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Estimation of the potential of *Lemna minor* for effluent remediation in integrated multi-trophic aquaculture using newly developed synthetic aquaculture wastewater

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Abstract

Aquaculture is an important source of animal protein and a key contributor to global food security. However, aquaculture can exert a negative effect on the aquatic environment due to the release of effluents containing high nutrient levels. In integrated multi-trophic aquaculture (IMTA), the waste produced by one species is the input for another, referred to as extractive species (ES). Potential ES include plants. In the present study, it was explored whether *Lemna minor* can be used to remove nitrogen and phosphorus from aquaculture wastewater. A representative synthetic wastewater was designed based on the composition of aquaculture effluents found in the literature. Synthetic wastewater was found to be a suitable medium for growth of *Lemna minor*, and plants readily took up NH_4^+-N, NO_3-N and PO_4^{3-}-P. In particular, NH_4^+-N concentrations rapidly decreased. The highest removal rates per square meter of water surface, calculated for NH_4^+-N, NO_3-N and PO_4^{3-}-P, were, respectively, 158, 206 and 32 $mg{\cdot}m^{-2}{\cdot}day^{-1}$, and these rates were achieved at a plant surface density of 80%. As removal of nutrients is essentially a surface area–related process, the effect of plant density on nutrient uptake was determined. Uptake of nutrients per square meter of surface area was highest at the highest plant density. Yet, when uptake rates were calculated per square meter of water area covered by *Lemna* fronds, the highest removal rates were found at the lowest plant density, and this is likely to be associated with a reduced intraspecific competition. The present work enables the calculation of potential nutrient uptake by *Lemna minor* and lays the foundation for a more scientific approach to the design of duckweed-based aquaculture wastewater treatment systems.

Keywords Lemnaceae · Aquaculture · Water restoration · Removal rate · Synthetic wastewater

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Introduction

Global food security refers to a scenario in which everybody has access to sufficient, safe and nutritious food (FAO 2001). Food quality is a major consideration in the concept of food security and, especially, the supply of quality protein is of concern (Tuomisto 2010). Fish in the broadest sense, i.e. including shellfish, is a key source of animal protein with some 4.5 billion people worldwide acquiring at least 15% of their intake from this source (Naylor et al. 2000). However, the stagnation in capture fisheries has resulted in a situation whereby supply is not meeting demand (Ahmed et al. 2019). This, in turn, has led to increased emphasis on fish farming to improve food security (Ahmed and Lorica 2002; Allison 2011; Jennings et al. 2016). The global production of farmed fish increased from 105.46 million tonnes in 2016 to 110.21 million tonnes in 2018 (Tacon 2018). The total global production is valued 243.26 billion of US dollars (FAO 2018). The growth of the aquaculture sector is set to continue, with the European Union having invested €3.4 billion in the aquaculture sector over the period 2000–2020 (Guillen et al. 2019).

Despite the importance of the aquaculture industry as a contributor to global food security, the sustainability of current aquaculture practises has been questioned. Negative impacts on coastal and marine habitats through, amongst others, waste disposal, fish escape and pathogen invasions have been reported (Valenti et al. 2018). Aquaculture can similarly be a major threat to the local freshwater environment (Wang et al. 2019). Effluent containing fish excrement and feed leftovers can impact on receiving waters, causing, amongst others, eutrophication and oxygen depletion (Correll 1998; Cornel and Whoriskey 1993). Regulatory authorities typically impose limits on the discharge of nutrient-rich effluents (e.g. Musacchio et al. 2020), and this necessitates some form of wastewater treatment, representing a substantial cost to the aquaculture industry (Turcios and Papenbrock 2014).

In response, integrated multi-trophic aquaculture (IMTA) has been developed. The principle of IMTA is that the waste produced by one species is the input (e.g. feed source or fertiliser) for another species, referred to as extractive species (ES). These ES include heterotrophic filter feeders and detritivores, as well as autotrophic seaweeds and a range of plant species. In essence, ES perform a form of environmental remediation. The biomass of the species used for bioremediation can comprise a second source of income for the industry (Ridler et al. 2007). As a result, IMTA systems can be mostly closed loop, with a reduced impact on the surrounding environment.

The concept of phytoremediation is well established (Chandra et al. 2017). Aquatic plant species with remediation potential are of particular interest for integration into aquaculture. Lemnaceae (common name duckweed) are a family of (mostly) free-floating freshwater plants that are known for having a high growth rate (duplication biomass in less than 2 days in optimal conditions (Ziegler et al. 2015) and high protein content (Oron et al. 1985; Mohedano et al. 2012). Moreover, ammonium, the prevalent form of nitrogen in aquaculture effluent, is the preferred form of nitrogen for Lemnaceae (Landolt and Kandeler 1987). Lemnaceae protein has the added advantage of a favourable amino acid profile, which means that biomass has potential value as a feed source (Zhou and Borisjuk 2019). The combination of remediation potential and valuable biomass makes Lemnaceae attractive candidates for the role of ES in IMTA.

In practise, a major challenge for IMTA is that a balance is needed between waste production by fish and remediation by the ES (Reid et al. 2008). Thus, it is important that the removal capacity of ES is well established, to underpin the design of IMTA systems. The aim of the present study was to develop a synthetic aquaculture wastewater and use it to investigate the quantitative capacity of *Lemna minor* to remove nutrients from aquaculture effluent. The development of the SAW is an important step to enhance reproducibility of experimental studies. This standardised synthetic wastewater facilitates generation of reproducible data that can be used to develop mathematical models on the balance between waste production and plant-based removal in IMTA. A standardised synthetic wastewater is a useful tool to test the effect of different conditions on the performance of duckweed in phytoremediation systems. For example, it can be used to verify the effect of light and temperature and to indicate the performance of these plants in different regions and/or in different seasons. Here, SAW is used to quantify the effect of nutrient availability and plant density on the removal efficiency.

Material and methods

Plant material and growth conditions

The strain of *Lemna minor* used for this study was collected in Blarney, Co. Cork, Ireland (51°56′25.7″N 8°33′49.1″W) and registered in the Rutgers Duckweed Stock Cooperative (www.ruduckweed.org) as strain number 5500, also referred to as MJ100. Axenic fronds were cultured on half-strength Hutner's medium (Hutner 1953), in a growth room at a temperature of 20°C, a light intensity of 40 μmol m^{-2} s^{-1} and a day/night regime of 16-h light and 8-h darkness.

Synthetic aquaculture wastewater

A synthetic aquaculture wastewater (SAW) was developed on the basis of published data. The search of peer-reviewed literature centred on studies that analysed the chemical composition of freshwater aquaculture effluents. The relevant literature was searched using the on-line scientific database Google Scholar. All searches were completed by January 2020. The principal search terms used to identify relevant publications were "freshwater, aquaculture and effluents". The first 40 search results were examined for papers providing analytical data on freshwater aquaculture effluents. Additionally, the citation lists of identified publications were inspected for further relevant papers. The papers were screened for the concentrations of NO_3-N, NH_4^+-N and PO_4^{3-}-P in aquaculture effluents. Variations in these nutrient concentrations were analysed in order to identify representative concentrations for SAW.

Experimental design

The nitrogen and phosphorus removal ability of *L. minor* in SAW was determined at four different plant densities: 20% (0.9 $g{\cdot}l^{-1}$), 60% (1.8 $g{\cdot}l^{-1}$), 40% (2.7 $g{\cdot}l^{-1}$) and 80% (3.6 $g{\cdot}l^{-1}$) of water surface area). The 100% density was not tested as, from a management point of view, it would be complicated to maintain this density at all times. In fact, growth would lead to a multi-layered mat of duckweed and cause degradation of the lower layers of duckweed and the release of nutrients in these fronds. In this paper, the plant density refers to the percentage of the surface area of the plastic culture container that was covered by fronds. The surface area of the culture containers in which the experiments were carried out was 36 cm^2 (6 × 6 cm), and the four densities were obtained by adding fronds such that they covered, respectively, 7.2, 14.5, 21.6 and 28.8 cm^2 of the available surface. These areas were covered using approximately 90, 180, 270 and 360 mg of fresh biomass, respectively. These weights were obtained using approximately 70, 140, 210 and 280 fronds of *L. minor*. Each culture container was filled with 100 ml SAW.

The experiment started with four culture containers for each plant density. Every 24 h, one culture container per density was used to perform water analysis. The concentrations of NO_3-N, NH_4^+-N and PO_4^{3-}-P were quantified in order to determine the amount of nutrients removed. In the other culture containers, the plant density was kept constant by removing every day as many fronds as had grown during the previous 24 h. The experiment lasted 4 days and was independently replicated twelve times. During the experiment, the plants were kept under the same growth conditions described in "Plant material and growth conditions" with the exception of the light intensity. The intensity during the experiment was increased to 300 μmol m^{-2} s^{-1}.

Water quality analysis

Nitrate, ammonia and phosphate concentrations in the medium were determined using a HACH DR2800 machine. NO_3 -N was determined by the cadmium reduction method (HACH method 8171) using NitraVer®5 Nitrate Reagent Powder Pillows (absorbance measured at 500 nm, detection range between 0.3 and 30.0 mg $\cdot l^{-1}$). The NH_4^+-N concentration was determined by the Nessler method (HACH Method 8038) using Nessler reagent, mineral stabilizer and polyvinyl alcohol dispersing agent (absorbance measured at 420 nm, detection range of 0.02–2.50 mg $\cdot l^{-1}$). PO_4^3 -P was determined by the ascorbic acid method (HACH method 8048) using PhosVer® 3 Phosphate Reagent Powder Pillows (absorbance measured at 880 nm, detection range of 0.02 to 2.50 mg $\cdot l^{-1}$).

Calculations of N and P uptake rates

The removal rate of NO^-_3-N, NH_4^+-N and PO_4^{3-}-P was calculated for plants kept at four different plant densities. Removal rates of N and P were calculated per square meter of water surface (covered by different plant densities) per day, as well as per square meter of water area covered by fronds of *Lemna minor* (at different densities) per day. The following formula was used to calculate the uptake rates:

$$RWS = (Ci - Cf) * V / Aw * t$$

where RWS is the removal rate per water surface, Ci is the initial concentration of the nutrient, Cf is the final concentration of the nutrient, V is the volume in litre, t is the time in days and Aw is the surface area of water available to the plants in square meter (area of the culture container).

$$RDS = (Ci - Cf) * V / Ad * t$$

where RDS is the removal rate per square meter of water area covered by *Lemna* fronds, Ci is the initial concentration of the nutrient, Cf is the final concentration of the nutrient, V is the volume in litre, t is the time in days and Ad is the water area covered by *Lemna* fronds in square meter.

The relationship between removal rates and concentrations of NO^{-}_{3}-N and NH_4^+-N in the SAW was analysed in more detail for the two highest plant densities (60% and 80%), for which removal rates per surface area were highest and significantly different from the removal rates at 20% and 40%. For PO_4^{3-}-P, all the four densities were included in the analysis.

Statistical analysis

All statistical analysis was performed using IBM SPSS statistics 26. A two-way repeated measures ANOVA was conducted to determine the effects of plant density and time on the removal of NO_3 -N, NH_4^+-N and PO_4^3 -P. For the simple two-way interactions and simple main effects, a Bonferroni adjustment was applied. Statistical significance was accepted with 95% confidence level.

The removal rates of NO_3 -N, NH_4^+-N and PO_4^3 -P at different plant densities were compared using a one-way ANOVA. The normality of the rates was assessed by the Shapiro-Wilk test and the homogeneity of variances by Levene’s test. Post hoc analysis was performed with a Tukey test.

Linear regression was used to explore the effects of different concentrations of NO_3 -N, NH_4^+-N and PO_4^3 -P on removal rates. The linearity of the relation between the two variables was assessed by visual inspection of the scatter plot, and homoscedasticity and normality of the residuals were verified. When the variables failed to show a clear pattern on the scatter plot, a Kendall tau-b test was carried out to confirm the lack of correlation. The proportion of the variation in the removal rate explained by the concentration was determined, and dependent variable values were predicted from new independent variable values.

Verification of predicted values

Real aquaculture wastewater was collected from a fish farm in Ireland, farming rainbow trout (*Oncorhynchus mykiss*) and European perch (*Perca fluviatilis*). The wastewater contained 3.75, 1.9 and 3.6 $mg \cdot l^{-1}$ of, respectively, NH_4^+-N, NO_3 -N and PO_4^3 -P. Duckweed at densities between 60 and 80% were placed in a culture container containing 100 ml of real aquaculture wastewater. The nutrient content was measured after 24 h, and the uptake rates were calculated in order to compare the rate of uptake from real aquaculture medium with that predicted from the experiments using synthetic aquaculture medium. The experiment was replicated 12 times.

Results

Development of a synthetic aquaculture wastewater

The literature was mined for data on the composition of freshwater aquaculture effluents and specifically concentrations of NO_3 -N, NH_4^+-N and PO_4^{3-}-P. A total of 16 articles contained relevant data for the purpose of the present study. These articles contained data on the effluents from farms culturing rainbow trout (*Oncorhynchus mykiss*), salmon smolt (*Salmo salar*), brown trout (*Salmo trutta*), arctic char (*Salvelinus alpinus*), European eel (*Anguilla Anguilla*), pike perch (*Sander lucioperca*), sturgeon (*Acipenser* sp.), Nile tilapia (*Oreochromis niloticus*), European perch (*Perca fluviatilis*), European sea bass (*Dicentrarchus labrax*), catfish (*Ictalurus* sp.) and bluegill (*Lepomis macrochirus*) (supplementary table I). There was substantial variation in effluent concentrations of NO_3 -N, NH_4^+-N and PO_4^{3-}-P (Fig. 1a–c). The mean values for NO_3 -N, NH_4^+-N and PO_4^{3-}-P , in the papers reviewed, were, respectively, 25.7, 5.7 and 2.7 mg $\cdot l^{-1}$. The highest concentration of NH_4^+-N was found in a study by Gendel and Lahav (2013). The authors farmed 105 tilapia in 500 l of medium and the concentration of NH_4^+-N in the water reached 20 mg$\cdot l^{-1}$. The highest concentration of NO_3 -N (200 mg$\cdot l^{-1}$) was found in a study by Dalsgaard et al. (2013) in which rainbow trout was farmed at a density of 50–80 kg$\cdot m^{-3}$. The highest concentration of PO_4^{3-}-P in effluent was found by Ng and Chan (2018). The authors observed a concentration of 19 mg$\cdot l^{-1}$ PO_4^{3-}-P in a fish farm in China; however, they did not specify which species was farmed and at which density.

Based on the information obtained from the literature (Fig. 1, Suppl. Table I), a synthetic aquaculture waste effluent was designed that contains 25, 8 and 2 mg $\cdot l^{-1}$ of NO_3-N, NH_4^+-N and PO_4^{3-}-P, respectively. These values are the rounded up mean values calculated for the papers reviewed. SAW was supplemented with common macro- and micronutrients, to resemble eutrophic wastewater (Table 1). Other elements necessary for the growth of Lemnaceae were added in the amount and proportions suggested by Hutner (1953). The pH of the synthetic wastewater was 5.5.

Lemna minor–mediated removal of nutrients from SAW

The growth of *L. minor* on SAW during the 4 days of experiment was substantial. Every day, a number of fronds had to be removed in order to maintain the chosen densities. Approximately 7, 14, 20 and 25 fronds of *L. minor* were removed daily from the containers with plants at the densities of, respectively, 20%, 40%, 60% and 80%. The NH_4^+-N, NO_3 -N and PO_4^3 -P concentrations in the SAW were measured every day. NH_4^+-N depletion was substantial (Fig. 2a). In most of the experiments with a plant density of 60% and 80%, the concentration of NH_4^+-N in the medium fell to just above zero after 4 days. The decrease in the concentration of NH_4^+-N ranged between 6.17 ± 0.33 (at a plant density of 20%) and 7.67 ± 0.13 (at a plant

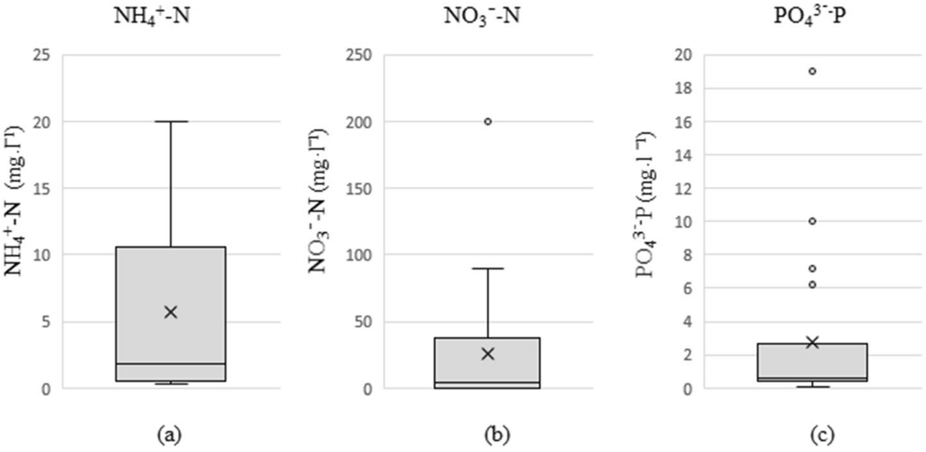


Fig. 1 Concentrations in freshwater aquaculture effluents of NH_4^+-N (a), NO_3 -N (b) and PO_4^{3-}-P (c) found in the literature. The data were collected from 16 articles (Supplementary Table I). All the concentrations were converted in milligrams per litre. The x indicates the mean, the horizontal line within the box indicates the median, and the lower and upper extremes of the box indicate respectively first and third quartiles. Grey circles are outliers

Table 1 Composition of synthetic aquaculture wastewater

	Concentration (mg · l^{-1})
(NH4)2SO4	40
KNO3	73
Ca(NO3)·4H2O	186.15
KH2PO4	8.7
CaSO4	328.6
$MgSO_4$·4H2O	738
K2SO4	471.5
ZnSO4·7H2O	1
MnSO4 H2O	0.1
CuSO4 5H2O	0.03
Na2MoO4 2H2O	0.1
Ferric citrate	1
EDTA Na	0.029
H3BO3	1

density of 80%) mg ·l^{-1}. The biggest decrease in the concentration of NH_4^+-N was observed during the first 24 h. Overall, less NH_4^+-N was removed from SAW at lower plant densities. A two-way repeated measures ANOVA revealed a significant interaction between time and density ($p < 0.01$). The simple main effects of the plant density and time on NH_4^+-N concentration were significant, with both $p < 0.01$. Plants at a density of 80% removed more NH_4^+-N than plants at density 40% ($p < 0.01$) and 20% ($p < 0.01$). Plants at a density of 60% removed more NH_4^+-N than plants at density 40% ($p < 0.001$) and 20% ($p < 0.01$). Plants at a density of 40% removed more NH_4^+-N than plants at density 20% ($p < 0.001$).

The concentration of NO_3 -N in the medium decreased slowly over the 4 days of monitoring (Fig. 2b). The concentration of NO_3 -N in the SAW reached values as low as 13.5 ± 1.6 mg·l^{-1} at the highest plant density and after 4 days. A two-way repeated measures ANOVA highlighted that there was significant interaction between time and density on NO_3 -N concentration ($p = 0.047$). The simple main effects of time and density were also significant ($p = 0.005$ and 0.02, respectively). Plants at a density of 80% removed more NO_3 -N than plants at density 20% ($p < 0.011$) and 40% ($p = 0.035$).

The daily decrease in PO_4^3 -P concentration was constant during the 4 days of experiment, (Fig. 2c). The concentration of PO_4^3 -P in the SAW reached values as low as 0.89 ± 0.31 mg·l^{-1} after 4 days and at the density of 60%. There was no significant two-way interaction between time and plant density on the PO_4^3 -P concentration. Only time had a significant effect, with $p < 0.001$.

Daily nutrient removal per square meter of water surface and per square meter of water area covered by *Lemna* fronds

The absolute nutrient removal rate during the first 24 h of the experiment was calculated based on the volume of SAW used in the experiment and expressed per square meter of water surface (Fig. 3a) or per square meter of water area covered by *Lemna* fronds (Fig. 3b). The rates calculated were plotted as a function of the plant density. When the removal rates of both forms of N were calculated per square meter of water surface, they increased with increasing plant density. The highest removal rates of NH_4^+-N were 157.61 ± 10.57 mg·m^{-2}·day^{-1}, achieved at a plant density of 80%. The lowest rate of NH_4^+-N removal was 99.12 ± 6.41

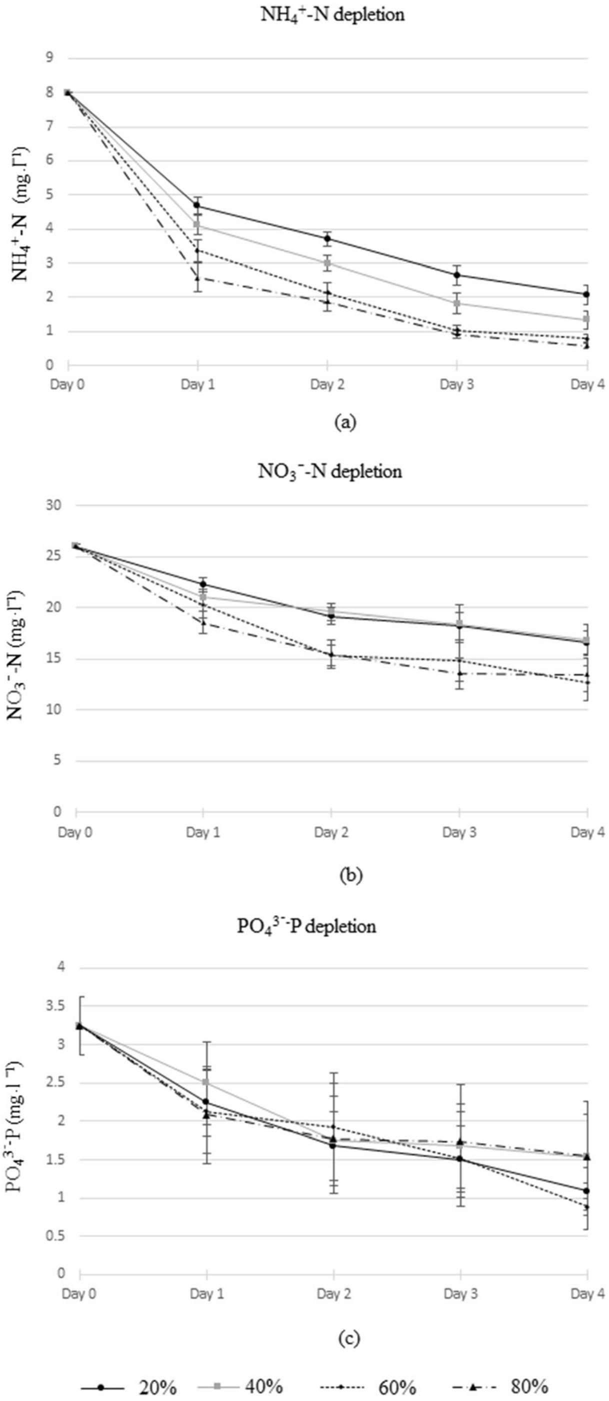


Fig. 2 Decrease over time of NH_4^+-N (a), NO_3-N (b) and PO_4^{3-}-P (c) in synthetic aquaculture wastewater with *L. minor* at four different densities (20%, 40%, 60% and 80%). Slightly higher nutrient concentrations on day 0 relate to carry-over from standard medium to experimental medium. N = 12, bars are standard errors

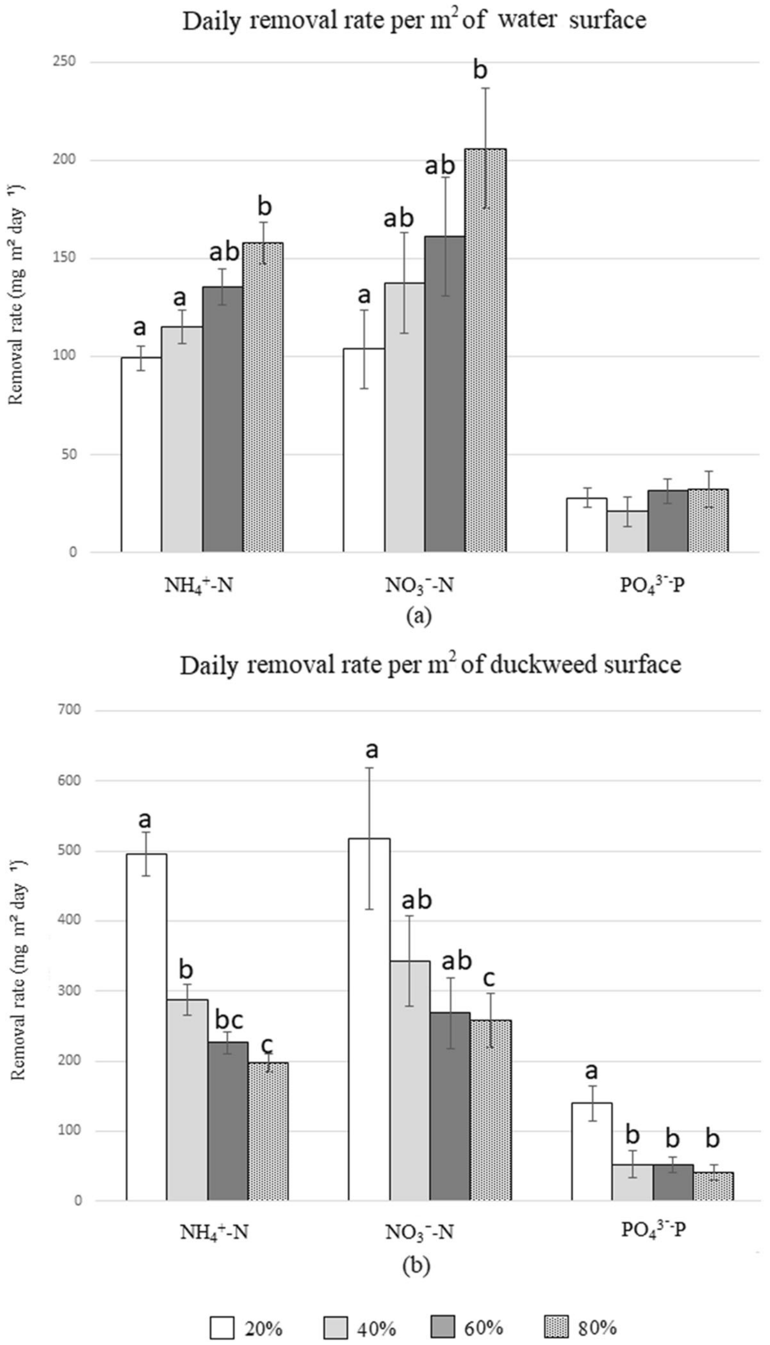
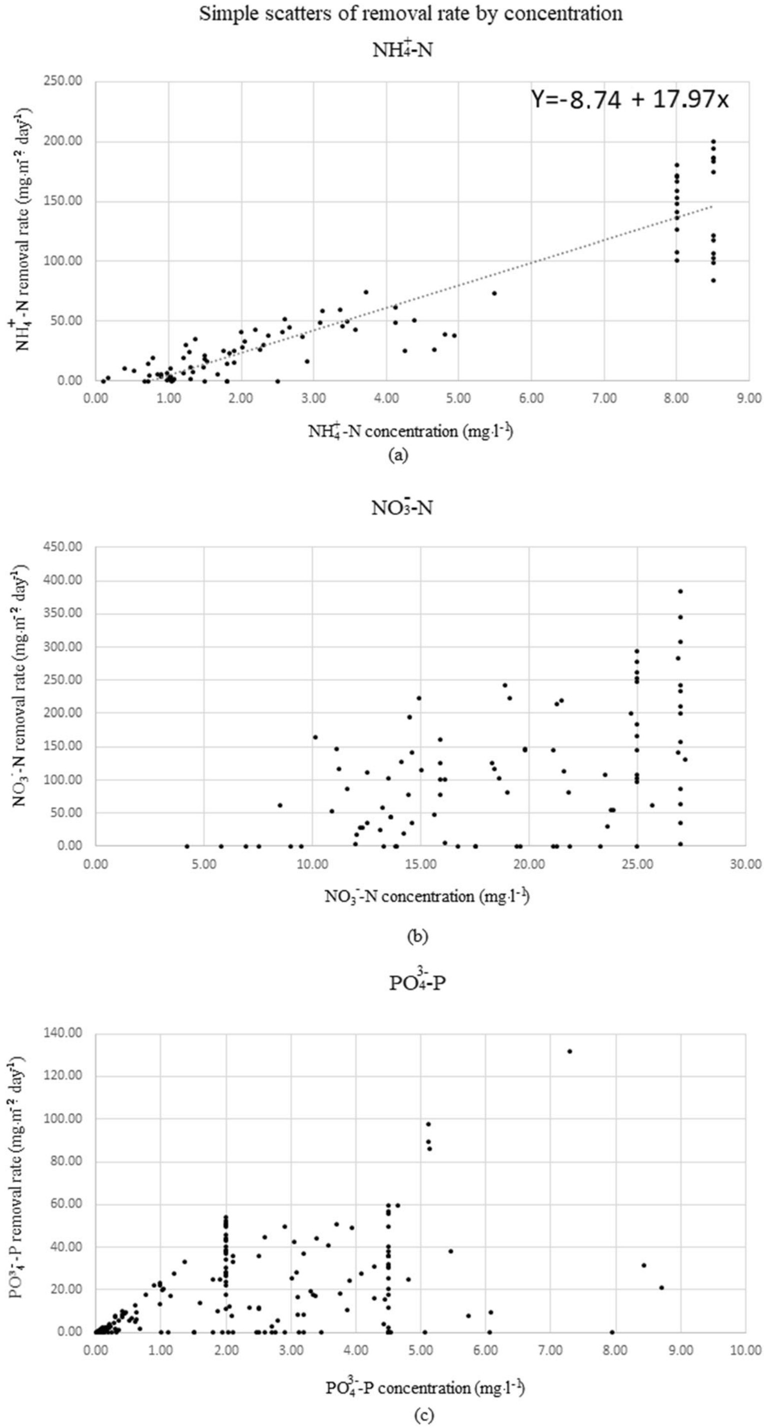


Fig. 3 Nutrient removal rate per square meter of water surface (a) and per square meter of *L. minor* covering the water surface (b), at different plant densities (20%, 40%, 60%, 80%) and measured during the first 24 h of experiments. Different letters indicate significant differences. N = 12, bars are standard errors


Simple scatters of removal rate by concentration
NH_4^+-N
Y=-8.74 + 17.97x
NH_4^+-N removal rate (mg·m^{-2}· day^{-1})
0.00
50.00
100.00
150.00
200.00
250.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
NH_4^+-N concentration (mg·l^{-1})
NO_3^--N
NO_3^--N removal rate (mg·m^{-2}· day^{-1})
0.00
50.00
100.00
150.00
200.00
250.00
300.00
350.00
400.00
450.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
NO_3^--N concentration (mg·l^{-1})
PO_4^{3-}-P
PO_4^{3-}-P removal rate (mg·m^{-2}· day^{-1})
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
PO_4^{3-}-P concentration (mg·l^{-1})

(a)

(b)

(c)

◀ **Fig. 4.** Simple scatter plot of daily NO_3 -N (a), NH_4^+-N (b) and PO_4^{3-}-P (c) removal rates expressed per square meter of water surface area as a function of nutrient concentration in the medium. For NH_4^+-N, the fit lines and the regression equations are also indicated. Dots represent individual measurements. NH_4^+-N concentrations of 8 $mg \cdot l^1$, NO_3 -N concentrations of 25 $mg \cdot l^1$ and PO_4^{3-}-P concentrations of 2.5 $mg \cdot l^1$ represent the initial concentrations used in uptake experiments. Concentrations higher to SAW concentrations are due to carry-over from standard medium to experimental medium

$mg \cdot m^{-2} \cdot day^{-1}$, achieved at a plant density of 20%. Plants at the density of 80% removed significantly more NH_4^+-N than plants at a density of 20% ($p < 0.01$) and 40% ($p < 0.01$). The highest removal rate of NO_3 -N was 205.99 ± 30.71 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at a plant density of 80%, while the lowest was 103.61 ± 20.27 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at the density of 20%. Plants at the density of 80% removed significantly more NO_3 -N than plants at the density of 20% ($p = 0.045$).

In contrast to what was observed for rate calculated per square meter of water surface, when the removal rate was calculated per square meter of plants covering the water surface, it decreased with the increasing plant density. The highest removal rates of NH_4^+-N were 495.6 ± 32.05 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at a plant density of 20%. The lowest rate of NH_4^+-N removal was 197.01 ± 13.21 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at a plant density of 80%. Plants at the density of 20% removed significantly more NH_4^+-N than plants with a density of 80% ($p < 0.01$), 60% ($p < 0.01$) and 40% ($p < 0.01$); the removal of NH_4^+-N at 40% was also significantly higher than the removal at 80% ($p = 0.025$). The highest removal rate of NO_3 -N was 518.05 ± 101.39 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at a plant density of 20%, while the lowest was 257.49 ± 38.38 $mg \cdot m^{-2} \cdot day^{-1}$, achieved at the density of 80%. Plants at the density of 20% removed significantly more NO_3 -N than plants at the density of 80% ($p = 0.045$).

The removal rate of PO_4^3 -P per square meter of water ranged between 20.87 ± 7.72 and 32.43 ± 9.1 $mg \cdot m^{-2} \cdot day^{-1}$, with no significant differences between densities. When calculated per square meter of plant covering the water surface, the highest removal rate, 139.46 ± 24.15 $mg \cdot m^{-2} \cdot day^{-1}$, was observed at the plant density of 20%. At this density, the removal rate was significantly higher than at 40% ($p < 0.01$), 60% ($p < 0.01$) and 80% ($p < 0.01$).

The detailed results of the statistical analyses are reported in tables contained in supplementary material 1.

Removal rates at different nutrient concentrations in the SAW

The daily nutrient removal rate for all sampling time-points and recalculated per square meter of water surface was plotted as a function of the measured concentrations of NH_4^+-N, NO_3 -N and PO_4^3 -P in SAW. For NH_4^+-N and NO_3 -N, data are presented for plants kept at 60 and 80%, at which densities the uptake rates are most substantial. For PO_4^3 -P, the removal rates at all the densities tested were included as no significant differences between densities were highlighted in the previous analysis. A visual inspection of the scatter plots highlighted a linear relationship between daily NH_4^+-N removal rate and NH_4^+-N concentration (Fig. 4a). This relationship was further analysed using regression analysis. The analysis shows that the concentration of NH_4^+-N in the medium significantly predicts the removal rate of the compound in the water ($p < 0.01$). The medium concentration accounted for 86% ($R^2 = 86\%$) of the variability in removal rate. A Kendall tau-b correlation was run to determine the relationship between concentration of NO_3 -N or PO_4^{3-}-P and daily removal rate. This test was selected as a visual inspection of the scatter plots failed to show a linear relationship between variables. There was a weak association between both nutrients and daily removal rate, which was not statistically significant (τb = 0.127, p = .681, and τb = 0.111, p = .702 respectively). Because of the lack of correlation between variables, the regression analysis was not carried out for NO$_3$ -N and PO$_4^{3-}$-P data (Fig. 4b, c).

The observed regression line was used to predict the uptake rate of NH$_4^+$-N from real, farm-collected, aquaculture wastewater (containing 3.75 mg·l^{-1} of NH$_4^+$-N). The predicted uptake rate was 58.8 mg·m^{-2}·day^{-1}. The observed uptake rate of NH$_4^+$-N by duckweed grown in the real aquaculture wastewater was 53.6 ± 0.4 mg·m^{-2}·day^{-1} (Fig. 5).

Discussion

A key challenge in the design of IMTA systems is the balance between fed and extractive species (Wei et al. 2017; Fang et al. 2019; Nardelli et al. 2019). Where ES are algae or plants, it is essential to consider their intrinsic growth and nutrient uptake characteristics. These characteristics vary in response to changes in the composition of the wastewater (Kwon et al. 2013), as well as in response to environmental parameters such as temperature and light (Iasimone et al. 2018). Growth and nutrient uptake are also species specific (Khatun et al. 2016; Yongpisanphop et al. 2017; Queiroz et al. 2020). Here, uptake of NO$_3$ -N, NH$_4^+$-N and PO$_4^3$ -P by *L. minor* was analysed under controlled conditions using a synthetic aquaculture wastewater and various plant densities, in order to obtain quantitative information on the remediation potential of this species.

Development of a synthetic aquaculture wastewater for controlled experiments

The development of synthetic wastewater to enable reproducible testing of the efficiency of different water remediation systems is increasingly prevalent (e.g. Visvanathan et al. 2008;

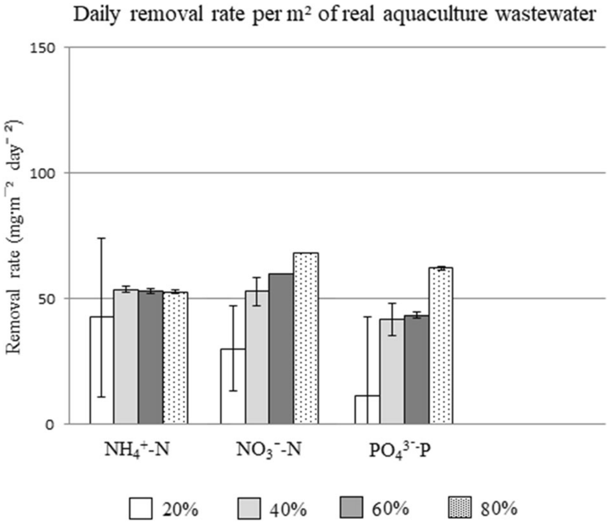


Fig. 5. Nutrient removal rate per square meter of real aquaculture wastewater surface at different plant densities (20%, 40%, 60%, 80%), measured during the first 24 h of experiments. The wastewater contained 3.75, 1.9 and 3.6 mg·l^{-1} of, respectively, NH$_4^+$-N, NO$_3$ -N and PO$_4^{3-}$-P

Saleem et al. 2011; Ansari et al. 2017; Barnharst et al. 2018; Walsh et al. 2020). The use of such a standardised medium facilitates studies on the impacts of other parameters, such as plant species, plant density, climatic factors, microbial populations and IMTA system design parameters. Previously, a SAW was used by Ng and Chan (2018) to test the phytoremediation ability of *Spirodela polyrhiza*, *Salvinia molesta* and *Lemna* sp. The authors based the NH_4^+-N and PO_4^{3} -P concentrations (20 and 19 mg·l^{-1} respectively) in their synthetic wastewater on the concentrations measured at a local fish farm. In the current study, a SAW was developed on the basis of realistic concentrations of NH_4^+-N, NO_3 -N and PO_4^{3} -P in freshwater aquaculture effluents. Selected papers refer to a variety of fish farms, farming different species at different densities. Thus, the developed SAW is a generic medium that can be used as a proxy representing effluent from a broad range of freshwater fish farms.

Removal of nutrients from SAW

In this study, the removal rate of nutrients was determined by measuring the concentration of the nutrients in the SAW every day. The experiment was carried out in aseptic conditions. Therefore, it is reasonable to assume that the nutrients removed from the SAW were taken up by the plants as the microbial activity was negligible. Given the small scale of the experiments, it was not possible to determine the amount of nutrients in the plant biomass.

The developed SAW is a suitable growth medium for *L. minor*, with both plant growth and nutrient removal being substantial. A comparison of the NH_4^+-N uptake rate from real farm aquaculture wastewater (58.8 mg·m^{-2}·day^{-1} at a concentration of 3.75 mg·l^{-1}) and the rates obtained using SAW (53.6 ± 0.4 mg·m^{-2}·day^{-1} at 2.4 mg·l^{-1}) shows good agreement. Thus, the developed SAW proved to be a reliable representative of real aquaculture wastewater.

Rapid removal of NH_4^+-N and PO_4^{3} -P from SAW was observed during the first 24 h of the experiment. Comparatively, the decrease of NO_3^--N in the SAW was lower. The relatively slow removal of NO_3^--N is most likely related to the presence of NH_4^+-N in the SAW. When both forms of nitrogen are present in a medium, Lemnaceae prefer to take up NH_4^+-N (Feller and Erismann 1971), probably as the direct conversion of ammonia into protein is a more energy-efficient process (El-Shafai et al. 2007). The measured removal rates of the two combined forms of nitrogen from SAW are slightly lower than those reported in the literature. For example, in the literature, values range from 500 to 2100 mg·m^{-2}·day^{-1} (Körner and Vermaat 1998; Cheng et al. 2002; Mohedano et al. 2012). The phosphorus removal rates measured in this study are also in the lower portion of the published range (i.e. from 20 to 590 mg·m^{-2}·day^{-1}) (Körner and Vermaat 1998; Cheng et al. 2002; Mohedano et al. 2012). The absence of bacterial and algal activity under the used experimental conditions is probably responsible for the relatively low removal of nutrients.

Following the first 24 h, removal rates of all nutrients decreased, and this was associated with lower nutrient concentrations in the used stationary system. To analyse the relationship between removal rates and nutrient concentration in the SAW in more detail, removal rates (mg·m^{-2}·day^{-1}of water surface) of NH_4^+-N, NO_3 -N and PO_4^{3} -P were plotted against the concentration of the compounds in the SAW. For NO_3 -N and PO_4^{3} -P, the relationships between removal rate and the concentration were not well defined. However, the removal rate of NH_4^+-N linearly decreased with decreasing concentration in the medium. These data cannot be directly interpreted as meaning that NH_4^+-N is limiting for growth, as growth is likely to be more closely related to internal redistribution of nutrients, rather than uptake (Kufel et al. 2012). Nevertheless, the ability to predict removal rates at different nutrient concentrations is of fundamental importance when designing duckweed-based phytoremediation system as it can inform attempts to achieve a balance between fed and extractive species.

Effect of plant density on the removal rates

As removal of nitrogen and phosphorus is essentially surface area–related processes, the plant density in the system needs to be considered. In the present study, the removal rate was calculated both per square meter duckweed covering the water surface and per square meter of water surface. The two different approaches reflect two different aspects of duckweed-based wastewater systems. The removal rate per square meter of water is an important tool to predict the efficiency of the system from the water-restoration point of view. On the other hand, the analysis of the removal rate calculated on the basis of plant surface reveals physiological characteristics that can potentially reflect on the nutritional values of the plants.

The duckweed density relates directly to system management and harvest protocols. More plants will result in more nutrient uptake capacity. Indeed, the data show that the removal rate per square meter of water surface was higher at higher plant densities. However, the removal rate per square meter of duckweed was highest at the lowest densities. It has been argued that the increase in plant density leads to intraspecific competition, which is associated with a slowdown in nutrient removal and/or growth (Clatworthy and Harper 1962; Rejmánek et al. 1989). The higher removal rate at low plant density could be associated with different biochemical compositions of the biomass produced, and this aspect should be investigated further. It has been shown that the most efficient plant density for nutrient removal is around 80% (Verma and Suthar 2015). Furthermore, it has been shown that at high-density plants not only maximise nutrient removal, but also block sunlight penetration in the water column, impeding growth of phytoplankton and other algae (Roijackers et al. 2004).

Nutrient removal by Lemnaceae needs to be analysed in more detail in terms of impacts on growth and nutritional value. Duckweed is considered a potential crop plant for animal feed as well as human nutrition (De Beukelaar et al. 2019), particularly in the context of the circular economy. The optimization of the nutritional value of these plants is therefore of major interest (Appenroth et al. 2018).

Conclusions

The present work lays the foundation for a more scientific approach to the design of duckweed-based aquaculture wastewater treatment systems. The results presented here highlight the importance to consider the load of nutrients in the wastewater and its effect on the plant's ability to remove them, in order to design an efficient system.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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Consent to participate Not applicable.

Consent to publish Not applicable.

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