of nitrogen; (B) duckweeds grown in media with ammonium as only source of nitrogen. Curves represent the general trend of the data upon parametric fitting of datasets to a substrate-inhibition kinetic mode (Haldane, 1965). In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the* $RGR_{IQ\text{-}score}$ *baseline (= 100). Data for L. aequinoctialis, L. gibba, L. minor, L. minuta and L. trisulca was retrieved from 1, 2, 3, 1 and 1 independent experiments respectively.*

There are two main mechanisms by which ammonium is toxic to plants. The first derives from the ease at which ammonium is transported across the cell membrane and the second from changes in pH as a result of ammonium uptake (Britto & Kronzucker, 2002). Both ammonium (NH_4^+) and its non-ionised form, ammonia (NH_3), are transported into the membrane by low affinity transporters, which activity is upregulated at high external nitrogen concentrations, resulting in increased influx of nitrogen (Cerezo et al., 2001; M. Y. Wang et al., 1993). As the ammonium uptake rate of the plant exceeds the assimilation rate or the storage capacity, the plant will actively transport ammonium back to the exterior (Hecht & Mohr, 1990; Husted et al., 2000). As a result, the energy demand for this process (Britto et al., 2001), together with a reduced influx of other cations (e.g., K^+, Mg^{+2}, Ca^{+2}) and increased uptake of anions (Cl^-, $SO_4^=$) may limit overall plant growth (Gerendás et al., 1997; Roosta & Schjoerring, 2007; Van Beusichem et al., 1988). A recent study on *Landoltia punctata* has shown that the coordination of carbon and nitrogen metabolism in duckweeds may act as ammonium detoxification mechanism, making duckweeds more tolerant to ammonium than other higher plants (Tian et al., 2021).

The second proposed mechanism by which ammonium is toxic to plants relates to external and internal pH changes (McQueen & Bailey, 1990; Schubert & Yan, 1997). Ammonium uptake by higher plants is linked to a cation counter-phase, to compensate for the charges on the cell membrane potential. This effect occasionally leads to the acidification of the culture medium in which the plant is growing (Brix et al., 2002; Ruan et al., 2007; Schubert & Yan, 1997). Moreover, nitrate reduction in plants is considered a sink for excess NADPH production by photosynthesis. When an already reduced source of nitrogen is supplied, like ammonium, the accumulation of NADPH can indirectly affect the internal cell pH by altering the reactive oxygen species and enzymes involved in maintaining the pH balance (Guo et al., 2007). In duckweeds, it has been found that the optimum pH value for growth is around 7 (Caicedo et al., 2000; Jones et al., 2023; McLay, 1976), so that, in cases where ammonium is the only available source of nitrogen, there is a double stress factor that reduces plant growth.

3.3.2. Phosphorus

Phosphorus (P) is a cellular constituent and an energy carrier. It is a component of the phospholipids that make up cell membrane and DNA, RNA, and ATP molecules (Maathuis, 2009). As a cellular constituent, P supports plant growth, particularly in the development of roots that have several adaptive responses to acquire P from the soil and aquatic environments. P also promotes flowering, fruit setting and seed formation (Maathuis, 2009). In wastewaters, phosphorus can be found in mineral form, mainly as orthophosphates (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$,) and, in a smaller amount, in organic form. The form at which mineral phosphorus can be found strongly depends on water temperature and pH. Furthermore, phosphorus is a non-renewable resource, that is unevenly distributed in the world, hence the importance of its recovery and reuse from waste streams (Slocombe et al., 2020).

Phosphorus concentration

The occurrence of phosphorus in wastewater is closely related to the sources of phosphorus. Industrial, agricultural and household activities have the greatest impact on the amount of phosphorus found in wastewater. As such, phosphorus concentration can be relatively low, as in domestic wastewater (0.2 – 20 mg P L^{-1}) or high, as in effluents from intensive crop and livestock production (12 – 780 mg P L^{-1}) (Carrillo et al., 2020). A particular case is that of aquaculture where the large volumes of water used for fish production dilute the phosphorus concentration to values below 1 mg P L^{-1}. In aquatic environments (fresh waters), phosphorus is usually considered as the limiting nutrient controlling growth of photosynthetic organisms. Therefore, the effect of low phosphorus concentrations on duckweed growth needs to be assessed.

Our results show that increasing phosphorus concentration of the culture medium improves duckweed relative growth rate, however, how this occurs depends on the nitrogen source used for the culture (Figure 9). On one hand, when ammonium is used as the sole source of nitrogen, *L. minuta* reaches a maximum growth rate at a phosphorus concentration of 1.5 mg P L^{-1}. Thereafter, higher P concentrations reduce the rate at which the plant grows (Figure 9A). On the other hand, in the presence of nitrate, the RGR of different duckweed species reaches a maximum at a phosphorus concentration of 1 mg P L^{-1}. In this case, the growth rate is not affected by further increases in phosphorus supply, remaining always above the $RGR_{IQ\text{-}score}$ baseline (Figure 9B).

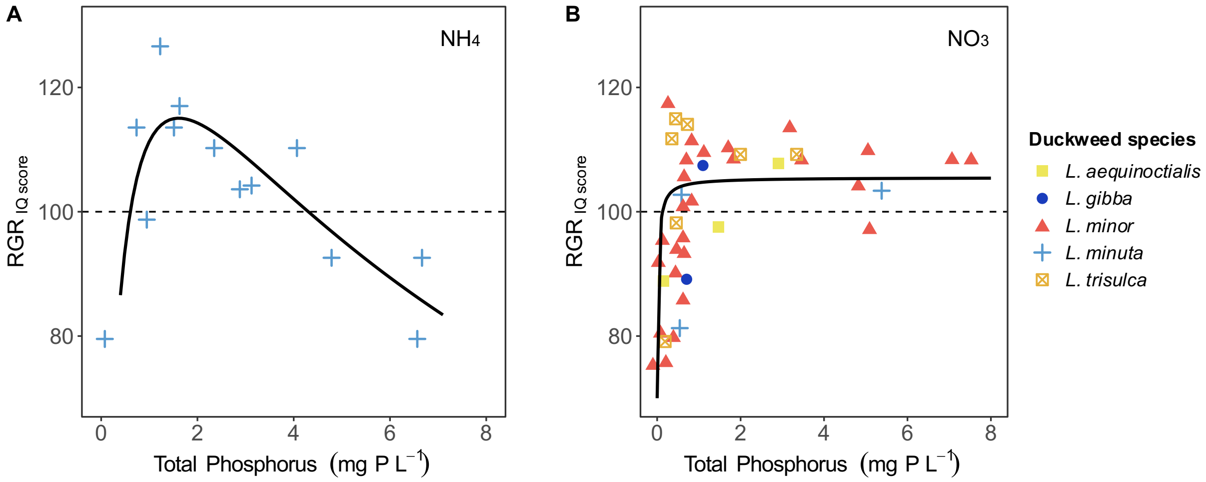

Figure 9. Effect of phosphorus supply on the relative growth rate (RGR) of duckweed in media with different nitrogen source. *(A) duckweeds grown in media with ammonium as only source of nitrogen (median N:P ratio = 7.0); (B) duckweeds grown in media with nitrate as only source of nitrogen (median N:P ratio = 6.5). Curves represent the general trend of the data upon non-parametric fitting of datasets. In all cases, RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the RGR IQ-score baseline (= 100). Data for L. aequinoctialis, L. japonica, L. minor and L. minuta was retrieved from 10, 2, 7 and 1 independent experiments respectively.*

In higher plants, phosphorus uptake and relocation are carried out by phosphorus transporter proteins (PHT) (Młodzińska & Zboińska, 2016). There is evidence that PHT proteins can be induced either at low (high-affinity) or high (low-affinity) external phosphorus concentration (Bayle et al., 2011). In a recent study, 73 PHT highly conserved genes have been identified in different duckweed species (X. Zhao et al., 2021). Within these, 21 belong to the PHT1 subfamily, responsible for P acquisition from the environment, suggesting that P uptake by duckweed follows similar mechanisms to those previously reported in terrestrial plants. In general, an excess supply of phosphorus does not negatively affect plant growth, unless the concentration of phosphorus in the plant tissues exceeds 1% of the plant dry weight, a phenomenon known as P_i toxicity (Marschner, 1996; Takagi et al., 2020). The estimated Michaelis-Menten constant (*Km)* for low-affinity and high-affinity PHT transporters suggest that saturation condition is reached at external P concentration of 0.1 and 1.5 mg P L^{-1} respectively (Nussaume et al., 2011). Also, there is a close link between nitrogen and phosphorus uptake, in which PHT proteins interact with nitrogen transport proteins to maintain nutrient balance in the plant (H. Feng et al., 2017). As a result, high external phosphorus concentrations induce higher P uptake and consequently higher N uptake, meaning that plant growth would not be affected by phosphorus but by the concentration and species of nitrogen being taken up. If ammonium is the nitrogen source (as in

Figure 9-A) we have that, at constant N:P ratios, the concentration of nitrogen can be such that duckweed growth is inhibited, as explained in the previous section.

Nitrogen and phosphorus supply balance

The nitrogen to phosphorus supply ratio (N:P ratio) is important for plants nutriiton as it is a parameter that indicates the availability of phosphorus and nitrogen for plant growth. The assessment of the N:P ratio allows to establish the condition in which plant growth can be limited by low availability of a nutrient, or the appropriate proportion of nutrients for biomass production. The optimal N:P ratio for plant growth can vary depending on the plant species and the environmental conditions.

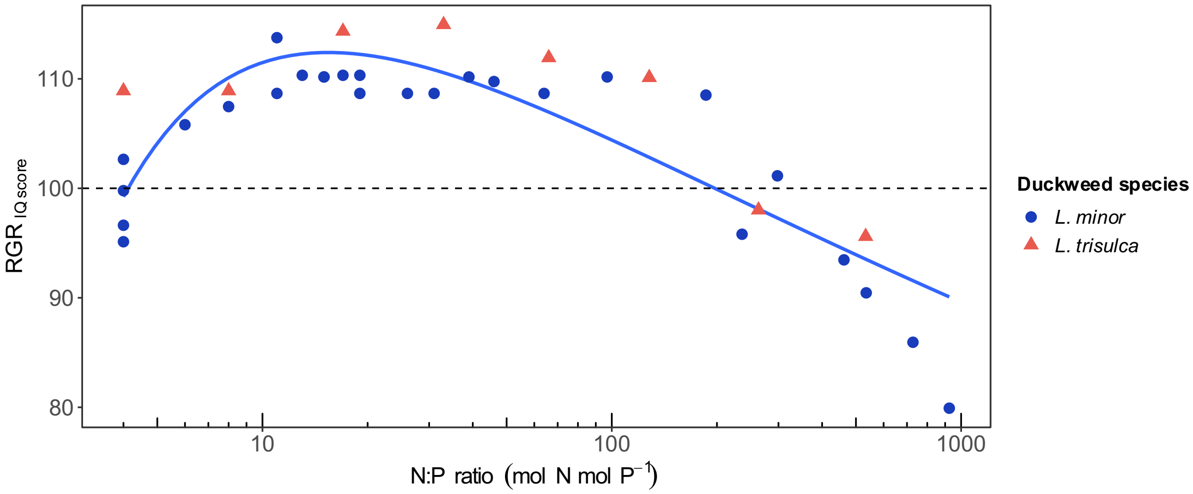

Figure 10. Nitrogen and phosphorus balance affect the relative growth rate (RGR) of duckweeds. *Nitrogen to phosphorus ratio (N:P ratio) was calculated only for those experiments carried out between 25 – 27°C using nitrate as the sole source of nitrogen. The curve represents the general trend of the data upon non-parametric fitting of datasets. RGR is expressed as the IQ scores from each independent experiment. Dashed line represents the RGR IQ-score baseline (= 100). Data for L. minor and L. trisulca was retrieved from 2 and 1 independent experiments, respectively.*

In the case of two different *Lemna* species, we found that the optimal N:P supply ratio that maximises plant growth is 15:1 (Figure 10). At lower N:P ratios (the nutrient imbalance causes plants to undergrow due to lack of nitrogen, and at higher ratios the lack of phosphorus and excess nitrogen cause plant growth to be limited or inhibited. It has previously been reported that the optimal N:P molar ratio for plant growth is 15:1 (7:1 masss ratio) (Koerselman & Meuleman, 1996) which is consistent with that found for *Lemna* species. Similar values were also found for grain legumes (Sadras, 2006) and microalgae (Liu et al., 2011). In wastewater, the molar N:P ratio varies on the type of wastewater and usually fluctuates between 11:1 and 22:1 (5:1 – 10:1, mass ratio) (de Godos et al., 2016; L. Wang et al., 2010), suggesting that wastewater can be used for duckweed cultivation without the need for additional nutrient supply. In conventional wastewater treatment nutrient balance is also an important parameter as it influences microbial activity responsible for the removal of organic matter and oxygen consumption.

4. LIMITATIONS OF THE STUDY

The limitations of this study are noteworthy, particularly concerning the absence of data on additional factors influencing duckweed growth beyond temperature, light, and nutrient availability. Firstly, there is a scarcity of studies addressing variables such as pH, plant mat density, interactions with other microorganisms, and others, making it challenging to draw meaningful comparisons and conclusions. Secondly, the difficulty in extracting quantitative data from existing literature can be attributed to a lack of standardization in result presentation, complicating the calculation of comparison indicators for a more comprehensive analysis. Lastly, the focus of the study was been deliberately narrowed to ensure a more in-depth analysis of the specified variables, sacrificing a broader understanding of the multifaceted aspects affecting duckweed growth. Consequently, these limitations emphasize the need for future research to explore the interplay of a wider array of factors to enhance the comprehensiveness of findings in the field of duckweed cultivation and its applicability for wastewater treatment.

5. CONCLUSIONS

Duckweed-based systems for wastewater treatment and nutrient recovery have the potential to provide sustainable and cost-effective solutions for water pollution and nutrient management. However, careful consideration must be given to various factors that affect the growth and nutrient uptake of duckweeds, such as temperature, light, and nutrient supply. These factors can be controlled and optimized through proper design, construction and operation of duckweed-based systems. Temperature is a critical factor that affects the growth and development of duckweeds, and the selection of the appropriate duckweed species for the local climate is essential. While temperature controls the rate of chemical reactions and influences the growth rate of duckweeds, light is the primary energy source for photosynthesis. Light intensity and photoperiod are crucial in regulating the total amount of radiation that reaches the plants and understanding the effect of these factors on duckweed growth can help optimize cultivation conditions and inform new technology developments, particularly for indoor cultivation using artificial light. Nutrient supply, especially nitrogen and phosphorus, significantly affects duckweed growth and nutrient uptake. Nitrogen plays a crucial role in the growth and development of duckweeds, while phosphorus is an essential component of cellular structure and an important energy carrier in plants. The concentration of these nutrients in wastewater can vary depending on the source of the wastewater; careful control of nutrient supply is essential for optimal duckweed growth, but typical N:P ratios in wastewater are sufficient to support duckweed growth. The recovery of nitrogen and phosphorus from wastewater is particularly crucial due to its non-renewable nature and P uneven distribution in the world. Duckweed-based systems can provide a sustainable solution for nutrient recovery from wastewater and overall nutrient management in catchments if use as an alternative for wastewater remediation. With the right design, construction and operation, duckweed-based systems can offer a cost-effective and sustainable alternative to conventional wastewater treatment methods. Overall, the implementation of duckweed-based systems for wastewater treatment and nutrient recovery requires a comprehensive understanding of the various factors that affect duckweed growth and nutrient uptake. By considering temperature, light, and nutrient supply in the planning and design of these systems, sustainable and cost-effective solutions can be developed for water pollution and nutrient management.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available as part of supplementary materials.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version when published.

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Table S2. Summary statistics for the thermal models shown in Fig 2.

The effect of temperature to the relative growth rate of duckweed was adjusted to Hinshelwood's thermal model.

$$\mathrm{RGR_{IQ-Score}} = \mathrm{a} * \exp\left(-\frac{\mathrm{E}}{\mathrm{RT}}\right) - \mathrm{b} * \exp\left(-\frac{\mathrm{E_h}}{\mathrm{RT}}\right)$$

Parameter	Estimate	Std. Error	t value	P
Landoltia				
a	6.810e+06	9.053e+07	0.075	0.942
E	2.793e-01	3.270e-01	0.854	0.426
b	3.981e+32	5.513e+34	0.007	0.994
E_h	1.855e+00	3.671e+00	0.505	0.631
Residual standard error: 10.44 on 6 degrees of freedom				
Lemna				
a	6.810e+06	9.053e+07	0.075	0.942
E	2.793e-01	3.270e-01	0.854	0.426
b	3.981e+32	5.513e+34	0.007	0.994
E_h	1.855e+00	3.671e+00	0.505	0.631
Residual standard error: 10.44 on 6 degrees of freedom				
Spirodela				
a	6.810e+06	9.053e+07	0.075	0.942
E	2.793e-01	3.270e-01	0.854	0.426
b	3.981e+32	5.513e+34	0.007	0.994
E_h	1.855e+00	3.671e+00	0.505	0.631
Residual standard error: 10.44 on 6 degrees of freedom				