Size matters – Microalgae production and nutrient removal in wastewater treatment high rate algal ponds of three different sizes

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Abstract

High rate algal ponds for coupled wastewater treatment and resource recovery ~~has~~ have been the focus of much international research over the last 15 years. Microalgal biomass productivity reported in full-scale studies (1-ha or greater) have often been substantially lower than that reported from smaller scale ponds in similar climates, regardless of the season or the dominant microalgal species used. The disconnect between smaller-scale and full-scale productivity is unclear and uncertainty remains regarding the applicability of smaller scale studies to full-scale systems. In order to better understand the differences in reported productivity, the performance of three different size wastewater treatment high rate algal ponds (5 m^2, 330 m^2 and 1-ha) were assessed with respect to nutrient removal and microalgal productivity over three seasons. Both daily areal nutrient removal and biomass production were affected by the size of the pond. NH_4-N removal via nitrification~~/~~/denitrification decreased with increasing pond size, with the highest removal rate in the 5 m^2 pond and the lowest in the 1-ha. Microalgal areal productivity was maximal in the 330 m^2 pond, suggesting that a combination of mixing frequency and higher photosynthetic potential under low light conditions were the main drivers of enhanced productivity in this pond compared to the 5 m^2 (mesocosm) and 1-ha (full-scale) ponds. The lowest daily nutrient removal and biomass production occurred in the 1-ha (full-scale) pond. Our results suggest that, based on the current design and operation of high rate algal ponds, the optimum size for maximum productivity is considerably smaller than the current full-scale systems. This has implications for commercial scale systems, with respect to capital and operational costs.

Keywords: Microalgal productivity; ~~f~~Full-scale HRAP; ~~w~~Wastewater treatment; ~~n~~Nutrient removal; ~~o~~Open raceway ponds

~~1.~~1 Introduction

Over the past decade, there has been much global interest in the use of microalgae as a feedstock for a range of products including, but not limited to, renewable energy, biopharmaceuticals, nutraceuticals, bioplastics, pigments and fertiliser [1–3]. While high value microalgal products, such as biopharmaceuticals and nutraceuticals, are economically viable, for low values products, lifecycle production costs currently preclude microalgal-based biorefinery from being an economically viable option [4]. However, coupling wastewater treatment and resource recovery using microalgal~~/~~/bacterial based high rate algal ponds (HRAPs) makes low value microalgal biorefinery more commercially realistic [4–6]. Coupling wastewater treatment and microalgal biorefinery addresses the needs of circular economy by utilising nature's cycles for recovering materials, energy and nutrients for economic re-use [7].

Nevertheless, potential production rates have often been estimated based on either theoretical maximum microalgal biomass yields, or from biomass measured from cultures grown under optimised conditions at lab, or small scale, with microalgal production estimated to be in the order of 73 t of biomass ha^{-1} y^{-1} [8–10].

While wastewater treatment and resource recovery using microalgal-based HRAPs is an established technology, reported ranges of combined microalgal~~+~~/bacterial biomass production in wastewater treatment HRAPs has varied from 2.0–25.0 g m^2 d^{-1} for organic matter and from 5.7–30.0 g m^2 d^{-1} of total suspended solids, well short of the theoretical maximum of 50–60 g m^2 d^{-1} (e.g. [11–18]). Due to the limited number of full-scale wastewater treatment HRAP systems worldwide, many of these reported studies have been undertaken at mesocosm or pilot-scale. For those studies in full-scale HRAPs, microalgal biomass productivity have been notably lower (3.7–11.5 g m^2 d^{-1} for organic matter) compared to pilot-scale or mesocosm scale studies in similar climates, regardless of the season, or the dominant microalgal species used (e.g. [12,18,19]). The disconnect between small-scale and full-scale productivity is unclear and, to date, there has been no published investigation comparing coupled wastewater treatment and microalgal biomass production across a range of HRAPs sizes, including full-scale. As a result of this disconnect, uncertainty remains regarding the applicability of smaller scale studies to full-scale systems and such contrasting biomass yields has huge implications for industry investment when high yields from small-scale studies are applied to full-scale predictions.

The aim of this research was to evaluate if microalgal productivity and~~+~~/or nutrient removal varied across different size wastewater treatment HRAP ponds and to better understand the disconnect between small-scale and full-scale systems. We compared microalgal photosynthesis and biomass production as well as nutrient removal efficiency of both nitrogen and phosphorus across three different size wastewater treatment HRAPs, ranging from 5 m^2 to 1-ha in surface area, over three seasons. The findings of this work may help in the design and techno-economic evaluation of full-scale microalgal wastewater treatment and biorefinery systems.

~~2.~~2 Methods

~~2.1.~~2.1 Study site, ponds and environmental variables

The study was conducted at the Cambridge Wastewater Treatment Plant (WWTP), North Island, New Zealand (37° 53′ 54.63″ S, 175° 26′ 17.15″ E). The experimental system consisted of paired, single-loop, raceway HRAP ponds in three sizes. The mesocosm pond had a surface area of 5 m^2 and a total treatment volume of 1.5 m^3, the pilot-scale pond had a surface area of 330 m^2 and a total treatment volume of 90 m^3, while the full-scale pond had a surface area of 1-ha and a total treatment volume of 2~~,~~900 m^3 (Fig~~ure~~. 1). All ponds had an operation depth of 300 mm and a hydraulic retention time (HRT) of eight days. All three ponds were kept on the same HRT throughout the experiment to a) avoid comparative issues with changing HRT with changing season and b) to avoid comparative issues between ponds of different sizes. A single paddlewheel in each of the ponds was used to mix the wastewater and microalgae-bacteria consortia around the pond at a horizontal velocity of 0.2 m s^{-1}, which gave a single circuit time of 0.5 ~~minutes~~min, 9.2 ~~minutes~~min and 65 ~~minutes~~min, in the mesocosm, pilot-scale and full-scale HRAPs, respectively.

~~Figure 1.~~**Fig. 1** The different sized high rate algal ponds used in the experiment. A) ~~m~~Mesocosm pond with a total treatment volume of 1.5 m^3, B) pilot-scale pond with a total treatment volume of 90 m^3 and C) two full-scale ponds with a total treatment volume of 2~~,~~900 m^3 each.

alt-text: Fig. 1

All HRAPs were inoculated with pond water and microalgae~~+~~/bacteria consortia from the full-scale HRAPs and were operated on an eight day HRT before any measurements were taken, to allow algae to acclimate to the new ponds. During late spring, a grazer bloom throughout all the ponds resulted in 'pond crashes' and loss of algal biomass. The spring monitoring was terminated at this point and the ponds were re-inoculated from the full-scale HRAP, following a week of recovery. During the winter and spring monitoring periods, the microalgal community was dominated (>85% of the total biovolume) by the green microalga *Ankistrodesmus falcatus* (Corda) Ralfs, while during the summer monitoring (following re-inoculation) the microalgal community was dominated by the green microalga *Micractinium pusillum* Fresenius.

The influent for all HRAPs was digested effluent from a covered anaerobic pond treating municipal wastewater (see [18] for further details). Pond operation parameters are summarised in Table 1.

~~Table 1.~~**Table 1** Seasonal pond operation parameters and environmental variables in HRAPs operated at different depths. Data are medians ± standard deviations.

alt-text: Table 1

Parameter	Winter			Spring			Summer		
	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale
Sampling months	June-–August			September-–November			December-–February		
Daily inflow ($m^3\ d^{-1}$)	0.19	11.25	362.50	0.19	11.25	362.50	0.19	11.25	362.50
Pond Temp. (o°C)	8.9 ± 1.9	11.1 ± 1.6	11.0 ± 1.5	21.1 ± 2.3	19.3 ± 1.6	19.3 ± 1.7	25.1 ± 4.2	22.9 ± 2.1	23.0 ± 2.2
Pond pH	7.5 ± 0.7	7.6 ± 0.3	7.3 ± 0.3	7.7 ± 0.4	7.9 ± 0.2	7.5 ± 0.3	8.7 ± 0.8	8.7 ± 0.6	8.1 ± 0.4
Pond conductivity ($\mu S\ cm^{-1}$)	385 ± 40	382 ± 48	382 ± 50	314 ± 72	310 ± 72	314 ± 68	318 ± 33	319 ± 38	318 ± 42

The HRAPs were sampled twice weekly for analyses of dissolved nutrients, total suspended solids (TSS), organic matter and chlorophyll *a* (chl-*a*), while pond light climate was measured weekly. Samples were collected for a total of eight weeks for each season, resulting in 32 samples per pond size. These measurements were taken at the same time each day, between 09:00 and 10:00 am NZST, since, for a number of variables, the morning value is similar to the diurnal median value in HRAPs [20].

~~2.2.~~2.2 Nutrients and nutrient removal efficiency

Methods used were those described in Sutherland et al. ~~2014~~ [5]. Briefly, dissolved nutrient samples were filtered through filters (Whatman GF/F) and concentrations of ammonium (NH_4-N), nitrate-nitrite (NO_x-N) and dissolved reactive phosphorus (DRP) were determined colourimetrically according to standard methods [21]. The efficiency of nutrient removal from the water per unit biomass of the microalgal~~/~~/bacterial consortia, termed nutrient removal efficiency (*NRE*), was determined by:

$$NRE = \frac{(Influent\ concentration - effluent\ concentration)}{Organic\ matter} \times \frac{1}{retention\ time\,(d)}$$

where nutrient concentrations were corrected for any increase or decrease in pond daily flow as a result of evaporative losses and~~/~~/or precipitation gains. For nitrogen, NRE was determined based on dissolved inorganic nitrogen (DIN) concentrations, to include for any nitrification processes.

~~2.3.~~2.3 Total suspended solids, organic matter and chlorophyll *a* biomass

Methods used were those described in Sutherland et al. ~~2014~~ [5]. Briefly, a known volume of pond water was filtered through a pre-rinsed, pre-combusted and pre-weighed filter (Whatman GF/F), oven dried (105o°C) and weighed, once cooled, to determine the total suspended solids (TSS) concentration. Filters were then combusted at 450o°C for 4 ~~hours~~h, cooled in a desiccator, and re-weighed to determine the ash concentration. Organic matter was estimated as the difference between TSS and ash concentrations. For chl-*a* a known volume of pond water was filtered onto filters (Whatman GF/F) and the filters boiled in 100% methanol at 65.5o°C for 5 ~~minutes~~min then extracted at 4o°C, in the dark, for 12 ~~hours~~h. Samples were then centrifuged at 3000 rpm for 10 ~~minutes~~min and the absorbance of the supernatant read on a spectrophotometer (Shimadzu UV-2550). Chl-*a* concentrations were estimated using the trichromatic equations for methanol [22].

~~2.4.~~2.4 HRAP light attenuation and climate

Light attenuation through each HRAP water column were measured using 2π underwater sensors attached to a Quantum logger (Li-Cor Biosciences, Lincoln, Nebraska, USA). The vertical light attenuation coefficient (K_d) was calculated from the regression of log-transformed downwelling irradiance versus depth (Kirk 1994). Depth of the euphotic zone where subsurface light was 1% ($Z_{euphotic}$) was estimated from K_d [23]. The total light experienced by a cell moving up and down through the water column per day (E_{mix}) was calculated as:

$$E_{mix} = \left(100 \times \left(1 - e^{-K_d Z_{mix}}\right)\left(K_d Z_{mix}\right)^{-1}\right) \times daily\ surface\ irradiance$$

where Z_{mix} is the HRAP depth. Mean E_{mix} based on the 4-day period prior to biomass sampling was determined from total daily surface irradiance. Daily surface irradiance was recorded at an adjacent weather station.

~~2.5.~~2.5 Photosynthesis

Primary productivity (P) versus irradiance (E) curves were determined for each size HRAP by measuring the rate of oxygen evolution along a gradient of increasing irradiance. HRAP culture aliquots were placed in a oxygen chamber and irradiance levels were controlled through the oxyLab32 software programme (Hansatech Instruments Ltd., UK). Oxygen production was measured using a Clark-type fast response micro-sensor calibrated in 0 % and 100% air-saturated water (Unisense, Denmark). The total incubation time for each irradiance curve was kept to 15 ~~minutes~~min, to avoid rapid shifts in pH due to dissolved carbon depletion in the chamber. Photosynthetic measurements were undertaken during winter and summer.

The photosynthetic parameters α and P_{max} were estimated from replicate P-E curves by fitting the formula of Platt et al. (1980) using Sigmaplot graphing software (v 12.0):

$$P = P_{\max} \times \left[1 - \exp\left(\frac{-\alpha \times E}{P_{\max}}\right)\right]$$

where α is the slope of the linear portion of the P-E curve and shows the efficiency of photosynthesis under light-limiting conditions, while P_{max} is the point where the P-E curve levels off and represents the maximum rate of photosynthesis under light-saturated conditions [23]. E_k, the minimum saturation light intensity, defined as the light level at which photosynthesis shifts from light limitation to light saturation, was derived from the equation:

$$E_k = \frac{P_{\max}}{\alpha}$$

~~2.6.~~2.6 Statistical analyses

Statistical analyses were performed using analysis of variance (ANOVA). All statistical analyses were carried out using Statistica software (Statsoft Inc., Tulsa, OK, USA).

~~3.~~3 Results

~~3.1.~~3.1 Environmental variables

During winter, the morning temperature of the ponds was significantly lower ($p <$ ~~0~~.05) in the mesocosm HRAP than either the pilot or full-scale HRAPs, while in spring and summer, morning temperatures were consistently higher in the mesocosm HRAP than the pilot and full-scale HRAPs, but there differences were not significant (Table 1). Both HRAP pH and conductivity did not differ significantly between the three different size HRAPs (Table 1). Morning pH varied seasonally, with summertime being significantly higher ($p <$ ~~0~~.05) than winter, regardless of the HRAP size (Table 1). A similar seasonal trend was observed for temperature, with winter being significantly lower ($p <$ ~~0~~.01) than spring and summer, in all three size HRAP (Table 1).

~~3.2.~~3.2 Nutrient removal and removal efficiency

Regardless of the season, the NH_4-N concentrations in the full-scale HRAP effluent were significantly higher ($p <$ ~~0~~.01) than both the mesocosm and pilot-scale HRAPs, which did not differ from each other (Table 2). The effluent NH_4-N concentrations in all HRAPs during all seasons were significantly lower than the influent concentration (Table 2). In contrast, for all three seasons the effluent NO_3-N concentrations were significantly higher ($p <$ ~~0~~.01) in the mesocosm HRAP compared to the pilot-scale and full-scale HRAPs, which did not differ significantly from each other, nor the influent NO_3-N concentrations (Table 2). Mesocosm effluent NO_3-N concentrations increased from winter to summer, with summer concentrations significantly higher ($p <$ ~~0~~.01) than winter (Table 2). In order to assess the total removal rates of dissolved nitrogen, NH_4-N and NO_3-N were summed to give the dissolved inorganic nitrogen (DIN) concentration. In winter, the percentage of DIN removed was significantly lower ($p <$ ~~0~~.05) in the full-scale HRAP compared to the pilot-scale HRAP but not the mesocosm HRAP. In spring, the percentage DIN removed in the full-scale HRAP was significantly lower ($p <$ ~~0~~.01) than both the mesocosm and pilot-scale HRAPs and in summer the percentage removal rates were significantly lower than the mesocosm ($p <$ ~~0~~.05) and the pilot-scale ($p <$ ~~0~~.01) HRAPs (Table 2). The percentage DIN removed in the pilot-scale HRAP was significantly higher in both spring ($p <$ ~~0~~.05) and summer ($p <$ ~~0~~.01) compared with the mesocosm HRAP (Table 2).

~~Table 2.~~Table 2 Seasonal variation in nutrient removal in different sized HRAPs. Data are means ± standard deviations, $n = 32$. NH_4-N = ammoniacal nitrogen, NO_3-N = nitrate, DIN = dissolved inorganic nitrogen (NH_4-N + NO_3-N), DRP = dissolved reactive phosphorus.

alt-text: Table 2

Parameter	Winter			Spring			Summer		
	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale
Influent									
NH_4 (g m^{-3})	38.2 ± 5.8	38.2 ± 5.8	38.2 ± 5.8	35.3 ± 2.5	35.3 ± 2.5	35.3 ± 2.5	56.8 ± 8.3	56.8 ± 8.3	56.8 ± 8.3
NO_3 (g m^{-3})	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
DIN (g m^{-3})	40.2 ± 5.7	40.2 ± 5.7	40.2 ± 5.7	36.4 ± 2.4	36.4 ± 2.4	36.4 ± 2.4	57.7 ± 8.3	57.7 ± 8.3	57.7 ± 8.3
DRP (g m^{-3})	6.6 ± 1.1	6.6 ± 1.1	6.6 ± 1.1	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.5 ± 0.8	5.5 ± 0.8	5.5 ± 0.8
Effluent									
NH_4 (g m^{-3})	18.8 ± 4.8	20.1 ± 1.3	25.2 ± 3.7	10.4 ± 3.7	13.1 ± 1.5	19.8 ± 3.2	5.9 ± 2.5	4.7 ± 2.4	16.9 ± 3.0
NO_3 (g m^{-3})	5.1 ± 2.1	0.9 ± 0.3	0.9 ± 0.3	6.8 ± 2.0	1.3 ± 0.2	0.9 ± 0.2	8.2 ± 1.2	0.7 ± 0.1	0.9 ± 0.1
DIN (g m^{-3})	23.9 ± 3.9	21.0 ± 2.2	26.0 ± 3.5	17.1 ± 2.2	14.3 ± 1.4	20.7 ± 3.1	14.1 ± 2.4	5.5 ± 2.4	17.8 ± 2.8
DRP (g m^{-3})	4.3 ± 0.8	3.5 ± 1.2	4.4 ± 0.8	3.0 ± 0.3	3.0 ± 0.2	4.2 ± 1.0	3.2 ± 1.0	3.3 ± 1.7	3.1 ± 1.4
Percentage removal									
DIN	37 ± 3	45 ± 7	32 ± 5	52 ± 9	61 ± 4	41 ± 4	76 ± 5	90 ± 4	69 ± 7
DRP	32 ± 18	50 ± 25	32 ± 17	39 ± 6	40 ± 10	14 ± 24	42 ± 12	38 ± 28	42 ± 19
Nutrient removal efficiency (NRE)									
DIN	$0.018 \pm 2.4 \times 10^{-4}$	$0.022 \pm 1.1 \times 10^{-4}$	$0.018 \pm 4.2 \times 10^{-4}$	$0.021 \pm 1.3 \times 10^{-4}$	$0.021 \pm 1.4 \times 10^{-4}$	$0.018 \pm 2.3 \times 10^{-4}$	$0.029 \pm 5.4 \times 10^{-4}$	$0.030 \pm 3.1 \times 10^{-4}$	$0.033 \pm 2.1 \times 10^{-}$
DRP	$0.003 \pm 5.3 \times 10^{-5}$	$0.003 \pm 3.1 \times 10^{-5}$	$0.003 \pm 8.2 \times 10^{-5}$	$0.002 \pm 1.8 \times 10^{-5}$	$0.002 \pm 2.5 \times 10^{-5}$	$0.0009 \pm 5.0 \times 10^{-5}$	$0.0015 \pm 9.1 \times 10^{-5}$	$0.001 \pm 7.4 \times 10^{-5}$	$0.002 \pm 3.6 \times 10^{-}$

During winter and summer, the effluent dissolved reactive phosphorus (DRP) concentrations did not differ significantly between the HRAPs, while in spring, the DRP concentration in the full-scale effluent was significantly higher ($p < 0.05$) compared to both the mesocosm and pilot-scale HRAPs, which did not differ significantly from each other (Table 2). The percentage removal of DRP relative to the influent concentration was significantly lower ($p < 0.05$) in the full-scale HRAP compared to the other HRAPs during spring, but did not differ significantly between the HRAPs during winter and summer (Table 2).

The nutrient removal efficiency of DIN (NRE-DIN), i.e. the amount of dissolved nitrogen removed per unit organic matter, did not vary between the HRAPs regardless of the season (Table 2). The nutrient removal efficiency of DRP (NRE-DRP) showed similar patterns to NRE-DIN during winter and summer, with all similar removal efficiencies in all three HRAPs. However, during spring, the NRE-DRP was significantly lower ($p < 0.05$) in the full-scale HRAP compared to the other two scale ponds (Table 2).

3.3 Total suspended solids, organic matter and chlorophyll *a* biomass production

For all three seasons, both the total suspended solids (TSS) and organic matter concentrations were significantly lower ($p < 0.01$ except wintertime $p < 0.05$ compared to pilot-scale) in the full-scale HRAP compared to the mesocosm and pilot-scale HRAPs (Table 3). During summer, the TSS and organic matter concentrations were significantly higher ($p < 0.01$) in the pilot-scale HRAP compared to the mesocosm HRAP, but they did not differ significantly from each other during winter and spring (Table 3). During spring, the proportion of organic matter to the TSS was significantly lower ($p < 0.05$) in the mesocosm HRAP compared to the other two size HRAPs, which did not differ significantly from each other, while for the other two seasons, there was no difference amongst the HRAPs (Table 3). Chl-*a* biomass concentration was significantly lower ($p < 0.05$ in winter, $p < 0.01$ in spring and summer) in the full-scale HRAP compared to the mesocosm and pilot-scale HRAPs (Table 3). Chl-*a* biomass in the pilot-scale HRAP was significantly higher ($p < 0.01$) in the pilot-scale HRAP compared to the mesocosm HRAP during spring and summer, but did not differ significantly during winter (Table 3). Organic areal productivity (the amount of organic biomass produced per square meter of pond per day) was significantly lower in the full-scale HRAP compared to the other two size HRAPs, during winter ($p < 0.05$), spring ($p < 0.01$) and summer ($p < 0.01$) (Figure. 2). During summer, the organic areal productivity was significantly higher ($p < 0.01$) in the pilot-scale HRAP compared to the mesocosm HRAP, but did not differ significantly during winter and spring (Figure. 2). Chl-*a* areal productivity was significantly lower ($p < 0.05$ in winter, $p < 0.01$ in spring and summer) in the full-scale HRAP compared to both the mesocosm and pilot-scale HRAPs for all seasons, while the mesocosm was significantly lower ($p < 0.01$) than the pilot during spring and summer (Figure. 3).

Table 3. Table 3 Seasonal variation in microalgal productivity and light climate in different sized HRAPs. Data are means ± standard deviations, $n = 32$. K_d is the light attenuation co-efficient, $Z_{euphotic}$ is the depth above which photosynthesis > respiration, E_{mix} is the total amount of light to which a circulating cell is exposed.

alt-text: Table 3

Parameter	Winter			Spring			Summer		
	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale
Pond total suspended solids (g m^{-3})	131 ± 32	124 ± 31	107 ± 13	147 ± 17	144 ± 14	119 ± 20	211 ± 10	237 ± 25	168 ± 34
Pond organic matter (g m^{-3})	114 ± 19	111 ± 35	96 ± 11	120 ± 22	129 ± 14	106 ± 15	191 ± 11	215 ± 17	152 ± 30
Percentage organic matter	87 ± 8	90 ± 7	88 ± 5	82 ± 3	90 ± 4	89 ± 4	90 ± 3	91 ± 4	91 ± 5
Pond Chl *a* (mg m^{-3})	1996 ± 271	1808 ± 479	1541 ± 360	2306 ± 327	3001 ± 472	1602 ± 215	3627 ± 320	4301 ± 699	2600 ± 418
Ratio organic matter to Chl *a*	57	61	62	52	43	66	53	50	58
K_d (m^{-1})	22.0 ± 3.0	19.0 ± 5.6	13.4 ± 5.9	27.0 ± 6.4	31.1 ± 4.2	18.7 ± 2.7	34.4 ± 2.9	42.8 ± 6.3	27.5 ± 3.8
$Z_{euphotic}:Z_{total}$	0.70 ± 0.19	0.79 ± 0.18	1.38 ± 0.47	0.65 ± 0.25	0.50 ± 0.07	0.87 ± 0.16	0.49 ± 0.03	0.37 ± 0.06	0.57 ± 0.08
E_{mix} (mmol.d^{-1})	1.50 ± 0.71	1.68 ± 0.90	2.70 ± 1.44	3.41 ± 1.12	2.37 ± 0.33	3.92 ± 0.40	3.15 ± 0.40	2.23 ± 0.76	3.57 ± 0.33
E_{mix}:surface PAR	0.15	0.17	0.27	0.16	0.11	0.18	0.07	0.05	0.08

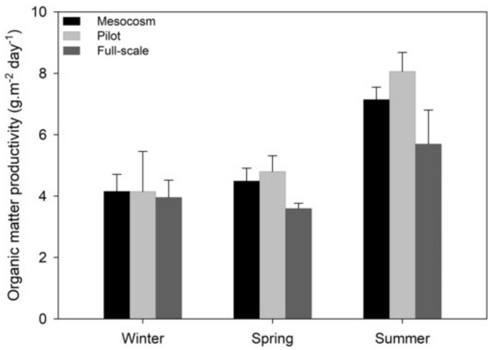
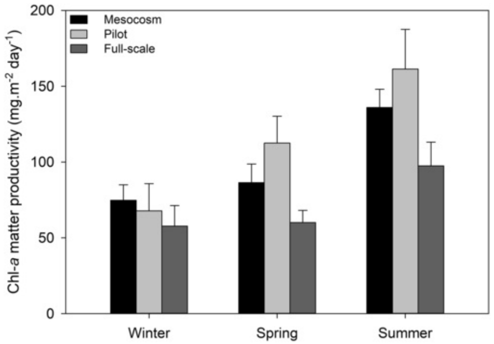


Figure 2. Fig. 2 Areal organic matter productivity in three different size HRAPs, over three seasons. $N = 32$, error bars indicate one standard deviation.

alt-text: Fig. 2



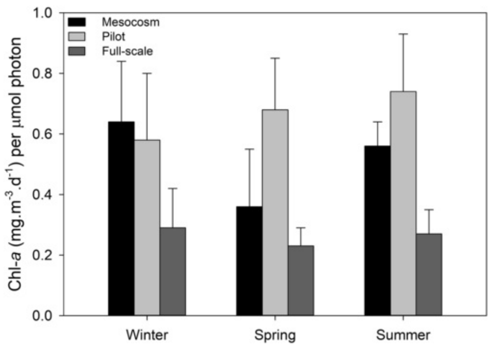
~~Figure 3.~~Fig. 3 Areal chl-*a* productivity in three different size HRAPs, over three seasons. $N = 32$, error bars indicate one standard deviation.

alt-text: Fig. 3

~~3.4.~~3.4 HRAP light climate and attenuation

K_d was significantly lower ($p<$~~0~~.01) in the full-scale HRAP compared to the other two size HRAPs for all three seasons, while K_d was significantly higher ($p<$~~0~~.01) in the pilot-scale HRAP compared to the mesocosm HRAP during summer (Table 3). For all three seasons, the proportion of $Z_{euphotic}$ to Z_{total} was significantly greater ($p<$~~0~~.01), in the full-scale HRAP compared to the other two size ponds, with $Z_{euphotic}$ exceeding Z_{total} during winter. This meant that there was sufficient light reaching the bottom of the full-scale HRAP as a result of the lower biomass and subsequently lower K_d (Table 3). For the pilot-scale HRAP, the proportion of $Z_{euphotic}$ to Z_{total} was significantly greater ($p<$~~0~~.01) than the mesocosm HRAP during spring and summer only (Table 3).

The mean daily irradiance within the water column, E_{mix}, was significantly higher ($p<$~~0~~.01) in the full-scale HRAP compared to the other two size HRAPs, while during spring and summer, the E_{mix} in the mesocosm HRAP was significantly higher ($p<$~~0~~.01) than in the pilot-scale HRAP (Table 3). The proportion of E_{mix} to pond surface PAR was low in all HRAPs ranging from 5~~–~~ to 27%, depending on the pond and the season (Table 3). The conversion efficiency of E_{mix}~~–~~, defined as the amount of chl-*a* biomass produced per unit of light in the water column, was significantly lower ($p<$~~0~~.01) in the full-scale HRAP compared with the other two size HRAP for all three seasons (Fig~~ure~~. 4). The conversion efficiency was higher in the pilot-scale HRAP than the mesocosm, during spring and summer but did not differ from each other during winter (Fig~~ure~~. 4).



~~Figure 4.~~Fig. 4 Seasonal conversion efficiency of light to algal biomass (defined as chl-*a* concentration) in differed size HRAPs. $N = 32$, error bars indicate one standard deviation.

alt-text: Fig. 4

~~3.5.~~3.5 Photosynthesis

In winter, both P_{max} (the maximum rate of photosynthesis) and E_k (the minimum saturating irradiance and photosynthesis is maximal) did not differ significantly between the three different size HRAPs (Table 4). α (a measure of photosynthetic efficiency at low light) was significantly lower in the full-scale HRAP compared to the pilot-scale ($p<$~~0~~.01) and the mesocosm scale ($p<$~~0~~.05) HRAPs, which did not differ significantly from each other (Table 4). In summer, P_{max} was significantly higher ($p<$~~0~~.05) in the full-scale HRAP compared to the pilot-scale HRAP, but neither pond differed significantly from the mesocosm HRAP (Table 4). α was significantly higher in the pilot-scale HRAP compared to the full-scale (p < ~~0~~.01) and mesocosm (p < ~~0~~.05) HRAPs, which also differed significantly (p < ~~0~~.05) from each other (Table 4). Summer-time E_k did not differ significantly between the three HRAPs (Table 4).

~~Table 4.~~**Table 4** Photosynthetic parameters during winter and summer in three different sized HRAPs. P_{max} is the maximum rate of photosynthesis, α is the efficiency of photosynthesis under light limiting conditions and E_k is the saturating light level. Data are means ± standard deviations, n = 12.

alt-text: Table 4

Parameter	Winter			Summer		
	Mesocosm	Pilot	Full-scale	Mesocosm	Pilot	Full-scale
P_{max} (µmol O_2 cm^{-2} ~~hr~~h^{-1})	4.8 ± 0.5	4.9 ± 0.4	5.2 ± 0.6	13.1 ± 0.7	12.8 ± 0.4	14.5 ± 1.1
α (µmol O_2 cm^{-2} ~~hr~~h^{-1}/µmol photons m^{-2} s^{-1})	0.034 ± 0.003	0.037 ± 0.002	0.029 ± 0.002	0.048 ± 0.003	0.053 ± 0.002	0.043 ± 0.002
E_k (µmol photons m^{-2} s^{-1})	173 ± 20	175 ± 24	181 ± 11	414 ± 27	400 ± 19	425 ± 23

~~4.~~4 Discussion

~~4.1.~~4.1 Nutrient removal efficiency

In wastewater treatment HRAPs, NH_4-N can be removed via three different pathways: i) nitrification (by nitrifying autotrophic bacteria), ii) assimilation into organic matter (by microalgae) and iii) by pH stripping when pH >9 [24]. pH mediated stripping of NH_4-N is not considered to be a major removal pathway for any of the three size HRAPs in the present study as daytime pH rarely exceeded 9, and for only short durations (data not shown). Nitrification played an increasing role in NH_4-N removal in the mesocosm HRAP, as indicated by the significantly higher nitrate-nitrate concentrations compared to the other two HRAPs and the reason for this is unclear. Nitrifying bacteria grow optimally between pH 7.5~~–~~-8.5 and are inhibited below pH 6.5 and above pH 10 [25,26]. In high rate algal mesocosms, Sutherland et al. [27] found that nitrification rates where highest when cultures were maintained between pH 7.0~~–~~-8.0 and lowest when cultures were maintained at either pH 6.5, or pH >9. Day-time pH values in all three ponds were within the range of 7.3~~–~~-9.3 over the course of the experiment (data not shown), suggesting that pH inhibition of nitrifying bacteria was not the reason for the differences between the ponds. In a study comparing the performance of two HRAPs under different operating regimes, García et al. [28] found that nitrification was promoted when pH-mediated NH_4-N stripping and algal uptake were both lowered. However, this was unlikely to be the explanation for the present study as both pH and algal NRE did not differ between the ponds. Similarly, Park & Craggs [29] found that nitrification rates increased under a combination of longer HRT and CO_2 addition due to higher bacterial biomass. However, this also does not appear to a likely explanation in the present study as ratios of organic matter to chl-*a* did not differ between the ponds. The final explanation for the higher nitrate concentrations in the mesocosm may be lower denitrification rates in this HRAP compared to the other two size HRAPs. Denitrification of nitrate to nitrogen oxide and~~/~~/or nitrogen gas, occurs under anoxic (low dissolved oxygen) conditions and, while rarely reported in HRAPs, has been reported occurring during the night in wastewater HRAPs, when the dissolved oxygen levels were < 2 mg/L (e.g. [13,29]). While night-time dissolved oxygen concentrations were not measured in the HRAPs during this study, it is plausible to suggest that the more frequent mixing of the wastewater in the mesocosm HRAP prevented dissolved oxygen levels dropping low enough to support denitrification. Further studies on the potential of night-time denitrification in wastewater HRAPs is needed to confirm, or not, this possible explanation.

Despite the significantly higher effluent DIN concentration in the full-scale HRAP compared to the other two HRAPs, the nutrient removal efficiency of DIN was similar across all three size ponds, for all seasons, suggesting similar nitrogen requirements by the cells in all three HRAPs despite the differences in the light climate and biomass between the ponds. Light limitation in dense microalgal cultures is often considered one of the main limiting factors in nitrogen uptake [24] and by this argument, we could have expected to see lower NRE-DIN in the HRAP with the highest K_d (pilot-scale) but this was not the case, despite the 1.6~~–~~-1.8 times difference in the E_{mix} between the HRAPs with the highest K_d and the lowest. Increased demand for nitrogen, such as an increased need for proteins by the photosynthetic apparatus as a result of increased photosynthetic potential at lower light [30], or the more favourable mixing frequency leading to faster growth rates may account for the similarities in NRE-DIN between the HRAPs despite differences in the light climate.

~~4.2.~~4.2 Total suspended solids, organic matter and chlorophyll *a* biomass production

For all three seasons, in all three ponds, organic matter (algal-bacterial) biomass concentrations were within, but at the lower end of, the reported range of biomass from previous studies in other temperate climate regions (e.g. [11,12,14,19,28]). Algal productivity was well below the theoretical maximum, regardless of the size of the HRAP. Higher biomass productivity would likely have been achieved in all three size HRAPs in this study with the addition of CO_2, with reported increases in algal-bacteria productivity increasing between 50 and 150 % compared to ponds without CO_2 addition in similar temperate climates (e.g. [16,29]). However, even with successful CO_2 addition across all three size HRAPs, algal productivity would have still been well below theoretical maximum. Reasons for this are explored in a number of publications on enhancing microalgal productivity in wastewater treatment HRAPs (e.g. [31]).

Both organic matter and chl-*a* biomass productivity responded to changes in HRAP pond size, with the lowest organic matter and chl-*a* biomass measured in the largest (full-scale) HRAP, regardless of the season. However, as HRAP size decreased, biomass production followed a unimodal distribution rather than a linear one. Light-limited microalgal biomass production in wastewater treatment HRAPs can be enhanced through modifications to the pond operation, including nutrient load, pond operation depth, vertical mixing frequency and hydraulic retention time [31]. Of these features, only vertical mixing frequency, or how often a parcel of water travels through the paddlewheel, which generates the vertical mixing, varied between the three size ponds. Vertical mixing occurred 130 times more frequently in the mesocosm than in the full-scale and occurred 7 times more frequently in the pilot-scale than the full-scale HRAPs.

Increased frequency of mixing can lead to increased biomass accumulation via two potential mechanisms. The first potential mechanism is decreased sedimentation losses of algal/bacterial biomass in the two smaller HRAPs compared to the full-scale HRAP. The density of most microalgae is greater than water, meaning that, without sufficient turbulence, they will passively sink out of the water column and settle on the pond floor, over time [32]. As HRAP size increases and the frequency of vertical mixing decreases, laminar flows and dead zones develop in the long channels, which can enhance sedimentation of cells on the pond floor [33]. On occasions, greater deposition of organic biomass has been observed in the full-scale HRAP compared to the pilot-scale and mesocosm HRAPs (authors own observations). In a high rate algal mesocosm based experiment, Sutherland et al. [34] demonstrated that biomass yields and sedimentation losses were directly related to the frequency of mixing events, with biomass increasing with increased frequency of mixing at the expense of sedimentation. These results are consistent with our findings with respect to the full-scale HRAP compared to the other two HRAPs; however, it does not account for the higher biomass in the pilot-scale compared to the mesocosm.

The second potential mechanism for enhanced biomass is that the increased mixing frequency resulted in the microalgal cells experiencing more favourable light conditions more frequently leading to higher net photosynthesis [35]. This is discussed further in Section 4.4.

~~4.3.~~4.3 HRAP light climate

Light is considered to be one of the main limiting factors in wastewater treatment HRAPs [36,37]. Over 80% of light absorption in wastewater HRAPs is by microalgal pigments [5], meaning that as microalgal biomass increases in the ponds, so too does light attenuation. In both the pilot-scale and mesocosm HRAPs, light was rapidly attenuated in the water column for all three seasons, with between $^1/_4$ and $^2/_3$ of the water column was at light levels below 1% of surface irradiance. In contrast, the full-scale HRAP had, at times, sufficient light to support photosynthesis throughout the entire water column. Reduced biomass concentration allows more light to penetrate deeper into the water column and increases the efficiency with which incident photons are used within the culture [37]. However, this was not the case in the present study, with the highest light utilisation efficiency recorded in the pond (pilot-scale HRAP) with the lowest E_{mix}, indicating that microalgae became more efficient at both light capture and utilisation, to compensate for the reduced light climate. These findings are consistent with previous studies on light absorption and utilisation across E_{mix} gradients in mass algal cultures [35,38,39]. This finding is discussed further in Section 4.4.

~~4.4.~~4.4 Photosynthesis

During both winter and summer, as light attenuation in the water column increased, there was an improvement in photosynthetic efficiency at low light (higher α), with the pilot-scale HRAP showing the highest α during both seasons. This resulted in increased conversion efficiency of light to biomass with increasing α, with the lowest conversion efficiency measured in the full-scale HRAP and the highest in the pilot-scale HRAP. Under low light intensity the photosynthetic rate is proportional to the photon flux, meaning that the quantum yield and light conversion efficiency remain maximal and microalgae with a higher α are more likely to have higher conversion efficiency under low light conditions than microalgae with a lower α [40]. One plausible explanation for this is that the increased frequency of vertical mixing resulted in more favourable fluctuations of medium light~~-~~/dark cycles for the cells in the smaller HRAPs compared to the full-scale HRAP. Improved photosynthesis at lower light levels under medium frequency light~~-~~/dark cycles is consistent with previous studies, where higher photosynthesis and biomass production occurred under low and moderate irradiances [34,35,41].

Differences in α between the pilot-scale and mesocosm HRAPs during summer but not winter were reflected in differences in biomass production and may be related to the frequency of light~~-~~/dark cycles experienced by the cells. The higher mixing frequency in the mesocosm HRAP meant that the microalgae spent less time in the dark, or low light, following saturating light exposure, compared to the microalgae in the pilot-scale HRAP. The dark period allows for the relaxation of the quenching processes and recovery of the electron transport rate [42]. Insufficient time in the dark may have left the cells from the mesocosm HRAP in a state of chronic photoinhibition, compared to cells in the pilot-scale HRAP, leading to decreased photosynthesis and biomass production [43].

During summer, the higher P_{max} in the full-scale HRAP suggested that the microalgae in this pond were better acclimated to higher irradiances than microalgae from the other two ponds. This was most likely due a combination of improved light climate within the pond (less attenuation) and a higher proportion of time cells spent in the upper proportion of the water column, due to reduced vertical mixing. However, the higher P_{max} did not appear to be sufficient to overcome the improved efficiencies at lower light in the other two HRAPs, as indicated by the lower biomass production in the full-scale HRAP.

For the pilot-scale and mesocosm HRAPs, improved photosynthetic efficiency at lower light levels, coupled with more frequent movement through more favourable light gradients and potential reduction in sedimentation rates, are all factors that most likely contributed to the higher biomass concentrations compared to the full-scale HRAP, despite the lower E_{mix} and P_{max}.

~~5.~~5 Conclusions

This study has demonstrated that both daily areal nutrient removal and biomass production were affected by the size of the HRAP. Areal NH_4-N removal was lowest in the full-scale system compared to the mesocosm and pilot-scale HRAPs, although nutrient removal efficiency did not differ amongst the different size pond. Nitrification~~/~~/denitrification was an important NH_4-N removal process in the smallest HRAP but became less important as HRAP size increased. Microalgal areal productivity was maximal in the medium size (pilot-scale) HRAP, suggesting that a combination of mixing frequency and higher photosynthetic efficiency under low light conditions were the main drivers of enhanced productivity in this HRAP compared to the mesocosm and, particularly, the full-scale HRAPs. Results from this study indicate that the differences in the daily wastewater treatment efficiency and biomass productivity per land area has implications for predictions from scale-up systems both in terms of both biomass yield and capital~~/~~/operational costs. Our results suggest that the optimum size for maximum productivity and wastewater treatment is considerably smaller than the full-scale systems based on its current design and operation. Further investigations on cost-effective optimisation of the microalgal performance in full-scale HRAPs is required in order to better predict the application of HRAP technology to industries producing low-value microalgal products.

Declaration of author contributions

DLS & RJC designed the study, while DLS, SH & JP carried out the experimental operation and data acquisition. DLS, PJR & RJC analysed and interpreted the data, DLS, PJR and RJC drafted the manuscript.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of competing interest

All authors declare there is no conflict of interest.

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Highlights

- Areal nutrient removal and biomass production varied with pond size.
- Nitrification~~-/-~~denitrification processes decreased with increasing pond size.
- Microalgal areal productivity was maximal in the 300 m^2 sized pond.
- Lowest nutrient removal and biomass production occurred in the 1 ha size pond.

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