Environmental Challenges 11 (2023) 100709

Contents lists available at ScienceDirect

Environmental Challenges

journal homepage: www.elsevier.com/locate/envc

# Maximizing duckweed biomass production for food security at low light intensities: Experimental results and an enhanced predictive model

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ARTICLE INFO

*Keywords:*
Duckweed
Growth
Temperature
Light intensity
Regression model
Biomass production

ABSTRACT

Aquatic macrophytes offer an excellent pathway to promote circular agricultural systems through the recovery and upcycling of waste nutrients into valuable agricultural products. The prolific aquatic plant and nutrient scavenger, duckweed (family *Lemnaceae*), is known not only for its wastewater treatment capabilities but also for its potential as a protein alternative compared to traditional plant and animal sources due to its high nutritional quality and low environmental impact. Although duckweed is known to grow under a wide range of environmental conditions, current models representing duckweed growth kinetics do not include variable(s) to quantify the effect of light intensity. In this work, data from our own experiments and from the literature were utilized to enhance an intrinsic duckweed growth model with a light intensity term, thereby dramatically improving the accuracy of specific growth rate predictions from an $R^2$ of 0.27 to 0.67. The resulting validated model helps advance our knowledge of duckweed's resilience to changing environmental conditions, with applications from enhancing food security under adverse climatic conditions to the optimization of vertical farming operations which strive to maximize food production while minimizing external inputs.

## 1. Introduction

Duckweed – a tiny floating aquatic plant of the family *Lemnaceae* – is a valuable agent for wastewater treatment due to its ability to capture nutrients that are otherwise known to cause harmful water quality impacts. There are 36 recognized duckweed species in five genera, including *Landoltia, Lemna, Spirodela, Wolffia,* and *Wolffiella* (Bog et al., 2019), with *Lemna* species often utilized as a model system for industrial applications (Van Hoeck et al., 2015). Although considered an invasive plant due to its rapid growth rate, studies have shown that duckweed can be successfully managed under controlled conditions, and the harvested biomass utilized to produce a variety of value-added products such as biofuels (Calicioglu et al., 2019; Cheng and Stomp, 2009), biofertilizers (Kreider et al., 2019), animal feed (Roman et al., 2021), and human food (Appenroth et al., 2018). In particular, as demand for sustainable protein-rich feed and food rises, duckweed has the potential to replace or supplement conventional protein sources.

One of the advantages of duckweed over other similar protein-rich plants is that it can grow under a broad range of environmental conditions, including temperature, light, pH, and nutrients (Ceschin et al., 2019; Leng, 1999). The rates at which duckweed grows and accumulates protein can vary widely depending on these external conditions and the type of duckweed. Some studies have found inhibitory effects on the growth of *Lemna* species at very high temperatures (~ 45 °C) and nutrient concentrations (> 60 mg N $L^{-1}$) (Filbin and Hough, 1985; Sońta et al., 2020). Generally, increasing light intensity is associated with higher duckweed growth and protein production; however, in a study where *Lemna aequinoctialis* was grown in a 1/2 Schenk-Hildebrandt growth medium at 23 °C and under varying light intensities ranging from 20 to 400 $\mu$mol $m^{-2}$ $s^{-1}$, optimal growth was found at an intermediate light intensity of 110 $\mu$mol $m^{-2}$ $s^{-1}$ (Yin et al., 2015). Even though a few studies have demonstrated the effect of light intensity on duckweed growth, the impact of very low light intensities ($\leq$ 25 $\mu$mol $m^{-2}$ $s^{-1}$) has only been examined to a limited extent on *Lemna* species (Ashby and Oxley, 1935; Yin et al., 2015).

With increasing focus on duckweed and its applications, there is value in understanding how duckweed performs when subjected to extreme growing conditions. This is important not only with regard to the changing climate but also in the unlikely but severe context of needing to grow food after a catastrophic event such as a nuclear war or super volcano eruption that would result in very dark and cold weather conditions (Turco et al., 1983). Growing duckweed yearlong in these scenarios for sustainable protein production would require an additional understanding of the ability of duckweed to grow under low light. This information would also be beneficial for large-scale indoor vertical farming applications, where optimizing energy consumption and cost relies heavily on the ability to grow duckweed under minimal external inputs without compromising on protein yields.

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https://doi.org/10.1016/j.envc.2023.100709
Received 1 November 2022; Received in revised form 13 March 2023; Accepted 20 March 2023

Duckweed growth modeling offers excellent opportunities both for predicting duckweed biomass yields under different growing conditions and for simulating its temporal growth pattern. Several models utilize simple first-order or exponential rate equations to fit duckweed growth data collected from experimental studies. Some of these models rely on Mitscherlich's form of equation in which plant yield rises exponentially with the increasing value of an external factor (such as aqueous nitrogen (N) concentration) before reaching a peak value and then decreasing linearly (Landesman et al., 2005; Briggs, 1975). A popular duckweed growth model by Lasfar et al. (2007) takes into consideration a range of parameters affecting duckweed growth such as temperature, N concentration, phosphorus (P) concentration, and initial mat density. The model, which was calibrated using experimental data on *Lemna minor* and validated with two other literature sources (which documented the performance of *Lemna, Spirodela,* and *Wolffia* species), simulates the intrinsic growth rate of duckweed under controlled conditions of external variables by fitting a Michaelis–Menten kinetics equation (assuming maximum uptake rate at a saturating nutrient concentration). However, it assumes a constant light intensity in accordance with the experimental setup and lacks a light intensity parameter in the equation.

The overall goal of this study was to improve our understanding of the effect of light intensity on duckweed growth using experimental data and an enhanced predictive growth model. Specifically, we did this by: (1) documenting the impact of low light intensities ($\leq$ 25 $\mu$mol $m^{-2}$ $s^{-1}$) on the growth of *Lemna japonica/minor* under different temperatures and nutrient concentrations; and (2) incorporating a new light intensity factor into an existing duckweed growth model and validating it using both experimental and literature data. The resulting enhanced duckweed growth model is expected to aid future studies, such as those focusing on duckweed production under extreme growing conditions and optimizing operating parameters in large-scale applications.

## 2. Methodology

### 2.1. Existing duckweed growth model

Lasfar et al. (2007) studied the effects of temperature, photoperiod, and nutrient (N and P) concentrations on the growth of *Lemna minor* and subsequently developed a global model to predict duckweed growth without measuring the mat densities over time (Eqs. (1) to ((3))). Their study relied on the effect of biotic and abiotic parameters (such as temperature, photoperiod, and nutrient concentrations) on the intrinsic growth rate ($r_i$) of duckweed which was modeled using Michaelis–Menten kinetics. The increase in duckweed mat density estimated using $r_i$ was then used to calculate the specific growth rate ($r_s$) following a first-order equation, as detailed in Eqs. (1) to ((3)).

$$r_i = R \cdot \theta_1^{\left((T-T_{op})/T_{op}\right)^2} \cdot \theta_2^{\left((T-T_{op})/T_{op}\right)} \cdot \theta_3^{\left((E-E_{op})/E_{op}\right)^2} \cdot \theta_4^{\left((E-E_{op})/E_{op}\right)} \cdot \frac{C_P}{C_P + K_P} \cdot \frac{K_{IP}}{K_{IP} + C_P} \cdot \frac{C_N}{C_N + K_N} \cdot \frac{K_{IN}}{K_{IN} + C_N} \quad (1)$$

$$D = \frac{D_L \cdot D_O}{\left(D_L - D_O\right) \cdot e^{-r_i \cdot t} + D_O} \quad (2)$$

$$r_s = \frac{1}{t} \cdot \ln\left(\frac{D}{D_O}\right) = \frac{1}{t} \cdot \ln\left(\frac{D_L}{\left(D_L - D_O\right) \cdot e^{-r_i \cdot t} + D_O}\right) \quad (3)$$

where $K_P$, $K_{IP}$, $K_N$, and $K_{IN}$ are the saturation and the inhibition constants of *P* and *N*, respectively; $C_P$ and $C_N$ are the *P* and *N* concentrations (mg $L^{-1}$), respectively; *R* is a constant (maximum intrinsic growth rate in $day^{-1}$); *T* is the temperature in °C with $T_{op}$ being the optimum temperature; *E* is the photoperiod (h); $r_i$ and $r_s$ are the intrinsic and specific growth rates ($day^{-1}$), respectively; $D_o$ is the initial mat density (g $m^{-2}$) of the duckweed; *D* is the instant mat density (g $m^{-2}$) (i.e., the duckweed biomass per square meter of covered water surface at a specific moment in time); and $D_L$ is the limiting mat density (i.e., the upper limit of the mat density beyond which the duckweed growth is strongly inhibited); *t* is the duckweed retention time (day); and $\theta_{1-4}$ are nondimensional constants.

The original Lasfar et al. (2007) model was calibrated using their own experimental data and validated with two literature sources. Light intensity used in their study (representing the photosynthetically active radiation) averaged 371 $\mu$mol $m^{-2}$ $s^{-1}$ and was assumed constant for all trials. In the present study, we collected additional literature and experimental data to incorporate a light intensity factor into the Lasfar et al. (2007) model.

### 2.2. Literature data

Four studies that grew duckweed (*Lemna* species) under various light intensities were selected to create the modeling dataset (Table 1). The initial list of articles was extracted with the help of 'Google Scholar' and 'Web of Science' databases using the search terms 'duckweed + light intensity + growth rate'. Although several studies were identified with this keyword search, the list was manually curated to identify only those that reported specific growth rates and crucial model parameters (temperature, photoperiod, N and P concentrations, initial mat density, and light intensity). Specific growth rates of duckweed (in $day^{-1}$) were either obtained directly from the papers or computed from their published data and/or graphs that showed an increase in duckweed biomass over time. The light intensities reported in these studies ranged from 15 to 450 $\mu$mol $m^{-2}$ $s^{-1}$. A total of 67 data points were generated from this literature review, out of which only 8 points accounted for duckweed grown under light intensities less than 25 $\mu$mol $m^{-2}$ $s^{-1}$. To increase the data at low light intensities, additional experimentation was performed as described in Section 2.3. Together, this literature and experimental data constituted the full dataset that was utilized in developing and testing the enhanced growth model (Table 1).

### 2.3. Experimental data

To expand the lower range of light intensities utilized in calibrating and validating the enhanced model, additional data were collected by conducting a laboratory-scale experiment with duckweed. Duckweed (previously identified as *Lemna japonica/minor* (Calicioglu and Brennan, 2018)) was harvested from a pilot-scale ecological wastewater treatment facility (Eco-Machine™), and grown in Hoagland media solution in the lab (Hoagland and Snyder, 1933). *Lemna minor* and *Lemna japonica* are indistinguishable using plastid barcoding and therefore are commonly referred to together as a single species (Borisjuk et al., 2015; Braglia et al., 2021). Hoagland media was chosen based on its successful use in several duckweed growth experiments in the past (Cox Cox Jr et al., 2022; Frédéric et al., 2006; Utami et al., 2018). A preliminary trial was initially run to determine the feasible range of light intensities for the main experiment. The trial was with light intensities of 1, 7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$ at room temperature (20–22 °C) revealed that duckweed showed negligible growth at 1 $\mu$mol $m^{-2}$ $s^{-1}$. Consequently, only the three higher light intensities (7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$) were adopted for the main experiment. The experimental setup and results from the preliminary trial are explained in detail in the Supporting Information (**Figures S1, S2, and S3**).

To study the combined effects of low light intensity, temperature, and nutrient concentrations, the main experimental design consisted of duckweed exposed to different sets of growing conditions (Table 2). While the experiment was initially designed for three temperature conditions (5, 15, and 22 °C), preliminary trials indicated minimal to no duckweed growth at 5 °C; therefore, 15 and 22 °C were selected as the two temperature settings for this study. For each temperature-nutrient media combination shown in Table 2, duckweed was grown in triplicate under three different light intensities (7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$) which fall within the range of light intensities observed on a typical

**Table 1**
List of studies used to curate the dataset for developing the enhanced duckweed growth model with a light intensity factor.

| Study | Duckweed Species | Number of data points | Growing Conditions |
|---|---|---|---|
| Yin et al. (2015) | *Lemna aequinoctialis* 6000 | 18 (3 outliers) | 36 mg N $L^{-1}$; 140 mg P $L^{-1}$; 23 °C temperature; 12 hr, 16 hr, and 24 hr photoperiod; six different light intensities (20, 50, 80, 110, 200, and 400 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are triplicate averages. |
| Landesman et al. (2005) | *Lemna obscura* | 8 | Four varying nutrient concentrations (0–54.6 mg N $L^{-1}$ and 0–12.5 mg P $L^{-1}$); 16.6 °C temperature; 16 hr photoperiod; two different light intensities (315 and 653 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are quadruplicate averages. |
| Ashby & Oxley (1935) | *Lemna minor* | 40 (1 outlier) | 112 mg N $L^{-1}$; 24.8 mg P $L^{-1}$; 12 hr photoperiod; five different temperatures (10–29 °C); eight different light intensities (15, 28, 65, 93, 139, 167, 204, and 296 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are singlet values. |
| Tabou et al. (2013) | *Lemna minor* | 6 (1 outlier) | 10 mg N $L^{-1}$; 1 mg P $L^{-1}$; 21 °C temperature; 12 hr photoperiod; six different light intensities (200, 250, 300, 350, 400, and 450 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are singlet values. |
| This study – Preliminary Trial | *Lemna japonica/minor* | 4 | Hoagland media solution; 22 °C temperature; 16 hr photoperiod; four different light intensities (1, 7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are triplicate averages. |
| This study -Main Experiment | *Lemna japonica/minor* | 15 | Varying nutrient concentrations (22.8–114.2 mg N $L^{-1}$ and 2.4–11.9 mg P $L^{-1}$); varying temperatures (22 °C and 15 °C); 16 hr photoperiod; three different light intensities (7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$); Data points are triplicate averages. |
| Total | | **86 (without outliers)** | |

**Table 2**
Nutrient concentrations corresponding to different Hoagland media strengths and their corresponding reactor labels with rounded nitrate-N ($NO_3$-N) concentrations. For each temperature-nutrient media combination shown, duckweed was grown in triplicate under three different light intensities (7, 13, and 25 $\mu$mol $m^{-2}$ $s^{-1}$).

| Temperature ( °C) | Hoagland media strength (media: deionized water) | Nutrient Concentration (mg/L) | | | | | Reactor $NO_3$-N Label |
|---|---|---|---|---|---|---|---|
| | | $NH_4^+$ | $NO_3^-$ | $PO_4^{2-}$ | $Ca^+$ | $K^+$ | |
| 22 ± 2 °C | 1:1 | 16.2 | 97.9 | 11.9 | 80.1 | 117.3 | 100 mg N $L^{-1}$ |
| | 1:10 | 3.2 | 19.6 | 2.4 | 16 | 23.5 | 20 mg N $L^{-1}$ |
| | 1:100 | 0.32 | 1.9 | 0.24 | 1.6 | 2.35 | 2 mg N $L^{-1}$ |
| 15 °C | 1:1 | 16.2 | 97.9 | 11.9 | 80.1 | 117.3 | 100 mg N $L^{-1}$ |
| | 1:3.33 | 9.7 | 58.8 | 7.2 | 48.1 | 70.4 | 60 mg N $L^{-1}$ |
| | 1:10 | 3.2 | 19.6 | 2.4 | 16 | 23.5 | 20 mg N $L^{-1}$ |

overcast day (Schlyter, 1972; Thimijan et al., 1983). The growth media was replaced every 3–7 days (with longer intervals toward the end of the experiment when duckweed growth slowed), and the duckweed fresh mass was recorded during the sampling event. Each time, after weighing the duckweed, the entire biomass was returned to the reactor without any intermittent harvesting. Additional details regarding the main duckweed growth experiments are provided in the Supporting Information (**Tables S1 and S2, and Figures S4 and S5**).

The experiments were allowed to run until the duckweed growth curve plateaued or stabilized. Plateau was determined by visually inspecting the growth curves after each sampling event. The experiment was stopped when the curve deviated from the linear growth line, or when the duckweed mass did not show noticeable difference between two sampling events. The 22 and 15 °C experiments were carried out for 40 and 106 days, respectively. Growth rates were estimated by calculating the slope of the growth curve (mass versus time) for the time period when duckweed exhibited the highest growth. The first few weeks of the experiment (until day 15 for the 22 °C reactors, and until day 30 for the 15 °C reactors) were considered an acclimation period or pre-linear growth phase and were omitted from growth rate calculations. This was done via visual inspection of the growth curve to ensure that the estimated growth rates correspond to only the active growing phase of duckweed, eliminating any impacts from transfer shock. Growth rates were expressed in two units as shown in Eqs. (4) and (5). Direct calculation of the slope of the growth curve yields the growth rate in units of g $day^{-1}$ (showing the absolute increase in biomass over a given time). Typically, the specific growth rate ($r_s$, expressed in $day^{-1}$) computed from logarithmically transformed values of biomass is used in plant and microbial growth studies.

$$Growth\ rate\ \left(g\ day^{-1}\right) = \frac{Increase\ in\ mass\ (g)}{Time\ (days)} \tag{4}$$

$$Specific\ growth\ rate\ \left(day^{-1}\right) = \frac{\ln\left[\frac{m_t}{m_0}\right]}{t} \tag{5}$$

where $m_t$ is the mass at the end of $t$ days, and $m_0$ is the initial mass.

### 2.4. Modifying the existing duckweed growth model

Non-linear regression modeling was used to add a light intensity term to the existing Lasfar et al. (2007) model shown in Eq. (1). The dataset for model calibration and validation included a combination of literature and experimental data, as explained in Sections 2.2 and 2.3. For each study, the preliminary intrinsic growth rate based on Lasfar et al. (2007) (relabeled here as $r_{iO}$) was first calculated using Eq. (1) by knowing the temperature, photoperiod, and N-P concentrations. The constants in the equation were set to default values: $K_P = 0.31$, $K_{IP} = 101$, $K_N = 0.95$, $K_{IN} = 604$, $R = 0.62$, $T_{op} = 26$ °C, $\theta_1 = 0.0025$, $\theta_2 = 0.66$, $\theta_3 = 0.0073$, $\theta_4 = 0.65$. In developing the new regression equation, $r_{iO}$ and light intensity (LI) were used as the explanatory or Y variables (Eq. (6)). The dependent variable was the observed intrinsic growth rate derived from experiments ($r_i$). Since $r_i$ is not measured directly from experiments like $r_s$, it was back-calculated using Eq. (3) with the measured $r_s$ (Eq. (5)), initial mat density, and duckweed retention time, as well as a constant value of limiting mat density (176 g $m^{-2}$ as specified in Lasfar et al. (2007)):

$$r_i = f\left(r_{iO},\ LI\right) \tag{6}$$

where $r_i$ is measured intrinsic growth rate (back-calculated from Eq. (3)), $r_{iO}$ is the intrinsic growth rate using the original Lasfar et al. (2007) model, and LI is the light intensity value.

The full modeling dataset containing 86 points from all the literature and experimental data was randomly ordered and split into calibration and validation datasets, each containing 43 data points. The calibration dataset was used to develop the new equation using the

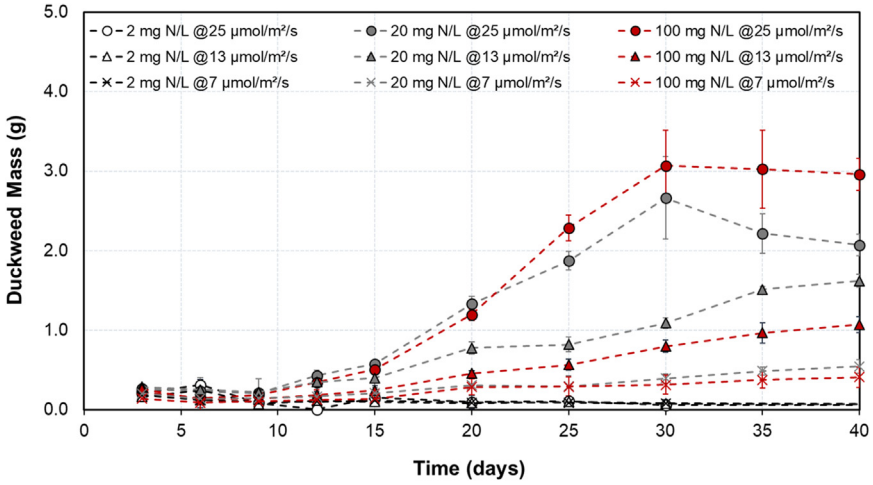


**Fig. 1.** Duckweed growth curves (fresh mass over time) for the experiment conducted at 22 ± 2 °C under different light intensities and nutrient concentrations (mg N $L^{-1}$ corresponds to N as $NO_3^-$). Data points are triplicate averages; error bars represent one standard deviation.

IBM® SPSS® Statistical Tool and subsequently tested with the validation dataset. Using the curve estimation feature within the software, the relationship between LI and $r_i$ was initially studied. This was useful in understanding whether any variable transformations (such as logarithmic or exponential) were required to better fit the model. With a trial and error approach, varying the number of parameters and the equation structure, the final form of the enhanced non-linear regression equation was derived. For each trial, the model performance was evaluated using the coefficient of determination ($R^2$), with $R^2 = 1$ indicating the best model fit between measured and modeled $r_i$ values. When multiple models yielded similar $R^2$ values, the model with the simplest form of the equation, or the least number of parameters, was selected to minimize parameter uncertainty. An additional metric known as the Root Mean Squared Error (RMSE) was also used to further evaluate the best model's performance, with RMSE = 0 indicating an ideal model fit. Since the specific growth rate, $r_s$, is commonly used in duckweed studies, modeled and measured $r_s$ values were also compared to further examine the model's ability to predict actual duckweed growth observed in experiments. This was done by estimating modeled $r_s$ for all the data points by substituting newly modeled $r_i$ in Eq. (3), and comparing these to measured $r_s$ values.

## 3. Results and discussion

### 3.1. Experimental results

Overall, the higher the light intensity, the higher the duckweed growth rates and total biomass accumulation for both the 22 °C and 15 °C experiments (Figs. 1 and 2). At 22 °C and the highest light intensity (25 $\mu$mol $m^{-2}$ $s^{-1}$), duckweed cultured in 100 mg N $L^{-1}$ exhibited a higher growth rate (0.14 g $day^{-1}$) compared to 20 mg N $L^{-1}$ (0.12 g $day^{-1}$) (Fig. 1). The duckweed cultured in 2 mg N $L^{-1}$ did not show any growth; therefore, it was discontinued after day 45 and omitted from further analysis in this study. In contrast to the growth under the highest light intensity, duckweed cultured in 20 mg N $L^{-1}$ at the two lower light intensities (13 and 7 $\mu$mol $m^{-2}$ $s^{-1}$) showed relatively better growth (0.05 and 0.01 g $day^{-1}$, respectively) and overall biomass accumulation than duckweed cultured in 100 mg N $L^{-1}$ (0.03 and 0.008 g $day^{-1}$). A very high nutrient concentration (100 mg N $L^{-1}$) likely inhibited plant growth when the duckweed growth was hindered under low light conditions. This is in agreement with other studies that showed a slight decline in growth rate for duckweed cultured in media with very high N concentrations (> 60 mg N $L^{-1}$) due to factors such as ammonia toxicity (Sońta et al., 2020; Caicedo et al., 2000). A study by (Zhang et al., 2010) has additionally shown that the combination of low light and high nutrients can have more additive or interactive effects on the carbon-nitrogen balance in macrophytes than the two factors acting alone. Such a limiting environment can generate less biomass, similar to that observed in our experiment at 7 $\mu$mol $m^{-2}$ $s^{-1}$ and 100 mg N $L^{-1}$.

At a lower temperature (15°C), the inhibitory effect of excess N was even more evident, with duckweed cultured in 60 mg N $L^{-1}$ consistently showing the highest growth rates (0.12, 0.07, and 0.03 g $day^{-1}$ at 25, 13, and 7 $\mu$mol $m^{-2}$ $s^{-1}$, respectively) compared to the 20 and 100 mg N $L^{-1}$ duckweed (Figs. 2 and 3a). The difference in growth rates at different N concentrations was, however, most prominent at the highest light intensity (25 $\mu$mol $m^{-2}$ $s^{-1}$) while the low light samples showed very small differences in growth rates for different N concentrations.

Using a two-way analysis of variance (ANOVA) test, we found that when temperature and nutrient concentrations are held constant, duckweed growth rates are statistically different at different light intensities (p-value < 0.05). Specific growth rates were in the range of: 0.02–0.06 $day^{-1}$ at 7 $\mu$mol $m^{-2}$ $s^{-1}$, 0.05–0.11 $day^{-1}$ at 13 $\mu$mol $m^{-2}$ $s^{-1}$, and 0.09–0.14 $day^{-1}$ at 25 $\mu$mol $m^{-2}$ $s^{-1}$, for the different temperatures and nutrient concentrations studied. In comparison, a previous study found a much lower optimal growth rate of *Lemna minor* when grown at higher light intensities: 0.07 $day^{-1}$ at 50 $\mu$mol $m^{-2}$ $s^{-1}$ and 0.13 $day^{-1}$ at 150 $\mu$mol $m^{-2}$ $s^{-1}$ (Petersen et al., 2022). However, other studies with this species have observed higher growth rates of up to 0.42 $day^{-1}$ at optimal light intensities, which proportionally matches the results we obtained (Ziegler et al., 2015).

It is important to note that the results obtained here are indicative of the *L. minor* species. The type of duckweed species can significantly affect how they grow and photosynthesize under different light conditions. For example, *L. minor* tends to grow marginally faster at low light intensities compared to *L. minuta*, which prefers brighter light (Paolacci et al., 2018). While both the species could display a rising growth rate with increasing light intensities (between 6 and 1000 $\mu$mol $m^{-2}$ $s^{-1}$), they do not show significant differences in growth rates below 40 $\mu$mol $m^{-2}$ $s^{-1}$. Another study reported peak growth rates of 0.19 $day^{-1}$, 0.18 $day^{-1}$, and 0.15 $day^{-1}$ by *L. aequinoctialis, L. punctata,* and *Spirodela polyrhiza*, respectively, when exposed to the same growing conditions.

By increasing the light input by 257% (from 7 to 25 $\mu$mol $m^{-2}$ $s^{-1}$), the resulting climb in growth rate in our study was anywhere between 124 and 449%, depending on temperature and nutrient concentrations. The lowest increase in growth rate (124%) was observed at 15 °C for the 100 mg N $L^{-1}$ reactors, and the highest increase in growth rate (449%) was seen at 22 °C for the 60 mg N $L^{-1}$ duckweed samples. Petersen et al. (2022) examined *L. minor* growth rates between 50 and 150 $\mu$mol $m^{-2}$ $s^{-1}$ and found that the rates increased by 67% for a corresponding 200% increase in light input. Another similar study found

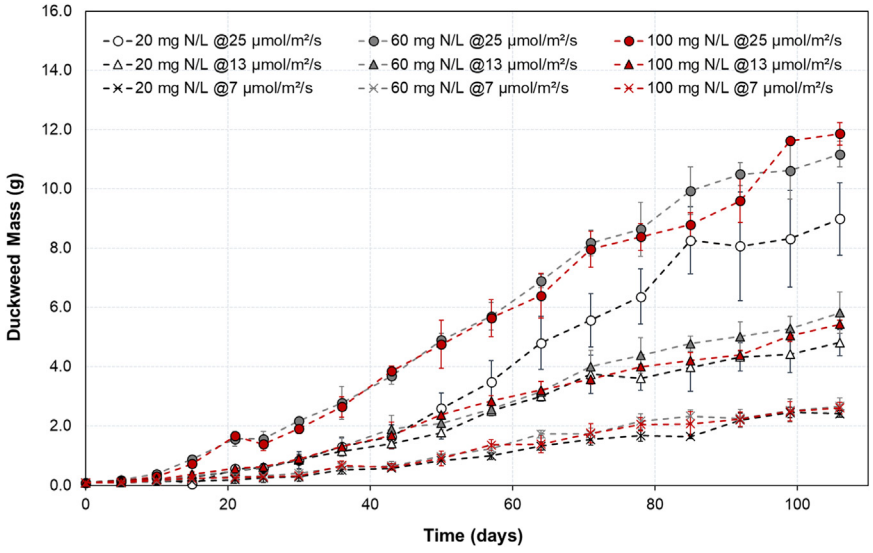


**Fig. 2.** Duckweed growth curves (fresh mass over time) for the experiment conducted at 15 °C under different light intensities and nutrient concentrations (mg N $L^{-1}$ corresponds to N as $NO_3^-$). Data points are triplicate averages; error bars represent one standard deviation.

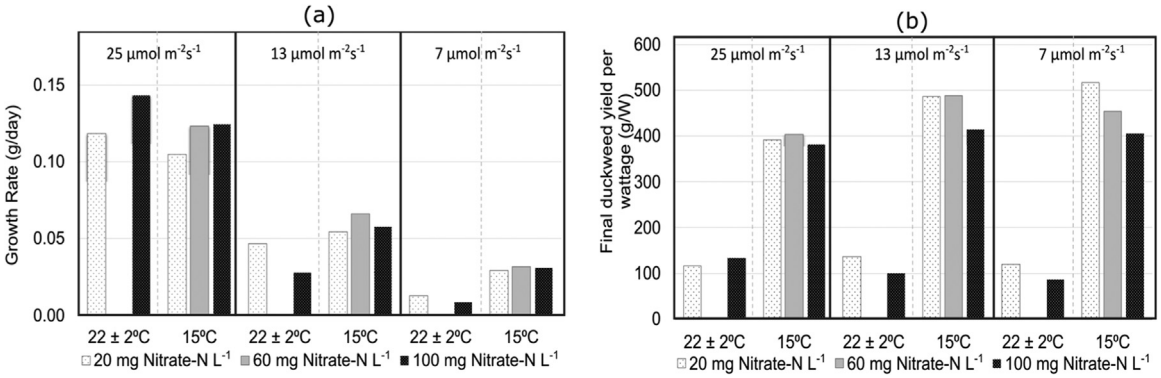


**Fig. 3.** (a) Duckweed growth rates calculated from the slopes of the growth curves for the periods when duckweed growth was highest, and (b) final harvested duckweed yield per wattage, both based on triplicate average values of duckweed fresh mass.

a 25% increase in the growth rates of *L. gibba* when light intensity was increased from 100 to 700 $\mu$mol $m^{-2}$ $s^{-1}$ (Stewart et al., 2020). Comparing these results, we could infer that the increase in duckweed growth rates with increasing light input is much more significant under low light conditions, and it becomes less distinct at higher light intensities.

At a given light intensity, temperature and nutrients did not have a statistically significant effect on duckweed growth rates within the range of growing conditions examined in this study. For example, the specific growth rate of duckweed cultured under 25 $\mu$mol $m^{-2}$ $s^{-1}$ and 20 mg N $L^{-1}$ at 22 °C (0.12 $day^{-1}$) was only 1.3 times higher than that observed at 15 °C. Similar results were obtained by Ashby and Oxley (1935)who showed a 1.2–1.5 times increase in growth rates between duckweed samples grown under 18 °C and 21 °C for eight different light intensities studied (15–296 $\mu$mol $m^{-2}$ $s^{-1}$). Considerable differences in growth rates ranging from 0.01 to 0.42 $day^{-1}$ were found, however, by Lasfar et al. (2007) when a wider range of temperatures (5–32 °C) was used. In addition, the very small variations in growth rates found in our experiments for duckweed grown under different nutrient concentrations with the same light intensity corroborate well with other similar studies. Lasfar et al. (2007), for instance, showed that growth rates tend to stabilize between 10 and 80 mg N $L^{-1}$, and the lowest rates were observed in media with less than 5 mg N $L^{-1}$.

In addition to light intensity and temperature, other factors like the type of nutrient media and light spectrum could also impact duckweed growth rates. For example, *L. minor,* showed proportional increases in growth as light intensities were increased from 50 to 850 $\mu$mol $m^{-2}$ $s^{-1}$ in an optimal laboratory medium but not in synthetic wastewater (Walsh et al., 2021b). This finding is attributed to the higher concentration of certain elements in wastewater causing changes in plant physiology and photosynthetic yield. Limited work with a few different duckweed species has shown that light spectrum did not have a significant effect on duckweed growth rate (Gallego et al., 2022; Petersen et al., 2022). But more research on this topic is needed to arrive at a definite conclusion. Since the study presented here only used laboratory media and a fixed light spectrum, a valuable expansion of the work would involve conducting similar studies with different types of wastewater media and using a range of light spectra.

With an initial duckweed mass of 0.10 g, the peak biomass accumulation for the different nutrient concentrations at 22 °C ranged from 2.66 to 3.07 g, 1.20–1.62 g, and 0.56–0.77 g at 25, 13, and 7 $\mu$mol $m^{-2}$ $s^{-1}$, respectively. Comparing duckweed masses on similar days at the two different temperatures, duckweed at 22 °C showed higher masses than at 15 °C due to the relatively slower growth at lower temperatures. However, by the end of the experiment (on day 106), the total biomass accumulated at 15 °C was considerably higher, ranging across the different nutrient concentrations from 8.99 to 11.86 g, 4.82–5.82 g, and 2.41–2.67 g for 25, 13, and 7 $\mu$mol $m^{-2}$ $s^{-1}$, respectively. This demonstrates that duckweed can thrive well even at low light and low temperature, and if given ample time, biomass can be harvested in large quantities similar to, or even greater than, that achieved at normal temperature and light conditions.

An important variable influencing duckweed growth is the mat density or the mass of duckweed per unit area. It can affect growth rates, biomass accumulation, and nutrient uptake, as demonstrated by others (Driever et al., 2005; Frédéric et al., 2006; Walsh et al., 2021a). Maintaining optimal mat density ensures no light or nutrient limitation due to overcrowding, while avoiding excessive growth of algae and other competing species. At 22 °C in this study, duckweed reached peak yield on day 30 at a density of 725 $g_{wet}$ $m^{-2}$ (**Figure S6,** Supporting Information). This is within the range of optimal mat density of 400-1600 $g_{wet}$ $m^{-2}$ reported by others (Alaerts et al., 1996; Frédéric et al., 2006; Koles et al., 1987; Skillicorn et al., 1993). It is worth mentioning that these studies were conducted under optimal temperature conditions (19–33 °C).

Interestingly for the 15 °C experiment conducted here, duckweed continued to grow well even beyond 100 days when the mat density was greater than 2500 $g_{wet}$ $m^{-2}$ (**Figure S7,** Supporting Information). Through visual inspection, it was evident that even though the thickness of the duckweed mats increased, the growth phase was still linear. This is unlike the room temperature reactors, which showed a fast decline in growth rates once the surface saturation levels were attained. It is, therefore, possible that sub-optimal conditions could alter how surface saturation affects duckweed growth dynamics and biomass accumulation. Since this study did not evaluate the nutrient uptake potential of duckweed, it is difficult to infer whether we would see similar trends with N and P uptake.

In terms of power input to duckweed mass output (and using a conversion factor of 1 $\mu$mol $m^{-2}$ $s^{-1}$ = 4.6 W $m^{-2}$), the final harvested duckweed yields per watt of light intensity were considerably higher for the 15 °C samples (318–516 g $W^{-1}$) compared to the 22 °C samples (86–136 g/W) (Fig. 3b). The low temperature (15 °C) and lowest light intensity (7 $\mu$mol $m^{-2}$ $s^{-1}$) resulted in the highest duckweed yield production per watt (516 g $W^{-1}$) at the end of 106 days. For the 22 °C experiment, lack of harvesting could have led to the growth vessels becoming saturated with duckweed and eventually causing duckweed death and a decline in total mass. Indeed, at later times in the 22 °C experiment, some biomass (roots and dead fronds) was left behind in the growth media when the duckweed was removed with the net for weighing. With regular harvesting, there is potential for even higher biomass accumulation over time. A key limitation of this study is that the procedure used for measuring the duckweed mass involved some manual removal of excess water from the nets which may have induced human errors in the measurements. Averaging the duckweed masses from triplicate samples was one of the ways we accounted for this limitation.

### 3.2. Enhanced duckweed growth model with light intensity term

After several trials on the calibration dataset with different forms of non-linear equations, a new enhanced duckweed growth model with light intensity term was developed as shown in Eq. (7). During the initial curve fitting procedure, the SPSS® Statistics Tool automatically eliminated 5 data points as outliers. The final regression equation yielded the best model performance ($R^2$ = 0.71 and RMSE = 0.04 $day^{-1}$ in calibration) among all the trials conducted. Interestingly, the light intensity ($LI$) term in the new equation retained a form that is slightly similar to the power terms corresponding to temperature and photoperiod in the original model. Although the trial runs included equations of the form $\theta_x^{((LI-LI_{op})/LI_{op})}$, $\theta_x^{((LI-LI_{op})/LI_{op})^2}$, and $\frac{C_{LI}}{C_{LI}+K_{LI}}$ to imitate the existing equation structure in the original model, these trials did not provide an $R^2 \geq 0.5$ and hence were omitted. The new regression model includes three constants in addition to a log-transformed value of light intensity.

$$r_i = \left( R \cdot \theta_1^{((T-T_{op})/T_{op})^2} \cdot \theta_2^{((T-T_{op})/T_{op})} \cdot \theta_3^{((E-E_{op})/E_{op})^2} \cdot \theta_4^{((E-E_{op})/E_{op})} \cdot \frac{C_P}{C_P+K_P} \cdot \frac{K_{IP}}{K_{IP}+C_P} \cdot \frac{C_N}{C_N+K_N} \cdot \frac{K_{IN}}{K_{IN}+C_N} + A_0 \right) \cdot A_1 \cdot \left( \frac{\ln(LI)-A_2}{A_2} \right) \quad (7)$$

where $A_0$ = 0.222, $A_1$ = 0.05, $A_2$ = 0.681.

The value of $r_i$ modeled using the original Lasfar et al. (2007) model was consistently lower than the measured $r_i$ calculated from experimental data. The coefficient $A_o$ was hence used to compensate for the over-predicted model values. The effect of low light intensity on duckweed growth is further represented by the coefficients $A_1$ and $A_2$. Regression coefficients help understand the relative contribution of each parameter to the overall model predictions. Although $A_0$ and $A_1$ coefficients were critical in fine-tuning the modeled $r_i$ values, their relative contribution to the explained variance in measured values was only 20% compared to the added light intensity term ($\frac{\ln(LI)-A_2}{A_2}$) which had a 51% contribution. In other words, eliminating the $A_0$ and $A_1$ coefficients from the regression equation (Eq. (7)) still resulted in an $R^2$-value of 0.51 for the same calibration dataset.

On testing the model's performance with validation data, the $R^2$ between measured and modeled $r_i$ was 0.50 and the corresponding RMSE was equal to 0.04 $day^{-1}$. In the original Lasfar et al. (2007) model, a very high value of $R^2$ (0.96) was reported in predicting $r_i$ using 11 validation data points. The relatively lower value obtained in our study can be attributed to the larger number of data points and a wider range of experimental variables used.

In terms of specific growth rate ($r_s$), which is a more commonly used growth parameter in duckweed studies, the $R^2$-values obtained with the new model were even higher at 0.78 in calibration (RMSE = 0.03 $day^{-1}$) and 0.59 in validation (RMSE = 0.04 $day^{-1}$). A high $R^2$-value (closer to 1) indicates that the enhanced model was able to explain a major portion (78%) of the variance in measured growth rates. Furthermore, the modeled regression line closely matches a 1:1 reference line representing a perfect model fit, which demonstrates that there are no signs of consistent under- or over-prediction by the new model (Fig. 4). Unlike $R^2$, RMSE has the same unit as the dependent variable ($r_i$ or $r_s$) and therefore there is no standard threshold set for optimum RMSE value. Typically, RMSE is used to compare similar models having the same dependent variables, wherein a lower RMSE indicates a better model. When the Lasfar et al. (2007) model was used to predict growth rates for the same dataset used in our study, a comparatively higher RMSE (0.11) was obtained. This demonstrates the enhanced prediction capability of the new model for the extent of $r_s$ values studied (0.007 to 0.22 $day^{-1}$ with an average of 0.11 $day^{-1}$). In addition, the $R^2$-values obtained in our study are within the range reported in some of the prior duckweed growth models (0.36–0.99) and above 0.60 which is considered a high $R^2$ in biological sciences (Hatano and Shoji, 2008; Caicedo et al., 2000; Landesman et al., 2005; Overinterpreting High R2, 2022).

For the combined calibration and validation datasets used in this study, the original Lasfar et al. (2007) model yielded an $R^2$ of only 0.27 for predicting the specific growth rate. In contrast, for the same datasets, the enhanced model developed here produced an $R^2$ of 0.67, demonstrating that the additional parameters and light intensity variable greatly improved model prediction accuracy. The new model performs fairly well in capturing the effect of different environmental variables on duckweed growth dynamics (Fig. 4).

For most of the studies considered here, the enhanced model was able to capture the variability in duckweed growth rate with changing light intensity (in the range of 7–650 $\mu$mol $m^{-2}$ $s^{-1}$) (Fig. 5). At lower light intensities (< 60 $\mu$mol $m^{-2}$ $s^{-1}$), even though a few data points show that the model under-predicts $r_s$, the deviations from measured values are within ± 0.03 $day^{-1}$ for 86% of the data points and within ± 0.01 $day^{-1}$ for 68% of data points. For comparison, the original Lasfar et al. (2007) model using their experimental data from growing duckweed at 371 $\mu$mol $m^{-2}$ $s^{-1}$ predicted $r_i$ with a deviation less than 0.03 $day^{-1}$ at a 95% confidence level. In our analysis, two sets of data points from Ashby and Oxley (1935) for duckweed grown at 10 °C and 29 °C were particularly notable since they were outside the

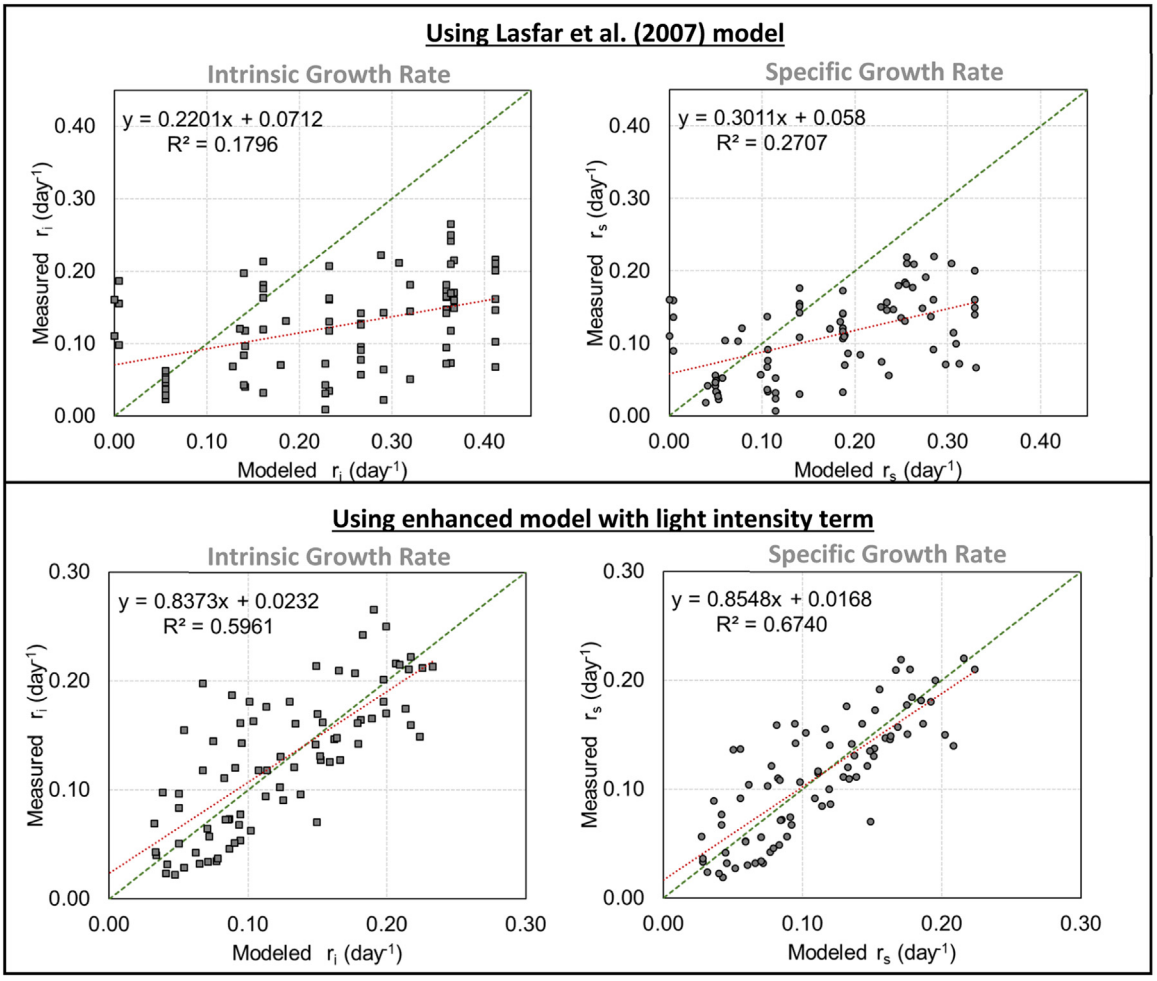


**Fig. 4.** Plots of measured and modeled intrinsic growth rates (on the left) and specific growth rates (on the right), showing all of the calibration and validation data points. The top panel shows values generated using the Lasfar et al. (2007) model, and bottom panel shows values estimated using the enhanced model developed in this study. The red dotted line is the modeled regression line, and the green dashed line is a reference 1:1 line representing a perfect model fit.

range of predicted values (Fig. 5). Our new model over-predicts the growth rates for points corresponding to a lower temperature (10 °C) and under-predicts the rates for those corresponding to higher than normal room temperature (29 °C). This indicates that fine-tuning the temperature parameters could enable better model calibration to predict duckweed growth across all temperatures. Although the original Lasfar et al. (2007) model considers an optimum temperature of 26 °C and inhibits growth above this temperature, further reduction in modeled growth rates for extremely low and high temperatures is warranted based on this finding.

Except for the Tabou et al. (2013)study which found duckweed growth inhibition at light intensities > 250 $\mu$mol $m^{-2}$ $s^{-1}$, there was no definite pattern of growth inhibition at very high light intensities across all the data analyzed. It should be noted, however, that the different light spectra or range of wavelengths used in these studies may have affected the duckweed growth differently. While our experimental data and that from Yin et al. (2015) used wide spectrum fluorescent tube lamps, Lasfar et al. (2007) and Tabou et al. (2013) used 400 W high pressure sodium lamps that are known to produce more intense light (with higher lumen/watt ratio) compared to fluorescent grow lights. This may explain the growth inhibition at light intensities > 250 $\mu$mol $m^{-2}$ $s^{-1}$ observed by Tabou et al. (2013), whereas Yin et al. (2015) reported no such inhibitory effects up to 400 $\mu$mol $m^{-2}$ $s^{-1}$. Since the two other studies (Landesman et al. (2005) and Ashby & Oxley (1935)) failed to report the exact spectrum of lights used, a thorough analysis of light intensity inhibition on duckweed growth was difficult.

Considering the effect of different light spectra on the vegetative growth of plants, an additional wavelength parameter would potentially improve the duckweed growth representation within the model. In addition, the dataset used for our model development had very few data points in the lower light intensity range (and were primarily from the experiments conducted in this study). With additional data demonstrating duckweed growth under extreme (very low and very high) light intensities, enhancements can be made to the proposed regression equations to make the model more universal. Incorporating the new light intensity term in the growth model helps us: (1) understand the effect of changing light conditions on duckweed growth; and (2) validate the existing Michaelis-Menten kinetics that illustrate negligible increases in growth rates beyond a certain light intensity, which according to the new model, occurs around 200 $\mu$mol $m^{-2}$ $s^{-1}$ (Fig. 5). This agrees with existing duckweed literature suggesting that light saturation of duckweed occurs in the range of 166.5–350 $\mu$mol $m^{-2}$ $s^{-1}$ depending on the growing conditions (Docauer, 1983; Landolt and Kandeler, 1987).

The proposed model offers an opportunity to investigate the combined effect of parameters like light intensity and temperature on duckweed production. This can be beneficial in understanding duckweed's photosynthetic responses and in large-scale system optimization to maximize duckweed yield. The interactive effect of temperature and light intensity on duckweed growth is well documented in the literature

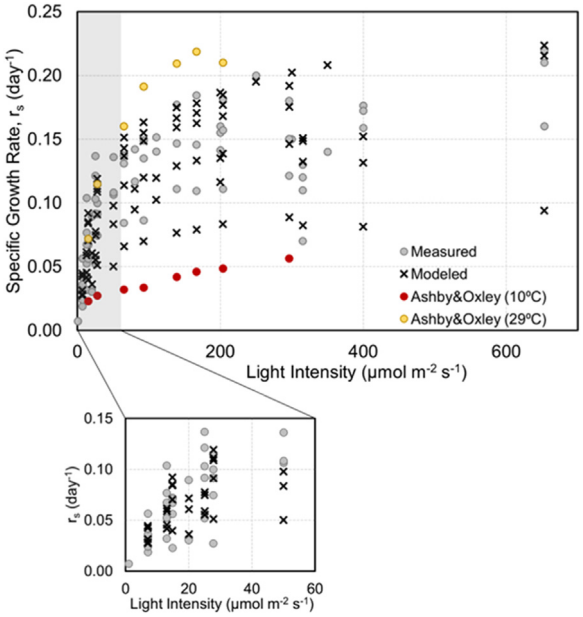


**Fig. 5.** Graph showing measured and modeled specific growth rates ($r_s$) at different light intensities. The plot includes all the data points aggregated in this study with a zoomed portion of points corresponding to light intensities $< 60$ µmol $m^{-2}$ $s^{-1}$.

(Coughlan et al., 2022). Whether duckweed is a $C_3$ or $C_4$ plant (a classification made based on photosynthetic response to temperature and light) is still a debated question. While $C_3$ plants thrive well with 1/3–1/2 of full sunlight, $C_4$ plants need full sunlight for attaining photosynthetic saturation. Filbin & Hough (1985) have referred to duckweed as a $C_4$ plant due to its tolerance to high temperature and light. In contrast, duckweed's ability to achieve peak growth well below full sunlight and under lower temperatures indicated its closer match to $C_3$ plants (Wedge and Burris, 1982). Our model results agree with this finding, validating that the light saturation levels of duckweed are much below the full sunlight value of 1400 $\mu$mol $m^{-2}$ $s^{-1}$.

## 4. Conclusions

This study validated the high tolerance of duckweed for extreme weather conditions and demonstrated its ability to thrive well under low light intensities and low temperatures. A series of laboratory-scale experiments showed that lowering the light intensity, temperature, and available nutrients can negatively impact duckweed growth rates, but that a considerable amount of biomass can still be accumulated over an extended period under these limiting environmental conditions. The interactive effect of environmental parameters was evident in our results which indicated lower growth rates at excessively high nutrient concentrations, especially when subject to limiting conditions of light and temperature.

Using the experimental data collected here together with other available datasets enabled the derivation of an enhanced duckweed growth model with an added light intensity term. This enhanced model effectively captured duckweed growth responses for a wide range of light intensities and also reasonably predicted specific growth rates ($R^2 = 0.67$). Light saturation levels derived from the model support past research outcomes placing duckweed's photosynthetic pathway in between that of $C_3$ and $C_4$ plants. The outliers observed in the modeling process indicate the need for additional experiments to represent the effect of extreme temperatures on duckweed growth. While we used synthetic laboratory media to isolate the effect of the light intensity parameter, an extension of this work could involve growing duckweed on natural wastewaters to eventually test the model with that growth data. The inclusion of a wavelength parameter to characterize the range of the light spectrum used could also further strengthen the model representation of duckweed growth dynamics. The enhanced model developed here not only improves our understanding of the effect of light intensity on duckweed growth, but also identifies optimal conditions for duckweed production and may be used to help minimize energy consumption and cost in large-scale commercial applications.

## 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 will be made available on request.

## Acknowledgments

We extend our sincere gratitude to Open Philanthropy Project for funding this research. Gregory House and Erin Anderson are also thanked for their help in setting up the experiments and collecting the duckweed growth data.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envc.2023.100709.

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