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Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study

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Abstract

Effluent water from shrimp ponds typically contains elevated concentrations of dissolved nutrients and suspended particulates compared to influent water. Attempts to improve effluent water quality using filter feeding bivalves and macroalgae to reduce nutrients have previously been hampered by the high concentration of clay particles typically found in untreated pond effluent. These particles inhibit feeding in bivalves and reduce photosynthesis in macroalgae by increasing effluent turbidity. In a small-scale laboratory study, the effectiveness of a three-stage effluent treatment system was investigated. In the first stage, reduction in particle concentration occurred through natural sedimentation. In the second stage, filtration by the Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley), further reduced the concentration of suspended particulates, including inorganic particles, phytoplankton, bacteria, and their associated nutrients. In the final stage, the macroalga, *Gracilaria edulis* (Gmelin) Silva, absorbed dissolved nutrients. Pond effluent was collected from a commercial shrimp farm, taken to an indoor culture facility and was left to settle for 24 h. Subsamples of water were then transferred into laboratory tanks stocked with oysters and maintained for 24 h, and then transferred to tanks containing macroalgae for another 24 h. Total suspended solid (TSS), chlorophyll *a*, total nitrogen (N), total phosphorus (P), NH_4^+, NO_3^-, and PO_4^{3-}, and bacterial numbers were compared before and after each treatment at: 0 h (initial); 24 h (after sedimentation); 48 h (after oyster filtration); 72 h (after macroalgal absorption). The combined effect of the sequential treatments resulted in significant reductions in the concentrations of all parameters measured. High rates of nutrient regeneration were observed

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in the control tanks, which did not contain oysters or macroalgae. Conversely, significant reductions in nutrients and suspended particulates after sedimentation and biological treatment were observed. Overall, improvements in water quality (final percentage of the initial concentration) were as follows: TSS (12%); total N (28%); total P (14%); NH_4^+ (76%); NO_3^- (30%); PO_4^{3-} (35%); bacteria (30%); and chlorophyll *a* (0.7%). Despite the probability of considerable differences in sedimentation, filtration and nutrient uptake rates when scaled to farm size, these results demonstrate that integrated treatment has the potential to significantly improve water quality of shrimp farm effluent. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Quantitative comparisons of shrimp farm influent and effluent water have demonstrated that effluent can contain elevated concentrations of dissolved nutrients, phytoplankton, bacteria, and other suspended organic and inorganic solids (Ziemann et al., 1992). The potential adverse environmental impacts from untreated effluent have raised concerns about the sustainability of shrimp farming (Phillips et al., 1993; Primavera, 1994). This has prompted the search for cost-effective methods of improving effluent water quality prior to discharge into receiving waters.

Traditional methods of wastewater (sewage) treatment are ineffective and prohibitively expensive for application in treating shrimp pond effluent (Hopkins et al., 1995). A potentially viable alternative is biological treatment of the effluent using oysters and macroalgae to remove suspended particulates and nutrients (Shpigel et al., 1993). The organic component of pond effluent can provide a rich source of food for bivalves (Newell and Jordan, 1983). Bivalves, such as oysters, can also facilitate the removal of fine inorganic matter from suspension by coagulating this material into larger, more settleable particles and egesting them as pseudofaeces (Tenore and Dunstan, 1973).

Previous studies have shown that filtration by oysters can significantly reduce the concentrations of bacteria, phytoplankton, total nitrogen (N) and total phosphorus (P), and other suspended particles in shrimp pond effluent (Jones and Preston, 1999). However, if sediment loads are too high, oyster filtration can be reduced or cease completely (Loosanoff and Tommers, 1948). Other studies have observed the problems associated with a high concentration of suspended solids on the health of oysters (Hopkins et al., 1993). These studies indicate the need to reduce the concentration of suspended particles by sedimentation prior to filtration by oysters.

Regular water exchange in shrimp ponds is required to maintain adequate water quality for optimal growth conditions. In particular, the toxic effects of high ammonia concentration on the shrimp (Chien, 1992) can be a critical factor in determining water exchange rates (Wajsbrot et al., 1989; Hopkins et al., 1993). Although oysters can reduce the concentration of some particulate and dissolved nutrients (Manahan et al., 1982; Dame, 1996), they can also increase the concentration of NH_4^+ through excretion (Hammen et al., 1966). Of the N absorbed by oysters, approximately 10% goes to

growth, 10% to gametes, 2% to byssal threads, with 50% lost as biodeposition and 27% as excretion (Dame, 1996).

Various species of macroalgae can rapidly assimilate large quantities of dissolved organic and inorganic nutrients, usually with a preference for NH_4^+ (e.g., D'Elia and DeBoer, 1978; Harlin, 1978; Ryther et al., 1981). Rhodophyta (red macroalgae) are particularly efficient at taking up nutrients rapidly and have mechanisms for storing large reserves of nutrients (Vergara et al., 1993). For example, the red macroalga *Gracilaria edulis* rapidly assimilates NH_4^+ (Jones et al., 1996), and another red macroalgae *Kappaphycus alvarezii* has been effectively used to assimilate waste nitrogen from the pearl oysters *Pinctada martensi* (Qian et al., 1996).

In addition to improving the water quality of shrimp effluent water, macroalgae and oysters can provide an additional source of income for shrimp farmers. For example, trials into tank culture of *Gracilaria chilensis* supplied with salmon seawater effluent have demonstrated production rates of four times those in wild beds and up to double the agar content (Retamales et al., 1994). The incorporation of oysters and macroalgae into a polyculture system may provide the potential for additional income; however, the costs associated with culture and maintenance of these crops must be considered. These organisms have specific requirements for growth and the level of management may be comparable to the principal aquaculture species. For example, macroalgae require sufficient water flow and periodic harvesting to ensure rapid growth and nutrient removal, and oysters are subject to fouling by barnacles, tube worms and oyster spat, requiring cleaning before sale.

This study used controlled laboratory experiments to test the combined efficiency of a three-stage system for treating pond effluent. The water quality after sedimentation, filtration of particulates by oysters (*Saccostrea commercialis*) and absorption of nutrients by macroalgae (*G. edulis*) were assessed.

2. Materials and methods

2.1. Experimental design

Shrimp pond effluent was collected from a commercial *Penaeus japonicus* farm in Moreton Bay, Australia. The farm had a total of 8 ha of ponds and at the time of sampling, the shrimp biomass in the ponds was approximately 3 t ha^{-1}. Ninety five percent of the suspended particles in the effluent from this farm are small (2–4 μm) clay particles (Jones et al., unpublished data). A 60-l water sample was collected from the effluent channel and transported to the laboratory in a plastic drum (35-cm diameter × 60-cm height). In the laboratory, any settled particles in the sample were resuspended and 3 replicate 1-l samples were collected for analysis of total suspended solids (TSS) (and percent organic of particulates), chlorophyll *a*, bacterial concentration, total Kjeldahl nitrogen (TKN) and total phosphorus (TP), and dissolved N (NH_4^+ and NO_3^-/NO_2^-), and dissolved P (PO_4^{3-}). A suite of physico-chemical parameters (temper-

ature, pH, salinity, dissolved oxygen, and turbidity) were determined with a Horiba U-10 water quality meter (CA, USA). A 30-ml sample of water was collected in test tubes, incubated in the dark for 30 min and subsequently placed into a Turner Designs Fluorometer 10-005 (Sunyvale, CA, USA) for determination of in vivo fluorescence (as a measure of chlorophyll *a*).

The effluent was left to settle in the dark for 24 h in the collection drum, with physico-chemical parameters, and in vivo fluorescence measured at over 24 h. The remaining parameters, TKN, TP, dissolved N (NH_4^+ and NO_3^-/NO_2^-), dissolved P (PO_4^{3-}), bacterial concentration, chlorophyll *a* and TSS were analysed after 24 h only. A sediment trap (23×15 cm) was placed on the bottom of the drum to determine the amount of settled material per litre (after 24 h) and the percent organic content of the settled particles.

After 24 h, 10 l of the settled sample was drained into each of four, 11-l aerated tanks ($26 \times 17 \times 25$ cm), one as a control (with dead oyster shells), and three for replicate oyster filtration treatments. Experimental tanks (Fig. 1) were maintained at 20–23°C and exposed to light on a 12:12 h light/dark cycle using daylight fluorescent tubes which provided approximately 250 μmol quanta m^{-2} s^{-1}. The 11-l tanks were each stocked with 16 oysters with a mean wet weight of 40 g. Dead oyster shells were used in the control tanks to negate any effects on water flow and consequent differences in settling rates. Oysters were placed on plastic mesh, which was situated on top of the sediment
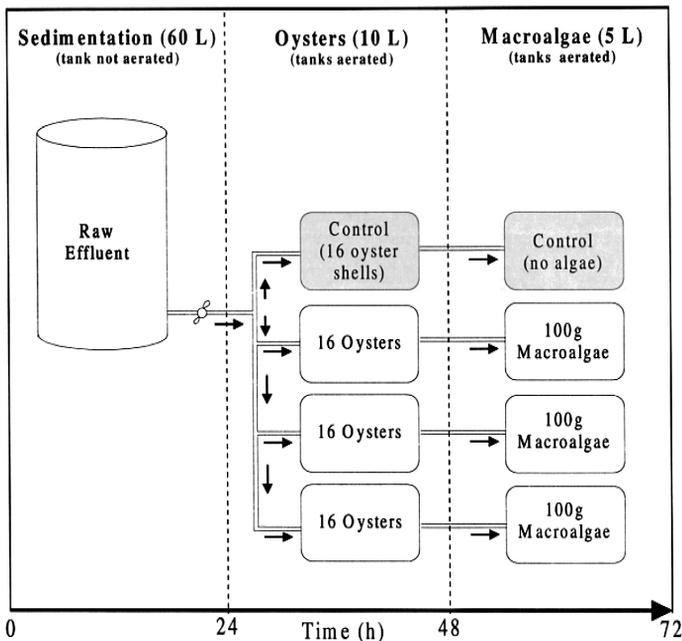
Fig. 1. Design of the integrated system for treatment of shrimp pond effluent and stocked with oysters (40 g *S. commercialis*) and macroalgae (*G. edulis*).

trap. Physico-chemical parameters, and in vivo fluorescence, were measured from 24 to 48 h. The remaining parameters, TKN, TP, dissolved N (NH_4^+ and NO_3^-/NO_2^-), dissolved P (PO_4^{3-}), bacterial concentration, chlorophyll *a,* TSS and sediment traps were analysed after 48 h.

After 24 h of oyster filtration, 5 l from each tank was drained into each of four more 11-l aerated tanks, with one as a control (empty), and three as replicate macroalgal absorption treatments (100-g wet weight of macroalgae per 5 l) (Fig. 1). The water from the oyster control tank was transferred into the macroalgal control tank, and the water from the replicate oyster treatment tanks was transferred into the corresponding macroalgal treatment tanks (Fig. 1). Both the oyster and macroalgal control tanks acted as controls for the combined biological treatment process using both the oysters and macroalgae. Consequently, the effluent in the macroalgal control tank contained higher concentrations of phytoplankton, bacteria and other suspended solids, because the oysters had not treated it yet. Physico-chemical parameters, dissolved N (NH_4^+ and NO_3^-/NO_2^-), and dissolved P (PO_4^{3-}) were measured from 48 to 72 h. The remaining parameters, TSS, TKN, TP, chlorophyll *a*, bacterial concentration and sediment traps were analysed after 72 h.

At the end of each 24-h period, three replicate 1-l samples were collected from each tank and filtered for chlorophyll *a* extraction, TSS calculation and the percent organic. Subsamples were taken for TKN and TP, dissolved N (NH_4^+ and NO_3^-/NO_2^-), dissolved P (PO_4^{3-}), and bacterial concentration. At all times excepting 0, 24, 48 and 72 h, chlorophyll *a* and TSS were determined as in vivo fluorescence and turbidity (NTU), respectively. Preliminary analysis determined correlations (± 2) between in vivo fluorescence and chlorophyll *a* to be 0.86, and between turbidity (NTU) and TSS to be 0.9. In vivo fluorescence measurements were converted to chlorophyll *a* concentration and turbidity measurements were converted to TSS for those periods of additional sampling at 15, 30 min, 1, 2, 3, 6 and 12 h.

The use of one replicate sample for the sedimentation and control tanks was considered sufficient based on the low variability in water quality parameters measured between replicates during previous studies at the same shrimp farm (Burford, 1997; Jones and Preston, 1999). Typically, the main variability in water quality of shrimp pond effluent is on a temporal scale (both diel and seasonal variability).

2.2. Analytical procedures

Chlorophyll *a* was determined by filtering a known volume of water sample through Whatman GF/F filters, which were immediately frozen. Acetone extraction and calculation of chlorophyll *a* concentration was performed using the methods of Clesceri et al. (1989) and Parsons et al. (1989).

The filtrate collected from filtering for chlorophyll *a* analysis was collected in 120 ml polycarbonate containers and immediately frozen. NH_4^+ and NO_3^-/NO_2^- and PO_4^{3-} were determined by the NATA-accredited Queensland Health Analytical Services Laboratory (QHASL) in accordance with the methods of Clesceri et al. (1989) using a Skalar autoanalyser (Norcross, GA, USA).

Unfiltered samples for nutrient analysis (total Kjeldahl nitrogen and total phosphorus) were collected in 120 ml polycarbonate and immediately frozen. They were subsequently analysed within 2 weeks by the QHASL in accordance with the methods of Clesceri et al. (1989) using a Skalar autoanalyser (Norcross, GA, USA).

TSS concentrations were determined using the methods of Clesceri et al. (1989). A known volume of water was filtered onto a pre-weighed and pre-dried (110°C, 24 h) Whatman GF/C glass fibre filter. The filter was then oven dried at 60°C for 24 h and TSS calculated by comparing the initial and final weights. Volatile suspended solids were determined as loss on ignition by combusting samples in a muffle furnace for 12 h at 525°C (Clesceri et al., 1989).

Bacteria samples were preserved with 2% formalin and kept at 4°C until analysis. A known volume (0.5–1 ml) of sample was stained with acridine orange, filtered onto a stained (Irgalan Black) 2-μm poretics filter and mounted on a slide. Bacteria were counted using epifluorescence microscopy (Hobbie, 1977).

For oyster filtration and macroalgal absorption treatments, three replicate tanks were analysed and means and standard errors were calculated. Differences between treatments were tested for significance using one way analysis of variance (ANOVA) and Tukey's Test for multiple comparison of means at a significance level of 0.05 using Minitab 12.1 software (State College, PA, USA).

3. Results

3.1. Chlorophyll a

Sedimentation reduced the mean chlorophyll *a* concentration from 180 to 130 μg l^{-1}. In the oyster control, the concentration of chlorophyll *a* was further reduced from 130 to 100 μg l^{-1} due to continued settling. The reduction in the chlorophyll *a* concentration from 130 to 11 μg l^{-1} in the oyster treatment was significantly greater ($P < 0.001$) than the control. The concentration of chlorophyll *a* decreased from 100 to 51 μg l^{-1} in the macroalgal control (no macroalgae) and from 11 to 1.5 μg l^{-1} in the macroalgal treatment. These decreases indicate additional settling of phytoplankton (Fig. 2B, Table 1).

3.2. Suspended solids

The initial concentration of TSS was 0.60 g l^{-1}. After sedimentation, the mean concentration was reduced to 0.17 g l^{-1}. Oyster filtration further decreased the concentration to 0.02 g l^{-1}. There was no change in the oyster control, indicating that material with a specific gravity greater than water had already settled out of suspension during the initial 24 h sedimentation period. There was no significant change ($P > 0.05$) in the concentration of TSS in the macroalgal control and treatments (Fig. 2A, Table 1). During sedimentation, 1.9 g of material was collected in the sediment traps. In the oyster
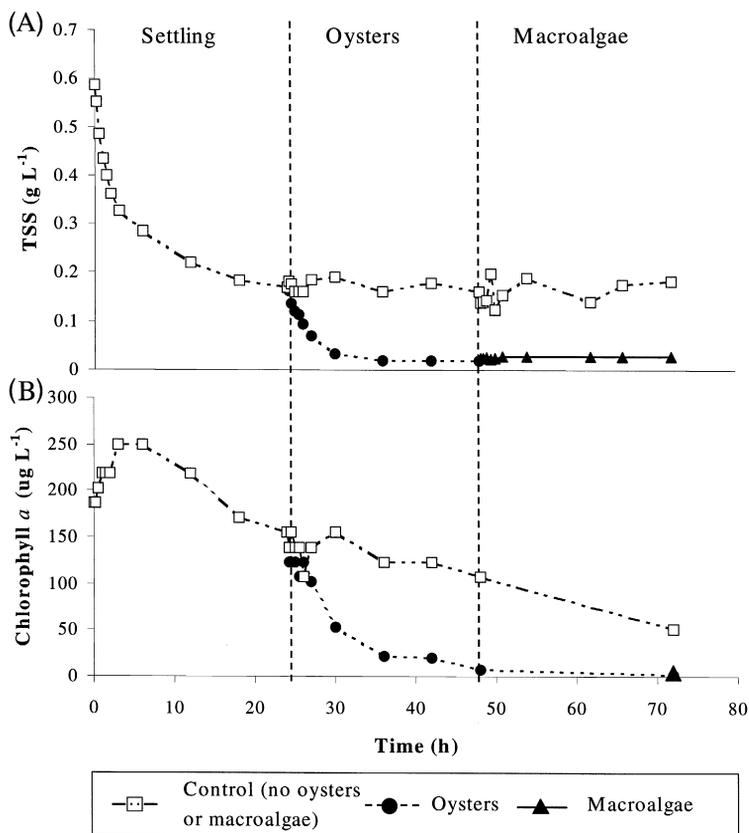
Fig. 2. Changes in total suspended solids (A) and phytoplankton biomass (chlorophyll *a*); (B) from sedimentation, oyster filtration and macroalgal absorption of shrimp pond effluent. Standard error bars have been plotted, but are too small to be visible.

treatment, 0.42 g was collected vs. 0.02 g in the oyster control. This indicates significant ($P < 0.001$) biodeposition of faeces and pseudofaeces by the oysters (Fig. 3).

3.3. Organic content

In the first 24 h, sedimentation removed a greater proportion of inorganic particles than organic particles. The relative organic content of the remaining suspended solids increased from 23% to 31%. The organic content of the settled material collected in the sediment traps was 21%, which was lower than the initial water column content of 23%. Sedimentation in the oyster control tanks did not change the organic content. Oyster filtration was more effective at removing organic material (phytoplankton, bacteria and detritus), decreasing the relative organic content of the suspended solids from 31% to 24%. The organic content of the settled sediment at the end of the oyster treatment was

Table 1
Final percentages of the initial and highest recorded concentrations of various water quality parameters of shrimp pond effluent after settling, filtration by oysters and filtration by macroalgae. Levels of significant difference from the initial and highest recorded concentrations are indicated

	Percentage of highest concentration (%)	F-value	Percentage of initial concentration (%)	F-value
TSS $(g\,l^{-1})$	12	1338[***]	12	1338[***]
TSS % Organic $(g\,l^{-1})$	12	507[***]	12	507[***]
TKN (μM)	28	609[***]	28	609[***]
TP (μM)	14	4629[***]	14	4629[***]
NH_4^+ (μM)	2.3	2987[***]	76	0.69
NO_3^- (μM)	2.2	1176[***]	30	30[**]
PO_4^{3-} (μM)	4.8	162[***]	35	19.6[*]
Bacteria (no. per l)	30	107[***]	30	107[***]
Chl a $(\mu g\,l^{-1})$	0.6	202[***]	0.8	202[***]

[*] $P \leq 0.05$.
[**] $P \leq 0.01$.
[***] $P \leq 0.001$.

31%, compared to 21% at the end of the sedimentation treatment (Fig. 4B). These changes mirrored the changes in the organic content of the suspended particles (Fig.
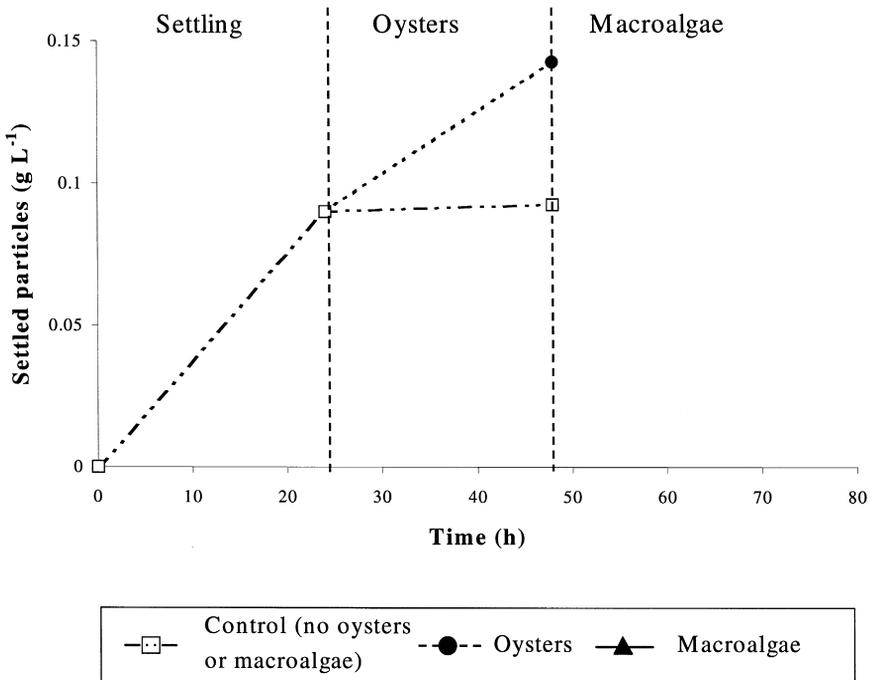
Fig. 3. Concentration of particles settled per litre from sedimentation and oyster filtration of shrimp pond effluent.
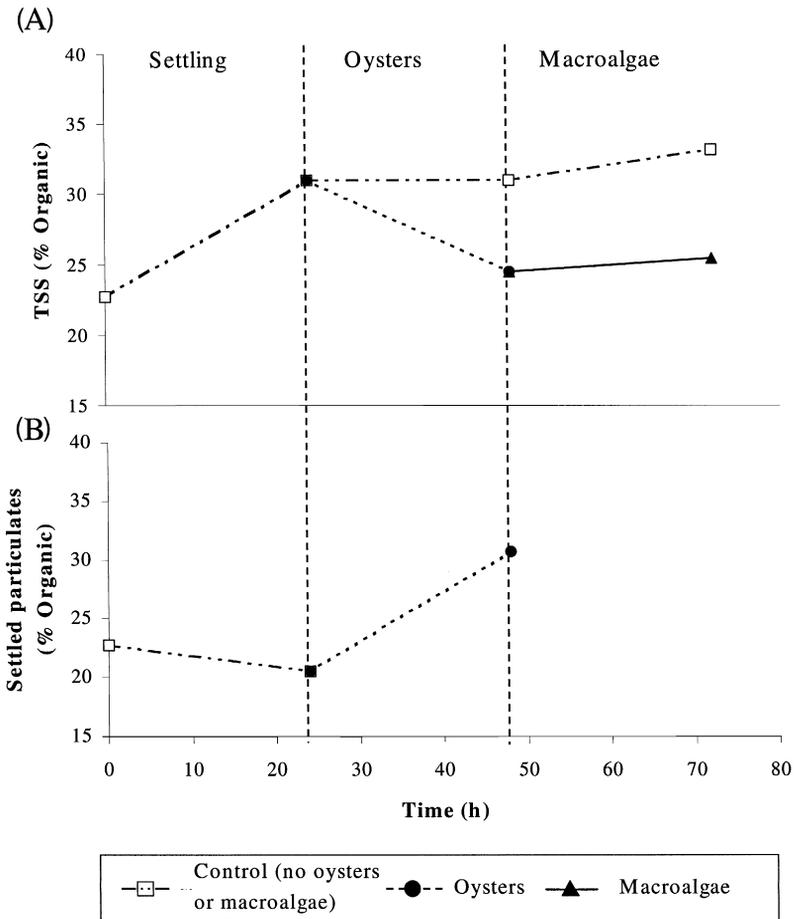
Fig. 4. Changes in the organic content of the (A) TSS and (B) settled particles in the shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption. Standard error bars have been plotted, but are too small to be visible.

4A). In the macroalgal treatment and control, the organic content of the suspended solids remained unchanged (Fig. 4A).

3.4. Bacteria

During sedimentation, bacterial numbers did not change significantly ($P > 0.05$), whereas the oyster treatment significantly ($P < 0.001$) reduced the mean concentration of bacteria from 19 to 6×10^{10} 1^{-1}. There was no significant change ($P > 0.05$) in bacterial numbers in the macroalgal controls and treatments (Fig. 5, Table 1).

3.5. Dissolved oxygen

Dissolved oxygen was 6.3 mg 1^{-1} immediately following the initial resuspension of any settled particles prior to sedimentation. After 24 h of sedimentation without stirring
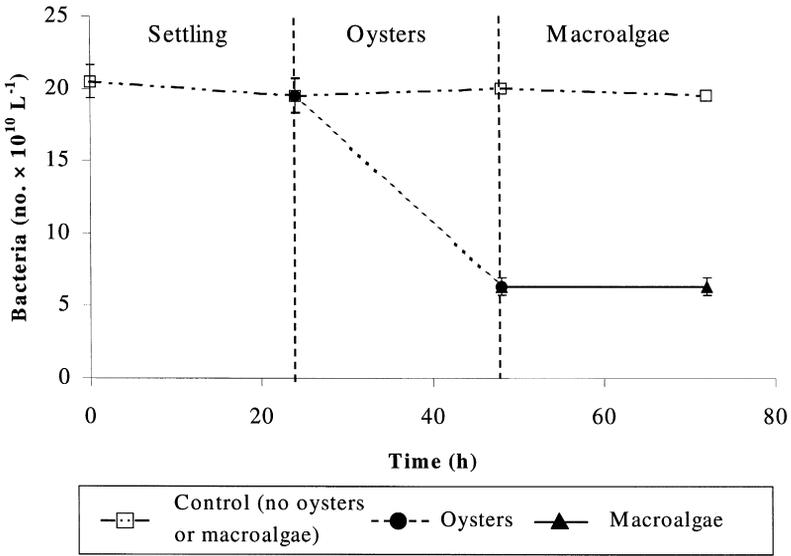
Fig. 5. Changes in bacterial numbers of shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption.

or aeration, respiration by bacteria and plankton reduced the mean concentration to 2.6 mg l^{-1}. Within 2 h of aeration being applied in the oyster tanks, the concentration of dissolved oxygen was up to 6.3 mg l^{-1}. The concentration peaked at between 7 and 8 mg l^{-1} in the macroalgal treatment and control tanks (Fig. 6).
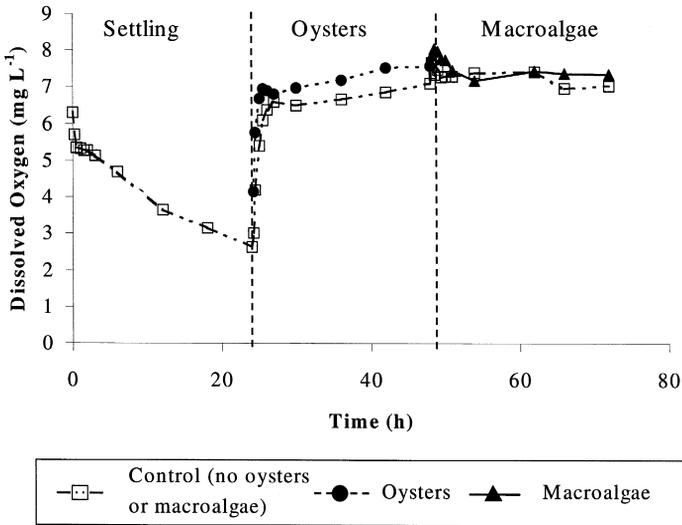
Fig. 6. Changes in water column dissolved oxygen concentrations of shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption. Standard error bars have been plotted, but are too small to be visible.
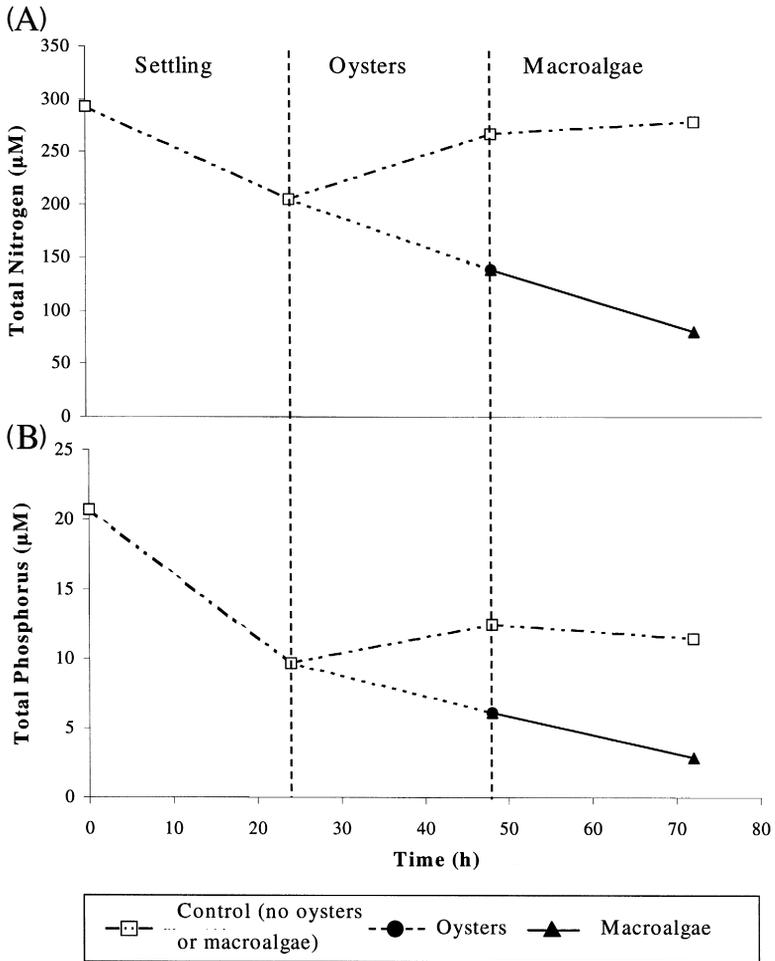
Fig. 7. Changes in water column total N (A) and P (B) concentrations of shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption. Standard error bars have been plotted, but are too small to be visible.

3.6. Total nitrogen

The sedimentation treatment reduced the mean concentration of total Kjeldahl nitrogen (TKN) from 290 to 205 μM. The oyster treatment further reduced the concentration to 138 μM, while in the control the concentration increased to 266 μM. The macroalgal treatment reduced the concentration from 138 to 81 μM, while in the control the concentration increased from 266 to 279 μM (Fig. 7A, Table 1).

3.7. Total phosphorus

Total phosphorus (TP) also decreased during sedimentation from 21 to 9.7 μM. The oyster control increased the mean concentration to 12.5 μM, whereas the oyster
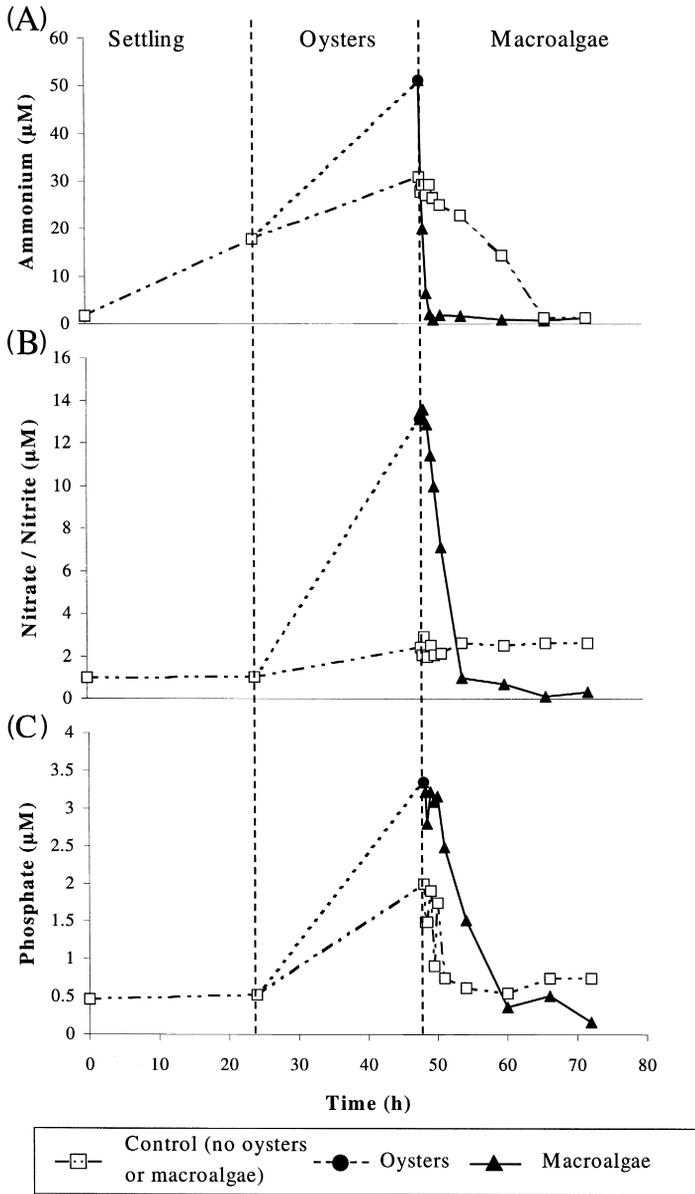
Fig. 8. Changes in water column NH_4^+, NO_3^-, PO_4^{3-} concentrations of shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption. Standard error bars have been plotted, but are too small to be visible.

treatment reduced the concentration to 6.1 μM. The macroalgal control reduced the concentration from 12.5 to 11.5 μM and the macroalgal treatment reduced the concentration from 6.1 to 2.9 μM (Fig. 7B, Table 1).

Table 2
Nutrient uptake and release rates for sedimentation, oyster filtration and macroalgal absorption. Negative symbols represent nutrient uptake and positive represent nutrient release. The top value for each treatment is the gross value, the middle value is the control and the bottom value (in bold type) is the net value after correction for nutrient changes in the control tanks. The last row of results represent the rates of macroalgal nutrient uptake over the first hour, when nutrient concentrations were still saturating uptake kinetics. nd = no data

Treatment	TKN μmol h^{-1}	TP μmol h^{-1}	NH$_4^+$ μmol h^{-1}	NO$_3^-$ μmol h^{-1}	PO$_4^{3-}$ μmol h^{-1}
Sedimentation					
Gross	−220	−28	+40.5	+0.05	+0.18
Oysters					
Gross	−28	−1.5	+14	+5.0	+1.2
Control	+26	+1.15	+5.7	+0.6	+0.6
Net	**−54**	**−2.65**	**+8.3**	**+4.4**	**+0.6**
Macroalgae					
Gross	−12	−0.65	−10.4	−2.8	−0.66
Control	+2.5	−0.2	−5.8	0.0	−0.26
Net	**−14.5**	**−0.45**	**−4.6**	**−2.8**	**−0.4**
Macroalgae over 1 h					
Gross	nd	nd	−225	−10	−3.4
Control	nd	nd	−7	+1	−2.1
Net	**nd**	**nd**	**−218**	**−11**	**−1.3**

3.8. Ammonium

The mean concentration of water column NH$_4^+$ increased during sedimentation from 1.7 to 18 μM due to regeneration. Regeneration rates were calculated as μmol h^{-1} based on the effluent volumes used during each treatment period (i.e., 60 l for sedimentation, 10 l for the oyster treatment, and 5 l for the macroalgal treatment). Thus, the observed rate of regeneration during sedimentation was 40.5 μmol h^{-1}, although this may have been substantially higher if the loss due to volatilisation and uptake by bacteria and phytoplankton was taken into account. The rate of NH$_4^+$ regeneration in the oyster control was considerably lower (5.7 μmol h^{-1}) increasing from 18 to 29 μM. This was probably a result of the decrease in the concentration of TSS, thereby reducing the source of remineralisation. The mean NH$_4^+$ concentration in the oyster treatment increased from 18 to 51 μM. During the macroalgal treatment, the NH$_4^+$ concentration was reduced from 51 to 1.3 μM (to 1.0 μM after 2 h). The uptake rate during the first hour (when N concentrations were still saturating uptake kinetics) was 225 μmol h^{-1} for the 100 g of macroalgae. The concentration of NH$_4^+$ in the macroalgal control decreased from 29 to 1.2 μM, but at a much slower rate (only to 26 μM after 2 h) than in the macroalgal treatment (Fig. 8A; Table 1 and Table 2). The reduction in NH$_4^+$ in the macroalgal control suggests volatilisation, nitrification or uptake by the high concentration of bacteria and phytoplankton. The dramatic reduction in NH$_4^+$ in the control at the start of the macroalgal treatment suggests that the concentrations actually

peaked sometime during the oyster treatment and were already declining before transfer to the macroalgal control tanks.

3.9. Nitrate / nitrite

The mean concentration of NO_3^-/NO_2^- was unchanged during the sedimentation period, where the effluent was not aerated, but increased from 1.0 to 1.4 μM in the oyster control and from 1.0 to 13 μM in the oyster treatment. There was no change in the NO_3^-/NO_2^- concentration in the macroalgal control, but the macroalgae in the treatment tanks significantly ($P < 0.001$) reduced the concentration from 13 to 0.3 μM (Fig. 8B, Table 1).

3.10. Phosphate

Phosphate was also unchanged during sedimentation, but increased from 0.5 to 2 μM in the oyster control and from 0.5 to 3.3 μM in the oyster treatment. The mean concentration in the macroalgal control decreased from 2 to 0.7 μM, and in the macroalgal treatment the concentration of P decreased from 3.3 to 0.16 μM (Fig. 8C, Table 1).

These reductions in PO_4^{3-} equate to a removal rate of 0.66 μmol h^{-1} in the macroalgal treatment, while loss in the control was only 0.26 μmol h^{-1}. During the first hour of macroalgal filtration, the concentration of PO_4^{3-} in the control decreased at a greater rate than in the macroalgal treatment. This may have been due to the presence of significantly more phytoplankton and bacteria in the control tanks, because the control water had not been treated by the oysters. Consistent with the NH_4^+, there was a dramatic reduction in PO_4^{3-} at the start of the macroalgal treatment. This suggests the concentrations actually peaked sometime during the oyster treatment and were declining before transfer to the macroalgal control tanks.

3.11. Nutrient uptake rates and ratios

During the macroalgal treatment, NH_4^+ was taken up in the first 2 h (hour 48 to hour 50), NO_3^- in the next 4 h (from hour 50 to hour 54), and PO_4^{3-} over 10 h (from hour 50 to hour 60). N in both forms was taken up at faster rates than PO_4^{3-}.

The TKN/TP ratio in the water column increased during sedimentation from 6.4 to 9.6, and then during oyster filtration from 9.6 to 10.2, probably due to adsorption of P to settling particulates. There was no change in the oyster control, but the macroalgal control showed a further increase to 10.9, and the macroalgal treatment increased to 12.6 (Fig. 9A).

The ratio of dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN/DIP) increased from 2.6 to 16.2 during sedimentation, predominantly as a result of NH_4^+ remineralisation. During the oyster treatment and control, the ratio dropped to 8.7 and 7.5, respectively. The macroalgal control increased the ratio to 18.8 after 6 h and then decreased to 2.3, whereas the macroalgal treatment decreased the ratio to 0.8 after 6 h and then increased it to 4.5. These responses coincided with the rapid drop in the
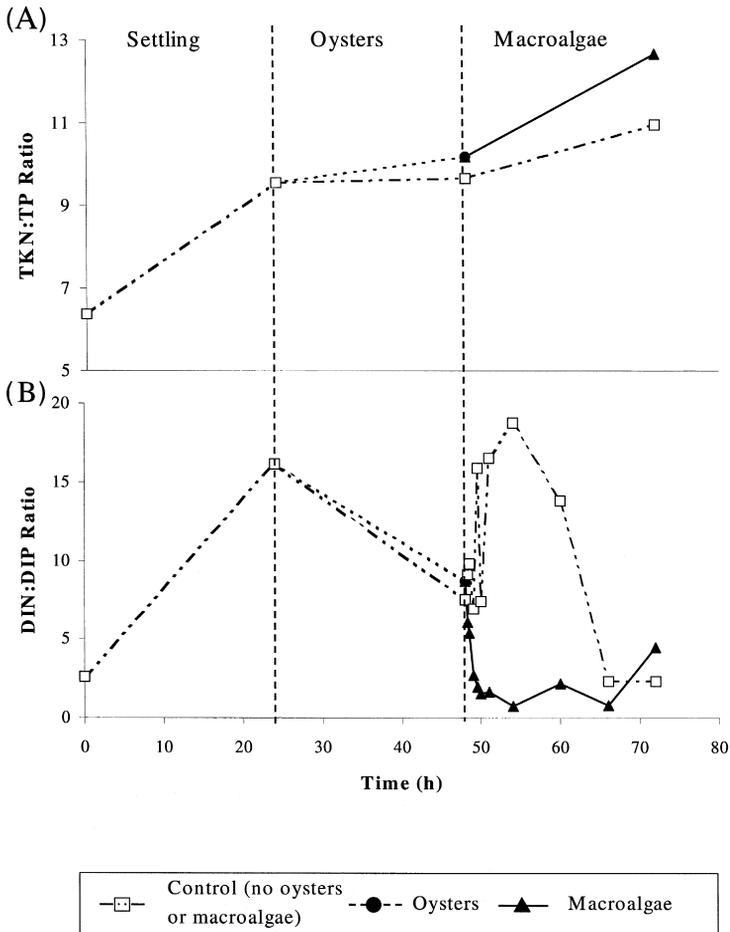
Fig. 9. Changes in water column total N/P ratio (A) and dissolved inorganic nitrogen: dissolved inorganic phosphorus ratio; (B) of shrimp pond effluent from sedimentation, oyster filtration and macroalgal absorption. Standard error bars have been plotted, but are too small to be visible.

PO_4^{3-} in the macroalgal control in the first 6 h, and the rapid uptake of NH_4^+ in the macroalgal treatment (Fig. 9B).

4. Discussion

4.1. Water quality

Previous studies on the use of sedimentation, bivalves and macroalgae to improve effluent water quality have generally examined each component separately. For exam-

ple, Shpigel et al. (1993) examined three separate regimes for treating fish pond effluent and concluded these could be more closely integrated. Our study advanced the concept to the point of laboratory tests to examine the combined efficiency of sedimentation, biofiltration by oysters and nutrient uptake by macroalgae.

The ability of a biofilter system to improve the quality of shrimp effluent is likely to vary depending on the initial water quality. The water quality of shrimp farm effluent depends on a number of factors, including pond soil type, quality of influent water, stage of growout season and management practices employed (Ziemann et al., 1992). Our study was conducted in November, early in the growout season when the concentration of NH_4^+ was relatively low (1.7 μM), compared to later in the growout season (65 μM) (Jones et al., unpublished data). However, there were very high concentrations of TSS and phytoplankton in the effluent at the time of the study. The concentrations of TSS (0.6 g l^{-1}), chlorophyll *a* (180 μg l^{-1}) and bacteria (20 × 10^{10} l^{-1}) were, respectively, 4.5, 5 and 10 times the values measured from the same shrimp farm in the previous growout season (Jones and Preston, 1999).

4.2. Sedimentation

Sedimentation of shrimp pond effluent was very effective at reducing the TSS load. The concentration was reduced to 12% of the initial concentration with proportionally more inorganic particles being removed from suspension. Additionally, there were significant ($P < 0.05$) reductions in TKN to 70%, TP to 47%, and chlorophyll *a* to 72% of the initial concentration. The results indicate that the majority of N in the effluent was associated with phytoplankton and bacteria, rather than being bound to readily settleable inorganic particles or detritus. In contrast to this, a relatively high proportion of the TP appeared to be associated with the readily settleable particles.

In this study, the rates of TKN removal by sedimentation and oyster filtration were not significantly different ($P > 0.05$); however, the rate of TSS removal was much slower during oyster filtration compared with sedimentation. This may be a result of higher N content in the small (< 5 μm) unsettleable particles (Cripps, 1992) removed by the oysters. Alternatively, it may simply reflect relatively slow settlement rates of phytoplankton compared to TSS. Despite the reduction in TKN and TP during sedimentation, there was a marked increase in the concentration of NH_4^+ in the water column, most likely due to mineralisation of particulate organic matter (Briggs and Funge-Smith, 1993).

Treatment for the removal of suspended solids must be adapted specifically for the target effluent. The size and density of the particles, along with the flow rates, surface area and retention time affects the efficiency of sedimentation (Chien and Liao, 1995). Effluent with high algal concentrations may require less sedimentation time, because the oysters can filter it more effectively.

4.3. Oyster filtration

Pre-sedimentation of suspended particles is highly beneficial to oyster filtration. In particular, the relative increase in the proportion of organic particles (from 23% to 31%)

may enhance the oysters' ability to assimilate particulate nutrients, as well as filter and egest the smaller inorganic clay particles as pseudofaeces (Loosanoff, 1949). In the present study, the concentration of TSS was reduced from 0.6 to 0.17 g l^{-1} after sedimentation. However, even at this reduced concentration of suspended solids the oyster pumping rates may have been inhibited. For example, a concentration of 0.1 g l^{-1} has been observed to reduce the pumping rate of oysters by up to 87% (Loosanoff and Tommers, 1948).

Ninety five percent of the suspended particulates in the effluent from this shrimp farm were 2–4 μm (Jones et al., unpublished data). During filtration, oysters sort particles by size, weight (Yonge, 1926) and chemical composition (Loosanoff, 1949; Menzel, 1955). Oysters preferentially ingest organic material, reject inorganic material, and preferentially ingest N rich over C rich particles (Newell and Jordan, 1983). Rejected material is expelled as pseudofaeces, and when food concentration exceeds the digestive capabilities of the gut, pseudofaeces may contain some digestible food.

In addition to rejecting small inorganic particles, oysters may facilitate removal of these particles from suspension by coagulating them into larger, more settleable particles before egestion as pseudofaeces. In addition to the fine inorganic particles, oysters filter small organic rich particles, such as bacteria and very small phytoplankton that would otherwise stay in suspension (Loosanoff, 1949; Kautsky and Evans, 1987). Oysters can filter particles as small as 1 μm, although their efficiency in removing smaller particles (1–3 μm) is substantially lower than for larger particles (Haven and Morales-Alamo, 1970).

The oysters removed large concentrations of phytoplankton, bacteria and other suspended solids from the water column, and produced faeces and pseudofaeces at a rate of 78 mg (g dry wt.)$^{-1}$ d^{-1}. This rate is within the range of 66–246 mg (g dry wt)$^{-1}$ d^{-1} observed for the Pacific oyster, *Crassostrea gigas* (Boucher and Boucher-Rodoni, 1988).

Decreases in TP were observed in the oyster treatment due to removal of particulate P (bacteria, phytoplankton and clay particles with adsorbed P) at a rate greater than the inputs of PO_4^{3-}. Shrimp pond effluent often contains a high proportion of small clay particles (Hopkins et al., 1995) rich in phosphate (Pomeroy et al., 1965). Bacteria may compete with sediments for phosphate thereby maintaining the phosphate in the water column (Pomeroy et al., 1965). The increase in PO_4^{3-} and TP observed in the oyster control was probably due to sediment release and the greater increase in PO_4^{3-} in the oyster treatment due to excretion by oysters (Asmus et al., 1995). Most of the P filtered by oysters is converted to biodeposits, with 8% being released as PO_4^{3-} (Dame et al., 1989), and only 3% absorbed and converted into biomass (Sornin et al., 1986).

In this study, oyster filtration reduced the concentration of TKN to 28% and TP to 14% of the initial effluent concentration. These reductions represent an uptake ratio of 20:1. Based on the Redfield ratio of 16:1 in phytoplankton, the oysters were removing more N than expected. Furthermore, this is in contrast to the results of Dame et al. (1989) who observed an N/P uptake ratio by oysters (*C. gigas*) of 2:1. This disparity suggests that the oysters were probably not discriminating between phytoplankton and other particles such as bacteria, detritus and sediments. Therefore, their N/P uptake ratio may be indicative of the combined N/P ratio of the suspended particulates. The

high N content of shrimp feed pellets may be one of the sources responsible for elevating the N/P ratio taken up by the oysters.

During oyster filtration, there was a net decrease in the concentration of particulate nutrients, and a net increase in the concentration of dissolved inorganic nutrients. In addition to particulate matter, oysters can also take up dissolved organic and inorganic nutrients and dissolved organic matter (DOM), such as dissolved free amino acids, from the water column (Manahan et al., 1982; Dame, 1996). In particular, oysters can assimilate PO_4^{3-} directly from the water column for carbohydrate metabolism, energy transfer and shell deposition (Pomeroy and Haskin, 1954).

A net NH_4^+ excretion rate of 0.9 μmol g^{-1} DW h^{-1}, which is in the range described in the literature (Boucher and Boucher-Rodoni, 1988), was measured in the oyster treatment tanks. This takes into account inputs from regeneration, but does not incorporate the losses to phytoplankton and bacterial uptake of NH_4^+, volatilisation and nitrification which may significantly enhance these rates (Boucher and Boucher-Rodoni, 1988).

4.4. Macroalgal absorption

G. edulis in this study achieved 87% of its total NH_4^+ uptake within the first hour. A similar rate (80%) was observed for the red macroalga, *K. alvarezii*, incubated in the nutrients excreted by the pearl oyster *P. martensi* (Qian et al., 1996). The initial rate of NH_4^+ uptake in our study was 218 μmol h^{-1} per 100 g wet wt. (Table 2), which is very close to the rate observed by Qian et al. (1996) for 200-g wet wt. for *K. alvarezii*, and is within the range observed for *Gracilaria* (Rivers and Peckol, 1995) and other species of macroalgae (Taylor et al., 1998). The NO_3^- uptake rates in this study, however, were considerably lower, with a maximum rate of 15%/h compared to 66%/h for the *K. alvarezii*. Low light and high concentrations of NH_4^+ (D'Elia and DeBoer, 1978) can inhibit NO_3^- uptake. The ability to assimilate NO_3^- can also be species specific. Consistent with the findings for *G. edulis*, uptake of NO_3^- by *Ulva lactuca* was found to be minimal (Krom et al., 1995). Preferential uptake of NH_4^+ before NO_3^- is typical for most species of macroalgae and is consistent with the results of Oki and Fushimi (1992) who found that as the concentration of NH_4^+ decreased, the uptake rate of NO_3^- increased.

In this study, the fastest rates of net uptake of NH_4^+, NO_3^- and PO_4^{3-} by macroalgae were 2.6, 2.5 and 2.2 times higher than the net oyster release rates, respectively. The net rate takes into account losses to phytoplankton and bacterial uptake, volatilisation and nitrification (Table 2). The proportion of NH_4^+ to NO_3^- taken up by the macroalgae varied, being 20:1 during the period of fastest uptake of NH_4^+ uptake (in the first hour), but only 1.6:1 over the entire 24 h. This overall rate is comparable to the oyster release rates of NH_4^+ and NO_3^-, which were 1.9:1. The uptake of NH_4^+ and NO_3^- by *K. alvarezii* was in similar proportions to the release rates of the pearl oyster (Qian et al., 1996).

Macroalgae did not significantly ($P > 0.05$) change the $NH_4^+ : NO_3^-/NO_2^-$ ratio, but the uptake of N markedly increased the DIN/DIP on a short time scale, due to the saturating availability of N, and the ability of *G. edulis* to store reserves of N for times

of nutrient limitation (Gerloff and Krombholz, 1966; Jones et al., 1996). This reduction in the DIN/DIP ratio may have implications for water recirculation and downstream environmental impacts. Analysis of bioindicators downstream of shrimp farms has indicated responses typical of high NH_4^+ rich effluent (Jones et al., unpublished data).

Despite the rapid removal of N from the effluent, this uptake rate is substantially lower than recorded literature values for other species of *Gracilaria* (Friedlander and Dawes, 1985; Peckol et al., 1994), suggesting that removal rates could be improved. Nutrient removal efficiency of macroalgae may be improved with increased light, particularly for nitrate uptake (Hanisak, 1979; Falkowski, 1983), increased water flow to reduce the boundary layer (Wheeler, 1980), and higher stocking densities (Ugarte and Santelices, 1992). Our study was conducted indoors under experimental conditions. Under full solar radiation, photosynthetic rates could be considerably higher and nutrient uptake rates correspondingly higher. In a biological treatment pond, water flow rates would be determined by the physical volume of water being pumped through the pond and the water movement due to the action of paddlewheels or other aerators. In this study, the macroalgae were stocked at 2.3 kg m^{-2}, whereas maximum growth rates of *G. chilensis* have been achieved by stocking at 4 kg m^{-2} in winter and 8 kg m^{-2} in summer (Ugarte and Santelices, 1992).

Given the low concentrations of NH_4^+ in the effluent from the shrimp farm at the time of the study (early in the shrimp growout season), the total reduction in the concentration of NH_4^+ was lower than expected. The 24% reduction in NH_4^+ from 1.7 to 1.3 μM may not be indicative of the real potential for this system to remove NH_4^+. Due to addition of NH_4^+ to the system through the decay of epiphytes and macroalgal tissue, a concentration of approximately 1 μM may be the equilibrium point between uptake and input from decay. Based on the fast rates of uptake observed in this study, it can be expected that during the late phase of the growout season, when the concentrations of NH_4^+ are considerably higher (up to 60 μM) (Jones et al., unpublished data), that overall removal efficiency will be considerably higher. Conversely, the macroalgae may reach a saturation threshold with no further ability to store nutrients. This may mean that further NH_4^+ could only be taken up at a rate proportional to growth.

The percent reductions by macroalgal nutrient absorption have been expressed as overall reductions from the initial concentration in the shrimp effluent. However, because remineralisation and oyster excretion significantly ($P < 0.001$) increased the concentration of NH_4^+, NO_3^-, and PO_4^{3-}, the efficiency of nutrient removal can be expressed as a percentage of the concentration in the water received by the macroalgae. Based on these calculations, the concentrations of NH_4^+, NO_3^- and PO_4^{3-} were reduced to 2.3%, 2.2% and 4.8%, respectively (Table 1).

4.5. Nutrient regeneration

During sedimentation, the rate of NH_4^+ regeneration was 1.8 mmol m^{-2} day^{-1}, although this did not take into account loss of NH_4^+ by phytoplankton and bacterial uptake, or by volatilisation. The NH_4^+ regeneration rate declined markedly after transfer to the aerated oyster control tanks. This may have been due to the reduction in particulates, or the result of increased aeration. These results are consistent with those of

Kamiyama et al. (1997), who observed increases in NH_4^+ release during low dissolved oxygen conditions and NO_3^- release under high dissolved oxygen conditions. The NH_4^+ regeneration rates during sedimentation were markedly lower than the sediment NH_4^+ release rates of between 8.4 and 18 mmol m^{-2} day^{-1} that have been recorded in fish ponds (Riise and Roos, 1997). In our study, the NH_4^+ uptake rate by the macroalgae was 118 mmol m^{-2} day^{-1}. At this rate, the macroalgae would be able to remove up to 6.5 times the NH_4^+ released from the fish ponds observed by Riise and Roos (1997).

During sedimentation when the concentration of dissolved oxygen was very low, there was no production of NO_3^-, despite high amounts of NH_4^+ remineralisation. This is typical of anaerobic aquaculture ponds (Blackburn et al., 1988). Once under aerobic conditions in the aerated oyster controls, nitrification was observed, although rates were still very low. In the oyster treatment tanks, there was significant ($P < 0.001$) production of NO_3^-, which is consistent with the nutrient release by the pearl oyster *P. martensi* (Qian et al., 1996).

Oysters excrete NH_4^+, amino acids, urea, uric acid (Hammen et al., 1966) and PO_4^{3-} (Pomeroy and Haskin, 1954; Dame, 1996; Dame et al., 1989). The nitrogen release rates by the pearl oyster was 0.52 μmol h^{-1} of NH_4^+ and 0.44 μmol h^{-1} of NO_3^- per oyster (Qian et al., 1996), compared to 0.52 and 0.28 μmol h^{-1}, respectively, for *S. commercialis* in this study. It has been suggested that the observed NO_3^-/NO_2^- release from oysters is due to nitrifying bacteria in the digestive tract of the organism (Saijo and Mitamura, 1971; Boucher and Boucher-Rodoni, 1988). There could also be free-living nitrifying bacteria in the water column, although there was only negligible production of NO_3^- in the oyster and macroalgal control tanks.

The apparent enhancement of nitrification by the oysters resulted in more NO_3^- being available for denitrification. By increasing the proportion of NO_3^-–N relative to NH_4^+–N, bivalves such as oysters can enhance nitrification/denitrification coupling, promoting greater removal of N from the effluent. Rates of denitrification in mussel beds have been observed to be 21% greater than bare sediment (Kasper et al., 1985) and combined with the mussel harvest, this represented a 68% greater loss of nitrogen than bare sediment.

Bivalves are known to significantly increase the rates of carbon deposition (Doering and Oviatt, 1986; Doering et al., 1986) and the rates of benthic flux of DIN (Doering et al., 1987). Consequently, the sediment NH_4^+ and organic N pools under bivalves are significantly higher than those in bare sediments (Kasper et al., 1985). Bivalves enhance movement of organic nitrogen to the sediments, where it decomposes. This decomposition in the aerobic surficial sediments yields NH_4^+ (remineralisation) and NO_3^-/NO_2^- (nitrification) and, in the deeper anaerobic sediments, NO_3^- is converted into N_2 gas (denitrification) (Dame, 1996). Stimulation of nitrification by oysters can result in more NO_3^- present in the water, and less NH_4^+ (Boucher and Boucher-Rodoni, 1988).

By stimulating nitrification, the oysters significantly ($P < 0.001$) reduced the NH_4^+: NO_3^-/NO_2^- ratio from 13:1 to 4:1. This has significant management implications, especially in relation to the possibilities for recycling of treatment pond effluent back into the production ponds. Aeration and uptake by phytoplankton and bacteria in the macroalgal control tanks reduced the NH_4^+/NO_3^- ratio to 0.5:1. Although the ratio of NH_4^+/NO_3^- was lower in the control, the total concentration of DIN was significantly ($P < 0.001$) lower in the macroalgal treatment.

Greater improvements in water quality may be possible by combining the oysters and macroalgae in the same treatment pond. Rapid uptake of the nutrients resulting from remineralisation of oyster biodeposits (Kelly and Nixon, 1984), may reduce potential stimulation of phytoplankton production. There may be a balance between nutrient enhanced phytoplankton productivity and the removal of phytoplankton biomass by oysters (Zeitzschel, 1980).

For co-culture of macroalgae and oysters to be successful, the growth requirements of each species need to be optimised. In particular, if the temperature, water flow rate, or light availability is not adequate for macroalgal growth, there may be more biomass decaying than being produced. This would result in a decline in water quality and consequently lower oyster growth rates (Qian et al., 1996).

5. Conclusions

The results from this study detail the ability of sedimentation, oysters and macroalgae to substantially reduce the concentrations of water column nutrients, suspended solids, phytoplankton and bacteria of shrimp pond effluent and highlights the relationships between these components of the effluent water quality. These laboratory trials provided detailed information regarding potential sedimentation and nutrient regeneration rates, oyster filtration rates, and nutrient uptake rates. However, scaling this integrated system to farm size may result in considerably different improvements to effluent water quality.

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