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The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture

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ABSTRACT

The low volume batches of highly-concentrated wastewater discharged from land-based marine recirculating aquaculture systems are ideally suited for treatment by halophyte planted constructed wetlands. To evaluate the role of plants and the effect of planting density on yield and performance in small-scale saline constructed wetlands (CWs), $NH_4^+ + NO_3^- + NO_2^-$ = total dissolved inorganic nitrogen (TDIN) and dissolved inorganic phosphorus (DIP) were measured at regular intervals over 24 h periods. CWs were planted with the halophyte *Salicornia europaea* at high- and low-densities and were compared to the performance of unplanted controls. *S. europaea* plants were cropped regularly to assess potential commercial yield at the two densities. There was no significant effect of planting density on performance or crop yields and planted beds consistently outperformed the control beds removing 62.0 ± 34.6 mmol N m^{-2} d^{-1} (34–73% of influent TDIN) compared to 23.0 ± 26.8 mmol N m^{-2} d^{-1} (−1% to 41% of influent TDIN) by control beds. Results for DIP were less clear, significant removal occurred only once, with reduction of 18.3 ± 5.0 mmol P m^{-2} d^{-1} by planted beds and 18.1 ± 2.6 mmol P m^{-2} d^{-1} by the unplanted controls. The results demonstrate the effectiveness of halophyte-planted CW in treatment of marine aquaculture wastewater.

1. Introduction

Aquaculture production currently contributes almost half of all global fish production (60 million MT), and is expected to increase by a further 60–100% over the coming decades (FAO, 2010). The development of marine aquaculture, particularly of fish and crustaceans, has largely focused on the intensive production of carnivorous or omnivorous species in coastal cages and ponds. The intensification of production in land-based intensive marine recirculating aquaculture systems (RAS) is a relatively recent development and offers the potential for bio-secure, environmentally-contained production (McCarthy and Gardner, 2003; Park et al., 2008; Tal et al., 2009). This represents a step away from more traditional 'flow through' or 'open systems' that can discharge high volumes of wastewater to the environment, which are difficult to remediate (Folke and Kautsky, 1992). In contrast, recirculating aquaculture systems produce relatively small batches of highly concentrated effluents that are more amenable to treatment prior to discharge (Piedrahita, 2003), and that may be suitable for treatment in constructed saline wetlands.

Constructed wetlands (CW) are increasingly applied for the treatment of numerous types of effluent from agriculture, heavy industry, municipal and intensive freshwater land-based aquaculture (Vymazal, 2005a,b). There has been considerable research into optimal wetland design, plant assemblages and sediment types (Tanner, 1994, 1996; Tanner et al., 2012; Vymazal, 1996, 2002, 2005a,b; Lin et al., 2002a–c, 2005; Schulz et al., 2003; Haddad et al., 2006), however, much of this work has been into systems planted with glycophyte plant species for remediation of freshwater effluents which is not directly transferable to saline systems. There is growing interest into the potential of constructed wetlands planted with facultative or obligate halophytes for the remediation of saline effluent and marine intensive land-based aquaculture wastewaters (Brown et al., 1999; Lin et al., 2002a,b, 2003, 2005; Lymbery et al.,

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2006, 2013; Sousa et al., 2011; Calheiros et al., 2012; Webb et al., 2012).

Since plant roots are the major source of oxygen in subsurface flow (SSF) wetlands, aside from atmospheric diffusion (Brix, 1994) the role of higher plants is crucial in establishing a successful CW. Plants in CWs not only increase microbial assemblages in the root zone, release oxygen into the sediment and assimilate nutrients, but they also maintain the hydraulic conductivity of the substrate (Brix, 1994; Haberl et al., 1995). Additionally they can provide a commercial crop that can result in above-ground biomass being almost completely removed from the system (Webb et al., 2012). Reported nitrogen uptake by macrophytes in CWs varies greatly, but is generally in the range of 10–30% of influent concentrations (Koottatep and Polprasert, 1997; Burgoon et al., 1991; Haddad et al., 2006; Lin et al., 2002c; Brown et al., 1999). However, much higher values have been reported with direct plant uptake of nitrogen reaching 85–100% of influent total dissolved inorganic nitrogen (TDIN) (Rogers et al., 1991 as cited in Hunter et al., 2001; Romero et al., 1999; Webb et al., 2012).

Previous research into CWs planted with emergent macrophytes (e.g. *Phragmites* spp., *Typha* spp.) for treatment of saline/brackish wastewater is limited (Lin et al., 2002a,b; 2003; 2005; Lymbery et al., 2006; Klomjek & Nitisoravut, 2005), and there are even fewer studies of the use of halophyte planted CWs for saline wastewater remediation (Brown et al., 1999; Brown & Glenn, 1999; Sousa et al., 2011; Calheiros et al., 2012). The present study and a previous work (Webb et al., 2012) report findings from a 3 year investigation evaluating the potential of constructed wetlands planted with the halophyte *Salicornia europaea* agg. (L) for use as a biofilter for wastewater discharged from a commercial pilot RAS unit growing marine shrimp (*Litopenaeus vannamei*) at high stocking densities. Recent results with *S. europaea* indicate that high levels of nutrient removal can be achieved (Webb et al., 2012) and the present study is a more detailed investigation into rates of removal over daily treatment cycles, CW effectiveness under previously simulated high nutrient loading and the role of the halophyte *S. europaea* in the CW system.

The objectives were to:

1. Investigate the effects of planting density on removal rate of TDIN and dissolved inorganic phosphate (DIP).
2. Evaluate the effect of planting with *S. europaea* agg. (L) through comparison of overall uptake and removal rates of TDIN and DIP to unplanted control CWs.

2. Materials and methods

2.1. Filter bed construction

To investigate the effect of planting density on nutrient uptake, 9 small scale replicate subsurface flow CWs were installed in a single span polytunnel, 5 m × 20 m ($W \times L$) on an intensive marine fish farm in Pwllheli, North Wales, UK. Each CW had a 4 m^2 surface area and 1.2 m^3 volume (1 m × 4 m × 0.3 m, $W \times L \times H$). The CWs were scaled down replicas of those described in detail by Webb et al. (2012) and were laid out in a randomised block design, with the polytunnel divided into 3 sections and each CW type (high-/low-density planting and unplanted control) was represented in each of these (Fig. 1). In those beds designated for high-density planting, the mixed M grade quarry sand layer was reduced to 60 mm depth. In addition, the original irrigation and drainage system was modified enabling the separate supply of waste water to and discharge from each group of 3 CW (Fig. 1).

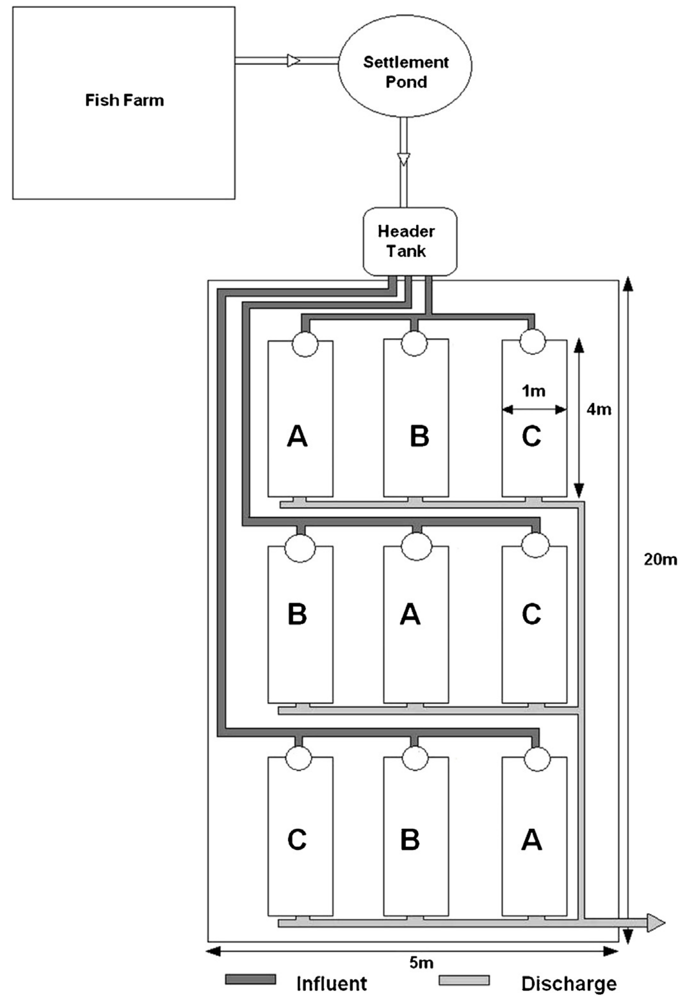

Fig. 1. A schematic representation of the layout of the replicate constructed wetlands. The polytunnel was split into 3 blocks and each constructed wetland type (A = high-density, B = low-density, C = control) was present in a section of each.

2.2. Plant production

Germination and early growth took place in a controlled environment greenhouse using *S. europaea* agg. (L) seeds sourced from 3rd generation cultivated plants under a 16:8 light:dark photoperiod. The minimum temperature was maintained at 25 °C during the light period and 20 °C during the dark period.

2.3. High-density planting system

In the high-density planting treatment, injection moulded polypropylene 'Empot' carrier trays, 555 mm × 310 mm × 30 mm ($L \times W \times H$) (http://www.lbsgardenwarehouse.co.uk, Ref. DTRS918) were lined with 17 g m^{-2} geotextile (http://www.lbsgardenwarehouse.co.uk, frost protection fleece Ref. R-F2050) and filled with ≤60 mm, mixed M grade quarry sand. *S. europaea* seeds were sown directly onto the sand surface to replicate high-density aggregations and irrigated with fresh water until germination at which point they were grown hydroponically in a Tropicmarin© saline solution (salinity of 10) with Phostrogen© soluble plant feed (N:P:K 14:10:27 + trace elements; Bayer CropScience Ltd., Cambridge, UK). Two months after germination, trays were transported to the experimental system and placed in three of the nine CWs, 21 trays (7 × 3), covered the surface of a single CW giving a density of approximately 10,000 plants m^{-2}.

2.4. Low-density planting

For the low-density planting, seeds were sown onto the surface of Desch Plantpak P576 plug trays filled with ≤6 mm, mixed M grade quarry sand, and irrigated with fresh water. Two weeks after germination, the seedlings were thinned out to 1 plant per plug and grown hydroponically in a saline solution (salinity of 10) using with Phostrogen soluble plant feed (N:P:K 14:10:27 + trace elements; Bayer CropScience Ltd., Cambridge, UK). Two-month old plants were transplanted into the CWs at a density of 200 plants m^{-2} on 19th May 2008.

2.5. Plant growth

Three weeks after introduction into the system, on June 9, planted CWs were cropped, with all growth 10 cm above the sediment surface being removed. The total plant biomass removed from each filter bed was termed "plant yield". Cropping was repeated every three weeks, giving a total of four harvests.

2.6. Wastewater

Wastewater was generated by a commercially-operating intensive recirculating marine aquaculture facility (Llyn Aquaculture Ltd.) growing marine shrimp (*L. vannamei*) at high stocking densities. Seawater supply into the facility was pumped from Cardigan Bay, UK to a large outdoor concrete holding tank prior to pumping into the farm system. Standard water management for the aquaculture facility (Llyn Aquaculture Ltd.) involved once-daily discharge of batches of wastewater flushed from sediment traps and weekly discharge of batches of wastewater produced from the back-flush of the biofilter and bead filter units into an outdoor, uncovered settlement/treatment pond system. The experimental CWs were testing the feasibility of halophyte CWs as a 'down-stream' wastewater treatment system processing batches of water pumped (400 W 240 V automatic dirty water pump; www.screwfix.com) from the initial settlement pond (10 m × 4 m × 0.5 m) into a covered and lined 12 m^3 header tank, via a 300 L vortex separation tank.

2.7. Irrigation

From the header tank, wastewater was supplied to the irrigation system by (3 400 W 240 V automatic dirty water pumps; www.screwfix.com) via 25 mm pipe. Each pump supplied a lateral block of 3 CWs. The irrigation system consisted of a capillary system of flexible pipe (JG speedfit BPEX barrier pipe) running overhead before dropping down and terminating in an isolating tap/flow regulator and an inline Kent V110 25 mm volumetric flow meter at each filter bed. The CWs were filled to just below the surface of the upper sand layer to prevent algal fouling of the filter surface. The perforated pipe at the end of each CW housed the drainage point was fitted with a removable stand pipe/overflow that could be removed to allow complete draining. During filter operation, water was continuously circulated below the surface within each filter bed, using an 18 W submersible pump (Hozelock Cascade 700) fitted within one of the vertical perforated pipes to move water via a 15 mm garden hose from one to the other, drawing water horizontally through the subsurface stone layer of the filter. The CWs were drained after 48 h prior to refilling with the next batch of wastewater.

2.8. Environmental monitoring and water sampling

Ambient air temperature and humidity were measured on a Tinytag View 2 Int Temp/RH (TV-4500) data logger (http://www.geminidataloggers.com). The *in situ* water pH and temperature measurements were taken with a Hanna HI 98127 pH meter and along with water samples were taken from each of the 9 CWs at 0, 3, 6, 9, 12 and 24 h after filling. Based on previous findings (Webb et al., 2012), 24 h was deemed an optimum time frame to observe a linear rate of reduction in inorganic nitrogen. Sampling was done shortly after a harvest and shortly before the next one over a 57 d period. Prior to filling, the CWs were drained back into the treatment pond system to remove water remaining from the previous flooding. Immediately after re-filling, triplicate 20 ml water samples were taken from each filter bed using a 20 ml disposable sterile plastic syringe fitted with a 30 cm length of Teflon tubing. Sample water was flushed through the syringe and tubing twice before the sample was then passed through a Whatman GD/X syringe filter (25 mm, 0.45 μm pore size) into an acid washed (10% HCL) 20 ml plastic bottle. Samples were frozen (−20 °C) until analyses. Sample salinity was measured during analysis using a WTW-Tetracon 325 conductivity meter.

2.9. Water analysis

Dissolved ammonium (NH_4^+) was determined with the fluorimetric method of Holmes et al. (1999) using a Hitachi F2000 fluorescence spectrophotometer. The other major dissolved inorganic nutrients, nitrate, nitrite and phosphorus, were determined using standard colourimetric methodology (Grasshoff et al., 1983) as adapted for flow injection analysis (FIA) on a 5–channel Lachat Instruments Quick-Chem 8000 autoanalyzer (Hales et al., 2004). These analyses were monitored daily using reference oceanic water (batch 54, Scripps Institute of Oceanography, University of California, San Diego) as an external standard and riverine water collected on 24/04/07 from River Clwyd, UK, as an internal standard. The external standard yielded: $[NO_2^-] = 0.02 \pm 0.02\ \mu mol\ kg^{-1}$ ($n = 34$; reported $[NO_2^-] = 0.00\ \mu mol\ kg^{-1}$), $[NO_3^-] = 1.27 \pm 0.15\ \mu mol\ kg^{-1}$ ($n = 31$; reported $[NO_3^-] = 1.25\ \mu mol\ kg^{-1}$), $[DIP] = 0.34 \pm 0.03\ \mu mol\ kg^{-1}$ ($n = 27$; reported $[DIP] = 0.39\ \mu mol\ kg^{-1}$). The internal standard yielded, $[NO_2^-] = 1.70 \pm 0.12\ \mu mol\ kg^{-1}$ ($n = 12$), $[NO_3^-] = 117 \pm 3\ \mu mol\ kg^{-1}$ ($n = 17$), $[DIP] = 2.60 \pm 0.18\ \mu mol\ kg^{-1}$ ($n = 11$).

2.10. Statistical analysis

Initially, TDIN ($NO_3^- + NO_2^- + NH_4^+$) and DIP data were analysed using the Pearson product moment correlation coefficient. Parametric data that showed a linear relationship underwent further analysis using the general linear model (GLM). Where there was no significant linear relationship i.e. a linear change in nutrient concentration over time, further analysis was not required. The GLM analysis gave rate of removal (slope), and starting concentration (intercept) for each planting density and the control and allowed comparison of slopes and intercepts between the planting densities and the control. Following each GLM analysis residuals were checked for approximate normality and tested for homogeneity of variance. For DIP, where data sets showed a linear relationship and were normally distributed and had homogeneity of variance they underwent further analysis using the general linear model (GLM). For all other data sets (3, 10 and 29 July) there was no significant change in DIP concentrations over time, and further analysis was not required.

Table 1
The mean (±SD) daily water temperature (°C) pH and salinity and the concentration (μmol l^{-1}) of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), TDIN and DIP in the influent entering constructed wetlands planted with *Salicornia europaea* on sample dates July 3, July 10, July 29 and October 8.

	July 3	July 10	July 29	October 8
Temp (°C)	19.6 ± 1.4	20.6 ± 2.2	23.7 ± 1.5	14.6 ± 1.6
pH	7.6 ± 0.3	7.5 ± 0.4	7.8 ± 0.2	7.6 ± 0.2
Salinity	23.9 ± 0.4	28.1 ± 0.7	31.7 ± 0.6	26.0 ± 0.8
NH_4^+	904.6 ± 75.4	792.0 ± 49.4	1689.1 ± 228.5	1163.9 ± 90.4
NO_3^-	106.0 ± 21.2	2409.9 ± 224.5	3.3 ± 1.7	6.5 ± 1.5
NO_2^-	53.3 ± 15.1	419.9 ± 53.4	0.8 ± 0.4	0.4 ± 0.1
TDIN	1063 ± 66.6	3621 ± 210.6	1692 ± 228.9	1170.7 ± 89.8
DIP	366.1 ± 129.6	329.1 ± 39.1	300.4 ± 28.4	318.8 ± 45.5

For TDIN, with the exception of the control beds on two sampling dates, all data sets exhibited a linear relationship and underwent further analysis using the general linear model (GLM). Where the data sets for the control beds were not linear, they were included in the GLM analysis alongside the planted data, but only the planted data sets were compared.

To analyse changes in concentrations of NH_4^+, NO_2^- and NO_3^- data sets were each tested for normality then underwent linear regression to check for significant change ($p < 0.05$) over the sampling period.

To compare mean yield (FW kg m^{-2}) each data set (high/low) were checked for approximate normality and tested for homogeneity of variance, then analysed with ANOVA.

Removal efficiency was calculated by dividing the difference between the influent and effluent concentrations by the influent concentration, then multiplying by 100.

3. Results and discussion

3.1. Environmental variables

Air temperature in the polytunnel showed a consistent trend: daily maxima were measured between 12:00 and 15:00 (hours 3 and 6) and daily minima at around 24:00 (hour 12). Throughout July, the daily temperature ranged from 12.5 to 54.2 °C dropping to a range of 7.3–38.5 °C in October (Fig. 2).

3.2. Wastewater characteristics

The influent wastewater nutrient concentrations (μmol l^{-1}) and the mean daily water temperature salinity and pH in the constructed wetlands is summarised in Table 1. Within the aquaculture facility, biofilters maintain the nitrogen at safe levels for the farmed species, converting toxic NH_4^+–NO_3^-. Although backflushing produces NO_3^--rich wastewater, following discharge to the settlement pond this may be converted back into NH_4^+ so that overall, with the exception of July 10, NH_4^+ was the major constituent of the influent TDIN with NO_3^- and NO_2^- as minor components. The high NO_3^- values observed on July 10 reflect the variability in nutrient composition associated with actual fish-farm wastewater, with ratios of N-forms in the initial receiving pond varying according to residence time and frequency of discharge of new wastewater. The observed nutrient concentrations are similar to the average values observed following addition of artificial fertiliser in a similar system (Webb et al., 2012). Concentrations fall within the ranges reported in previous RAS studies (Brown and Glenn, 1999; Pagand et al., 2000; Lin et al., 2002a–c, 2005), but are high in comparison to values for several other RAS systems (Lymbery et al., 2006; Zachritz et al., 2008; Sousa et al., 2011).

3.3. Comparison of planting density

Overall there was no significant difference between the mean removal rates of 2.8 ± 1.2 mmol N m^{-2} h^{-1} by high-density beds and 2.5 ± 0.8 mmol N m^{-2} h^{-1} by those at low-density. Equally, there was no difference in the mean uptake of 65.3 ± 34.4 and 58.8 ± 35.7 mmol N m^{-2} d^{-1} (high- and low-density respectively) (Table 1). This indicates that planting density had no apparent effect on the rate of TDIN removal with no significant difference ($p > 0.05$) in performance of the CWs planted at high- and low-density.

The plant yield data, supports these findings: however, Fig. 3 illustrates that at Harvest 1 (June 9), three weeks after transplantation into the system, 1 month prior to the first water sampling date, the mean yield of 3.1 ± 0.4 FW kg m^{-2} taken from the beds planted at a high-density was significantly higher ($p < 0.05$) than the 0.4 ± 0.2 FW kg m^{-2} taken from the low-density beds. The difference in the yields between the two planting densities is most likely to be a reflection of the greater plant biomass at start-up when using the tray system. However, by harvest 2, five days after the first water sample was taken, this 'headstart' was no longer evident and the yields did not differ significantly ($p > 0.05$) between

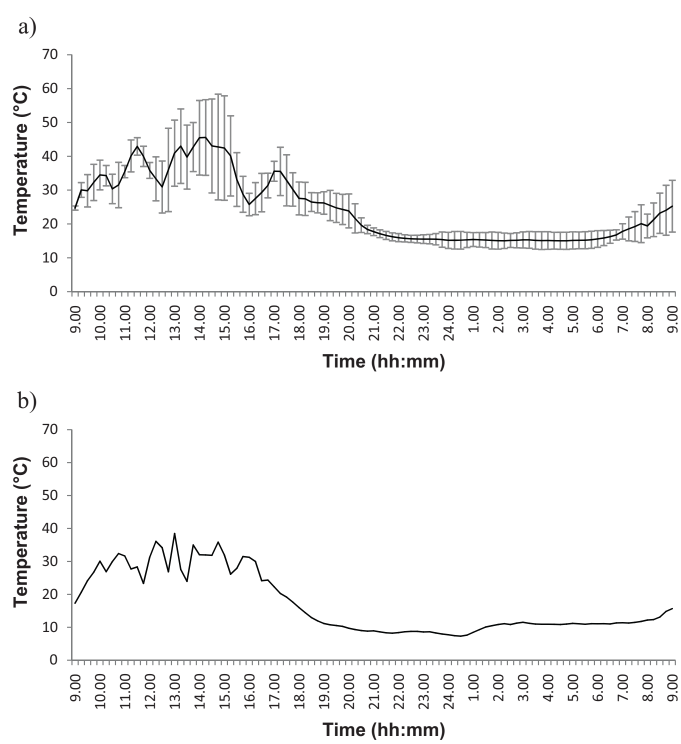

Fig. 2. The air temperature (°C) in the polytunnel (a) mean (±SD) for sampling periods July 3, 10 and 29 and (b) October 8. Temperature was logged at 9 am then every 15 min for 24 h.

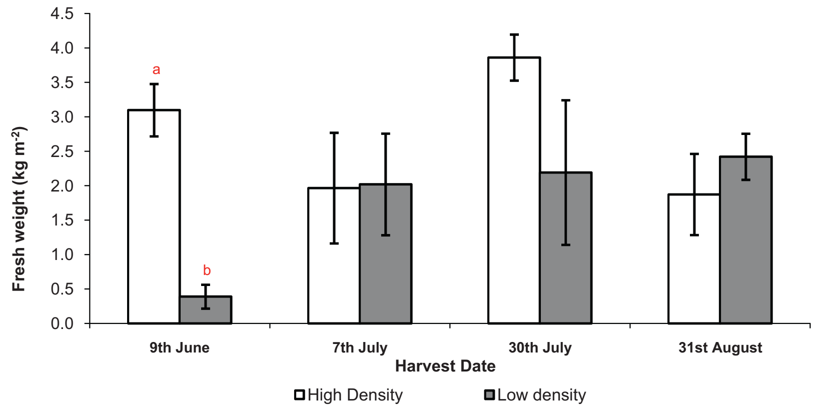

Fig. 3. The mean (±SD) yield (FW kg m^{-2}) of *Salicornia europaea* agg. (L) harvested every 3 weeks from triplicate constructed wetlands, planted at high (<10,000 m^{-2}) and low (200 m^{-2}) density. For each harvest date, means with different letters indicate significant difference (1-way ANOVA $p < 0.05$) an absence of letters indicates no significant difference between planting densities.

the two planting densities (see Fig. 3). If calculated over the four harvests, the cumulative high-density bed yield (sum of each harvest yield) was 10.1 ± 0.1 FW kg m^{-2} significantly higher than the 7.0 ± 2.0 FW kg m^{-2} produced by the low-density beds. However, if the first harvest (June 9) yield data is removed from the calculation, and only July harvests considered, there were no significant difference between the 7.7 ± 1.0 and 6.6 ± 1.8 FW kg m^{-2} produced by the high- and low-density planting. Due to the lack of significant difference between the removal rates of the high- and low-density planted beds, these data were pooled, termed 'planted' and compared directly with the control.

3.4. Performance of planted beds

Pooled uptake (Table 2) show planted beds significantly outperformed control beds removing 62.0 ± 34.6 mmol N m^{-2} d^{-1} (738 ± 318 μmol TDIN l^{-1} d^{-1}) over the experimental period compared to the 23.0 ± 26.8 mmol N m^{-2} d^{-1} (224 ± 212 μmol TDIN l^{-1} d^{-1}) by the control beds. These figures compare well with those measured by Webb et al. (2012) where CWs planted with *S. europaea* removed between 243.7 ± 67.5 μmol TDIN l^{-1} d^{-1} and 1833 ± 816 μmol TDIN l^{-1} d^{-1} under low and high TDIN loading regimes. There was a linear relationship between TDIN loading rate and removal rate, with removal rates increasing with increasing nitrogen loading (Fig. 4). However, the percentage removal efficiency (Table 2) shows that under the hydraulic operating system applied, the beds were unable to completely assimilate the high concentrations of TDIN

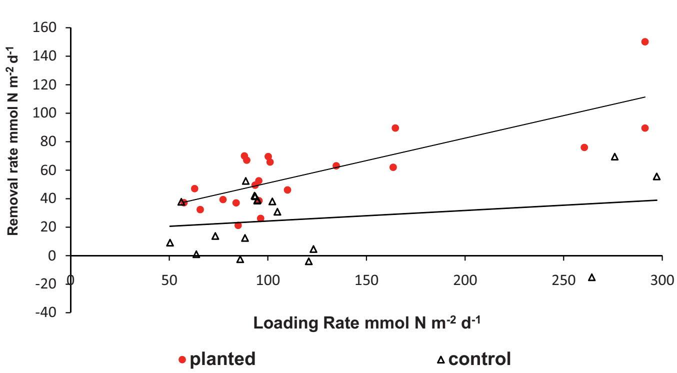

Fig. 4. The relationship between the TDIN loading rate and removal rate in wastewater over a growing season in 2008 (planted $R^2 = 0.6248$, control $R^2 = 0.0549$). NB. Points have been included from unreported sampling dates.

with efficiency (in terms of residual TDIN concentration in the effluent) declining with increasing N loading.

A maximum removal rate of 105 mmol N m^{-2} d^{-1} was achieved by the CW during a period of peak N loading, when influent TDIN ranged between 277.2 ± 7.2 and 284.3 ± 12.6 mmol N m^{-2} d^{-1} and is in line with the findings of other studies (Lin et al., 2002a; Webb et al., 2012). However, this was also the lowest removal efficiency seen with planted beds removing 33.9% of influent TDIN with discharge levels of 180.8 mmol N m^{-2}. The greatest efficiency was measured under the lowest loading of 83.9 mmol N m^{-2} with planted beds removing 73.1% of influent TDIN with only 20.3 mmol N m^{-2} discharged to waste. Planted beds had the slowest removal rate of 1.75 mmol N m^{-2} h^{-1} in the late season (October 8). Control beds also had peak TDIN removal rates during the period of peak TDIN loading with a removal rate of 69.6 mmol N m^{-2} d^{-1} being measured at an N loading of 276 mmol N m^{-2} d^{-1}.

Over the vegetative growth period the planted beds consistently outperformed the control beds with planted beds removing between 34% and 73% of influent TDIN and control beds removing between −1% and 27% of influent TDIN. This is consistent with other studies in freshwater or low salinity systems: Sousa et al. (2011) found net removal of nitrogen was greater in planted beds while Lin et al. (2002c) achieved 85–97% reduction of NO_3^- in planted beds compared to 10% and 36% in covered and uncovered control beds. Hunter et al. (2001) found beds containing vegetation removed 38% more NH_4^+ than those without vegetation. Similarly Koottatep and Polprasert (1997) and Burgoon et al. (1991) reported plant assimilation and uptake as between 10% and 30% of total nitrogen retention by the CW (as cited in Schulz et al., 2003).

Fig. 5 shows the change in TDIN concentration measured in planted and control beds over 24 h throughout the vegetative growth stage of the present experiment (July 3–29) and late season in October. During the July samplings, planted beds consistently had a significant decrease in TDIN concentration over each 24 h monitoring period (Fig. 5a–c). By comparison, control beds only had a significant removal of TDIN at the first sampling date (Fig. 5a). During the consecutive summer sampling periods there was no significant change in TDIN over time in the control beds (Fig. 5b and c). However, by October when the plants were entering senescence, and vegetative growth and associated N uptake was reduced, although there appears to be a difference in TDIN removal rates between planted and unplanted beds (Table 2) GLM output showed no significant difference between any of the CWs whether planted or not.

Throughout the high temperature periods in July there was no significant change ($p < 0.05$) in DIP concentrations over the

Table 2
The influent TDIN load, uptake and removal rate over a 24 h period by unplanted (control) constructed wetlands and by constructed wetlands planted with *S. europaea* at high (>10,000 m^{-2}) and low (200 m^{-2}) densities on sample dates July 3, July 10, July 29 and October 8.

	Influent load		Uptake		Removal efficiency		Removal rate	
	mmol m^{-2} d^{-1}	SE coef	mmol m^{-2} d^{-1}	SE coef	%	SD	mmol m^{-2} h^{-1}	SE coef
3 July								
High	81.10	3.1	70.28[a]	6.3	81.8	8.0	2.93	0.26
Low	86.61	2.6	57.05[ab]	5.8	64.5	6.7	2.38	0.24
Planted	83.86	0.2	63.57[c]	6.4	73.1	11.5	2.65	0.40
Control	85.48	3.7	34.97[b]	7. 4	12.7	20.4	1.46	0.31
10 July								
High	276.50	7.0	105.98	17.7	37.5	8.8	4.42	0.74
Low	284.26	12.6	85.06	25.5	30.3	24.5	3.54	1.06
Planted	280.38	3.9	99.54	18.8	33.9	16.9	3.98	0.62
Control	277.18	7.2	29.92*	14.4	0.3	16.3	1.54	0.61
29 July								
High	129.62	7.7	44.08	15.2	44.0	15.8	1.84	0.63
Low	131.84	8.9	52.39	17.9	46.6	13.4	2.18	0.75
Planted	130.73	0.8	46.28	17.7	45.3	13.8	2.01	0.25
Control	122.7	4.0	−4.91*	8.1	−0.7	5.0	−0.20	0.34
8 October								
High	97.68	3.9	43.47	7.8	44.9	8.7	1.81	0.33
Low	97.89	3.3	40.36	6.7	37.5	28.3	1.68	0.28
Planted	97.79	0.4	41.65	6.5	41.2	14.9	1.75	0.09
Control	95.66	2.4	26.73	4.8	26.9	11.8	1.11	0.20
Overall								
High	142.26	77.5	65.31	34.4	48.3	20.2	2.75	1.22
Low	143.74	81.2	58.75	35.7	45.2	20.6	2.45	0.79
Planted	143.00	78.0	62.0[a]	34.6	46.8	15.3	2.60	0.97
Control	137.71	82.9	23.0[b]	26.8	18.5	20.2	0.98	0.81

Different superscript letters indicate significant difference between CW types ($p < 0.05$).
* Indicates no linear change in concentration over time hence no comparative analysis between filter beds (significant difference is inferred).

24 h sampling period in any of the treatments, however by the late season (October 8) concentrations of DIP in the CWs reduced significantly with a mean DIP uptake of 15.9 ± 5.1 and 20.7 ± 4.4 mmol N m^{-2} d^{-1} by planted beds (high and low respectively) and 18.1 ± 2.6 mmol N m^{-2} d^{-1} by the unplanted controls (Table 3). There was no significant difference ($p > 0.05$) between the removal rates achieved by any of the beds whether planted or not and this uptake equated to a removal efficiency of 64.3 ± 13.8% of influent DIP averaged over the planted beds (64.3 ± 13.8% and 69.5 ± 10.1% high and low respectively) and 66.6 ± 7.2% of influent DIP in the control beds. This significant reduction in phosphate in all the CWs (Table 3) would call into question the role of the *Salicornia* plants in DIP removal. In a previous study (data not shown), Webb et al. (2012) found that at influent DIP concentrations of 35–90 μmol l^{-1} CWs planted with *S. europaea* removed 2.8 ± 1.2–6.6 ± 3.1 mmol m^{-2} d^{-1} DIP from influent waste water, 73% of which was retained in plant tissue. In this study, the much greater concentration of DIP in the influent (314.1 μmol l^{-1}) and variance in influent and effluent values may have masked rates of DIP removal in that range.

Although there is evidence to suggest that sorption of phosphorus to the CW filter media can play an important role in phosphorus removal this is usually related to the inherent characteristics of the substrate (Arias et al., 2001) and would therefore be apparent from the outset of the experiment.

3.5. Role of Salicornia in constructed wetlands

Research into the role of plants in CWs tends to have focussed on freshwater systems with wetland species such as *Phragmites* and *Typha* as being essential components of the CW ecosystem. However, the literature suggests that rather than assimilating inorganic nitrogen these plants, facilitate the establishment of the microbial communities responsible for nitrogen removal (Faulwetter et al., 2009) via the release of oxygen from their root system into the rhizosphere (Brix, 1997). This implies that actual plant uptake may account for a relatively small fraction of the overall N removal rate (Kadlec and Knight, 1996). Tanner et al. (2002) found that net plant N uptake by *Schoenoplectus tabernaemontani* represented less than 25% of the overall removal by a subsurface flow cascade CW. In a study comparing plant species and planted and unplanted CWs, Lin et al. (2002a–c) found with a loading rate of 1.35 g N m^{-2} d^{-1} (96.4 mmol N m^{-2} d^{-1}), *Phragmites* planted beds removed 73% of TDIN and of that, 11% was uptake by the plants. However, all of

Table 3
The influent DIP load, uptake and removal rate over a 24 h period on October 8 by unplanted (control) constructed wetlands and by constructed wetlands planted with S. *europaea* at high (>10,000 m^{-2}) and low (200 m^{-2}) densities on sample dates July 3, July 10, July 29 and October 8.

	Influent load		Uptake		Removal efficiency		Removal rate	
	mmol m^{-2} d^{-1}	SE coef	mmol m^{-2} d^{-1}	SE coef	%	SD	mmol m^{-2} h^{-1}	SE coef
8 October								
High	24.14	1.5	15.89	5.1	64.3	13.8	0.62	0.1
Low	28.13	1.1	20.70	4.4	69.5	10.1	0.81	0.1
Planted	26.13	0.1	18.30	5.0	66.9	11.2	0.71	0.1
Control	26.41	1.3	18.10	2.6	66.6	7.2	0.67	0.1

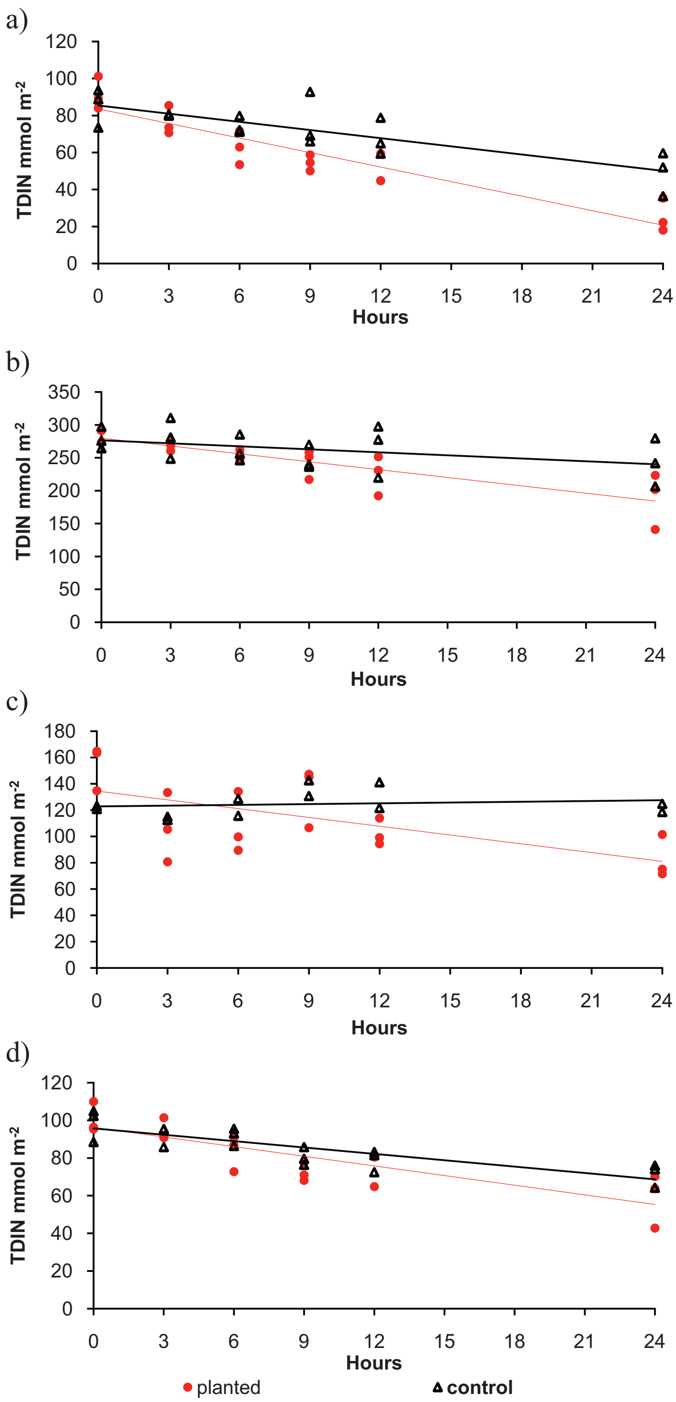

Fig. 5. The TDIN load ($mmol\ m^{-2}$) observed over a 24 h period in constructed wetlands planted with *S. europaea* agg (L) and in those left as unplanted controls on samples dates commencing (a) July 3, (b) July 10, (c) July 29 and (d) October 8.

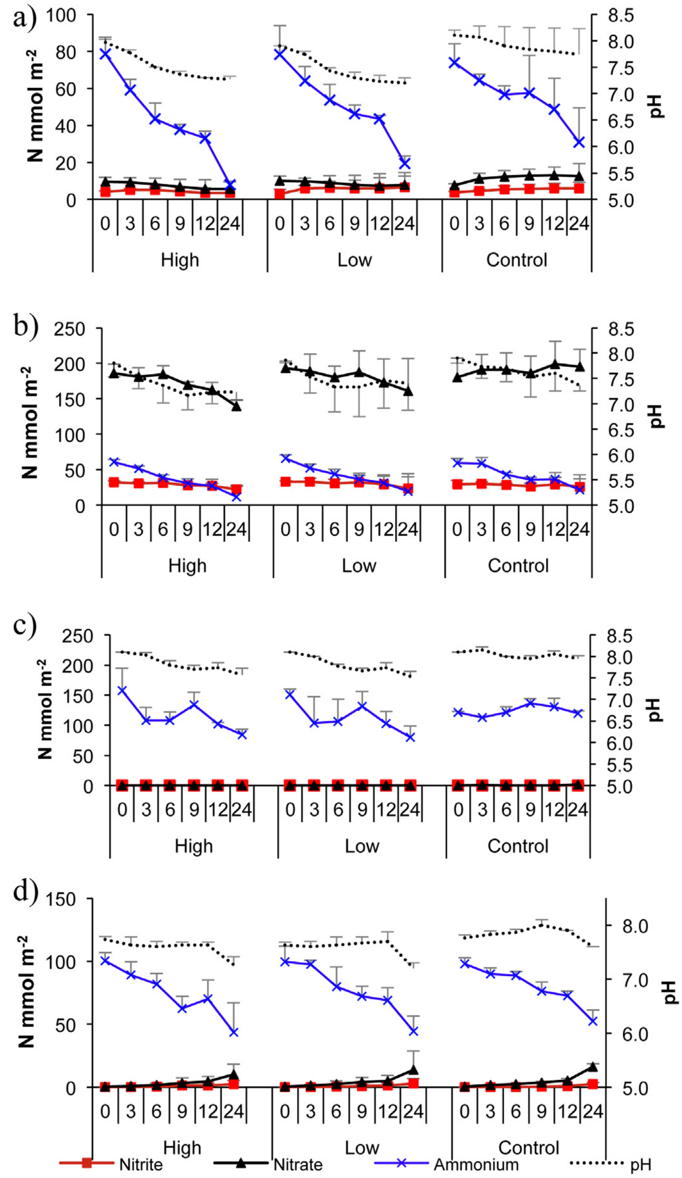

Fig. 6. The pH and load ($mmol\ m^{-2}$) of nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) in constructed wetlands planted with *S. europaea* at high- and low-densities and in unplanted control constructed wetlands over 24 h on samples dates commencing (a) July 3, (b) July 10, (c) July 29 and (d) October 8.

these systems were freshwater, or low salinity systems, planted with salt tolerant glycophytes and therefore are not directly comparable with the N uptake capacity of halophytes such as *Salicornia*.

According to Jefferies (1977), salt marsh halophytes are adapted to growth in an environment where nitrogen availability is highly variable both spatially and seasonally and growth responses to nitrogen addition differ significantly between, as well as within halophytic species, dependent on location on the saltmarsh. This unpredictable response to nitrogen would suggest that N uptake in CWs planted with *Salicornia*, should not be generalised from the wider literature on systems using glycophytes. For example, Brown et al. (1999) found that at a salinity of 10, although *Salicornia bigelovii* sequestered only 22% of the total nitrogen (inorganic + organic N) applied, 100% of the inorganic nitrogen was retained in plant tissue. Similarly, Webb et al. (2012) found that 85% of the TDIN removed by *S. europaea* CWs was retained in plant tissue.

Fig. 6 shows the concentration of ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) in the 3 bed types over 24 h on each of the four sampling dates, along with the corresponding change in pH. Overall, the major constituent of TDIN in the influent wastewater was NH_4^+, however on July 10 the NO_3^- load was $186.3 \pm 6.4\ mmol\ m^{-2}\ d^{-1}$, almost 60 times higher than the usual $0.1–12.8\ mmol\ m^{-2}\ d^{-1}$, and 3 times higher than the NH_4^+ load ($62.4 \pm 3.6\ mmol\ m^{-2}\ d^{-1}$). It would appear that on July 10 within the first 9 h of the sampling period, there was no significant change in NO_3^- concentrations which may be due to the preferential uptake of NH_4^+ by *S. europaea* with nitrate reduction apparent once NH_4^+ levels were reduced (Quintã, 2012). However, interpretation of relative uptake rates for the two nitrogen species is confounded by nitrification rates which were not quantified in this study.

3.6. Salicornia growth

Although planting density had little effect on nitrogen removal, it did influence plant growth form. *Salicornia* planted at low-densities were highly branched and grew laterally as well as vertically to fill the available space. In contrast those planted at high-density grew vertically showing little or no lateral growth/branching. Davy et al. (2001) reported that in the field, when present at low-densities, individual *S. europaea* plants become large, highly branched structures, whilst in populations of *S. europaea* at densities of >10,000 m^{-2}, individuals were unbranched with a single, terminal spike and showed little evidence of self-thinning. These observations suggest that within a physically constrained area, such as a CW, the morphological plasticity of *Salicornia* will produce growth patterns that result in similar total plant biomass per unit area irrespective of the initial planting density. Hence the choice of high- or low-density planting systems will largely depend on the relative cost and complexity of seeding beds by different methods, and the plant form favoured by the commercial market.

The cumulative harvest yields of 23.1 and 19.9 FW kg m^{-2}, and mean harvest yields of 2.6 ± 1.1 and 2.2 ± 0.7 FW kg m^{-2} (high- and low-densities respectively) compare well with the limited information available in other published studies for *Salicornia*. Ventura et al. (2011), using the same cropping method and a similar cropping regime (once every 4 weeks) achieved cumulative yields of *Salicornia* over 6 months of 13.4–16.0 kg m^{-2} with a mean yield per harvest of 2.2–2.7 FW kg m^{-2}. The results in the present study were greater than those reported by Webb et al. (2012) where overall mean yield per harvest was 0.9 kg m^{-2}, with nitrogen loading that was variable and for periods much lower than observed in the present study.

3.7. Role of microbial communities

The prevailing environmental conditions may have influenced plant performance and the establishment and subsequent success of nitrifying bacterial communities. In the control beds, observed increases in NO_3^- together with a decline in NH_4^+ on July 3 (Fig. 6a) indicate the early establishment of nitrifying bacterial communities, however, on the subsequent July sampling dates there is no significant change in NO_3^-, NO_2^- or NH_4^+ in the control beds. Nitrifying bacteria are generally temperature-sensitive, with optimum bacterial growth occurring at temperature ranges between 25 and 35 °C (Focht and Verstrate, 1977; Faulwetter et al., 2009). Above 42 °C nitrification drops off rapidly (Painter, 1970) and at 50 °C Sudarno et al. (2011) saw a minimal nitrite oxidation rate (NOR) which did not recover when the temperature returned to 22.5 °C. During the July sampling dates air temperature maxima within the polytunnel ranged from 45.2 to 54.2 °C peaking on July 29 at 54 °C and water temperature was the highest seen during the experimental period, never dropping below 20 °C (Table 1). Fig. 6b and c show that in July, when air temperatures exceeded 50 °C and water temperatures stayed above 20 °C there was no change in NO_3^- and NO_2^- in the unplanted control beds. By October, in comparison to levels seen in the previous month, all bed types had significant increases in both nitrite and nitrate concentrations during the 24 h monitoring periods. At this time, environmental conditions within the polytunnel were more suitable for successful establishment of nitrifying bacteria and daily air temperature maxima had decreased to 38.5 °C providing more optimal conditions for nitrifying bacterial growth within the filter bed surface (Focht and Verstrate, 1977; Fontenot et al., 2007; Faulwetter et al., 2009).

By October, plant vegetative growth and associated N uptake had reduced and there was no significant difference between the TDIN removal rates of the planted CWs and the unplanted controls (Table 2), confirming the role of plant nutrient uptake during the preceding summer period although it is difficult to directly compare the planted and unplanted beds due to the indirect effect of root structure on environmental condition within the sediment and hence microbial communities. As root oxygen release can influence and enhance microbial density, activity and diversity in the plant rhizosphere regions of subsurface flow CWs (Gagnon et al., 2007), the root-soil interface plays a significant role in nitrogen removal and N remediation from wastewater in shallow CW (Białowiec et al., 2012).

4. Conclusions

The present study demonstrates the efficiency of CWs planted with *S. europaea* in removing inorganic nitrogen from marine aquaculture wastewater discharged from an intensive RAS system. Results for phosphorous were less clear, with no significant removal observed on some days. Over the vegetative growth period, all the planted beds performed well as biofilters, and there were no significant differences between the performance of beds planted at the two densities tested. The behaviour of the control beds was inconsistent, removal rates never exceeded 35 mmol N m^{-2} d^{-1} and were significantly lower than those observed in the planted beds.

The late season results suggest that nutrient uptake by plants reduces as they flower and enter the senescent phase. Environmental conditions at that time of year were more suitable for nitrifying communities.

Due to the plasticity in morphological development of *Salicornia*, the plant crop yields did not differ between the two densities tested, resulting in similar biomass (per unit area) in both systems once cropping had started. This suggests that commercial systems should adopt the most-cost effective method for large-scale planting. Some further technical development is required to refine planting methods, dependent on the specific filter bed design being used. In the present study, the plant yields were very high (up to 23 kg m^{-2} over a three month period), and depending on market values represent a potentially significant secondary income from this form of waste water treatment.

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