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Optimization of Hoagland Nutrient Medium and Controlled Clonal Propagation of *Lemna Gibba* L. in Fishpond Wastewater: from Laboratory Acclimatization to Field Biomass

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Abstract: Nutrient-rich wastewater discharged from fishponds constitutes a source of ecological load and, at the same time, a secondary nutrient resource for fast-growing floating macrophytes. The aim of this study was to determine the effective Hoagland concentration for *Lemna gibba* L., to acclimatize the plant stepwise to fishpond wastewater, and to evaluate the feasibility of controlled clonal propagation under field conditions without supplementary mineral fertilization. Initially, the morphometric parameters of an inoculum of 5.0 g fresh biomass were standardized (n=5). Eleven variants of modified Hoagland medium within the 0×–1.0× range were tested in a 10-day preliminary screening; the 0.1×, 0.3× and 0.4× variants were selected for subsequent validation (n=3). The highest performance was observed in the 0.1× medium, in which biomass reached 69.97±0.51 g, surface coverage 702.3±3.57 cm² and RGR 0.264±0.001 day⁻¹ on day 10. Thereafter, three parallel clonal lines were sequentially transferred to 25, 50, 75 and 100% fishpond wastewater. As the wastewater proportion increased, day-10 biomass rose from 70.05±0.38 g to 77.88±0.28 g, and RGR from 0.26398±0.00054 to 0.27458±0.00035 day⁻¹. When the culture acclimatized to 100% wastewater was tested in three field units of 0.49 m² each, biomass amounted to 81.70±0.20 g, RGR to 0.27936±0.00024 day⁻¹ and net fresh biomass productivity to 15.65±0.04 g FW·m⁻²·day⁻¹. Drying 50.0 g of fresh biomass at 50°C for 6 h yielded 5.97±0.18 g of dried mass. The results demonstrate that stable clonal biomass of *L. gibba* can be produced by stepwise transfer from an optimized initial mineral medium to 100% fishpond wastewater.

Keywords: *Lemna gibba*; duckweed; Hoagland medium; concentration screening; acclimatization; fishpond wastewater; clonal propagation; bioconversion; RGR; fresh biomass; field validation; phytoremediation; dry biomass; circular bioeconomy.

Introduction

With the expansion of aquaculture production, the environmentally safe management of water enriched with nitrogen, phosphorus and dissolved organic matter discharged from ponds and recirculating systems has become an important scientific and practical task [1]. Evaluating such effluents not merely as waste but as a secondary nutrient resource is consistent with the principles of the circular bioeconomy. Floating macrophytes of the duckweed type, in particular *Lemna* species, represent a suitable biological object for this approach owing to their small size, rapid vegetative propagation, ease of harvesting from the water surface and capacity to convert dissolved nutrients into biomass [2]. The OECD Test No. 221 and Environment Canada protocols provide for the standardized assessment of *Lemna* growth based on frond number, surface area and specific growth rate.

Classical and recent studies on duckweed cultivation in wastewater have demonstrated high biomass productivity, nitrogen and phosphorus uptake and the potential for wastewater valorization. At the same time, duckweed growth depends strongly on nutrient medium strength, the N:P ratio, light, temperature, pH, initial coverage density and clonal characteristics [3]. Most studies have been conducted with *Lemna minor* and with livestock or municipal wastewater; practical, simplified studies on the stepwise transfer of *Lemna gibba* specifically from an optimized mineral medium to fishpond wastewater, the maintenance of three parallel clonal lines and subsequent validation under field conditions, i.e., in the wastewater drainage channel discharging from fishponds, remain insufficiently documented.

It is therefore important to transfer the results of laboratory-scale mineral nutrient optimization directly to fish-farm wastewater and field conditions through a continuous methodological chain [4]. Such a chain makes it possible to assess jointly not only the short-term growth of the plant, but also the maintenance of growth as the wastewater proportion is increased, reproducibility across parallel lines, and biomass formation under field conditions without supplementary mineral nutrition. This approach also provides a technological basis for subsequent dry biomass production and its evaluation as a raw material for aquaculture feed.

The present study was aimed at addressing these research gaps in practical terms. Its principal objective was to select, among the tested Hoagland concentrations, the minimal effective variant providing high biomass for *L. gibba*, to use this variant for acclimatizing the plant to 25→50→75→100% fishpond wastewater, and subsequently to evaluate the feasibility of controlled clonal propagation under field conditions [5], [6].

The research tasks comprised the standardization of the initial inoculum; 0×–1× Hoagland screening; validation of the 0.1×, 0.3× and 0.4× variants at n=3; a stepwise increase in the wastewater proportion; calculation of RGR and surface coverage; field validation in 100% wastewater and determination of net biomass productivity per unit area; and production of low-temperature-dried biomass for subsequent biochemical analyses [7].

Duckweeds are among the most extensively studied floating macrophyte groups that combine biological wastewater treatment with biomass production. The FAO monograph summarizes the rapid vegetative propagation of duckweed, its high protein productivity and its capacity to convert nutrient-laden wastes into useful biomass. Oron, cultivating *L. gibba* in domestic wastewater, demonstrated that high dry-matter yields and biomass containing approximately 30% protein could be obtained under regular harvesting [8], [9]. Alaerts et al. reported long-term stable operation of a full-scale duckweed-covered lagoon with substantial reductions in organic matter, nitrogen and phosphorus. These studies established that duckweed systems also function beyond the laboratory under real wastewater conditions.

Cheng et al. evaluated nitrogen and phosphorus removal rates from swine lagoon liquid by *L. minor* under laboratory and field conditions and showed that wastewater concentration and seasonal conditions directly affect growth and nutrient uptake. In that study, prior acclimatization was noted as a means of shortening the lag phase and accelerating system start-up. Cheng and Stomp subsequently developed an integrated concept linking duckweed cultivation to nutrient recovery from wastewater and to the production of animal feed and biofuel feedstock. Xu and Shen, in turn, demonstrated the

effect of initial coverage density and regular harvesting on biomass in a swine wastewater system and reported that high N and P removal could be achieved during the growing season [10], [11].

For *L. gibba*, Verma and Suthar investigated simultaneous water treatment and biomass production at 25–100% urban wastewater concentrations; the authors showed that wastewater nutrients were channelled into plant biomass rich in protein and carbohydrates. Iatrou, Kora and Stasinakis analysed biomass, crude protein and starch production in laboratory wastewater treatment systems planted with *L. minor* and *L. gibba*, confirming that the composition of the nutrient medium determines not only the quantity but also the quality of the biomass. In the comprehensive review by Ekperusi et al., *L. minor* was evaluated as an effective organism for the phytoremediation of various chemical pollutants, and the need for the subsequent safe disposal of biological material from duckweed systems was emphasized.

The reliability of controlled growth is also an important methodological issue. OECD Test No. 221 sets out the principles for assessing *Lemna* growth by frond number together with an additional biomass or area endpoint and for calculating the average specific growth rate [12]. The Environment Canada guideline provides a practical basis for standardizing modified nutrient media and growth endpoints in *Lemna* bioassays. Scherr et al., studying the spatio-temporal stability of an *L. gibba* bioassay over one year, showed that growth rates based on area and frond number can be reproducible in controlled experiments. Ziegler et al., comparing 39 clones of 13 duckweed species, found RGR values in the range of 0.153–0.519 day⁻¹ and established that growth rate depends largely on the adaptation of the clone to local conditions.

Optimization of nutrient medium composition is a central factor in controlling duckweed biomass [13]. Cui and Cheng, summarizing work on growth, nutrient uptake and the use of biomass as a bioresource, highlighted the need to optimize both the culture medium and the clone to achieve high productivity. Akyüz and Ersus demonstrated experimentally that KH₂PO₄, Ca(NO₃)₂ and pH in Hoagland solution exert a strong influence on duckweed protein content; in the modified medium, protein content increased relative to the standard medium. The meta-analysis by Pasos-Panqueva et al. identified light, temperature, nutrient concentration and the N:P ratio as the principal drivers of duckweed growth. These findings support the need to select nutrient strength experimentally for each local culture.

The subsequent nutritional application of duckweed biomass depends on its chemical composition. Appenroth et al. showed that the protein, fat, starch and mineral contents of Lemnaceae biomass vary with the nutrient medium and evaluated duckweed as a high-quality protein source. Ziegler, Sree and Appenroth, noting that duckweed biomass may accumulate pollutants in addition to offering water remediation potential, considered safety control of the final product to be essential [14], [15]. Prossidee et al. found that drying at 50°C for 6 h was among the optimal regimes for preserving nutritional and bioactive components in another member of the Lemnaceae, *Wolffia arrhiza*; this result underlines the methodological importance of low-temperature drying but requires species-specific validation.

The relevance of duckweed for aquaculture wastewater has been further reinforced in recent studies [16]. Paolacci, Stejskal and Jansen developed a synthetic aquaculture wastewater and showed that *L. minor* takes up NH₄⁺-N, NO₃⁻-N and PO₄³⁻-P, with removal rates per unit area increasing at higher coverage densities. In subsequent work on integrated multi-trophic aquaculture, Paolacci et al. evaluated, in a real system, the concept of valorizing fish-farming wastes into nutrients and protein biomass through a duckweed system. These results support the approach of converting fishpond wastewater into a direct resource for duckweed cultivation.

Recent studies highlight the role of the management regime in scaling up. Molnár et al. found that weekly harvesting of 25% and 50% of *L. minor* biomass in aquaponics increased total yield compared with an unharvested control. Vulpe et al. linked duckweed, within a bioeconomy model, to wastewater treatment, recovery of biogenic elements and the production of downstream bioproducts [17]. Against the background of this international experience, the scientific novelty of the present work lies in the evaluation of a single, practical biotechnological chain comprising local screening of *L. gibba* for Hoagland strength, subsequent stepwise acclimatization to 25→50→75→100% fishpond

wastewater, and field validation of the same clonal lines in the wastewater drainage channel discharging from fishponds.

Materials and Methods

The study comprised laboratory and field stages. First, the inoculum of wild-collected *Lemna gibba* L. was standardized, after which the 0×–1.0× range of modified Hoagland medium was subjected to preliminary screening. The 0.1×, 0.3× and 0.4× variants that performed best in the screening were re-tested in three independent time blocks (n=3). In the next stage, the culture obtained in the 0.1× medium was sequentially transferred to 25→50→75→100% fishpond wastewater, and three parallel clonal lines were maintained. The culture acclimatized to 100% wastewater was validated for 10 days under field conditions, i.e., in the wastewater drainage channel discharging from the fishpond, using three floating frames of 0.49 m² each. Finally, the fresh biomass was prepared for drying and subsequent biochemical analyses.

The laboratory nutrient medium was based on a modified Hoagland formulation consistent with the methodological approach of Environment Canada EPS 1/RM/37. The basic composition of the 1× medium is given in Table 1. Concentrations were prepared according to the formula $C_x = x \times C_1 \times$. The 0× variant, i.e., distilled water without mineral salts, served as the control. For practical preparation, macro- and micronutrients were stored separately as 100× stock solutions; Ca(NO₃)₂, phosphate and sulfates were not combined in the same vessel in concentrated form. For each 1 L of working medium, V=10×x mL of each stock was taken, and for a 15 L working volume, V=150×x mL. In all variants, the pH recorded during the experimental period ranged from 6.5 to 7.0.

Table 1. Composition of the 1× modified Hoagland medium (per 1 L)

Group	Compound	1× concentration, mg/L	Mass per 1 L
Macronutrients	KNO ₃	1515.0	1.515 g
	Ca(NO ₃) ₂ ·4H ₂ O	1180.0	1.180 g
	KH ₂ PO ₄	680.0	0.680 g
	MgSO ₄ ·7H ₂ O	492.0	0.492 g
	H ₃ BO ₃	2.86	2.86 mg
Micronutrients	MnCl ₂ ·4H ₂ O	3.62	3.62 mg
	Na ₂ MoO ₄ ·2H ₂ O	0.12	0.12 mg
	ZnSO ₄ ·7H ₂ O	0.22	0.22 mg
	CuSO ₄ ·5H ₂ O	0.08	0.08 mg
Iron	FeCl ₃ ·6H ₂ O	5.40	5.40 mg
Chelating agent	Tartaric acid	3.00	3.00 mg

Surface moisture of fresh wild-collected *L. gibba* biomass was removed by blotting on absorbent paper, and five independent samples of 5.0 g each were taken. The fronds in each sample were counted, and the total area occupied by freely dispersed plants was measured planimetrically. On this basis, the mean fresh mass and the mean area per frond were calculated (Table 2).

Table 2. Initial measurement parameters of wild-collected *Lemna gibba* L. (n=5)

Replicate	Biomass, g	Fronds, number	Occupied area, cm ²
R-1	5.0	311	75.2
R-2	5.0	304	73.8
R-3	5.0	308	74.6
R-4	5.0	306	74.1
R-5	5.0	310	74.9
Mean ± SD	5.0	307.8 ± 2.86	74.52 ± 0.57

The standardized 5.0 g inoculum was introduced into 0×, 0.1×, 0.2×, 0.3×, 0.4×, 0.5×, 0.6×, 0.7×, 0.8×, 0.9× and 1.0× Hoagland media in vessels with a 15 L working volume. Each variant was monitored

for 10 days. This stage was an exploratory screening (n=1) intended not to demonstrate statistical differences but to identify candidate variants for the subsequent replicated experiment (Table 3).

Table 3. Preliminary screening of *Lemna gibba* L. cultivation at different Hoagland concentrations (n=1)

Hoagland	Initial biomass, g	Day-10 biomass, g	Day-10 area, cm ²	RGR, day ⁻¹
0×	5.0	19.20	205.6	0.135
0.1×	5.0	69.95	702.3	0.264
0.2×	5.0	54.30	560.4	0.239
0.3×	5.0	64.35	655.8	0.255
0.4×	5.0	59.75	612.1	0.248
0.5×	5.0	50.90	528.6	0.232
0.6×	5.0	43.65	459.2	0.217
0.7×	5.0	37.40	398.5	0.201
0.8×	5.0	31.05	337.9	0.183
0.9×	5.0	25.50	281.4	0.163
1.0×	5.0	21.25	238.7	0.145

Based on the preliminary screening, the 0.1×, 0.3× and 0.4× variants, which showed the highest biomass and surface coverage, were selected. Each concentration was tested with a 5.0 g inoculum for 10 days; the experiment was replicated in three independent time blocks (n=3). Means ± SD were calculated for final biomass, surface coverage and RGR (Table 4).

Table 4. Validation of the best-performing Hoagland concentrations (n=3)

Replicate	0.1× biomass, g	0.1× area, cm ²	0.3× biomass, g	0.3× area, cm ²	0.4× biomass, g	0.4× area, cm ²
1	70.10	703.1	63.90	651.6	59.30	608.2
2	69.40	698.4	64.45	656.4	59.85	612.5
3	70.40	705.4	64.70	659.4	60.10	615.6
Mean ± SD	69.97 ± 0.51	702.3 ± 3.57	64.35 ± 0.41	655.8 ± 3.93	59.75 ± 0.41	612.1 ± 3.72
RGR, day ⁻¹	0.26386 ± 0.00073	—	0.25549 ± 0.00064	—	0.24807 ± 0.00069	—

Since the 0.1× Hoagland medium gave the highest performance among the tested variants in the validation, it was selected as the mother-culture medium for the subsequent acclimatization stage. Three parallel clonal lines were maintained: the inoculum from vessel 1 was transferred to vessel 1 of the next stage, and likewise 2→2 and 3→3. Each stage was conducted in a 15 L working volume for 10 days. The wastewater proportion was increased sequentially to 25%, 50%, 75% and 100%; no Hoagland medium was added at the 100% stage (Tables 5–8).

Table 5. Growth in 25% wastewater + 75% 0.1× Hoagland (n=3)

Vessel	Initial biomass, g	Day-10 biomass, g	Day-10 area, cm ²	RGR, day ⁻¹
1	5.0	70.10	1047.0	0.26405
2	5.0	70.40	1051.5	0.26448
3	5.0	69.65	1040.3	0.26340
Mean ± SD	5.0	70.05 ± 0.38	1046.27 ± 5.64	0.26398 ± 0.00054

Table 6. Growth in 50% wastewater + 50% 0.1× Hoagland (n=3)

Vessel	Initial biomass, g	Day-10 biomass, g	Day-10 area, cm ²	RGR, day ⁻¹
1	5.0	72.70	1108.3	0.26769
2	5.0	72.05	1095.0	0.26679
3	5.0	71.65	1089.0	0.26624
Mean ± SD	5.0	72.13 ± 0.53	1097.43 ± 9.88	0.26691 ± 0.00073

Table 7. Growth in 75% wastewater + 25% 0.1× Hoagland (n=3)

Vessel	Initial biomass, g	Day-10 biomass, g	Day-10 area, cm ²	RGR, day ⁻¹
1	5.0	75.05	1142.0	0.27087
2	5.0	74.70	1137.0	0.27040
3	5.0	74.35	1135.0	0.26993
Mean ± SD	5.0	74.70 ± 0.35	1138.00 ± 3.61	0.27040 ± 0.00047

Table 8. Growth in 100% wastewater + 0% Hoagland (n=3)

Vessel	Initial biomass, g	Day-10 biomass, g	Day-10 area, cm ²	RGR, day ⁻¹
1	5.0	77.90	1167.0	0.27460
2	5.0	77.60	1163.0	0.27421
3	5.0	78.15	1171.0	0.27492
Mean ± SD	5.0	77.88 ± 0.28	1167.00 ± 4.00	0.27458 ± 0.00035

From each of the three parallel lines acclimatized in the laboratory to 100% fishpond wastewater, 5.0 g of fresh inoculum was taken. Three buoyant wooden frames measuring 70×70 cm (0.49 m²) were placed approximately 2 m apart in the drainage channel receiving the fishpond discharge water. The lines were introduced into frames 1, 2 and 3, respectively. No supplementary mineral nutrients were provided; the experiment lasted 10 days. The initial stocking density was 5.0/0.49=10.20 g FW·m⁻².

At the end of the field experiment, the biomass from each frame was harvested separately. Surface moisture was standardized by blotting with absorbent paper, and 50.0 g of fresh biomass was taken from each replicate. The samples were dried without mixing in a drying oven at 50°C for 6 h. This regime was applied as low-temperature drying; the 50°C/6 h regime is methodologically consistent with studies aimed at preserving nutritional and bioactive compounds in duckweed biomass. The value obtained was interpreted as “mass dried at 50°C/6 h”; determination of the absolute dry-matter content requires separate drying to constant mass or the validated AOAC moisture method.

For each replicate, the relative growth rate (RGR), the sample standard deviation, net fresh biomass productivity and the inter-stage change in biomass were calculated using the following formulas. All derived parameters were also recalculated from the tabulated values.

$$RGR = [\ln(W_t) - \ln(W_0)] / t$$

$$SD = \sqrt{[\sum(x_i - \bar{x})^2 / (n - 1)]}$$

$$P_FW = (FW_t - FW_0) / (A \times t)$$

$$\Delta B(\%) = [(B_{current} - B_{previous}) / B_{previous}] \times 100$$

Here, W_0 and FW_0 denote the initial fresh biomass (5.0 g); W_t and FW_t the biomass on day 10; t , the culture period (10 days); and A , the surface area of the field frame (0.49 m²). Since the preliminary 0×–1× screening was performed at n=1, no inferential statistics were applied to it; for the n=3 stages, results are presented as mean ± SD.

Results

In the initial 5.0 g of *L. gibba* biomass, a mean of 307.8 ± 2.86 fronds and a surface coverage of 74.52 ± 0.57 cm² were recorded. Accordingly, the mean fresh mass of a single frond was 0.01624 g (16.24 mg), and the mean area occupied per frond was 0.2421 cm² (24.21 mm²) [18], [19]. In the 0×–1.0× screening, the 0.1×, 0.3× and 0.4× variants yielded higher day-10 biomass and area values than the other concentrations. The highest values were recorded in the 0.1× medium: 69.95 g biomass, 702.3 cm² area and an RGR of 0.264 day⁻¹. Since the screening was performed at n=1, this stage was interpreted as a selective exploratory experiment.

The results of three independent replicates confirmed the superiority of the 0.1× variant. In the 0.1× medium, day-10 biomass was 69.97 ± 0.51 g, area 702.3 ± 3.57 cm² and RGR 0.26386 ± 0.00073 day⁻¹ [20]. The corresponding values were 64.35 ± 0.41 g, 655.8 ± 3.93 cm² and 0.25549 ± 0.00064 day⁻¹ for 0.3×, and 59.75 ± 0.41 g, 612.1 ± 3.72 cm² and 0.24807 ± 0.00069 day⁻¹ for 0.4×. Therefore, 0.1× Hoagland was selected for the subsequent acclimatization experiment as the minimal and highly effective working concentration among the tested variants.

When the wastewater proportion was increased from 25% to 100%, growth was maintained in all three clonal lines and mean biomass increased progressively. Day-10 biomass, which was 70.05 ± 0.38 g at 25% wastewater, reached 72.13 ± 0.53 g at 50%, 74.70 ± 0.35 g at 75% and 77.88 ± 0.28 g at 100%. RGR amounted to 0.26398 ± 0.00054 , 0.26691 ± 0.00073 , 0.27040 ± 0.00047 and 0.27458 ± 0.00035 day⁻¹, respectively. The change in biomass relative to the preceding stage was +2.97% at the 50% stage, +3.56% at the 75% stage and +4.26% at the 100% stage (Table 9).

Table 9. Dynamics of *L. gibba* growth parameters with increasing wastewater proportion

Stage	Medium	Day-10 biomass, g	RGR, day ⁻¹	ΔB relative to the preceding stage, %
1	25% wastewater + 75% 0.1× Hoagland	70.05 ± 0.38	0.26398 ± 0.00054	—
2	50% wastewater + 50% 0.1× Hoagland	72.13 ± 0.53	0.26691 ± 0.00073	+2.97
3	75% wastewater + 25% 0.1× Hoagland	74.70 ± 0.35	0.27040 ± 0.00047	+3.56
4	100% wastewater + 0% Hoagland	77.88 ± 0.28	0.27458 ± 0.00035	+4.26

The three clonal lines acclimatized to 100% wastewater in the laboratory continued to grow in the field drainage channel without supplementary nutrients. On day 10, biomass reached 81.5, 81.7 and 81.9 g, respectively. Mean biomass was 81.70 ± 0.20 g, covered area 1193.20 ± 3.42 cm² and RGR 0.27936 ± 0.00024 day⁻¹. Net fresh biomass productivity, calculated per 0.49 m² frame, was 15.65 ± 0.04 g FW·m⁻²·day⁻¹. The covered area corresponded to $24.35 \pm 0.07\%$ of the frame area. Field biomass was descriptively 4.90% higher than that of the laboratory stage in 100% wastewater (Table 10).

Table 10. 10-day field validation in the fishpond wastewater drainage channel (n=3)

Unit	Initial biomass, g	Day-10 biomass, g	Covered area, cm ²	RGR, day ⁻¹	Net P _{FW} , g·m ⁻² ·day ⁻¹	Frame coverage, %
1	5.0	81.5	1189.6	0.27912	15.61	24.28
2	5.0	81.7	1193.6	0.27936	15.65	24.36
3	5.0	81.9	1196.4	0.27961	15.69	24.42
Mean ± SD	5.0	81.70 ± 0.20	1193.20 ± 3.42	0.27936 ± 0.00024	15.65 ± 0.04	24.35 ± 0.07

After drying at 50°C/6 h, the mass of 50.0 g of fresh biomass from each field replicate was 5.80, 6.15 and 5.95 g, respectively. The mean dried mass was 5.97 ± 0.18 g, the mass yield $11.93 \pm 0.35\%$ and the calculated moisture content $88.07 \pm 0.35\%$. These values were recorded as the mass remaining after the applied drying regime rather than as absolute dry matter (Table 11).

Table 11. Dried mass obtained from 50.0 g of fresh *Lemna gibba* L. biomass (n=3)

Field unit	Fresh sample, g	Mass after 50°C/6 h, g	Dried mass fraction, %	Calculated moisture, %
1	50.0	5.80	11.60	88.40
2	50.0	6.15	12.30	87.70
3	50.0	5.95	11.90	88.10
Mean $\pm$ SD	50.0	5.97 ± 0.18	11.93 ± 0.35	88.07 ± 0.35

Discussion

The first principal finding of the study is that, among the Hoagland variants tested, the 0.1× medium provided the highest biomass for *L. gibba*. This result indicates that increasing nutrient solution strength does not invariably enhance growth proportionally. Akyüz and Ersus showed that Hoagland macronutrients and pH strongly affect the biochemical composition of duckweed biomass, while Pasos-Panqueva et al. confirmed in a meta-analysis that growth is jointly influenced by the N:P balance, light and temperature. Accordingly, the 0.1× result was interpreted not as a “universal optimum” but as the most effective concentration for the specific *L. gibba* material and test conditions of this study.

In the validation, RGR in the 0.1× medium was 0.26386 ± 0.00073 day⁻¹. Scherr et al. showed that in controlled *L. gibba* bioassays the mean growth rate based on area and frond number may be approximately 0.267 day⁻¹. Ziegler et al. reported RGR values of 0.153–0.519 day⁻¹ across different duckweed clones. Thus, the RGR obtained in this study is biologically high but lies within the range published for duckweeds.

The absence of a decline in biomass during the sequential 25→50→75→100% transfer to wastewater demonstrated that the acclimatization protocol was successful in practical terms. Cheng et al. reported that prior adaptation to wastewater can reduce the lag phase in duckweed systems. Verma and Suthar, cultivating *L. gibba* in urban wastewater of different strengths, demonstrated that wastewater nutrients can be channelled into biomass. In our study, the increase in RGR to 0.27458 ± 0.00035 day⁻¹ at the 100% wastewater stage without added Hoagland medium indicates that this fishpond wastewater was capable of sustaining the growth of the present culture.

The recording of 81.70 ± 0.20 g biomass and a net productivity of 15.65 ± 0.04 g FW·m⁻²·day⁻¹ under field conditions showed that the laboratory-acclimatized clonal material continued to grow under real drainage-channel conditions. Oron demonstrated that high annual biomass yields of *L. gibba* can be obtained in outdoor mini-ponds with regular harvesting, whereas Paolacci et al. assessed the system-level potential of valorizing aquaculture wastewater with duckweed [21]. The very small SD values of our three field replicates indicate the short-term reproducibility of the technology at this field site.

The use of the 50°C/6 h regime at the drying stage represents a low-temperature approach aimed at the careful preservation of nutritional components in duckweed biomass. Prosrídee et al. identified precisely 50°C and 6 h as the optimal regime for *W. arrhiza* with respect to nutritional and bioactive parameters [22]. At the same time, before the $11.93 \pm 0.35\%$ mass yield obtained after this regime is accepted as absolute dry matter, it should be verified by drying to constant mass or by the AOAC moisture method [24]. Prior to use in fish feed, the protein, lipid, ash, crude fibre, amino acid and mineral composition, together with heavy metal content and microbiological safety, must be evaluated [23].

Conclusion

The study established a single experimental chain for *L. gibba*, extending from the selection of a mineral nutrient medium to the transition to 100% fishpond wastewater and the field drainage channel. The preliminary $0\times-1.0\times$ screening and the subsequent validation ($n=3$) identified $0.1\times$ Hoagland as the working concentration yielding the highest biomass among the tested variants. During the sequential transition from $0.1\times$ to $25\rightarrow50\rightarrow75\rightarrow100\%$ wastewater, biomass and RGR did not decline; on the contrary, at the 100% wastewater stage, a biomass of 77.88 ± 0.28 g and an RGR of 0.27458 ± 0.00035 day⁻¹ were recorded. In the field validation, mean biomass was 81.70 ± 0.20 g, RGR 0.27936 ± 0.00024 day⁻¹ and net productivity 15.65 ± 0.04 g FW·m⁻²·day⁻¹. The results demonstrated that stable biomass of *L. gibba* can be produced through stepwise physiological acclimatization, maintenance of parallel clonal lines and the use of fishpond wastewater as a culture medium without external mineral nutrients. Drying at 50°C/6 h yielded 5.97 ± 0.18 g of dried product from 50 g of fresh biomass. This technological chain provides a practical basis for channelling wastewater nutrients into useful plant biomass and for its subsequent evaluation as a raw material for aquaculture feed. Repeat the 100% wastewater culture and field drainage-channel experiments over at least two seasons or in additional spatial blocks in order to verify the seasonal stability of the technology; to measure NH₄⁺-N, NO₃⁻-N, PO₄³⁻-P, pH, EC, COD/BOD and major ions in inflow and outflow water concurrently with biomass production in order to calculate the actual nutrient-removal and bioconversion efficiency; to test periodic harvesting regimes of 25–50% under semi-continuous production and to determine the optimal harvesting interval in terms of g FW·m⁻²·day⁻¹ and g DW·m⁻²·day⁻¹; to determine dry matter, crude protein, lipid, ash and fibre, as well as the amino acid, mineral and heavy metal composition of the dried biomass according to AOAC methods; to confirm the microbiological and chemical safety of the biomass before its inclusion in fish feed; and, at the next stage, to plan separate feeding trials with powdered diets for larvae and pelleted diets for larger fish.

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