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Polychaete-assisted sand filters – prawn farm wastewater remediation trial.

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Summary

Medium bedding sand which is commonly available in coastal sedimentary deposits, and a marine polychaete-worm species from Moreton Bay recently classified as *Perinereis helleri* (Nereididae), were deployed in a simple low-maintenance sand filter design that potentially has application at large scale. Previous work had shown that this physical and biological combination can provide a new option for saline wastewater treatment, since the worms help to prevent sand filter blocking with organic debris and offer a profitable by-product. To test the application of this new concept in a commercial environment, six 1.84 m^2 Polychaete-assisted sand filters were experimentally tested for their ability to treat wastewater from a semi-intensive prawn culture pond. Polychaetes produced exclusively on the waste nutrients that collected in these gravity-driven sand filters were assessed for their production levels and nutritional contents.

Water parameters studied included temperature, salinity, pH, dissolved oxygen (DO), oxidation/ reduction potential (redox), suspended solids, chlorophyll *a*, biological oxygen demand (BOD), and common forms of nitrogen and phosphorus. Pond water which had percolated through the sand bed had significantly lower pH, DO and redox levels compared with inflow water. Suspended solids and chlorophyll *a* levels were consistently more than halved by the process. Reductions in BOD appeared dependant on regular subsurface flows. Only marginal reductions in total nitrogen and phosphorus were documented, but their forms were altered in a potentially useful way: dissolved forms (ammonia and orthophosphate) were generated by the process, and this remineralisation also seemed to be accentuated by intermittent flow patterns. Flow rates of approximately 1,500 L m^{-2} d^{-1} were achieved suggesting that a 1 ha polychaete bed of this nature could similarly treat the discharge from a 10 ha semi-intensive prawn farm.

Sixteen weeks after stocking sand beds with one-month-old *P. helleri*, over 3.6 kg of polychaete biomass (wet weight) was recovered from the trial. Production on a sand bed area basis was 328 g m^{-2}. Similar ($P>0.05$) overall biomass production was found for the two stocking densities tested (2000 and 6000 m^{-2}; $n = 3$), but survival was lower and more worms were graded as small (<0.6 g) when produced at the higher density (28.2 ± 1.5 % and approx. 88 %, respectively) compared with the lower density (46.8 ± 4.4 % and approx. 76 %, respectively). When considered on a weight for weight basis, about half of the worm biomass produced was generally suitable for use as bait.

The nutritional contents of the worms harvested were analysed for different stocking densities and graded sizes. These factors did not significantly affect their percentages of dry matter (DM) (18.23 ± 0.57 %), ash (19.77 ± 0.80 % of DM) or gross energy 19.39 ± 0.29 MJ kg^{-1} DM) ($n = 12$). Although stocking density did not affect the worms' nitrogen and phosphorus contents, small worms had a higher mean proportion of nitrogen and phosphorus (10.57 ± 0.17 % and 0.70 ± 0.01 % of DM, respectively) than large worms (9.99 ± 0.12 % and 0.65 ± 0.01 % of DM, respectively) ($n = 6$).

More lipid was present in large worms grown at the medium density (11.20 ± 0.19 %) compared with the high density (9.50 ± 0.31 %) and less was generally found in small worms (7.1-7.6 % of DM). Mean cholesterol and total phospholipid levels were 5.24

± 0.15 mg g^{-1} and 13.66 ± 2.15 mg g^{-1} DM, respectively (n = 12). Of the specific phospholipids tested, phosphatidyl-serine or sphingomyelin were below detection limits (<0.05 mg g^{-1}), whilst mean levels of phosphatidyl-ethanolamine, phosphatidyl-inositol, phosphatidyl-choline and lysophosphatidyl-choline were 6.89 ± 1.09, 0.89 ± 0.26, 4.04 ± 1.17 and 1.84 ± 0.37 mg g^{-1}, respectively (n = 12). Culture density generally had a more pronounced effect on phospholipid contents than did size of worms.

By contrast, worm size had a more pronounced effect on total fatty acid contents, with large worms containing significantly higher (P<0.001) levels on a DM basis (46.88 ± 2.46 mg g^{-1}) than smaller worms (27.76 ± 1.28 mg g^{-1}). A very broad range of fatty acids were detected with palmitic acid being the most heavily represented class (up to 14.23 ± 0.49 mg g^{-1} DM or 27.28 ± 0.22 % of total fatty acids). Other heavily represented classes included stearic acid (7.4-8.8 %), vaccenic acid (6.8-7.8 %), arachidonic acid (3.5-4.4 %), eicosapentaenoic acid (9.9-13.8 %) and docosenoic acid (5.7-7.0 %). Stocking density did not affect (P>0.05) the levels of amino acids present in polychaete DM, but there was generally less of each amino acid tested on a weight per weight basis in large worms than in small worms. This difference was significant (P<0.05) for the most heavily represented classes being glutamic acid (73-77 mg g^{-1}), aspartic acid (50-54 mg g^{-1}), and glycine (46-53 mg g^{-1}).

These results demonstrate how this polychaete species can be planted and sorted at harvest according to various strategies aimed at providing biomass with specific physical and nutritional qualities for different uses.

Introduction

The abatement of nutrients entering waterways is a difficult issue that all developing countries face in protecting the diversity and health of their natural aquatic ecosystems. Whilst many non-point anthropogenic sources like agricultural and domestic runoff can represent a large portion of the total nutrient loading in estuaries worldwide (Gordon, 2007; Whitall *et al*., 2007), they are typically very difficult to address and generally require social and systems management changes. On the other hand, point sources provide opportunities for the design of nutrient collection and mitigation schemes that have economic or intrinsic benefits and provide incentives for businesses and enterprises to place greater values on previously wasted nutrients.

The potential for environmental damage from nutrients in aquaculture discharge is also well recognised and many researchers around the world are involved with developing new technologies to reduce the environmental footprint of intensive systems (Folke *et al*., 1998; Crab *et al.*, 2007). Contingent on this work are the indirect potential benefits they may provide in identifying ways to deal with other non-point nutrient sources that are increasingly entering estuaries, since the enriched aquatic ecosystems in aquaculture farms provide a relevant model for the study of any eutrophication control or intensive nutrient stripping mechanism. Coastal semi-intensive prawn farms commonly operating on flow-through methodologies provide this model for brackish waters. Typically, such farms exchange large volumes (5-30 %) of water in production ponds on a daily basis (Vigneswaran *et al*., 1999). Up to 27 % of the nitrogen and 10 % of the phosphorus in these systems are typically wasted in effluent waters (Funge-Smith and Briggs, 1998). In addition, on-farm accumulation of

wasted nutrients occurs in various forms, which include organic sludge, algal and bacterial biomass (Brune *et al.,* 2003), and incidental plants (eg: macrophytes like *Enteromorpha* sp: Cohen and Fong, 2006) and animals (eg: various gastropods and arthropods, and encrusting organisms like barnacles, *Balanus* sp., and tube worms, *Polydora* sp: Fujioka et al, 2007). These non target species coexist with the target crop and prosper from available resources, but are presently of limited economic value to farmers and can become problematic with overpopulation. A far better approach would be to increasingly direct unutilised nutrient resources towards more controlled organisms which may be intrinsically valuable and offer functional advantages in the farming system.

Several species of segmented marine worms (Phylum: Annelida; Class: Polychaeta) have been documented growing in prawn farm environments in uncontrolled ways (eg: *Notomastus* sp. (Capitellidae) and *Perinereis* sp. (Nereididae): Meksumpun and Meksumpun, 1999; *Perinereis* sp: Fujioka *et al*, 2007), and sand filters are commonly used to treat waters in domestic (Campos *et al*., 2002) and aquaculture systems (Vigneswaran *et al*., 1999; Palacios and Timmons, 2001). Preliminary studies (DPI&F unpublished data) had demonstrated that these two concepts could be combined in a controlled way to sequester nutrients that would otherwise be wasted in prawn pond discharge waters, but before investing further in this pursuit there was a need to test the concept in a commercial environment. Most appropriately, this commercial testing environment would expose the proposed system to the typically variable and sometimes harsh climatic and environmental conditions of a prawn farm, where silt from earthen ponds and naturally occurring organisms would have a chance to impact on the system's efficacy.

Several species of polychaetes are also now being intensively cultured around the world for fishing bait, feed resources and several potential uses in aquaculture (Olive *et al*., 2002; Costa *et al.*, 2003). Some recent authors in this area have suggested the use of local species to avoid unnecessary introductions that may result in environmental problems (Costa *et al.*, 2006; Scaps, 2003). Most of these operations have focused on various species from the family Nereididae, which have proved amenable to intensive culture conditions (eg: *Perinereis nuntia*: Poltana *et al*., 2007) and been shown able to synthesize essential fatty acids necessary for the balanced nutrition of marine fish and prawns (Olive, 1999; Costa *et al.*, 2000).

Our challenge in the present work was to favourably treat prawn pond effluent and simultaneously grow a profitable crop of marine worms using only waste nutrients. The objectives of this study were to 1) assess the wastewater remediation properties of Polychaete sand filtration beds at a commercial prawn farm; 2) document the survival and production levels of one local inter-tidal polychaete species from the family Nereididae (*Perinereis helleri*) when stocked in such filtration beds at two densities; and, 3) assess their potential suitability for fishing bait and document their nutritional contents for potential use as prawn or fish feeds.

Materials and methods

Six experimental polychaete sand filter beds were implemented at the Bullock Creek Prawn Farm at Donnybrook in South East Queensland during the 2007 production season. Each sand bed was constructed on the bottom of a round flat-bottomed 2000-L high-density polyethylene (HDPE) tank to a depth of approximately 200 mm with 400 L (dry volume) of sand. These tanks had an average diameter of 1550 mm (mid water column), but were 1530 mm in diameter at the sands surface, giving each sand bed a surface area of 1.84 m^2. The sand used (Table 1) was medium bedding sand commonly used in the construction industry and reliably available in large volumes at reasonable cost ($AUD22 $tonne^{-1}$ at the time of the study). Slotted corrugated HDPE pipe with a diameter of 60 mm formed the subsurface drainage under each sand bed. This drainage pipe was wrapped in 4-5 layers of 90 % shade cloth to prevent sand infiltrating the drainage systems.

Table 1 Particle size characteristics* of medium bedding sand (product code BMS 2000) from Southern Pacific Sands Pty Ltd.

Sieve size (mm)	Medium sand (% retained in series)	Medium sand (% passing)
4.750	0.0	100
2.360	0.1	99.9
1.180	4.5	95.4
0.600	18.8	76.6
0.300	57.8	18.8
0.150	18.3	0.5
0.075	0.4	0.1
<0.075 (Pan)	0.1	-

* Modified from commercial product data sheet.

Figure 1 provides a schematic view of the experimental system. Water from the monk drain of a semi-intensive *Penaeus monodon* culture pond (see Tables A 1 and A 2 in Appendix) was pumped intermittently to a manifold of 25 mm HDPE supply pipes. A main-pressure relief valve in line after the manifold allowed simultaneous adjustments to tank supplies, and valves at the end of each supply pipe provided finer adjustments to equalise flows to each tank. Flows to each tank were regularly adjusted to targeted flow rates. Unused water passing through the pressure relief valve was recirculated back to the culture pond and sand-filtered and overflow waters were piped to the farm's settlement pond.

The targeted rate of pond-water supply to each tank was based on a farm design incorporating 1 ha of sand bed for every 10 ha of culture ponds. Assuming a 10% daily exchange rate for culture ponds with an average depth of 1.5 m, each 1 m^2 of sand bed would therefore need to treat 1500 L d^{-1}. Since each experimental sand bed had a surface area of ~1.84 m^2, each would therefore need to treat 2760 L d^{-1} for scaled commercial relevance. This water delivery rate was provided during two 6-h periods (9 am – 3 pm & 9 pm – 3 am) through a simple timer controlling the supply pump. This simulated a tidal system and generally allowed the beds to drain completely between pumping periods.

The targeted inflow rate for each tank was therefore 3.83 L min^{-1}, which was estimated during routine management by repeatedly measuring the flow being delivered every 10 s. In practice, flows were regularly adjusted to just over 640 mL 10 s^{-1} (640 - 650 mL). Volumes of filtered drainage water were cumulatively measured with flow meters (BIL DN 25 mm, 16 Bar, 50ºC, to 0.0001 m^3) fitted to the tank outlet, and drainage flows were restricted immediately following these meters with smaller pipe fittings (9 mm inside diameter). Small tubs (approx 10 L) collected this drainage water and provided a mechanism for sample collection and testing in clean 1 L plastic beakers. Each tank was covered with plastic solar weave to prevent rainfall entering the tanks, and these were vented at the top of the conical structure to allow the exit of sun-heated air. Figure 2 provides a series of photos showing different components of the experimental tank setup at the farm.

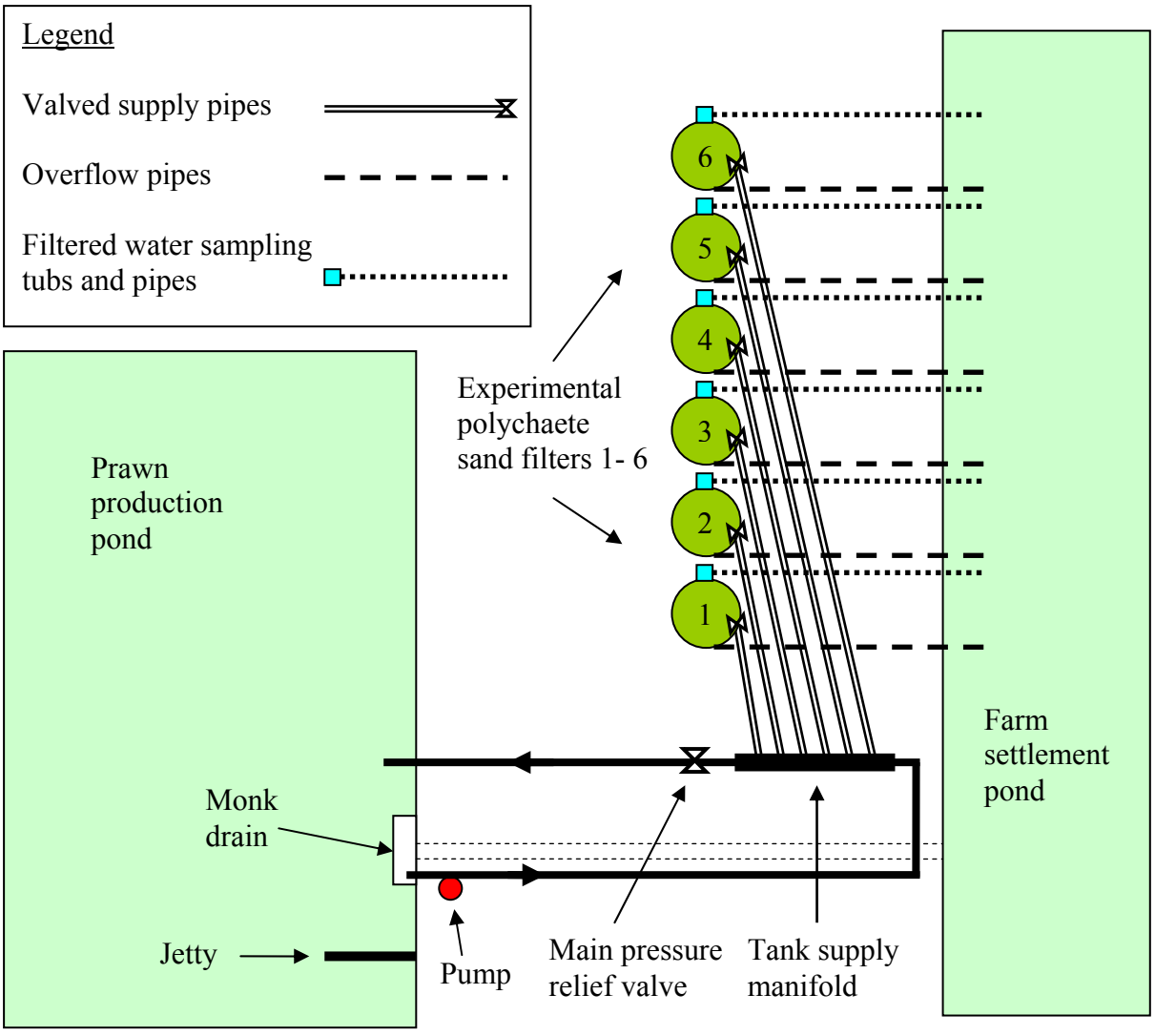

Figure 1 Schematic representation of the experimental polychaete sand filters and controlled water flows (not to scale).

Pond water supply to each sand bed began the day before stocking with polychaete juveniles (11th January 2007). During this short pre-stocking period, flow rates were approximately 50% greater than those later targeted, so that the entirety of each sand

bed was pre-soaked with pond water. Beds were drained completely immediately prior to stocking. A full record of the timelines for trial operation is provided in the Appendix (Table A 3).

All six experimental sand beds were stocked with one-month-old laboratory-reared mangrove worms from Moreton Bay in South East Qld (identified as *Perinereis helleri* by C. Glasby from Hutchings *et al*., 1991). Tanks 2, 4, and 6 were stocked on 12th January and tanks 1, 3 and 5 were stocked on 13th January. Stocking estimates were based on the transfer of a predetermined volume of sand from nursery beds for which the population densities of juveniles present had been estimated. These nursery estimates were undertaken on two consecutive days immediately prior to transfers. They consisted of stratified (surface, mid depth and lower depth) samples of sand (5 mL) taken from both sides of a central plane with a small spoon, and involved counting the number of juveniles in each sample under a low powered binocular microscope. The targeted stocking rates in applying this procedure (Table 2) were approximately 6,000 juveniles m^{-2} for filters 1, 3 and 5 (10,945 per tank), and 2,000 juveniles m^{-2} for filters 2, 4 and 6 (3,680 per tank). A degree of imprecision was associated with these high and medium stocking estimates, but they were assumed to be accurate to later derive survival estimates after harvest.

Figure 2 Experimental polychaete sand beds at the Bullock Creek Prawn Farm including the tank drainage system before filling with sand (top left), the positioning of tanks with covers on pond embankment (top right), the inside of a tank during operation (bottom left), and a tank's drainage water meter and collecting tub for sampling (bottom right).

Stocking involved gently spreading the prescribed amount of nursery sand over a small area (approx. 0.1m^2) of the damp bed surface. The inflow water was then brought on-line according to the normal operating conditions of the system (ie: on for 6 h, off for 6 h).

Table 2 Juvenile-density estimates in nursery beds and quantities of nursery sand transferred to each experimental polychaete sand filter tank.

Nursery tank	1st sample series* (juveniles in 5 mL)						2nd sample series* (juveniles in 5 mL)						Mean ± se density (mL^{-1})	Nursery sand transferred	
	Upper		Mid		Lower		Upper		Mid		Lower			Filter tank	Volume (mL)**
A	26	23	29	31	7	10	22	21	10	18	7	5	3.48 ± 0.65	1, 3 & 5	3142
B	7	16	4	8	7	2	10	2	1	2	4	7	1.17 ± 0.30	2	3154
C	21	32	5	8	2	11	9	3	4	2	7	4	1.80 ± 0.63	4	2044
D	22	13	4	5	1	3	10	13	2	6	1	5	1.42 ± 0.45	6	2598

* Two replicates taken from upper, mid- and lower sand depths
** Volume estimates added to the nearest 10 mL.

Water qualities, including temperature (ºC), salinity (g L^{-1}), dissolved oxygen (DO, mg L^{-1}), pH, and reduction/oxidation potential (redox, mV) were measured *in situ* using a hand held YSI 556 Multi Probe System. These parameters were measured on a weekly basis at 12 noon at the inflow and filtered discharge/outlet points and in the water column for each tank, as well as in the supply pond in the monk drain and off the jetty (when water depths permitted).

Water samples for nutrient analyses were collected over a four month period on a fortnightly basis from the 18 January 2007. They were taken between 12 noon and 1 pm from the intake points and filtered discharge collection tubs for each tank (see Figure 2). Samples were immediately stored on ice and all were transported to the BIARC laboratory for processing within 2 h. Parameters tested included total nitrogen (TN), total phosphorus (TP), total ammonia nitrogen (TAN), oxides of ammonia (NO_2 + NO_3 = NOx), orthophosphate (PO_4), total suspended solids (TSS), chlorophyll *a* (Chl*a*) and 5-day biological oxygen demand (BOD), using the standard methods described by APHA (1989). Nutrient analyses were performed with a Lachat QC8000 Flow Injection Analyser using methods described in the instrument manufacturer's methods (QuickChem Methods, Zellweger Analytics Inc. Milwaukee WI 53218). Chl*a* was studied using glass fibre filters (47 mm) with spectrophotometric determination using the trichromatic method. BOD determinations were undertaken using the Oxidirect® respirometric BOD measurement system from Lovibond, Germany.

Statistical analyses were undertaken using GenStat (2007). For all water quality and nutrient parameters tested, repeated measures analyses of variance (ANOVA) were performed. This was done, firstly to determine if the effect of worm density was significant in the treated (outflow) water, and if not, data for all tanks were pooled for comparisons of inflow, in-tank and outflow levels using a split-plot analysis. Mass balance estimates were calculated by adding the sequential mean differences between inflow and outflow concentrations multiplied by volumes filtered at discrete sampling occasions. These were corrected for net removal on a square metre of sand basis.

Polychaetes that survived and grew in the worm beds were harvested over one week at the beginning of May 2007, in the 16th week after stocking juveniles. These were

separated from the sand in 2 mm sieves. After removing sandy residues and silt-laden mucus, the worms from each tank were visually sorted into two size classes, namely (1) bait-sized individuals (≥ 0.6 g), and (2) those too small for easy use as fishing bait (< 0.6 g). The number of individuals in each of these groups was tallied to provide total survival estimates and proportional size-related production assessments. After a purging period of 3-4 h, each group was weighed on bulk after allowing free water to drain through a fine capture net for 20 s. Groups were then frozen in a -80ºC freezer in a thin flattened layer within sealed plastic bags.

Weights and survival percentages were subjected to one-way ANOVA using the tank as the experimental unit. The balance of small verses medium worms was tested using a Chi-square test on the contingency table of counts.

Each group was later assessed in terms of their proximate analysis including moisture, fat, ash, gross energy, nitrogen, phosphorus, lipid, cholesterol, phospholipid, and fatty and amino acid contents. These data were subjected to ANOVA. In all analyses, protected least significance difference testing (LSD) was conducted between means.

Results

Water volumes and flows

A total of 1,788,376 L of pond water was filtered during the trial. For at least the first half of the experiment, pond water delivery rates to each tank remained very consistent on a daily and weekly basis with few operational difficulties experienced. The cumulative volumes of pond water filtered by each sand bed (Figure 3) demonstrate the consistent ability of this water treatment method to handle the nominated commercially relevant water treatment rates (1,500 L m^{-2} d^{-1} or 2,760 L $tank^{-1}$ d^{-1}). Towards the end of the trial, a few difficulties developed in getting consistent flows (640 - 650 mL 10 s^{-1}) delivered into the tanks (see Figure A 1), and this resulted in targeted inflows falling slightly short of the target on several occasions (see Figure A 2).

These water supply difficulties and other bed management activities are described in Figure 4 along with the depths of water which prevailed in tanks during the sampling events. There was no clear evidence of worm density consistently affecting water depth in the tanks. Initially, pond water passed freely through each bed, merely wetting the sand and filling most of the pore space. However, after about one week free water began to accumulate over the sand beds when the inflows were operating.

The surfaces of all beds were raked for the first time after three weeks of operation when the water depths above beds were rising to 300 mm or more. This rapidly improved percolation rates so that all tanks continued to fully drain between inflow periods. Surface raking was again performed on later occasions when it was observed that beds had not fully drained between inflow periods (see Figure 4 and Table A 3 for details). By the end of February, the worm's activities in clearing phytoplankton from the leading surface layers of the filter were clearly visible, and this effect was most obvious in the area where the nursery-sand carrying the seed-worms had been deposited. Surface raking was not undertaken thereafter.

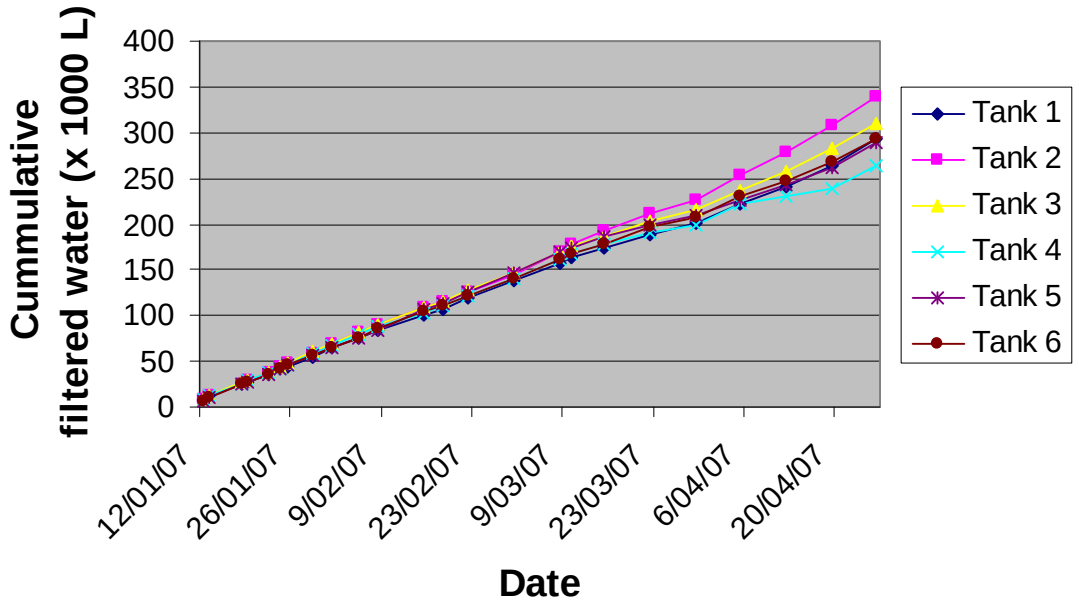

Figure 3 Cumulative volumes of pond water filtered by each sand bed.

Although all tanks were equipped with overflows, this generally only occurred on a few occasions when inflows had climbed above prescribed rates. Over the final month of operation however, all tanks began to fail to drain completely between inflow periods. The volumes of water which built above the sand beds during this time (see Figure 4) acted as a reservoir which mitigated flows so that relatively consistent volumes of pond water continued to be filtered by the sand beds on a daily basis during this time (see Figure 3).

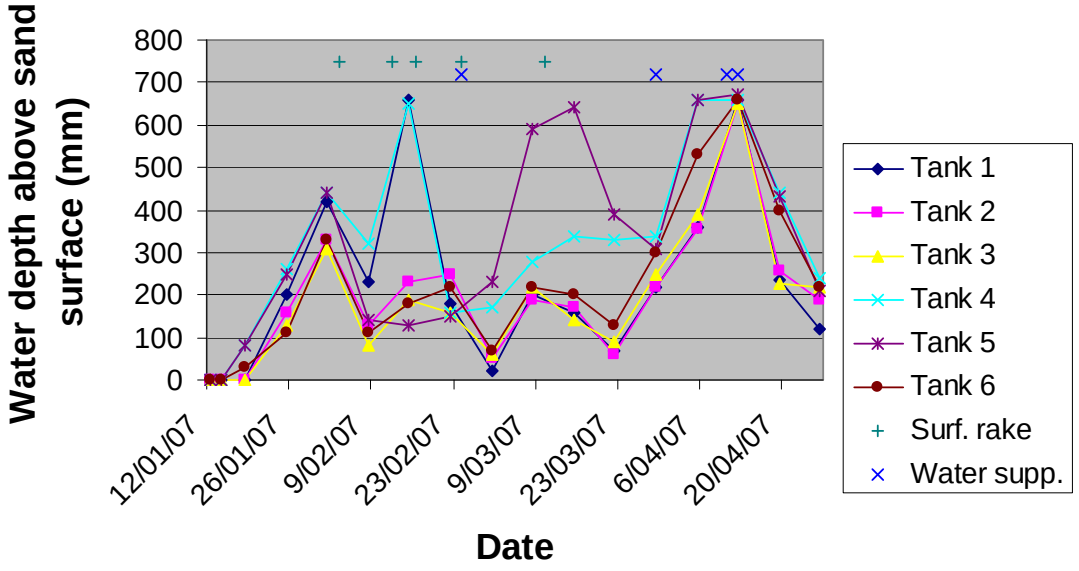

Figure 4 Water depths above sand bed surfaces during weekly sampling at 12 noon. Also shown are days when the bed surface was raked and when pond water supplies were inconsistent and likely to have affected water depths.

Water qualities and nutrients

The qualities of water flowing into the experimental tank system (inflow) varied with different pond management activities (see Tables A1, A2 and A3). Most notably, inflows after the 15th March were taken from the prawn pond after it had been almost completely harvested of prawn stock, and refilled to approximately 30 % of its original volume. This greatly reduced the phytoplankton bloom being delivered to tanks and also occasionally affected different aspects of the way the sand filters were operated. For example, on the night preceding the 29th March sampling date, the pump failed so that sand beds remained dry all night, and this may have particular relevance to effects seen on that date for some parameters including ammonia and orthophosphate (discussed later). Additionally, from the 10th April, inflows were sourced from the farms supply pond due to the need to fully harvest and empty the prawn pond. This again dramatically changed the nature of water being treated by the experimental system (also discussed later with relevant water quality parameters).

Water qualities were also typically affected by the prevailing weather conditions. Several particularly cold periods occurred during the 2006/07 summer season, causing water temperatures to vary uncharacteristically from one week to the next. This coupled with very limited rainfall early in the season, which caused relatively high initial salinities (up to 41 ppt. at the beginning of February at this farm), meant that prawn production levels were generally low at this farm and across the industry. However these relatively harsh environmental conditions conveniently challenged the polychaete-based water treatment system. The pH, DO and redox levels measured in the supplying prawn pond, and the prevailing green chlorophyte phytoplankton blooms mixed with varying levels of yellow/brown diatoms, were typical of effluents generated in subtropical semi-intensive prawn farming environments.

The tank inflow water just after stocking the juvenile polychaetes (12th & 14th Jan) had a temperature range of 29 to 31 ºC, a salinity range of 39.0 to 39.2 ppt., a pH range of 8.1 to 8.3, and a DO range of 7.9 to 8.0 (see Appendix). Weekly data from the 18th January to the 26th April are presented in the Figures that follow.

No differences ($P>0.05$) were found between tanks with different densities of worms for any water quality or nutrient parameter (ie. temperature, salinity, pH, DO, redox, TSS, BOD, Chl*a*, TN, NOx, TAN, TP or PO_4). This allowed the pooling of data from the medium and high density tanks to simply study the effects of the worm assisted sand filters (total of six replicates) on pond water qualities and nutrients as it passed through the treatment system. Data for each separate tank are presented in Figures A 3 through to Figure A 15.

For all parameters, except NOx, the effect of the tank on inflow levels, and/or the effect of the sand bed, differed significantly ($P<0.05$) over time. This effect of time can be expected since most parameters that were measured are heavily affected by the weather, either directly as in the case of ambient temperatures and sun strength affecting water temperatures, or indirectly through conditions which stimulate algal bloom developments and other biological processes which in turn mediate parameter changes.

Time had by far the most dominant effect on water temperatures and salinities. The highest temperatures recorded during the trial occurred in the first 2 weeks after

stocking and this peaked on the 25th Jan at 34.9ºC (tank water temperature). Water salinity also peaked at 40.4 ppt around this time. Cold snaps each month caused low points within a slowly declining temperature regime for the remainder of the experiment/season, whereas salinities returned to more reasonable levels with some moderate rainfall between mid-February and mid-March. The lowest temperature of 25.2ºC was recorded in the outflow on the 12th April, and the lowest salinity of 29.2 ppt. was recorded in the inflow on the 15th March.

On most occasions the temperature of water in the tank was significantly higher than that of the inflow and outflow (Figure 5). Since sampling was undertaken during the hottest part of the day (noon), the sun-generated heat contained in the air under the tank covers, and the insulative properties of the sand, undoubtedly affected these results. There was no consistent pattern of salinity effects from the tanks and sand filters (Figure 6).

The increasing pH levels of the tank inflows over time reflected the typical pattern of an increasing algal bloom in a prawn pond as the nutrient levels rise with stock feeding levels. Both the tank and sand bed treatments had a pronounced effect on water pH. On all but 2 sampling occasions the pH of water in the tank was significantly higher than the inflow, and the outflow was significantly lower than the inflow (Figure 7). Outflow pH levels remained comparatively stable and more neutral during the entire time that the system was operating with prawn pond water (up to 29th March). After this time the source was changed to the farm reservoir pond which had a much lower pH, thereby also depressing outflow levels.

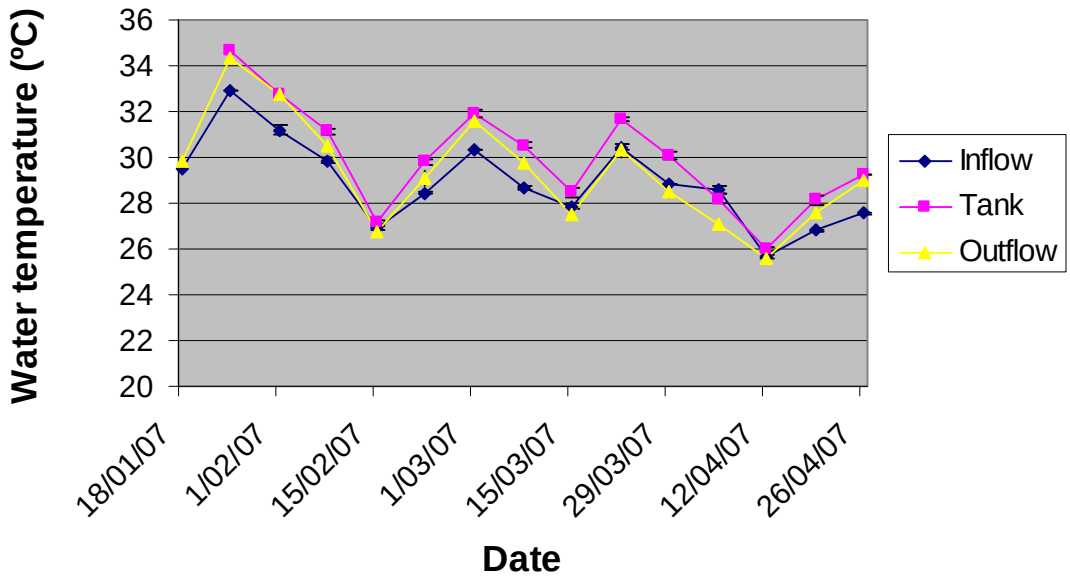

Figure 5 Mean (± se. *n* = 6) temperatures for inflow, tank and outflow (filtered) waters during weekly sampling at 12 noon (5% LSD between treatments = 0.47).

The DO levels of inflows were generally depressed by the delivery system. This was probably caused by a build-up of biofilms on the inside of delivery pipes where flow rates were relatively slow allowing lengthy contact times. Compared with the pond proper, DO levels were also reduced in the monk drain where the suction point for the delivery system was situated (see Tables A 1 and A 2). This was not unexpected due

to lower light and more limited water exchange inside the monk structure, and it was particularly pronounced before the 1st February when there had previously been limited discharge from the pond to clear settled debris, which also most likely had a high demand for oxygen.

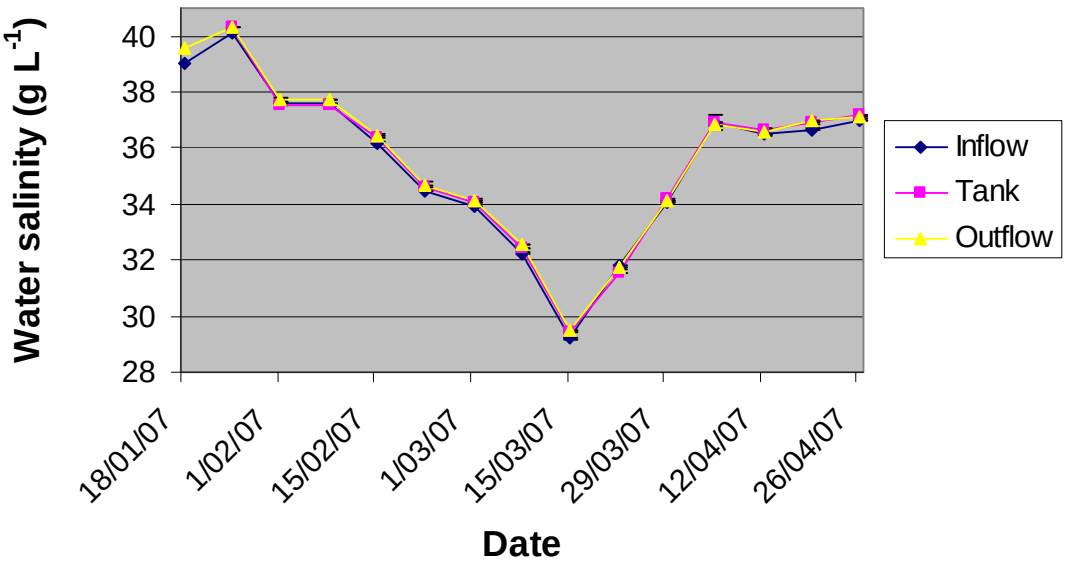

Figure 6 Mean (± se. *n* = 6) salinities for inflow, tank and outflow (filtered) waters during weekly sampling at 12 noon (5% LSD between treatments = 0.20).

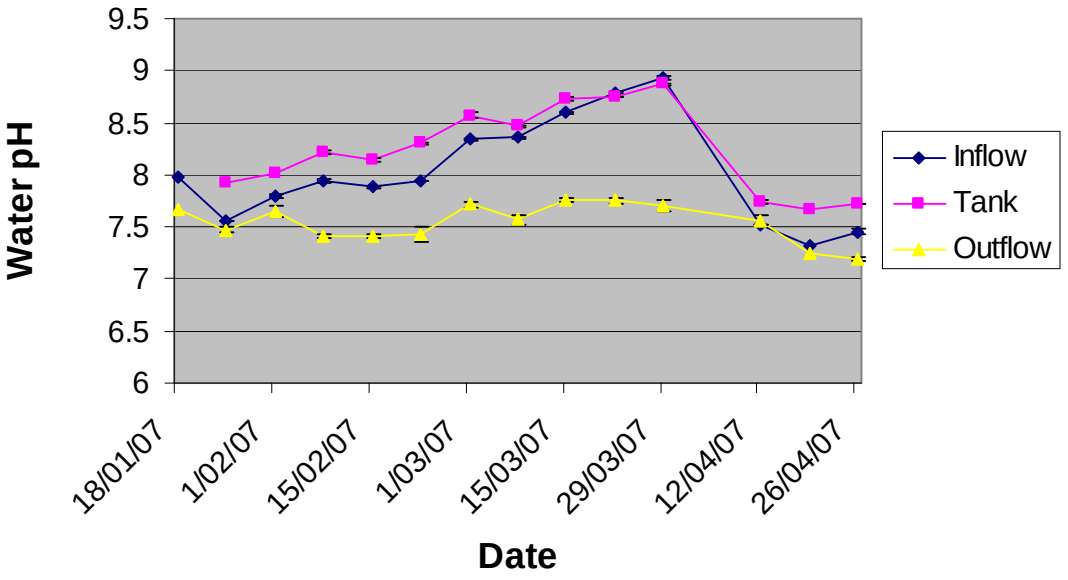

Figure 7 Mean (± se. *n* = 6) levels of pH for inflow, tank and outflow (filtered) waters during weekly sampling at 12 noon (5% LSD between treatments = 0.08).

DO levels in the tank's water tended to reflect more closely the levels in the pond and on most occasions were significantly higher than the inflow. Most noticeable was the highly significant effect of the sand bed on water percolating through it, whereby almost all dissolved oxygen was consistently stripped (Figure 8). Not surprisingly, from March onwards the outflow water had an anoxic (hydrogen sulphide) smell and

portions of the sediment turned black. The development of this reducing environment was also evident in the redox potential results (Figure 9). Again the sand bed had a pronounced affect causing extremely low readings after only one week of operation.

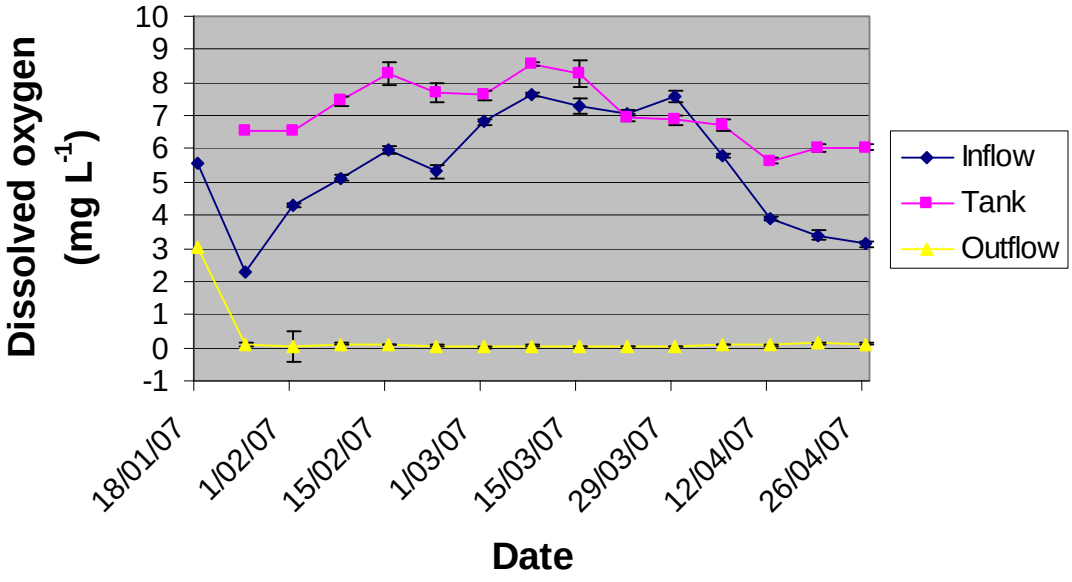

Figure 8 Mean (± se. *n* = 6) levels of dissolved oxygen for inflow, tank and outflow (filtered) waters during weekly sampling at 12 noon (5% LSD between treatments = 0.50).

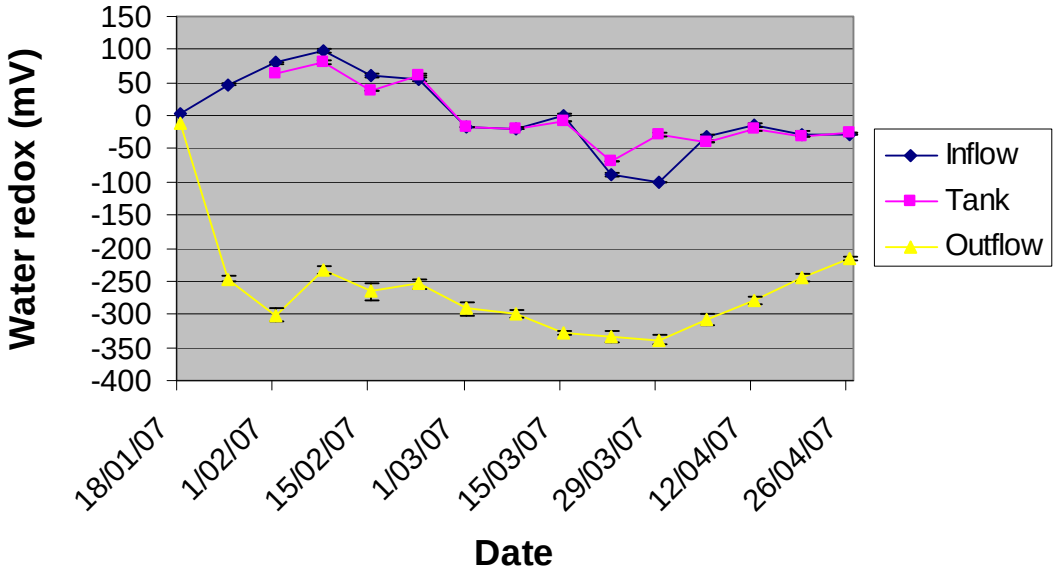

Figure 9 Mean (± se. *n* = 6) redox potential for inflow, tank and outflow (filtered) waters during weekly sampling at 12 noon (5% LSD between treatments = 15.72).

Although the effect of the sand bed on suspended solids varied significantly over time, it had a far more pronounced affect compared with time, with outflow levels consistently significantly lower than inflow levels (Figure 10). The overall mean outflow TSS level for all 8 sampling times (11.3 mg L^{-1}) was well under half of that of inflow levels (30.7 mg L^{-1}).

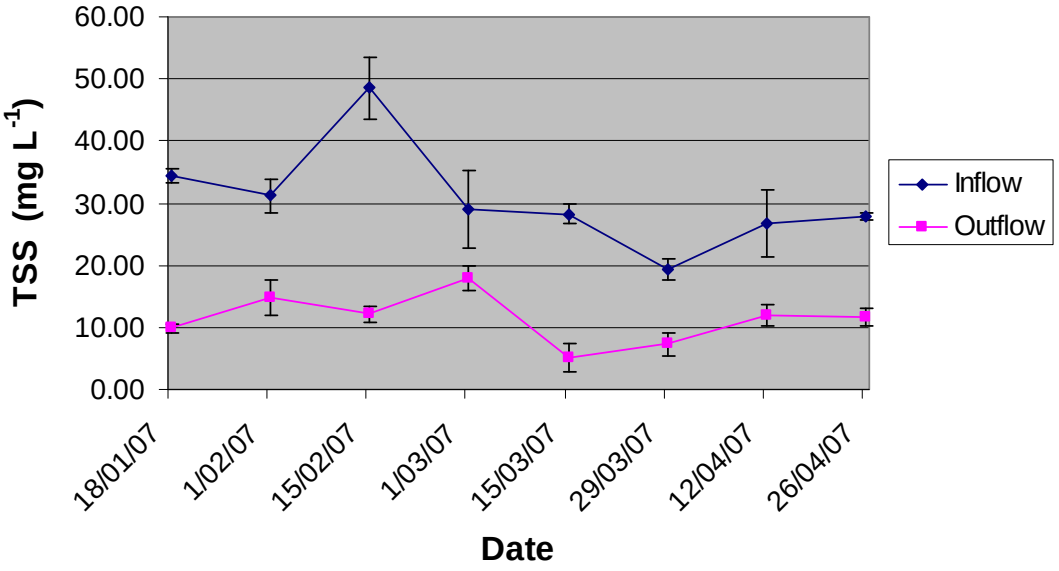

Figure 10 Mean (± se. *n* = 6) total suspended solids (TSS) in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 9.68).

Whilst the sand beds were engaged in the regular treatment of prawn pond water, BOD levels were consistently reduced (Figure 11), and this was by significant ($P<0.05$) levels for all but one sampling occasion (15/03/07). However, on the day that the water supply was interrupted the night before, the BOD of the outflow was significantly higher then the inflow. When the beds were operating with the lower ($P<0.05$) BOD water from the reservoir pond, no effect on BOD was apparent.

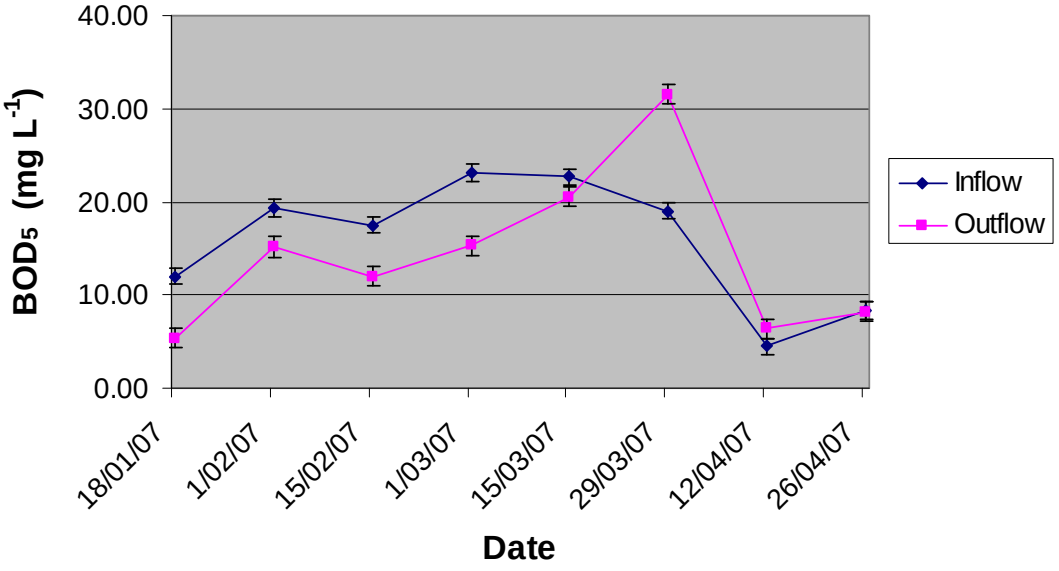

Figure 11 Mean (± se. *n* = 6) biological oxygen demand (BOD_5) of inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 3.97).

The effect of the sand bed on Chl*a* levels was highly significant ($P<0.01$), with consistent reductions when pond water rich in phytoplankton was being treated

(Figure 12). In contrast with BOD (above) and dissolved nutrients (see later results), this reduction was not impeded by less flow through the bed the night before (see corresponding results for 29/03/07).

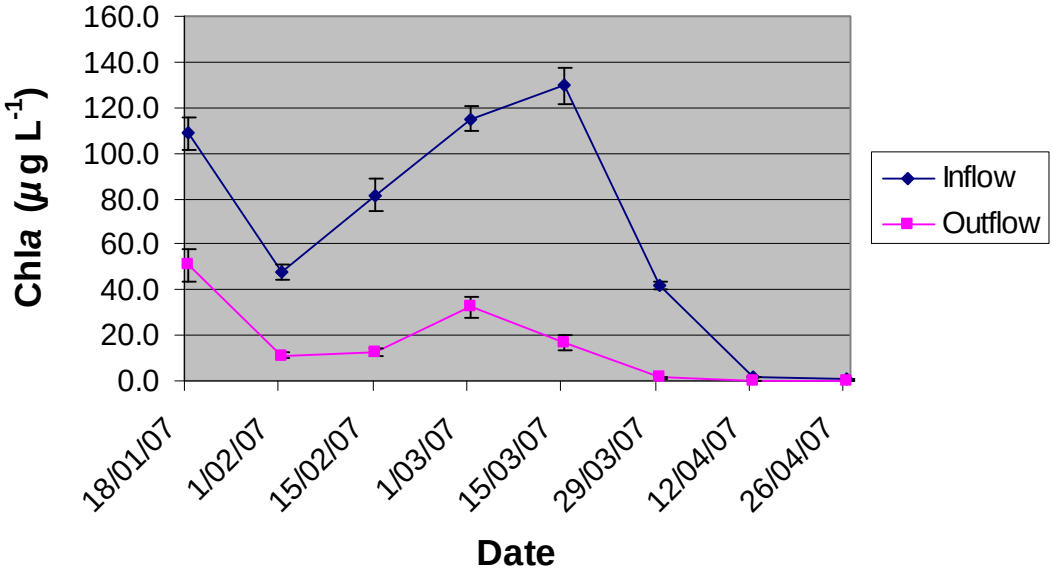

Figure 12 Mean (± se. *n* = 6) chlorophyll *a* (Chl*a*) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 12.93).

The effect of the sand bed on TN levels over time was not consistent (Figure 13). Although marginal reductions occurred on the first 4 sample days of the experiment, these were not significant (P>0.05). The significant (P<0.05) increase in TN levels on the 29/03/07 was caused by the sharp increase in the TAN fraction (Figure 14).

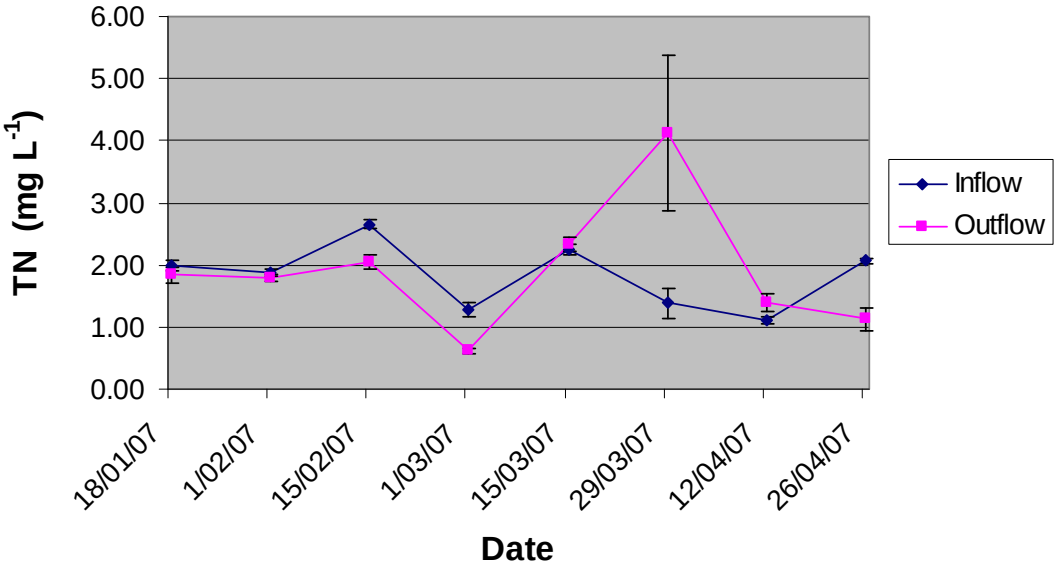

Figure 13 Mean (± se. *n* = 6) total nitrogen (TN) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 1.21).

On most occasions the levels of TAN in the outflow were higher than in the inflow, but these differences were only statistically significant ($P<0.05$) on the 15/03/07 and 29/03/07 (Figure 14). Regarding other common forms of nitrogen, analyses suggested that sand beds did not effect NOx levels during the trial ($P>0.05$). This was despite a significant ($P<0.05$) rise in levels on the last sampling occasion (Figure 15).

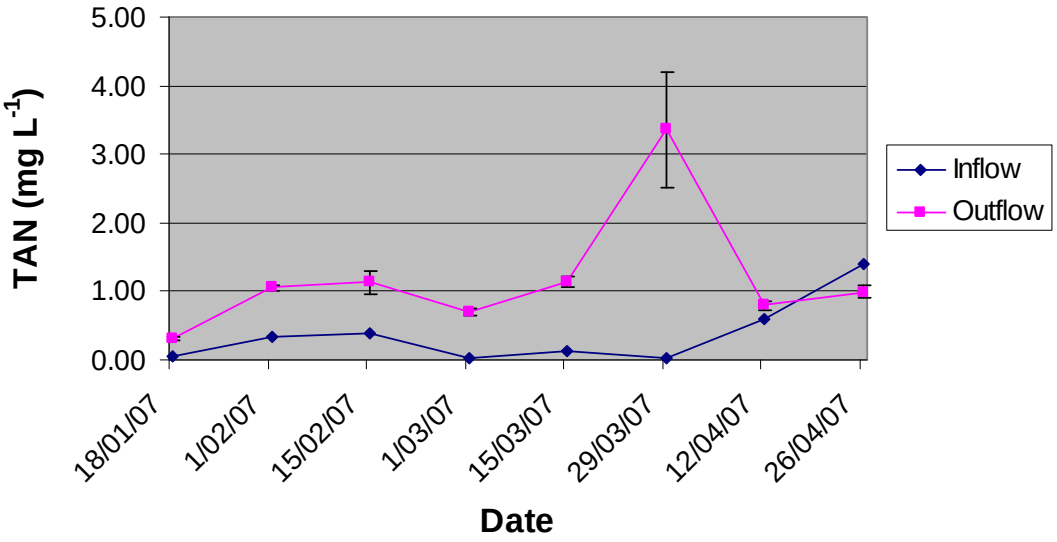

Figure 14 Mean (± se. *n* = 6) total ammonia (TAN) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 0.79).

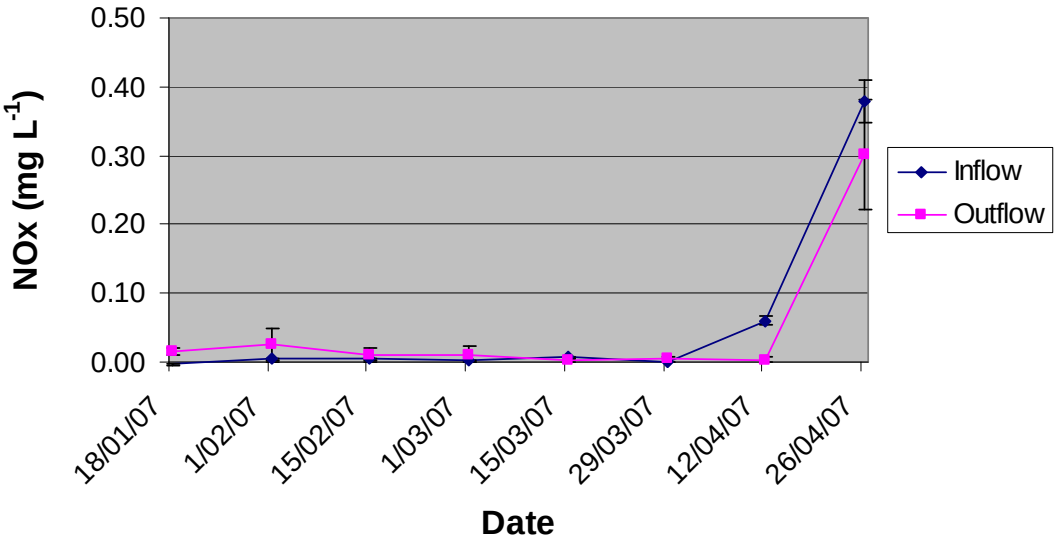

Figure 15 Mean (± se., *n* = 6) nitrate + nitrite (NOx) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 0.08).

The effect of the sand bed on TP levels (Figure 16) also fluctuated significantly ($P<0.01$) during the experiment, but the effect of time was more pronounced than that of the sand bed. Outflow levels were marginally lower than inflow levels on the first five sampling occasions, but these differences were not significant ($P>0.05$). As with the results for TN, TP was heavily impacted by dissolved forms, particularly after

disruption of the water supply the night before the 29/03/07 samples were taken. PO_4 levels in the sand bed-discharge peaked on that day (Figure 17). Continuous remineralisation of phosphorus was evidenced by the significant ($P<0.05$) differences between inflow and outflow PO_4 levels over several earlier weeks.

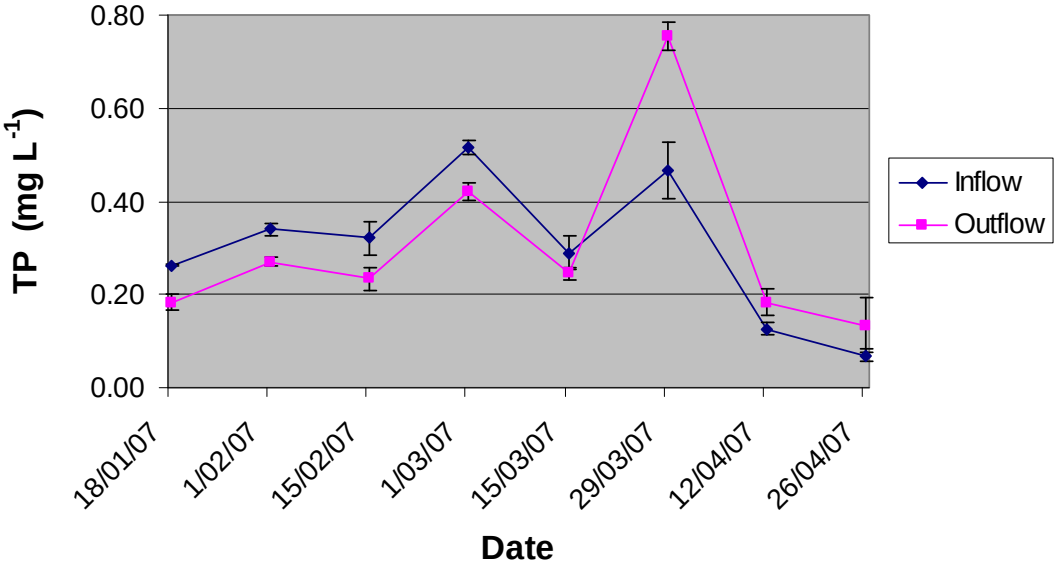

Figure 16 Mean (± se. *n* = 6) total phosphorus (TP) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 0.10).

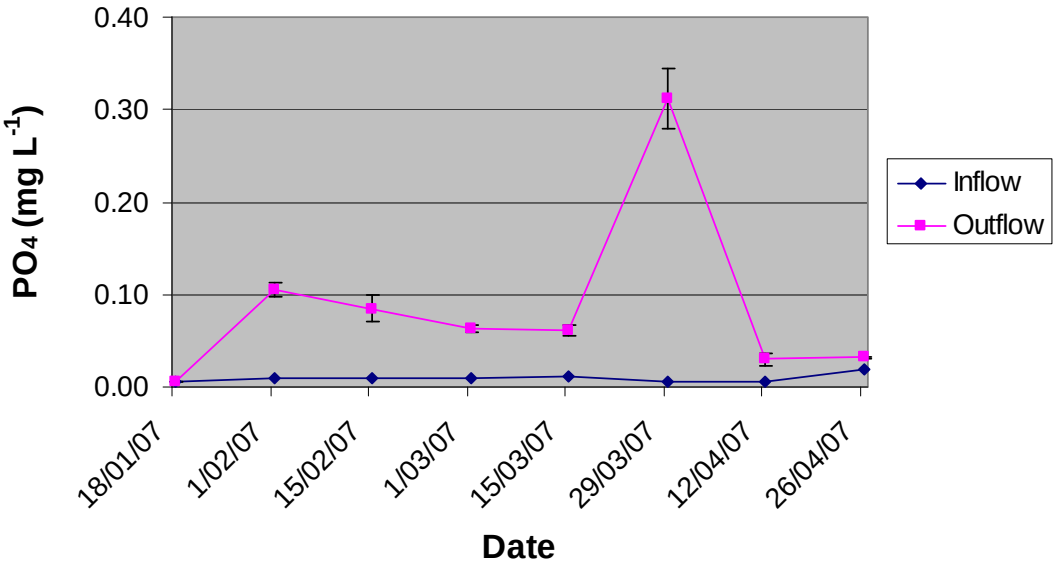

Figure 17 Mean (± se. *n* = 6) orthophosphate (PO_4) levels in inflow and outflow (filtered) waters during fortnightly sampling at 12 noon (5% LSD between treatments = 0.03).

Mass balance calculations demonstrated the continual interception of suspended solids (Figure 18) and chlorophyll *a* (Figure 19) from pond water, where by the end of the trial 3.12 kg and 7.88 g had been removed by each square metre of sand bed, respectively. The other nutrients also had compounding removals for several weeks

but lost ground during the second half of the trial after the disruption of water flows, and when remineralised forms were sporadically released by the beds. During the month after the 15th March, more nitrogen appeared to be released from the beds than was previously removed. This result was heavily impacted by the high nitrogen levels detected in outflow samples on the 29th March. However, caution should be exercised in placing too much onus on this part of mass balance calculations due to the potential for short-lived elevations in ammonia to incorrectly bias the results when applied to all the water treated during that period. Particularly since samples on the 29th March were taken soon after restarting the water flow after a period of drying.

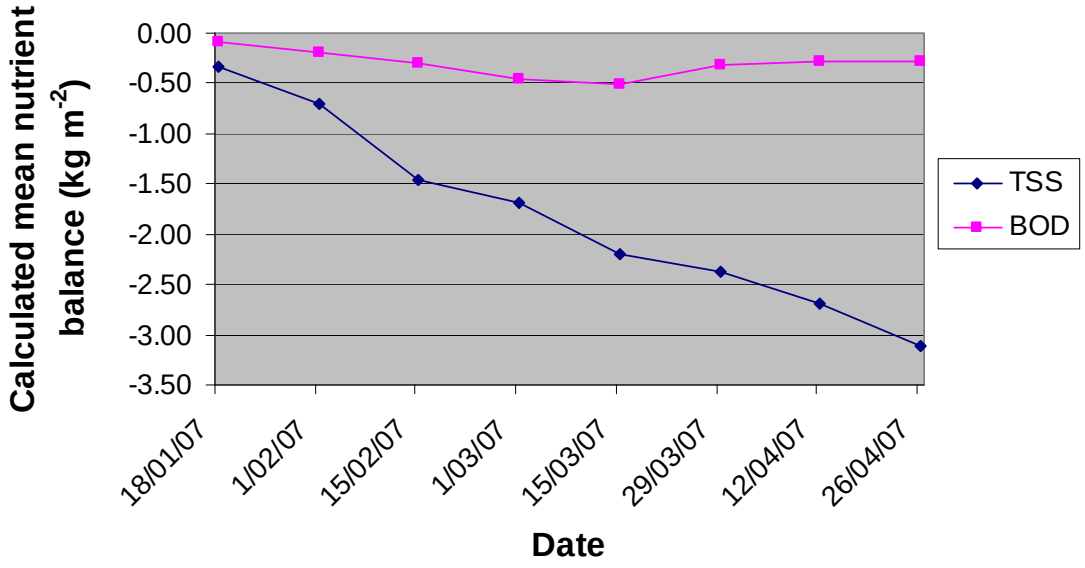

Figure 18 Calculated total suspended solids (TSS) and biological oxygen demand (BOD) mass balances based on mean volumes filtered and differences between inflow and outflow on fortnightly sampling dates.

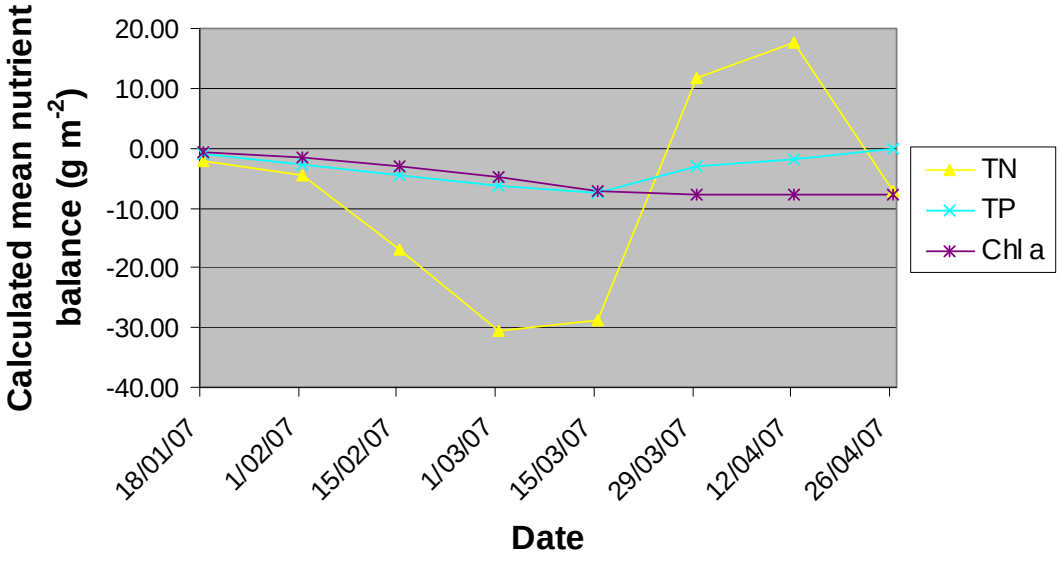

Figure 19 Calculated total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (Chl a) mass balances based on mean volumes filtered and differences between inflow and outflow on fortnightly sampling dates.

Polychaete biomass production and survival

The sizes of worm juveniles when stocked (one month old) were very small (approx. 5 mm long), and their transparent nature at this stage makes them almost impossible to detect with the naked eye whilst they are ranging freely in the substrate. However, after about three weeks their colour darkens and they generally grow to a length of 10 to 20 mm, making them easily detectable in subsamples of sand taken from beds. At that stage in the present trial they were particularly prevalent in subsamples taken from the area where the nursery sand carrying the seed-worms had been deposited. One month after stocking, small worm holes began to appear in the surfaces of the beds. These became enlarged and easily discernible at low tide after the second month.

Nine weeks after stocking many larger worm holes were apparent in beds 4, 5 and 6, lots of smaller holes were apparent in bed 3, and somewhat fewer variable sized holes were apparent in beds 1 and 2. These observations provided a general guide to the biomass and sizes of worms that were growing in particular beds, as evidenced by the harvest results collected after 15 weeks of culture in the sand filters (Tables 3 and 4). For example, the tank with the lowest total harvested biomass (Tank 2) had fewer apparent holes, and the tank which produced the highest number of small sized worms (Tank 3) had a large number of small holes. The presence of larger holes indicated the presence of a significant number of larger worms, although this was at times obscured by mound building activities on the surface, presumably from sand excavated from the network of burrows and being deposited on the sand surface at particular points.

A total of 3,623.7 g of worm biomass was recovered from the trial at harvest. Similar ($P>0.05$) wet weights were harvested from tanks that were previously stocked at different rates (Table 3), with the higher stocking densities only producing a marginally higher production level (346 g m^{-2}) compared with the medium stocking density (311 g m^{-2}). The overall mean production level of worm biomass during the 4-month trial was 328 g m^{-2} (604 g per tank).

Table 3 Total weights of live polychaetes harvested from tanks with different stocking densities. Means with different superscripts are significantly different ($P<0.05$).

Stocking density	Tank	Total weight (g)	Mean ± se
High	1	620.0	636.3^{a} ± 19.1
	3	674.4	
	5	614.6	
Medium	2	504.0	571.6^{a} ± 35.0
	4	621.0	
	6	589.7	

After visually grading harvested worms into smaller and larger specimens, the high density tanks on average yielded significantly higher ($P<0.05$) weights of small worms, and the medium density tanks yielded significantly higher weights of large worms (Table 4). This equated with 63 % and 42 % of worm biomass from the high and medium density tanks, respectively, that were generally too small for easy use as bait.

Table 4 Weights (g) of live polychaetes harvested from tanks with different stocking densities after manually grading into smaller (<0.6 g) and larger individuals. Means with different superscripts are significantly different (P<0.05).

Stocking density	Tank	Small	Large	Small Mean ± se	Large Mean ± se
High	1 3 5	364.0 465.5 373.8	256.0 208.9 240.8	$401.1^{c} \pm 32.3$	$235.2^{a} \pm 13.9$
Medium	2 4 6	257.0 232.2 233.3	247.0 388.8 356.4	$240.8^{ab} \pm 8.1$	$330.7^{bc} \pm 42.9$

The mean survival of worms in the high density tanks was significantly lower (P<0.05) than in the medium density tanks (Table 5). In terms of the numbers of worms in the different size grades, both stocking densities produced more small worms than large ones. The mean percentage of large worms in tanks stocked at medium densities was 23.7 %, which was significantly (P<0.01) higher than 11.6 % for tanks stocked with high densities (see Table A 4). Figure 20 provides pictures of the large graded size class of this polychaete species.

Table 5 Survival of polychaetes harvested from tanks with different stocking densities. Means with different superscripts are significantly different (P<0.05).

Stocking density	Tank	Stocking estimate	Number harvested	Survival (%)	Mean ± se % Survival
High	1 3 5	10,945 10,945 10,945	3,306 3,190 2,763	30.2 29.1 25.2	$28.2^{a} \pm 1.5$
Medium	2 4 6	3,680 3,680 3,680	1,406 1,940 1,822	38.2 52.7 49.5	$46.8^{b} \pm 4.4$

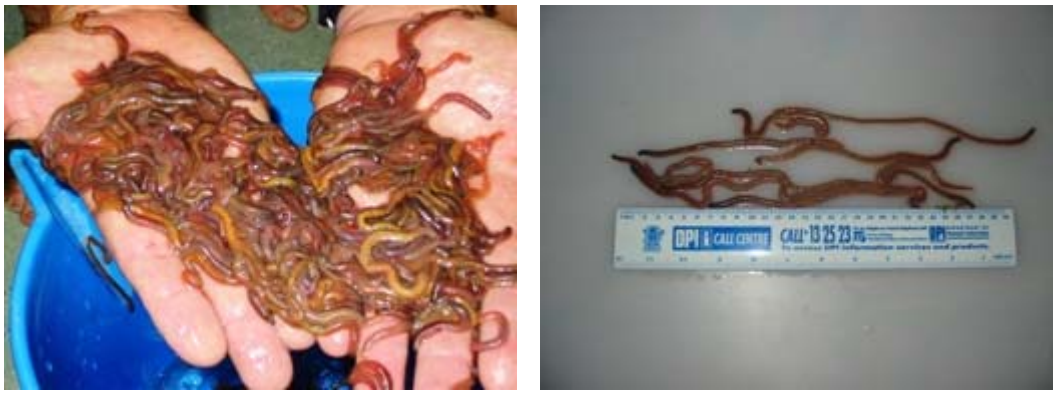

Figure 20 Pictures of some of the larger harvested specimens of this polychaete species *en mass* (left) and as individuals (right).

Polychaete biomass contents

Neither of the treatments (ie: graded size or stocking density) significantly affected the percentage of dry matter (see Table A 5 for raw data) or the percentage of ash (see Table A 6) in the worm samples. There was an average of 18.23 ± 0.57 % dry matter in the biomass samples taken across the trial, of which 19.77 ± 0.80 % was ash (n = 12). Size and density also had little effect on gross energy (P>0.05) (see Table A 7), with an average of 19.39 ± 0.29 MJ kg^{-1} found in the 12 samples analysed.

Size had a significant (P<0.05) effect on the nitrogen content of worm biomass on a dry matter basis (see Table A 8), where small worms had a higher mean proportion (10.57 ± 0.17 %) than large worms (9.99 ± 0.12 %) (n = 6). This result for nitrogen is consistent with amino acid results (shown below) since both are important components of biomass protein. Size also had a significant effect on phosphorus content on a dry matter basis (see Table A 9), whereby small worms had a higher mean proportion (0.70 ± 0.01 %) than large worms (0.65 ± 0.01 %) (n = 6). Density did not effect these nitrogen or phosphorus results (P>0.05).

The total lipid contents of worms harvested were affected by a significant interaction (P<0.05) of stocking density with size. More lipid was generally present in large worms compared with small worms, as well as in large worms grown at the medium density compared with the high density (Table 6). In contrast, the effects of density and size on cholesterol contents were not significant (P>0.05), where worms harvested had an overall mean of 5.24 ± 0.15 mg g^{-1} of dry sample (n = 12).

Table 6 Total lipid contents (% of dry sample) of graded (small and large) polychaetes harvested from experimental sand filter tanks stocked at different densities. Means with different superscripts are significantly different (P<0.05).

Stocking density	Tank	Small	Large	Small Mean ± se	Large Mean ± se
High	1	7.27	9.78	7.06^{a} ± 0.17	9.50^{b} ± 0.31
	3	6.71	8.89		
	5	7.19	9.82		
Medium	2	7.46	11.30	7.64^{a} ± 0.15	11.20^{c} ± 0.19
	4	7.52	10.83		
	6	7.93	11.47		

In phospholipids analyses, the levels of phosphatidylserine or sphingomyelin were below the detection limits (0.05 mg g^{-1}) of the test (see Table A 10). No significant (P>0.05) differences were detected between the levels of other specific phospholipids tested (Table 7), although some differences between worms from medium and high densities were close to this standardised significance threshold (eg: total phospholipids: P=0.056; phosphatidyl-ethanolamine: P=0.056; phosphatidyl-inositol: P=0.083; phosphatidyl-choline: P=0.076; lysophosphatidyl-choline: P=0.142). Despite this, the variance ratios observed in the analyses showed that density had a far more pronounced effect on phospholipid contents than worm size. The overall mean levels detected on a dry matter basis were 6.89 ± 1.09 mg g^{-1} for phosphatidyl-ethanolamine, 0.89 ± 0.26 mg g^{-1} for phosphatidyl-inositol, 4.04 ± 1.17 mg g^{-1} for phosphatidyl-choline, 1.84 ± 0.37 mg g^{-1} for lysophosphatidyl-choline, and 13.66 ± 2.15 mg g^{-1} for total phospholipids.

Table 7 Mean (± se, *n* = 3) phospholipid contents (mg g^{-1} of dry sample) of graded (small and large) polychaetes harvested from experimental sand filter tanks stocked at different densities. Within rows, means with different superscripts are significantly different (P<0.05).

Phospholipids	High density				Medium density			
	small		large		small		large	
	mean	± se	mean	± se	mean	± se	mean	± se
Phosphatidyl-ethanolamine	**9.17**^{a}	2.55	**9.10**^{a}	2.42	**4.83**^{a}	1.62	**4.45**^{a}	1.11
Phosphatidyl-inositol	**1.38**^{a}	0.63	**1.39**^{a}	0.64	**0.50**^{a}	0.37	**0.29**^{a}	0.21
Phosphatidyl-choline	**5.88**^{a}	2.35	**6.72**^{a}	3.61	**1.97**^{a}	0.95	**1.57**^{a}	0.52
Lyso-Phosphatidyl-choline	**0.96**^{a}	0.96	**1.53**^{a}	0.95	**2.08**^{a}	0.49	**2.80**^{a}	0.30
Total	**17.39**^{a}	4.58	**18.73**^{a}	5.73	**9.38**^{a}	2.40	**9.12**^{a}	1.72

A very broad range of fatty acids were detected in worm biomass analyses (see Table A 11). For the 14:1n-5, 19:0, 20:3n-6, 20:4n-3, 22:1n-11, and 22:3n-3 classes, all samples were below the detection limits of the test (<0.05 mg g^{-1}). Of the other 30 fatty acids detected, the 18:4n-3, 22:0, and 22:5n-6 classes were not significantly affected by the treatments, and were generally found at low mean levels (0.15 ± 0.02, 0.06 ± 0.00 mg g^{-1} and 0.13 ± 0.01 mg g^{-1}, respectively) representing only very small proportions of the total fatty acids present (0.41 ± 0.05 %, 0.13 ± 0.01 %, 0.38 ± 0.02 %, *n* = 12). Palmitic acid (16:0) was the most heavily represented class detected. There was a significant interaction of size with density in this and the uncertain 18:1n-? class (Table 8), where small worms from the high density treatment had the lowest proportions (18.38 ± 0.46 % and 4.40 ± 0.11 %, respectively) and large worms from the medium density treatment had the highest proportions (27.28 ± 0.22 % and 5.53 ± 0.04 %, respectively) (*n* = 3).

Table 8 Mean (± se, *n* = 3) fatty acid contents (mg g^{-1} of dry sample) of graded (small and large) polychaetes harvested from experimental sand filter tanks stocked at high and medium densities. Within rows, means with different superscripts are significantly different (P<0.05).

Fatty acid	High density				Medium density			
	small		large		small		large	
	mean	± se	mean	± se	mean	± se	mean	± se
16:0	**4.64**^{a}	0.36	**10.53**^{c}	0.34	**6.07**^{b}	0.30	**14.23**^{d}	0.49
18:1n-?	**1.11**^{a}	0.03	**2.15**^{c}	0.05	**1.40**^{b}	0.07	**2.88**^{d}	0.10

? = imprecise peak in the chromatogram but may have been 18:1n-12 (petroselenic acid) although no cis form was found.

Stocking density and/or size (after visual grading) had significant effects on all other fatty acid classes detected, but interactions of these factors were not significant (P>0.05). Large worms had significantly higher levels of the remaining fatty acids than small worms, and this was also true for total fatty acid levels (Table 9).

Table 9 Mean (± se, *n* = 6) fatty acid contents (mg g^{-1} of dry sample) of graded small or large polychaetes harvested from experimental sand filter tanks. Means within rows are significantly different (P<0.05).

Fatty acid	Graded size	
	Small	Large
14:0	0.16 ± 0.01	0.28 ± 0.02
15:0	0.16 ± 0.01	0.25 ± 0.02
16:1n-7	0.80 ± 0.06	1.56 ± 0.11
17:0	0.67 ± 0.03	0.98 ± 0.05
18:0	2.45 ± 0.11	3.48 ± 0.16
18:1n-7	1.91 ± 0.13	3.64 ± 0.24
18:1n-9	0.61 ± 0.04	1.15 ± 0.07
18:2n-6	0.61 ± 0.03	0.85 ± 0.04
18:3n-3	0.46 ± 0.06	0.79 ± 0.10
20:1n-6	0.36 ± 0.03	0.94 ± 0.06
20:1n-7	0.14 ± 0.01	0.19 ± 0.01
20:1n-9	0.48 ± 0.04	0.97 ± 0.06
20:1n-11	0.67 ± 0.04	1.28 ± 0.08
20:2n-6	0.45 ± 0.03	0.98 ± 0.07
20:3n-3	0.04 ± 0.01	0.20 ± 0.03
20:4n-6	1.21 ± 0.05	1.63 ± 0.07
20:5n-3	3.81 ± 0.15	4.62 ± 0.18
22:1n-7	1.93 ± 0.06	2.65 ± 0.06
22:1n-9	0.01 ± 0.01	0.08 ± 0.01
22:4n-6	2.40 ± 0.10	3.17 ± 0.13
22:5n-3	0.94 ± 0.05	1.17 ± 0.05
22:6n-3	0.40 ± 0.02	0.55 ± 0.04
24:1n-9	0.01 ± 0.01	0.05 ± 0.01
Total	27.76 ± 1.28	46.88 ± 2.46

Since the medium density produced on average larger worms, it also had higher levels for some fatty acid classes (Table 10). Along with palmitic acid (see discussion above), several other fatty acids were heavily represented. Their overall average proportions in small and large worms, respectively (*n* = 6), are listed below in general order of decreasing mean proportions:

13.8 and 9.9 % for eicosapentaenoic acid (20:5n-3) (EPA);
8.8 and 7.4 % for stearic acid (18:0);
6.8 and 7.8 % for vaccenic acid (18:1n-7);
7.0 and 5.7 % for docosenoic acid (22:1n-7);
4.4 and 3.5 % for arachidonic acid (20:4n-6) (AA);
2.9 and 3.3 % for palmitoleic acid (16:1n-7);
3.4 and 2.5 % for docosapentaenoic acid (22:5n-3);
2.2 and 2.5 % for oleic acid (18:1n-9);
2.4 and 2.7 % for eicosenoic acid (20:1n-11);
2.4 and 2.1 % for heptadecanoic acid (17:0);
2.2 and 1.8 % for linoleic acid (18:2n-6);
1.7 and 2.1 % for gondoic acid (20:1n-9);
1.6 and 2.1% for eicosadienoic acid (20:2n-6);
1.6 and 1.7 % for alpha-linolenic acid (18:3n-3);

1.3 and 2.0 % for eicosenoic acid (20:1n-6);
1.4 and 1.2% for docosahexaenoic acid (22:6n-3) (DHA).

The AA : EPA : DHA ratios were somewhat similar for small (3.0 : 9.5 : 1) and large (3.0 : 8.4 : 1) worms, differing only in the EPA content.

Table 10 Mean (± se, *n* = 6) fatty acid contents (mg g^{-1} of dry sample) of polychaetes harvested from experimental sand filter tanks stocked with high or medium densities. Means within rows are significantly different (P<0.05).

Fatty acid	Stocking density	
	High	Medium
14:0	0.19 ± 0.02	0.25 ± 0.03
16:1n-7	1.01 ± 0.14	1.36 ± 0.19
17:0	0.76 ± 0.06	0.89 ± 0.08
18:0	2.69 ± 0.19	3.25 ± 0.27
18:1n-7	2.39 ± 0.32	3.17 ± 0.44
18:1n-9	0.77 ± 0.11	0.99 ± 0.14
20:0	0.06 ± 0.00	0.08 ± 0.00
20:1n-6	0.57 ± 0.11	0.72 ± 0.15
20:1n-7	0.14 ± 0.01	0.18 ± 0.01
20:1n-9	0.62 ± 0.09	0.83 ± 0.13
20:1n-11	0.85 ± 0.11	1.09 ± 0.16
20:2n-6	0.62 ± 0.10	0.81 ± 0.14
20:3n-3	0.08 ± 0.03	0.16 ± 0.04
20:4n-6	1.32 ± 0.08	1.52 ± 0.11
22:1n-7	2.20 ± 0.13	2.38 ± 0.19
22:6n-3	0.42 ± 0.03	0.52 ± 0.04
24:0	0.04 ± 0.01	0.08 ± 0.01
Total	33.40 ± 3.42	41.24 ± 4.93

The most heavily represented amino acids found in the polychaete biomass were glutamic acid, aspartic acid and glycine. The levels of these and three other amino acids were significantly affected (P<0.05) by graded size, but none were affected (P>0.05) by stocking density. In general there were lower concentrations of each amino acid tested in large worms than in small worms, and for those listed in Table 11 this difference was significant (P<0.05). For those amino acids not shown in Table 11, the overall mean (± se, *n* = 12) levels (on a dry matter basis) are listed below in decreasing order:

37.59 ± 0.67 mg g^{-1} arginine;
36.35 ± 0.49 mg g^{-1} leucine;
29.70 ± 0.44 mg g^{-1} proline;
23.73 ± 0.30 mg g^{-1} threonine;
23.19 ± 0.34 mg g^{-1} valine;
21.77 ± 0.30 mg g^{-1} serine;
20.66 ± 0.29 mg g^{-1} isoleucine;
19.20 ± 0.36 mg g^{-1} phenylalanine;
17.26 ± 0.32 mg g^{-1} tyrosine;
9.08 ± 0.88 mg g^{-1} methionine;
6.05 ± 0.15 mg g^{-1} tryptophan;
5.67 ± 0.46 mg g^{-1} cystine.

Table 11 Mean (± se, *n* = 6) amino acid contents (mg g^{-1} of dry sample) of graded small or large polychaetes harvested from experimental sand filter tanks. Means within rows are significantly different (P<0.05).

Amino acid	Graded size	
	Small	Large
Glutamic acid	77.23 ± 1.03	72.57 ± 1.27
Aspartic acid	53.70 ± 0.74	50.36 ± 1.01
Glycine	52.62 ± 0.63	45.69 ± 0.64
Alanine	37.84 ± 0.60	34.49 ± 0.71
Lysine	32.11 ± 0.66	29.30 ± 0.54
Histidine	12.26 ± 0.36	11.10 ± 0.28

Discussion

In the early 70's, a research group working at the Woods Hole Oceanographic Institution made reference to the potential value of detrital food chains in integrated polyculture systems and methods for wastewater treatment (Ryther *et al*., 1972, 1975; Tenore *et al*., 1974). Their work involved seawater enriched with nutrients from treated sewage effluent. The marine phytoplankton which proliferated were channelled into various constructed ecosystems for seafood production and nutrient assimilation. Bivalve molluscs (oysters and clams), polychaete worms, and other invertebrates (amphipods) dominated in the benthic components which complemented fish and seaweeds that were also used as nutrient sinks. The models developed were ecologically stable, were productive of a diverse array of useful organisms, and almost completely removed inorganic nitrogen from waters discharged.

Unfortunately since then, large scale aquaculture systems have mainly developed as intensive monocultures. These typically involved unbalanced ecosystems that result in low utilisation of nutrient inputs. Intensive shrimp farms that use the flow-through model are an example, where relatively low percentages of nutrients applied to ponds are assimilated by stock (eg: 18-27% of N and 10-15% of P: Funge-Smith and Briggs, 1998; 22 % of N: Preston *et al*.,n 2000). This creates what is widely considered unacceptable ecological footprints (Folke *et al*., 1998) and involves inefficient use of natural marine resources (Deutsch *et al*., 2007).

Although several innovative groups around the world are striving to create more efficient, commercially relevant, zero exchange or recirculation systems for environmentally friendly seafoods production (eg. see Neori *et al*., 2000; 2004; 2007; Jones *et al.,* 2002; Brune *et al*., 2003; Avnimelech, 2007 and Crab *et al*., 2007), few are integrating benthic detrital systems to make use of their inherent productivities at large scale. This is despite wide recognition of the significant nutrients that are wasted in this zone as settled material and sludge. Animals which can grow in this zone, like Polychaeta, can make use of this portion of wasted nutrient to create additional profit. They may also improve the condition of the farm environment by improving pond bottom soil structures and promoting oxidation of reduced sulphides in sediments (Chareonpanich *et al*., 1994). In general they aid in the decomposition and conversion of organic matter into available nutrients for improved plant productivity, just as earthworms do in horticulture.

DPI&F are currently leading research efforts in Australia to stimulate the wide-spread uptake of polychaete technologies for the increased profitability and sustainability of tropical marine aquaculture systems. Of particular interest in this work is their application to broad-scale sand filtration beds. Two subtropical inter-tidal species from the family Nereididae were previously screened for this use in pilot work at BIARC in 2005 and 2006 using semi-intensive fish- and prawn-pond wastewaters. Both polychaete species demonstrated yearly production levels of about 400 g per m^2 in sand filtration beds, and functionally appeared to reduce sand clogging for prolonged filtration (DPI&F unpublished data). As demonstrated in the present study, this form of water treatment can provide moderate reductions of nitrogen and phosphorus, significant reductions in chlorophyll and suspended solids, and significant conversion of particulate based nutrients into dissolved forms.

The principle behind this new form of brackish water treatment lies in the burrowing and feeding activities of the worms in the sand, and their ability to survive and grow on the deposited organic material. They appear to help maintain flow through the sand filter whilst organic debris (algae and particulate matter) that is trapped by the sand directly and/or indirectly becomes their food. The worm biomass production levels are sufficiently valuable to fund these low-maintenance water remediation activities on a broad scale (sand bed construction, stocking with worm juveniles and worm biomass harvest), since the live worms produced are suitable for lucrative bait markets and their proximal and fatty acid analyses suggest they are also valuable for use as marine broodstock conditioning and maturation diets (for fish and crustaceans).

To build on these encouraging preliminary results at BIARC, there was a need to repeat the work in a commercial farm environment. Particularly since the extra silt loading in wastewaters from earthen ponds (BIARC has fully lined ponds) had the potential to block the sand filter beds, thus limiting water filtration efficiencies, and possibly affect the growth and nutritional content of the polychaetes produced. To assess the potential for these limitations, the Bullock Creek Prawn Farm at Donnybrook in South East Queensland was selected. It has operated for many years with earthen ponds and therefore generates challenging conditions in this regard.

Despite our previous work which showed that higher worm densities facilitated the passage of water through the sand substrate more freely, we did not find evidence of this effect in the present farm trial. However, the reason for this could be that both the medium and higher densities used were sufficient to provide this desired effect. This was certainly apparent towards the end of the trial when all sand beds were devoid of surface fouling and were obviously being grazed regularly by the inhabiting worms. These species are known to emerge from their burrows at night and when water levels over the substrate are low, when they graze clean all surfaces that they can access. Their ingestion of organic-rich sediments, and deposition of faecal mounds on the surface, acts to turn over or renew surface sediments on a regular basis (active bioturbation). In general the size of hole in the surface of the bed indicates the size of the worms that are present, and this is evidence of their surface foraging activities whilst partially remaining in their burrows. The mound building that was apparent in all tanks in this trial suggested that the populations were in some respects coordinating their sand deposition on the beds' surfaces. This may be an adaptation which helps adjacent animals from continually expending energy to evacuate sand

deposited in their burrow by their neighbours, and to ensure that some access to the surface remains, even when worm densities are high.

Our previous (as yet unpublished) work has also shown that this surface activity is insufficient to keep the surface clean when the worms are young and when their populations are underdeveloped. Surface blocking then occurs, and particularly when there are heavy organic loads such as coarse diatoms being deposited on the sand. It generally takes several weeks for worm populations to reach a critical level of biomass to provide an effective surface cleansing effect. If flow rates through the sand (filtration rates) are insufficient to meet the farms needs in the meantime, it is nevertheless possible to provide the temporary remedy of raking the surface. This is best undertaken when the bed is dry, so that fine silts do not immediately fall back into pore space after being physically resuspended in overlying water. Of course, this biological sand-cleansing effect can be overloaded at any time with excessive suspended matter, so it is important in the operation of this type of system to integrate a range of effluent treatment systems, and to regulate the flow. As demonstrated in this study, this could involve the normal deposition of the heaviest suspended solids in the middle of prawn ponds, with effluent to be treated by sand beds drawn in a metered fashion from monk drains around the periphery of ponds.

Many of the design principles of sand filters proposed for filtration of domestic stormwater (WSUD, 2006) serve as comparisons with this application. Both applications require provision of overflow capacity that is oversized to account for above design operation needed during heavy rainfall. Although sand depths of 600 mm are typically proposed for these "Water Sensitive Urban Design" sand filters (WSUDSF) and that this depth has been found effective in removing TSS, TN and TP (CRCCH, 2005), a much smaller effective sand depth was used in the present work (~ 200 mm total and 140 mm above the under-drain pipe) to minimise the cost of sand. This would be particularly important when applying this method at large scale. Since the nature of particle sizes that are to be intercepted from mariculture effluent are much smaller (eg: microalgae down to 1 μm) than in stormwater, finer filter media was also necessary. This reduced the pore size and the water infiltration rate, however the availability and cost of bulk sand supplies guided selection of a grade that allowed reasonable hydraulic conductivity and removed most small algae species. Assuming that this particular sand product had an approximate bulk density of 1.4 tonnes m^{-3}, and that each m^2 of bed had 0.2 m^3 (200 mm deep) of sand, each m^2 of bed used 0.28 tonnes of sand. At a cost of $A22 $tonne^{-1}$ (excluding delivery costs and bulk handling discounts) sand in the beds at larger scale would cost in the order of $A600 per 100 m^{-2}. This cost of sand is relatively low considering the potential quantities of polychaetes (approx. 33 kg based on present demonstrated average) coupled with their high value, as well as the wastewater remediation properties that the bed would simultaneously offer.

Another significant difference between the way WSUDSF are proposed to operate and that of the present study, are in the ways the beds are constructed and the filters operate. In WSUDSF a fine gravel drainage layer over the perforated pipe system helps prevent the finer filtration media from entering and blocking the drainage pipes. However this approach is unsuitable in polychaete sand filters because this course material would block sieves used to harvest the worms, and would add unnecessary complexity to the bed construction phase. Several textile materials wrapped around

the perforated pipes have provided adequate protection of drainage lines in this and our previous work which has used a single grade of sand in the bed. These include 3-5 layers of shade cloth, light geofabric (eg: Biddum by Geofabric Pty Ltd), and commercial ag-pipe gauze, and several other commercial products are available and would likely be suitable. Additionally, rather than using Darcy's equation (see below) to predict the maximum percolation rates possible through the sand filter, and then ensuring that the capacity of the under-drains is greater than this Q_{max}, filtration rates in our work have been restricted by the under-sizing of drain outlets. This primary choke in the system has been gauged to provide reasonable flow based on a 1:10 treatment area to production pond basis, and is designed to help prevent too much suction pressure developing which places pressure on the textile material protecting drainage pipe perforations. In general we have also tried to use pipe protecting material that has a pore size much greater than the largest particles which travel through the sand filter, so that it is the sand rather than this textile material which becomes the secondary choke in the system.

One of several mediating factors which affect filtration rate is the depth of water which builds over the sand filter as inflow exceeds drainage rates. Since the Polychaete filtration system is based on gravity driven percolation of water through the sand, filtration rates increase with head pressure above the mass of sand (identified as h_{max} in Darcy's equation below). In the present study, this factor did not remain constant due to the regular tidal simulation, but this pattern of use has practical application in mitigating variable flows from the production ponds at a prawn farm, particularly if the sand filter is built on the bottom of sedimentation ponds which receive intermittent (eg: daily) flows from the farm.

Darcy's equation

$$Q_{max} = k \,.\, A \,.\, (h_{max} + d) \,/\, d$$

where, Q_{max} is the maximum infiltration rate (m^3 /s)

k is the hydraulic conductivity of the soil filter (m / s)

A is the surface area of the sand filter (m^2)

h_{max} is the depth of pondage above the sand filter (m)

d is the depth of the filter media (m)

Another variable factor in this theoretical equation is the hydraulic conductivity of the sand filter (k). This can generally vary for different grades of sand from 1 mm s^{-1} to 0.1 mm s^{-1} (WSUD, 2006), and Palacios and Timmons (2001) have provided modifications to Darcy's equation which account for TSS loading. However, in the present work the drainage pipe outflow restrictors prevented filtration rates from ever achieving their maximum flows. As the relatively high loading of silt and organic debris clogged the pore space in the sand beds, k was undoubtedly reduced until the polychaetes' activities on the surface became significant. Furthermore, maximum daily filtration rates were not routinely achieved in the present experiment because the

6-hourly inflow patterns applied generally allowed sand beds to drain completely and remain dry for a period of time on a regular basis. The reasoning behind this was to provide this intertidal polychaete with more natural conditions, and to demonstrate the flexibilities of the filtration beds in terms of effectively treating wastewaters that are supplied on an intermittent basis. Future research will investigate whether this tidal simulation is necessary for the growth of this polychaete species, so that daily treatment rates can be increased via continuous filtration if necessary.

The present study sought to set a water handling rate for sand beds of 1,500 L m^{-2} d^{-1} (2,760 L $tank^{-1}$ d^{-1}), which generally was successfully adhered to for the majority of the trial (refer to Figures 3 and A 2) given the water supply failings and other related complications which prevailed during later periods of the experiment. This nominal amount was based on a 10 % daily exchange rate for culture ponds with an average depth of 1.5 m and assuming 1 ha of sand bed for every 10 ha of culture ponds. This water exchange rate, by all accounts is a reasonable exchange rate for intensive prawn culture ponds (5-10 % d^{-1}: Funge-Smith and Briggs, 1998; 5-30 % d^{-1}: Vigneswaran *et al*., 1999).

The percentage of farm area accounted for by sand beds in this design (ie. 10 %) is well under the areas currently required in government licensing arrangements in Queensland for settlement/treatment ponds (ie. 30% of farm area). This potentially allows for additional infrastructures (eg: seaweed culture beds, see below) which could be integrated with these sand-based water treatment measures for further nutrient recovery and removal from waste streams prior to release back to adjacent waterways. Rather than complete on-farm recirculation, an approach of flowthrough and treated release could prove better from a farming perspective, particularly since new phytoplankton seeds from adjacent natural waters are often useful for pond management options, and since high evaporation rates can cause excessive salinities when waters are continually recirculated on farms during dry seasons.

The consistent significant removal of suspended solids from pond waters by the sand bed was not surprising given that sand filtration has long been used for this purpose in municipal water treatment (Campos *et al*., 2002) and various aquaculture applications (Vigneswaran *et al*., 1999). In practical terms suspended solids are mainly captured in the surface layers of sand filters, and this is where resistance to water infiltration mainly occurs with prolonged use (Palacios and Timmons, 2001). Currently, Queensland EPA policy restricts discharge of suspended solids from existing marine prawn farms to 12 kg ha^{-1} d^{-1}, with a maximum of 75 mg L^{-1} for any isolated discharge event (Qld EPA, 2008). This total allowable daily load gives an average concentration of 8 mg L^{-1} if we assume (as above) that 10 % pond exchange is necessary each day. By comparison, the overall mean for the outflow TSS for all eight sampling times in the study was 11.3 mg L^{-1}, which is only slightly higher than this calculated mean discharge level, and not significantly higher using the 5 % LSD value derived for comparisons of treatments in the analysis (9.7 mg L^{-1}). Thus, in terms of TSS, the method offers existing prawn farmers the ability to adequately remediate wastewaters according to current EPA standards.

Chlorophyll *a* and BOD levels are not specified in the current EPA standards although these parameters were previously limited by licence conditions. Several years ago, before the present solids- and nutrient-load based system evolved, Chl*a* was generally

limited to a maximum of 40 μg L^{-1} and 5-day BOD to a maximum of 10 mg L^{-1}. The sand bed filtrate easily complied with these previous Chl*a* limits, but exceeded the previous BOD limits.

The effect of the experimental system on water temperatures was largely due to the tank covers which trapped solar radiation to heat water within the tanks. The sand beds then appeared to provide a degree of insulation to cool the water percolating through them, and this effect was more pronounced during the middle part of the day when the sun was at it hottest. Elevated temperatures (within natural ranges) have previously been shown to increase the sediment mixing and bioturbation of some polychaetes (Ouellette *et al*., 2004), so it is likely that an optimal temperature range exists which stimulates the surface activity and desirable cleansing actions of different species at the sand bed's surface. *Perinereis helleri* was shown in this study to tolerate temperatures up to 34.9ºC, and though its lower tolerances were not approached during this summertime study, they are not likely to be challenged in this regard in any coastal areas of Queensland, or indeed anywhere that tropical prawns are cultured.

Similarly, salinities of 40.4 ppt. were tolerated by this species, and this is likely not its upper salinity limit. Nereid polychaetes are also well known for their relatively low salinity tolerances, since they have been found to inhabit areas in close proximity to freshwater springs (Zipperle and Reise, 2005). These are important points for survival in prawn farming environments which can regularly and sometimes quite severely be affected by evaporation and precipitation.

The system also appears to offer a particularly effective way of tempering the pH fluctuations in discharge waters from prawn ponds, whereby outflow pH levels remained relatively stable and more neutral compared with climbing inflow levels as the stock biomass in the prawn production pond increased. EPA discharge standards require pH levels to be between 6.5 and 9.0, and this upper limit is often exceeded in eutrophic ponds supporting a strong algal bloom. Although dissolved oxygen levels in bed filtrate were below the EPA minimum of 4 mg L^{-1}, this could easily be rectified with simple methods of aeration (eg: gravity driven ripples or open flow in channels).

The treatment of pond waters in these types of sand beds may further offer several biosecurity advantages for mariculture systems by helping to break disease cycles. Primarily, most organisms which could carry these diseases are screened out with the fine particulate matter being trapped in the sand, so that biological vectors for disease transfer would be greatly reduced. Polychaetes, anaerobic bacteria and protozoans associated with their burrows would be the most likely organisms to leak from these systems. These are already very common in all estuaries and indeed in most aquaculture systems where sludge accumulates, so could not generally present additional risks to the health of receiving waters. Furthermore, very few organisms can withstand the temporarily hostile conditions created by stripping of all oxygen and combining the very low redox potential with the presence of sulphide rich sediments. Therefore, this form of in-line treatment of pond-water could protect natural environments or recirculated aquaculture ecosystems from amplified disease levels in intensive operations.

The polychaete burrowing activities in substrate adds further to the general health of ecosystems by oxygenating otherwise anaerobic sediments, which leads to a greater degree of aerobic decomposition of settled organic materials. Expansion of anaerobic/aerobic interfaces in the sediment increases the biological productivity of substrates and the diversity of benthic organisms it can produce. Such increased biological diversity can add directly to natural food chains within recirculated aquaculture systems and thus further improve nutrient use efficiencies. Conversion of waste carbon into benthic food organisms that are used by the target crop (eg: prawns) will reduce the accumulation and development of reduced organic materials (eg: sulphides) and will also reduce the release of greenhouse gases like carbon dioxide.

Low levels of sulphide (up to 0.3 mg L^{-1}) have been measured from time to time in this type of anaerobic discharge when samples are meticulously collected immediately after release from the sand bed. However, after a very short period (a few minutes) of exposure to dissolved oxygen (eg: by simply adding to an open water body), it is either volatised or converted to sulphate, loosing its smell and potential toxic effect. Neori *et al*. (2007) recently highlighted the rapid oxidation of sulphide in the boundary layers between anaerobic and aerobic sediments, where patches of white elemental sulphur were often visible. In the present system, this white material also typically forms in the immediate stream of water discharging from the sand beds, where the odour of hydrogen sulphide indicates the existence of anaerobic sediments within. However, this white material is generally combined with a bacterial slime or gel, which is likely associated with the bacteria involved in this sulphur deposition (eg: *Beggiatoa* sp., Nelson and Castenholz, 1981) and/or those utilising the sulphur after its transition to an oxidised form. Interestingly, this sulphur-rich gel is also likely to constantly form on the insides of the worms burrows, and probably provides, or is the base of, an important food source for inhabiting polychaetes and other benthic organisms (Reise, 1981), as it is in areas directly adjacent to deep ocean hydrothermal vents (Van Dover and Lutz, 2004).

Regarding the removal of nitrogen and phosphorus from prawn pond water, the results were disappointing compared with previous results in smaller systems and over shorter time frames. The EPA standards for existing farms relate to an average loading of 1.0 kg ha^{-1} d^{-1} with a maximum level of 3.0 mg L^{-1} for nitrogen, and 0.15 kg ha^{-1} d^{-1} with a maximum level of 0.4 mg L^{-1} for phosphorus (Qld EPA 2008). These total allowable daily loads give average concentrations of 0.67 mg L^{-1} of nitrogen and 0.1 mg L^{-1} of phosphorus if we assume (as above) that 10 % pond exchange is necessary each day. The sand filtrates generally had levels below these maxima but higher than the operational averages. Mass balance calculations showed that portions of these nutrients were effectively captured by the process for several weeks, but this collective benefit was lost during the second part of the trial when it appeared that the majority of nitrogen and phosphorus which had accumulated in the bed was released. It was not possible to determine if this result was more an artefact of the way water flows and sampling times were managed during the trial, or if this is an unavoidable function of nutrient accumulation followed by remineralisation and release. But most likely, our samples taken on the 29th March, detected a short-lived pulse of ammonia and orthophosphate which had accumulated in the sand bed during the period of limited flushing the night before.

However, the significant conversion of these nutrients into dissolved forms (available for plant uptake) suggests that integrated macrophyte production systems could be designed for further water polishing and diversification of product on prawn farms. This would be particularly useful if sand beds could be effectively managed to deliver a pulse of concentrated dissolved nutrients to such macrophyte systems. Several factors which have previously created difficulties for seaweed culture systems using raw prawn pond water (eg: suspended solids: Jones *et al*., 2002; phytoplankton and herbivorous invertebrates like amphipods: Palmer, 2005) are also effectively reduced by this sand filtration process. Preliminary work at BIARC has demonstrated the potential to grow very pure cultures of several naturally occurring seaweeds in the discharge stream of polychaete sand filters (see Figure 21). Even without the associated production and potential profitability of polychaete biomass, the sand bed's function of water clearing and break down of particulate organic matter to provide stabilised and available nutrient sources, creates major opportunities for new businesses and seafood products in the future. For example, the dried *Enteromorpha* sp. that has been grown at BIARC using this system has been shown to have useful protein and gross energy contents (22.8-25.9 % and 11 MJ kg^{-1}, respectively), and along with the polychaetes (also grown using waste nutrients) have been proposed as useful feed ingredients that could attain organic certification in the future (Slattery and Palmer, 2007).

Figure 21 Pictures of a naturally occurring seaweed (*Enteromorpha* sp.) growing vigorously in pond wastewater after polychaete sand bed treatment.

The polychaete species used in this study (*Perinereis helleri*) can be viewed as a suitable proxy for a range of locally available species that could similarly be used for this type of application in different parts of the world. However, there are likely to be many species which can equally or better utilise the growth conditions created. For example, although Meksumpun and Meksumpun (1999) documented naturally occurring *Perinereis* sp. in shrimp ponds in Thailand, other species (eg: *Notomastus* sp.) were more prevalent in, and were suggested as more suitable for controlling the organic enrichment of those sediments. Of particular importance in this pursuit is the economic value and potential commercial uses of the selected species, since the potential scale of production made possible by the simple low maintenance techniques described herein, could offer the means to address the over-harvesting of wild populations that is occurring for exploited polychaetes around the world (see Scaps, 2003; 2004).

These points are particularly relevant in light of some of the most recent work with a very similar species (*Perinereis nuntia*) in Thailand. Poltana *et al.* (2007) have recently documented this species' artificial production specifically for feeding to shrimp broodstock. Cultured population were screened for known prawn pathogens as they grew towards production levels of 3-4 kg m^2 over five months (stocked as nectochaetes at 6,000 m^2). This is ten times the production levels achieved in the present study, but this was also as the result of feeding the bed with fish meal up to three times per day. In Australia, where labour costs are higher, and where nutrient releases from mariculture enterprises are more heavily scrutinised, their production at more extensive levels using waste nutrients is likely to be preferred. Their on-farm production would avoid the dangers of disease introductions from wild sources, and ensure their extended availability and freshness for feeding to prawn broodstock.

*P.helleri'*s survival and production levels in the present study, and demonstrated high values as bait and high quality feed, appear sufficient to drive their economical implementation in the wastewater treatment methods that are documented. Although stocking levels in this study were assumed to be somewhat imprecise, due to the gregarious nature of juveniles in the nursery beds, the numbers recovered from replicate grow-out beds were surprisingly consistent adding confidence to the juvenile enumeration and stocking methods that were applied. Furthermore, the similarities of overall production levels at the two densities suggest that a carrying capacity exists for a given amount of sand that is receiving a consistent organic loading. Along with the documented differences in polychaete biomass contents at different sizes and densities, these findings may allow managers in the future to select for particular products that may be more suitable for specific uses. These factors add further confidence to the imminent commercialisation phase of these technologies in Australia

Conclusion

This project sought to begin investigations into the commercial application of beneficial production technologies for this new Class of animal in tropical Australia. It was anticipated that profitability and sustainability could be improved by integrating polychaete production and wastewater treatment systems within existing mariculture operations like semi-intensive prawn farms. The first step in this integration was to understand what effects the proposed system could have on wastewater. Results show how a 1 ha sand bed can treat wastewater from a 10 ha prawn farm using 10 % pond water exchanges rates each day. Suspended solids and chlorophyll *a* can be reduced to environmentally acceptable levels in one pass of a polychaete sand filter. Unlike the currently used settlement ponds, this can be a continuous treatment process that does not require settlement time delays, and it only uses 10 % rather than 30 % of farm area. Although this style of water treatment system could easily be overloaded with silt or algae which clog the filtration bed, its operation in conjunction with a normally circulating pond with monk drain outlet positioned on the periphery, affords it effective continuous operation for a full prawn cropping cycle.

Dissolved nutrients produced by the process reduced its potential to remove significant amounts of nitrogen and phosphorus on its own. However, this combined with solids and phytoplankton removal sets up particularly suitable conditions for the

integration of seaweed culture systems in the discharge. It also produces a commercially useful by-product, namely polychaetes suitable for various uses.

This study shows how profitability would be enhanced directly by adding the economic value of marine worm biomass produced from waste nutrients to aquaculture businesses. It also shows how mariculture operations may be able to improve their nutrient abatement activities and expand in an environmentally responsible way. These developments also offer several other sustainability aspects to natural resource managers, including reduced pressures on wild bait worm stocks and a reduction in the associated habitat disturbances of the natural environment. They offer potentially self-funding water treatment methods and provide by-products that can alternatively be internalised within the operation (eg: worms produced for use as supplemental feeds).

Without changing the presently accepted 70 % production to 30 % treatment area ratio, an idealised farm design in the future which takes the present results and discussion into consideration may practically look towards up to 10 % of farm area committed to discharge collection and volume mitigation in settlement channels. These settlement channels could then supply metered pre-settled wastewater to Polychaete sand beds occupying a further 10 % of farm area, which then feed sand filtered water enriched in dissolved nutrients to seaweed culture beds in the remaining 10 % of treatment/farm area. This design not only holds potential to improve water treatment activities so that the environmental footprint of intensive mariculture operations is reduced, but it would also significantly diversify the produce and add to farm productivity and profitability. This broad scale approach to treating pond mariculture effluent could also find application in the nutrient collection from, and treatment of brackish waters in estuaries that receive uncontrollable nutrients from other anthropogenic sources.

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Appendix

Table A 1 Source pond water qualities at the farm - samples taken off the jetty at depth of about 50 cm (see Figure 1)

Date	Time	Temp	Sal	pH	DO	Redox	Algal bloom comments
12 Jan	3.00 pm	29.0	39.2	8.3	8.0		Green bloom
18 Jan	12 noon	-	-	-	-	-	Greeny-brown bloom
25 Jan	1:00 pm	31.5	40.3	7.7	5.0	-25	Some vertical stratification in pond (paddle wheels off until 2 pm), greeny- brown bloom, dull weather, changing algae with bloom die off apparent
26 Jan	2:00 pm	-	-	7.6	5.0	-	Very pale green bloom
29 Jan	8:00 am	-	-	-	-	-	Green bloom looking more healthy
1 Feb	12 noon	30.7	41.0	7.9	5.3	69	Greeny-brown bloom
8 Feb	12 noon	28.4	37.8	8.1	6.7	80	Green bloom
15 Feb	12 noon	26.4	36.3	8.0	7.2	48	Green bloom
22 Feb	12 noon	27.0	34.6	8.1	6.9	61	Dull green bloom with pale silt from rain
1 Mar	12 noon	28.6	34.1	8.4	8.9	-19	Dull green bloom with pale silt from rain
8 Mar	12 noon	28.5	32.5	8.5	11.1	-15	Dull green bloom with pale silt from rain
15 Mar	12 noon	Readings not possible due to low pond level					
22 Mar	12 noon						Browny-green bloom

Table A 2 Source pond water qualities at the farm - samples taken from monk drain at depth of about 50 cm (see Figure 1)

Date	Time	Temp	Sal	pH	DO	Redox	Water control comments
25 Jan	1:00pm	30.9	40.3	7.6	3.0	-59	No discharge through monk causing reduced DO
1 Feb	12 noon	30.8	41.0	7.9	5.2	71	Discharge has reduced build-up of organic matter so that DO is not depressed.
8 Feb	12 noon	28.4	37.7	8.1	6.8	81	
15 Feb	12 noon	26.4	36.3	8.0	7.2	49	
22 Feb	12 noon	27.1	34.5	8.0	6.6	59	Pond level approx 200 ml lower due to partial harvest
1 Mar	12 noon	28.7	34.1	8.4	8.7	-21	
8 Mar	12 noon	28.2	32.4	8.5	10.3	-17	
15 Mar	12 noon	27.4	29.2	8.7	9.9	-5	Pond level to about 10 % of total volume due to harvest
16 Mar	-	-	-	-	-	-	Pond refilled to approx 30% of total volume
22 Mar	12 noon	28.8	31.8	8.9	9.9	-121	
29 Mar	12 noon	27.3	34.1	9.0	9.3	-117	
5 Apr	12 noon	27.4	37.0	-	7.1	-88	
12 Apr	12 noon	24.5	36.7	7.7	5.1	-16	no bloom
19 Apr	12 noon	26.6	37.0	7.5	4.1	-23	no bloom

Table A 3 Timelines for activities and general observations.

Date	Activity / observation
11 Jan	Pond water running freely through all sand beds.
12 Jan	Tanks 2, 4 & 6 stocked with Polychaetes. Water qualities in tanks measured at 6 pm.
13 Jan	Tanks 1, 3 & 5 stocked with Polychaetes.
14 Jan	Water qualities in tanks measured at 12 noon.
18 Jan	Water qualities in tanks measured and nutrient samples taken at 12 noon.
25 Jan	Water qualities in tanks measured at 12 noon. Water levels getting higher later in day.
29 Jan	Tanks 2, 3 & 6 draining more freely; algae on water and sand surfaces.
1 Feb	All tanks still draining freely. Juvenile worms found in beds of all tanks. Water qualities in tanks measured and nutrient samples taken at 12 noon.
3 Feb	Surfaces of all sand beds (all tanks) gently raked.
5 Feb	All tanks draining fully between scheduled water supply periods.
8 Feb	Tanks 1 & 4 not fully drained between water supply periods. Water qualities measured at noon.
12 Feb	Tanks 1 & 4 not fully draining - water off from 9am-3pm - surface of all tanks raked (water still over beds 1 & 4, ie. wet-rake); water back on at 9pm.
15 Feb	Water qualities in tanks measured and nutrient samples taken at 12 noon. Tanks 1 & 4 overflowing, all flow off @ 1pm after sampling and overnight.
16 Feb	All beds dry-racked, water supply back on @ 9 am.
18 Feb	All beds draining fully before scheduled inflows. Worm holes apparent in all tanks.
22 Feb	All beds fully draining before scheduled inflows; tanks 2 & 6 the lowest. Water qualities measured at noon. All inflows lower than target due to lower pond level - increased to target.
24 Feb	Tanks 2 & 4 not draining fully before scheduled inflows; water supply left off for daytime pumping period; surfaces of beds 2 & 4 (only) raked @ 1 pm.
1 Mar	All tanks fully draining. Water qualities in tanks measured and nutrient samples taken at 12 noon. Tank 5 intake readjusted.
5 Mar	All tanks fully draining except tank 5.
8 Mar	Water qualities in tanks measured at 12 noon. Inflows were too high so were reduced.
10 Mar	Pump off till 11.30 am due to harvest; all fully drained except tank 5; sand surface of tank 5 raked whilst submerged (wet-rake).
15 Mar	Water qualities in tanks measured and nutrient samples taken at 12 noon. Inflows were too low (due to lower pond level) so were increased to target.
17 Mar	Pump off till 11.30 am due to prawn harvest.
18 Mar	Pump off overnight due to loss of pump prime - prawn stuck in foot valve.
19 Mar	No tanks fully draining. Inflows highly variable due to debris dislodging from inside of supply pipes and clogging valves - all lines flushed and inflows reset at target.
20 Mar	Only tank 1 draining fully.
21 Mar	Only tank 1 draining fully.
22 Mar	Water qualities in tanks measured at 12 noon. Inflows lower than target due to prawns blocking foot valve – cleared and reset at target.
26 Mar	No tanks fully draining before scheduled inflow
29 Mar	Water supply failed over previous night due to foot valve failure. Lots of large worm holes apparent in beds 4, 5 & 6; many smaller holes in bed 3; fewer smaller holes in tank 1; fewer small & large holes in tank 2. Started am inflow as scheduled. Water qualities in tanks measured and nutrient samples taken at 12 noon.
5 Apr	No tanks draining completely. Water qualities in tanks measured at 12 noon. Tanks 4 & 5 overflowing.
10 Apr	Water supply changed to reservoir pond
12 Apr	All inflows much higher than target and all tanks overflowing. Water qualities in tanks measured and nutrient samples taken at 12 noon. Inflows reduced to target.
19 Apr	Water qualities in tanks measured at 12 noon.
24 Apr	Pump failed - new pump installed.
25 Apr	Intakes highly variable – all adjusted to target.
26 Apr	Water qualities in tanks measured and nutrient samples taken at 12 noon.
30 Apr	Harvested tank 1
1 May	Harvested tanks 2 & 3
2 May	Harvested tanks 4 & 5
3 May	Harvested tank 6

Table A 4 Numbers of smaller and larger polychaetes harvested from tanks with different stocking densities and mean percentages of totals harvested.

Stocking density	Tank	Small	Large	Mean ± se % Small	Mean ± se % Large
High	1	2,895	411	88.3 ± 1.2	11.7 ± 1.2
	3	2,893	297		
	5	2,395	368		
Medium	2	1,145	261	76.7 ± 2.4	23.3 ± 2.4
	4	1,439	501		
	6	1,358	464		

Table A 5 Percentages of dry matter for small and large polychaetes harvested from tanks with different stocking densities.

Stocking density	Tank	Small	Large
High	1	20.1	19.8
	3	19.8	16.3
	5	17.9	18.5
Medium	2	20.0	20.1
	4	18.6	18.0
	6	14.5	15.2

Table A 6 Percentages of ash (on dry matter basis) for small and large polychaetes harvested from tanks with different stocking densities.

Stocking density	Tank	Small	Large
High	1	21.7	21.5
	3	23.3	22.5
	5	17.9	18.2
Medium	2	23.5	20.7
	4	17.5	18.4
	6	16.8	15.2

Table A 7 Gross energy (MJ kg^{-1} dry matter) for small and large polychaetes harvested from tanks with different stocking densities.

Stocking density	Tank	Small	Large
High	1	18.20	18.97
	3	18.06	18.62
	5	19.52	20.13
Medium	2	18.05	19.65
	4	19.87	20.28
	6	20.04	21.26

Table A 8 Percentages of nitrogen (on dry matter basis) for small and large polychaetes harvested from tanks with different stocking densities.

Stocking density	Tank	Small	Large
High	1	10.51	9.91
	3	10.42	9.77
	5	10.82	10.12
Medium	2	9.86	9.56
	4	10.93	10.20
	6	10.89	10.35

Table A 9 Percentages of phosphorus (on dry matter basis) for small and large polychaetes harvested from tanks with different stocking densities.

Stocking density	Tank	Small	Large
High	1	0.70	0.66
	3	0.69	0.64
	5	0.71	0.66
Medium	2	0.66	0.62
	4	0.73	0.67
	6	0.71	0.62

Table A 10 Phospholipid contents* (mg g^{-1} of dry sample) of graded (small and large) polychaetes harvested from experimental sand filter tanks stocked at different densities (PTEA = Phosphatidylethanolamine, PTI = Phosphatidylinositol, PTS = Phosphatidylserine, PTC = Phosphatidylcholine, SM = Sphingomyelin, LPTC = Lyso-Phosphatidylcholine, TPL = Total Phospholipids).

Density	Tank	Worm size	PTEA	PTI	PTS	PTC	SM	LPTC	TPL
High	1	Small	11.05	1.82	-	7.87	-	0.00	20.73
		Large	13.44	2.54	-	13.74	-	0.00	29.72
Medium	2	Small	8.00	1.23	-	3.85	-	1.10	14.18
		Large	6.49	0.70	-	2.60	-	2.38	12.17
High	3	Small	12.35	2.18	-	8.57	-	0.00	23.10
		Large	8.76	1.28	-	4.72	-	1.31	16.06
Medium	4	Small	2.67	0.28	-	1.22	-	2.56	6.74
		Large	4.20	0.18	-	1.24	-	3.38	8.99
High	5	Small	4.12	0.13	-	1.20	-	2.89	8.34
		Large	5.09	0.35	-	1.71	-	3.27	10.42
Medium	6	Small	3.82	0.00	-	0.83	-	2.59	7.23
		Large	2.68	0.00	-	0.89	-	2.65	6.21

* "-" indicates <0.05 mg g^{-1}

Table A 11 Fatty acid* contents (mg g^{-1} of dry sample) of graded small (S) and large (L) polychaetes harvested from experimental sand filter tanks stocked at different densities.**

Fatty acid	High density						Medium density					
	Tank 1		Tank 3		Tank 5		Tank 2		Tank 4		Tank 6	
	S	L	S	L	S	L	S	L	S	L	S	L
14:0	0.16	0.24	0.10	0.23	0.16	0.26	0.16	0.29	0.20	0.34	0.19	0.33
14:1n-5	-	-	-	-	-	-	-	-	-	-	-	-
15:0	0.14	0.22	0.15	0.26	0.16	0.22	0.15	0.23	0.20	0.32	0.18	0.26
16:0	4.69	10.19	3.99	10.19	5.23	11.22	6.03	13.58	5.57	13.92	6.62	15.18
16:1n-7	0.72	1.30	0.54	1.25	0.76	1.48	1.00	1.84	0.83	1.72	0.95	1.80
17:0	0.59	0.87	0.58	0.92	0.68	0.90	0.65	0.95	0.79	1.16	0.73	1.08
18:0	2.25	3.16	2.05	2.99	2.40	3.27	2.51	3.81	2.65	3.64	2.86	4.01
18:1n-?	1.10	2.08	1.05	2.13	1.16	2.25	1.33	2.76	1.32	2.81	1.54	3.07
18:1n-7	1.59	2.96	1.48	3.12	1.81	3.35	2.03	3.86	2.23	4.29	2.32	4.28
18:1n-9	0.55	1.02	0.44	0.91	0.56	1.12	0.78	1.43	0.64	1.19	0.67	1.24
18:2n-6	0.57	0.79	0.51	0.72	0.67	0.91	0.66	0.92	0.59	0.80	0.68	0.94
18:3n-3	0.36	0.62	0.26	0.52	0.55	0.80	0.69	1.20	0.38	0.67	0.51	0.91
18:4n-3	0.10	0.12	0.05	0.08	0.16	0.21	0.22	0.30	0.09	0.13	0.15	0.22
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	0.06	0.06	0.05	0.06	0.07	0.08	0.07	0.08	0.07	0.08	0.08	0.09
20:1n-6?	0.31	0.82	0.24	0.75	0.37	0.95	0.49	1.17	0.32	0.93	0.40	0.99
20:1n-7	0.11	0.16	0.10	0.17	0.15	0.18	0.12	0.18	0.17	0.22	0.17	0.21
20:1n-9	0.44	0.86	0.32	0.76	0.43	0.88	0.60	1.13	0.48	1.04	0.58	1.15
20:1n-11	0.58	1.06	0.60	1.15	0.61	1.11	0.63	1.31	0.74	1.44	0.85	1.58
20:2n-6	0.39	0.81	0.33	0.79	0.43	0.95	0.52	1.13	0.44	1.01	0.56	1.19
20:3n-3	-	0.13	-	0.14	0.05	0.16	0.08	0.31	0.05	0.18	0.07	0.26
20:3n-6	-	-	-	-	-	-	-	-	-	-	-	-
20:4n-3	-	-	-	-	-	-	-	-	-	-	-	-
20:4n-6	1.07	1.45	1.06	1.42	1.27	1.61	1.25	1.75	1.23	1.65	1.37	1.86
20:5n-3	3.83	4.43	3.33	4.35	3.79	4.15	3.41	4.42	4.36	5.22	4.15	5.16
22:0	-	-	-	-	0.05	0.06	0.05	0.07	-	-	-	0.06
22:1n-7?	1.87	2.52	1.78	2.49	1.97	2.56	1.75	2.65	2.08	2.80	2.13	2.86
22:1n-9	-	0.07	-	0.06	-	0.07	-	0.09	-	0.08	0.05	0.10
22:1n-11	-	-	-	-	-	-	-	-	-	-	-	-
22:3n-3	-	-	-	-	-	-	-	-	-	-	-	-
22:4n-6	2.24	2.94	2.28	3.06	2.37	2.94	2.11	2.98	2.54	3.31	2.88	3.77
22:5n-3	1.01	1.19	0.90	1.23	0.88	1.00	0.74	1.02	1.07	1.30	1.04	1.30
22:5n-6	0.09	0.12	0.11	0.15	0.13	0.15	0.11	0.13	0.14	0.18	0.14	0.15
22:6n-3	0.33	0.45	0.34	0.52	0.38	0.49	0.41	0.53	0.48	0.71	0.45	0.56
24:0	0.06	0.07	-	-	0.06	0.08	0.07	0.10	0.06	0.08	0.09	0.09
24:1n-9	0.05	0.06	-	-	-	0.05	-	0.06	-	0.06	-	0.06
TOTAL	25.30	40.83	22.79	40.57	27.42	43.47	28.70	50.27	29.86	51.34	32.50	54.77

* "?" indicates some uncertainty regarding the specific fatty acid identified
** "-" indicates <0.05 mg g^{-1}

Table A 12 Amino acid contents (mg g^{-1} of dry sample) of graded small (S) and large (L) polychaetes harvested from experimental sand filter tanks stocked at different densities.

Amino acid	High density						Medium density					
	Tank 1		Tank 3		Tank 5		Tank 2		Tank 4		Tank 6	
	S	L	S	L	S	L	S	L	S	L	S	L
Alanine	39.00	34.63	35.79	33.60	37.86	35.86	36.29	31.50	38.83	35.02	39.27	36.34
Arginine	38.00	34.44	38.72	36.00	36.15	35.47	35.80	36.02	42.29	38.71	40.53	38.94
Aspartic acid	56.41	51.08	51.06	50.07	52.92	51.55	53.20	45.68	53.92	50.95	54.72	52.85
Cystine	5.11	2.26	6.54	3.07	6.73	5.95	5.27	5.69	7.01	7.67	5.93	6.83
Glutamic acid	80.82	72.68	74.52	71.74	74.70	72.99	76.06	67.37	78.69	73.69	78.61	76.94
Glycine	54.62	46.69	52.08	46.08	51.95	46.64	50.96	43.46	54.46	47.25	51.65	44.06
Histidine	11.57	9.78	12.28	11.09	12.59	11.47	10.93	11.08	13.39	11.72	12.78	11.49
Isoleucine	20.71	19.50	20.51	20.33	20.93	20.46	19.42	19.35	22.09	20.63	22.35	21.65
Leucine	37.07	34.51	36.21	35.49	36.41	35.55	34.92	33.69	38.96	36.26	39.01	38.06
Lysine	32.11	28.46	31.98	29.73	32.22	29.15	29.17	27.28	33.85	30.13	33.35	31.07
Methionine	7.67	2.89	10.07	4.40	10.51	10.24	7.78	8.35	12.23	13.26	10.63	10.94
Phenylalanine	19.35	16.81	19.01	18.66	19.75	18.98	18.23	17.80	21.45	19.60	20.63	20.14
Proline	31.07	28.14	28.47	28.18	31.01	31.46	28.57	27.97	30.73	28.60	32.18	29.99
Serine	22.37	20.23	21.79	20.90	21.74	21.29	21.25	20.46	23.87	21.80	22.97	22.54
Threonine	23.86	22.19	23.39	23.09	24.05	23.69	22.62	22.50	25.50	24.17	25.11	24.57
Tryptophan	5.49	5.74	6.57	5.94	6.65	6.25	6.05	5.18	6.68	5.87	5.57	6.63
Tyrosine	17.28	15.71	17.09	16.79	17.91	16.97	16.10	15.73	19.50	17.86	18.36	17.80
Valine	23.78	21.70	22.91	22.33	23.98	22.86	22.41	21.22	24.94	23.05	24.92	24.14

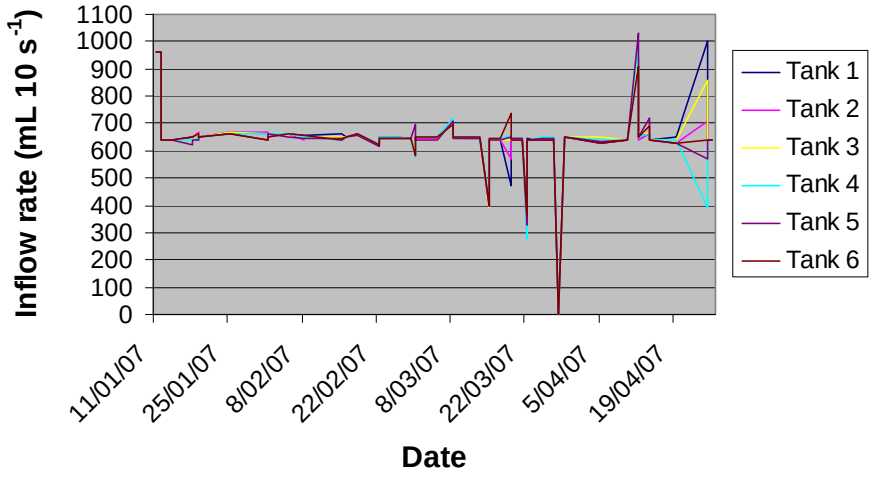

Figure A 1 Rates of inflow measured for each tank prior to regular readjustment to 640 - 650 mL 10 s^{-1}.

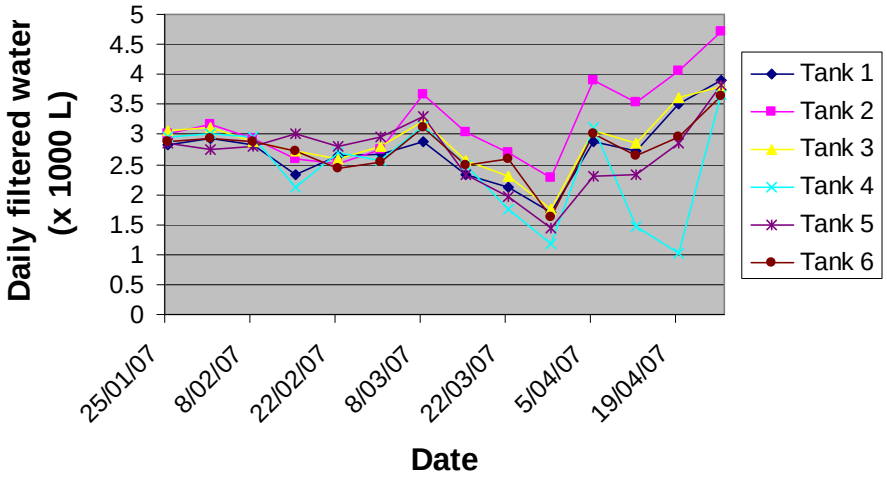

Figure A 2 Daily volumes of pond water filtered by each sand bed. Data were generated by dividing weekly increments to cumulative totals (see Fig. 4) by 7.

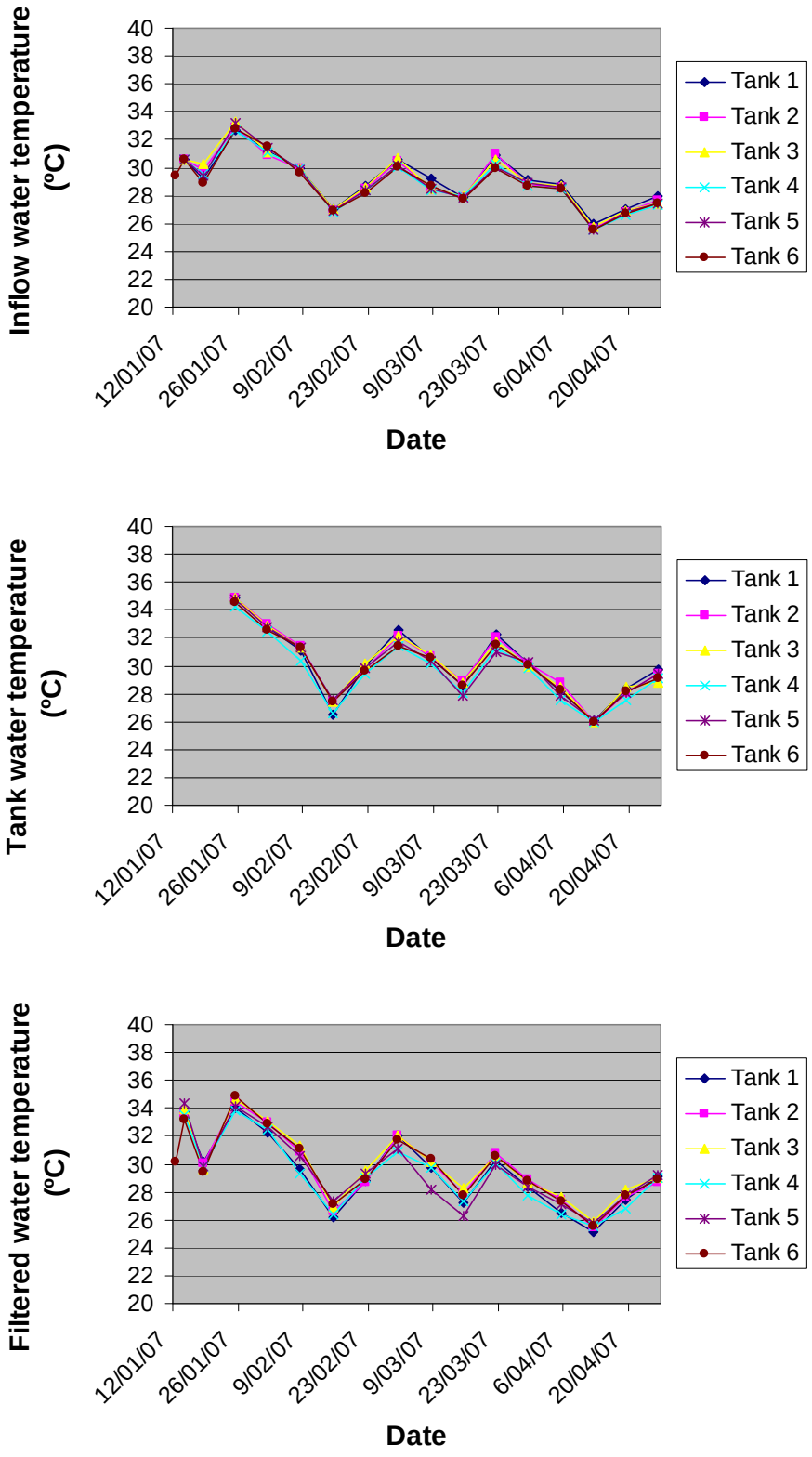

Figure A 3 Temperatures of the inflow, tank and outflow (filtered) waters for each sand filter during weekly sampling at 12 noon.

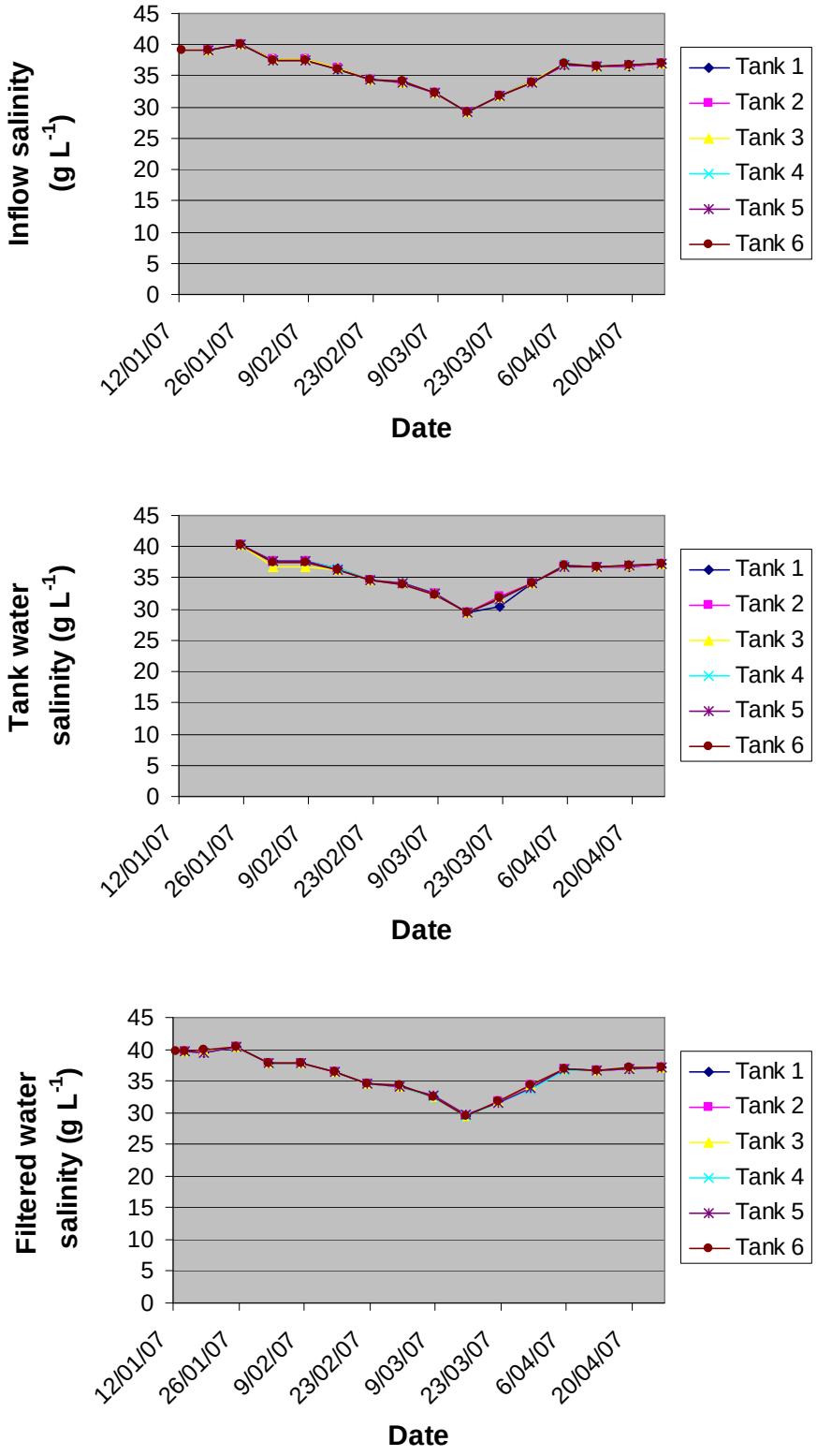

Figure A 4 Salinities of the inflow, tank and outflow (filtered) waters for each sand filter during weekly sampling at 12 noon.

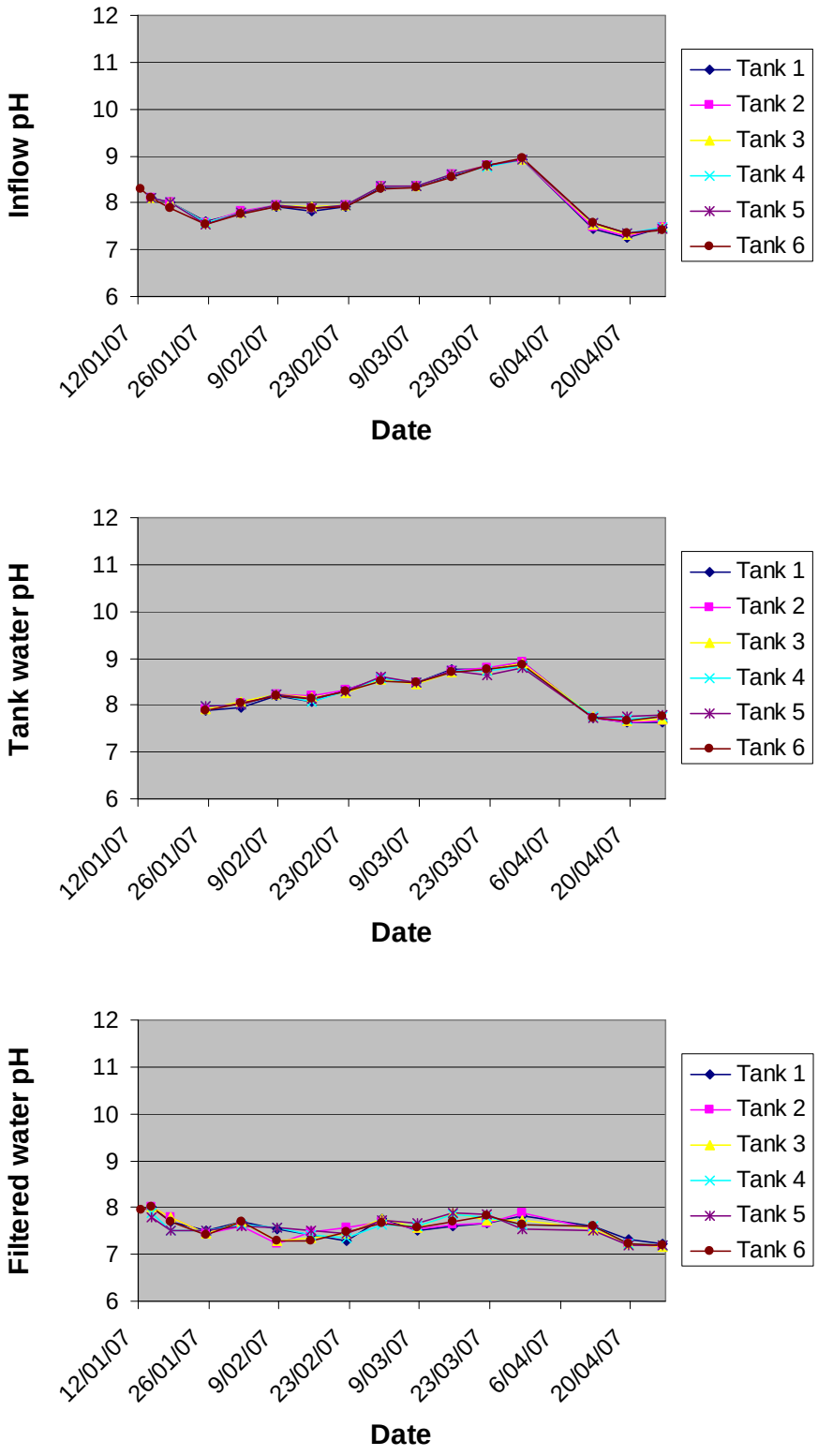

Figure A 5 Levels of pH for the inflow, tank and outflow (filtered) waters for each sand filter during weekly sampling at 12 noon.

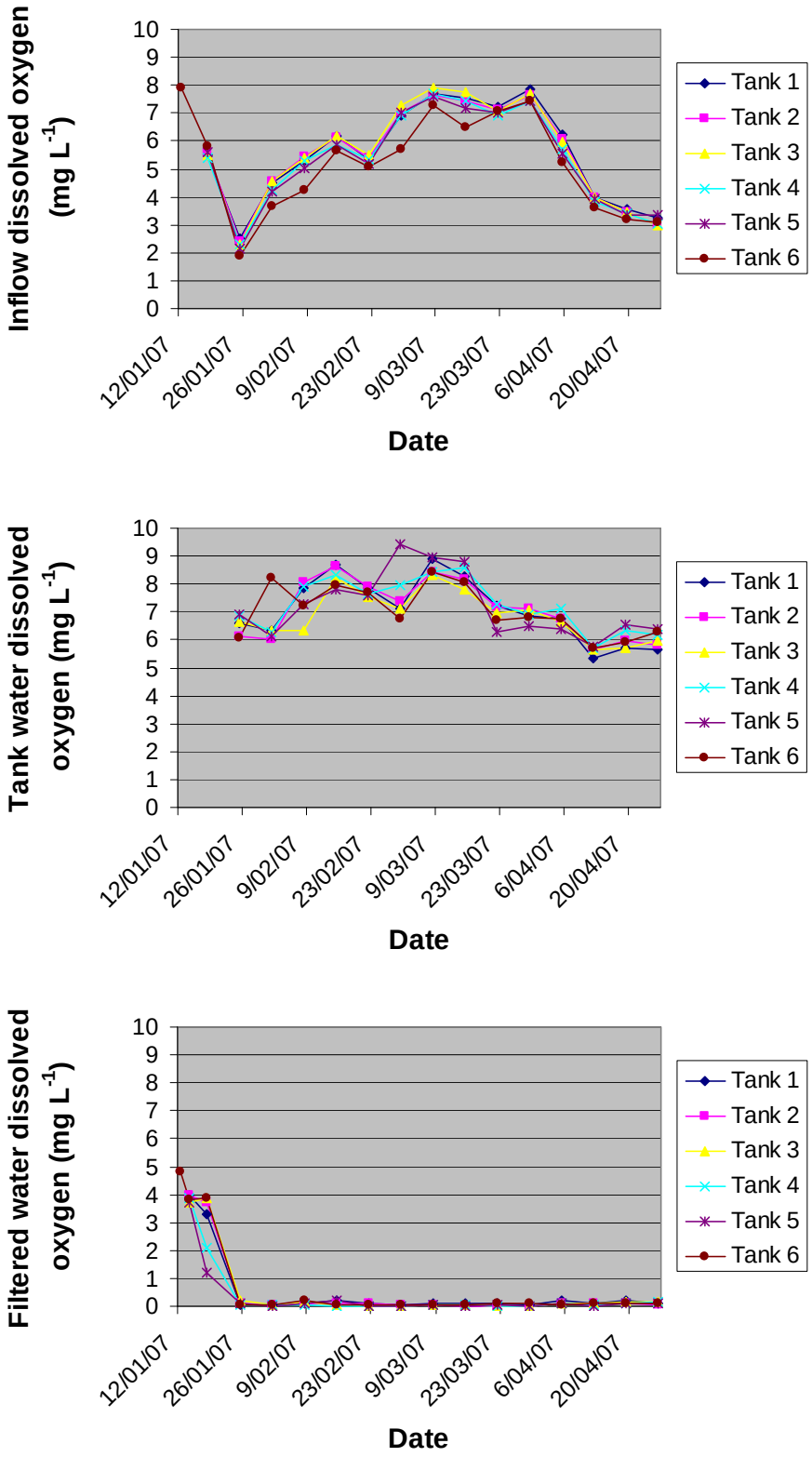

Figure A 6 Levels of dissolved oxygen for the inflow, tank and outflow (filtered) waters for each sand filter during weekly sampling at 12 noon.

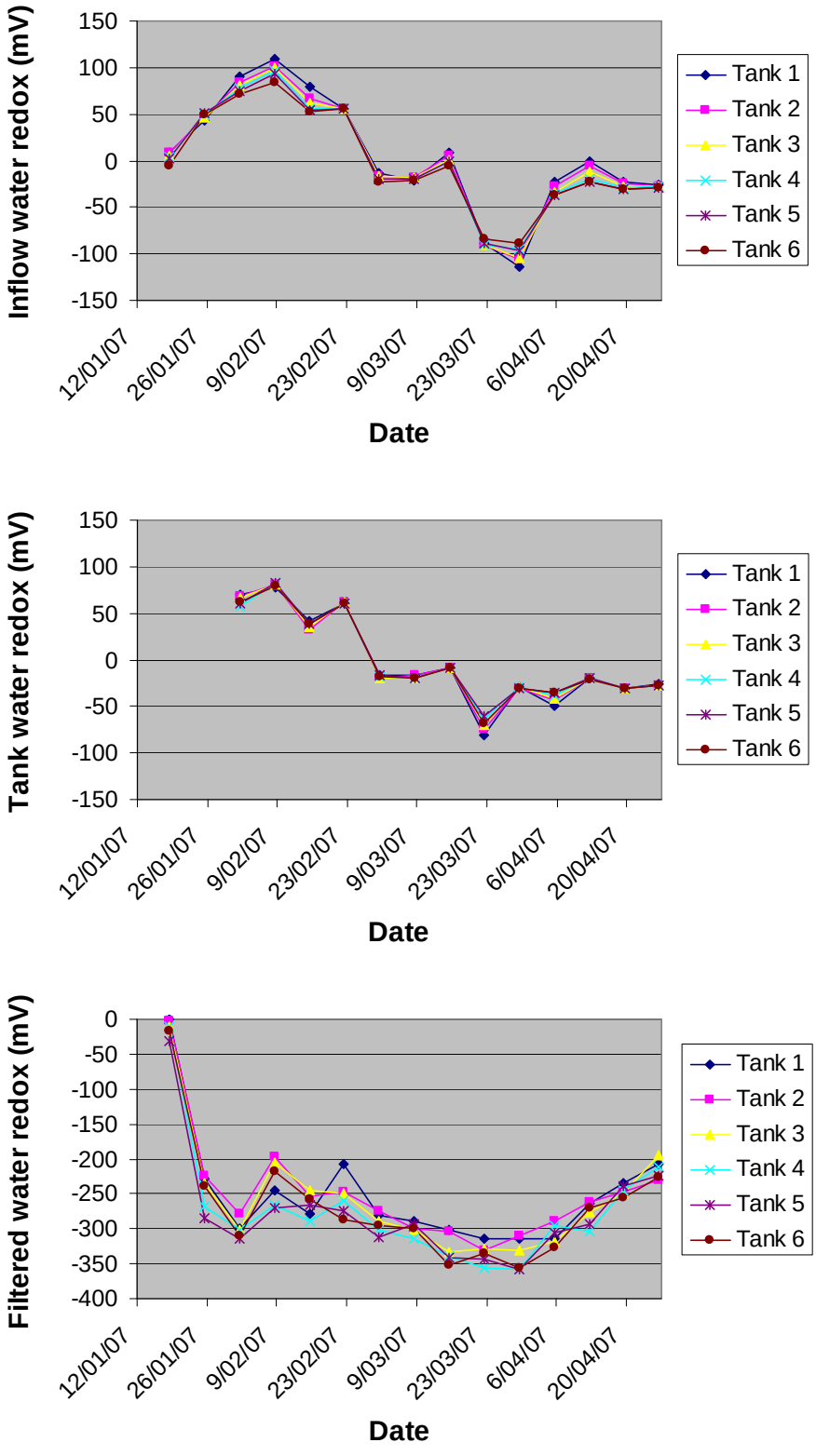

Figure A 7 Reduction/oxidation potential (redox) for the inflow, tank and outflow (filtered) waters for each sand filter during weekly sampling at 12 noon.

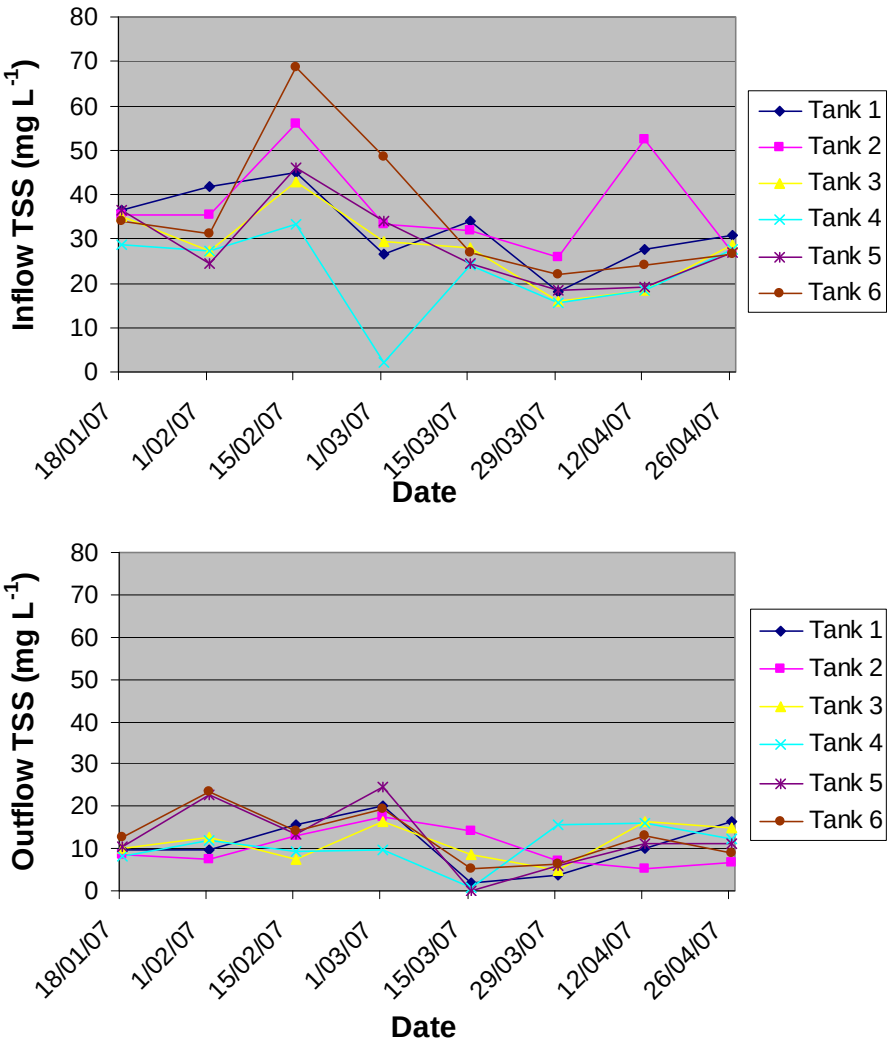

Figure A 8 Total suspended solids (TSS) in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

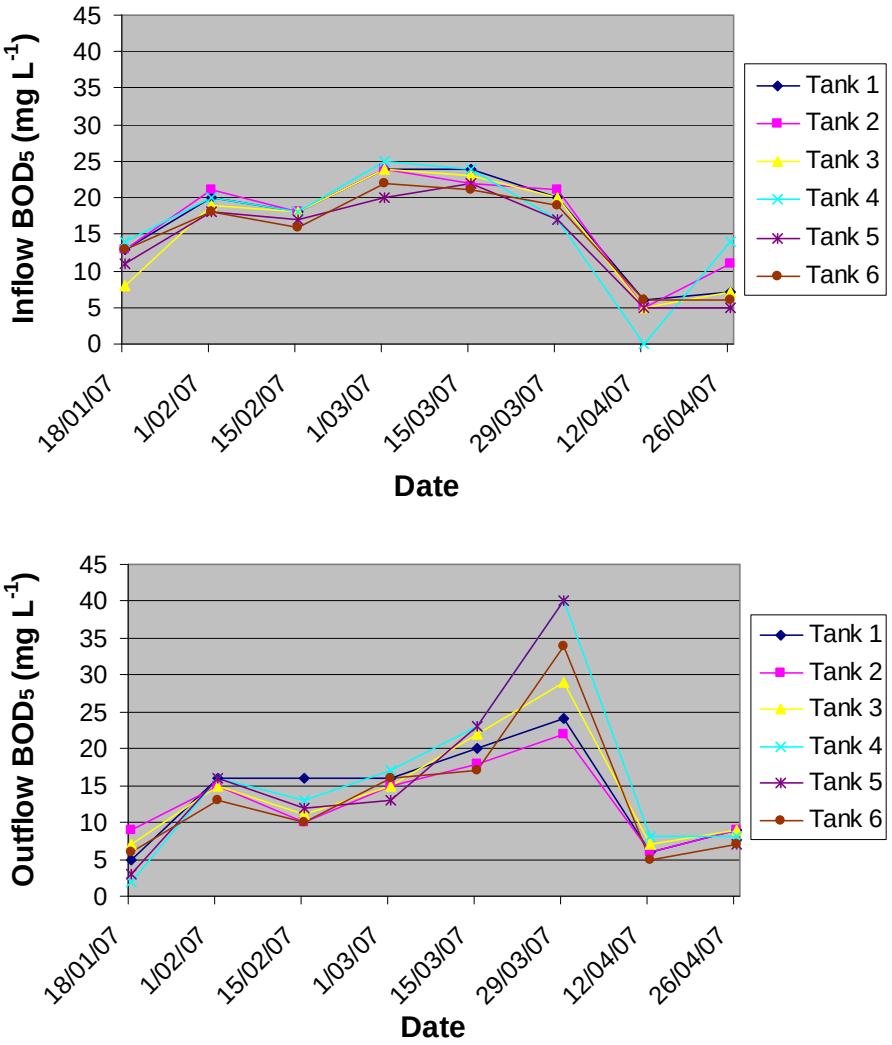

Figure A 9 Biological oxygen demand (BOD_5) of inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

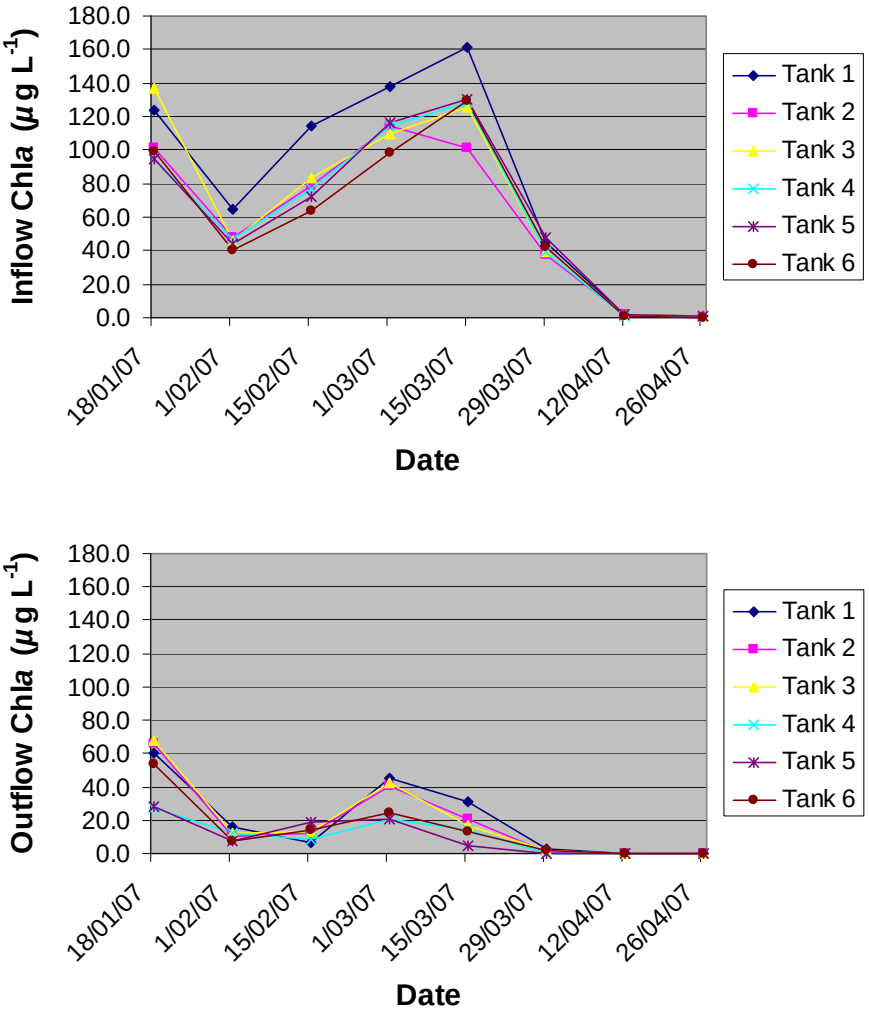

Figure A 10 Chlorophyll *a* (Chl*a*) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

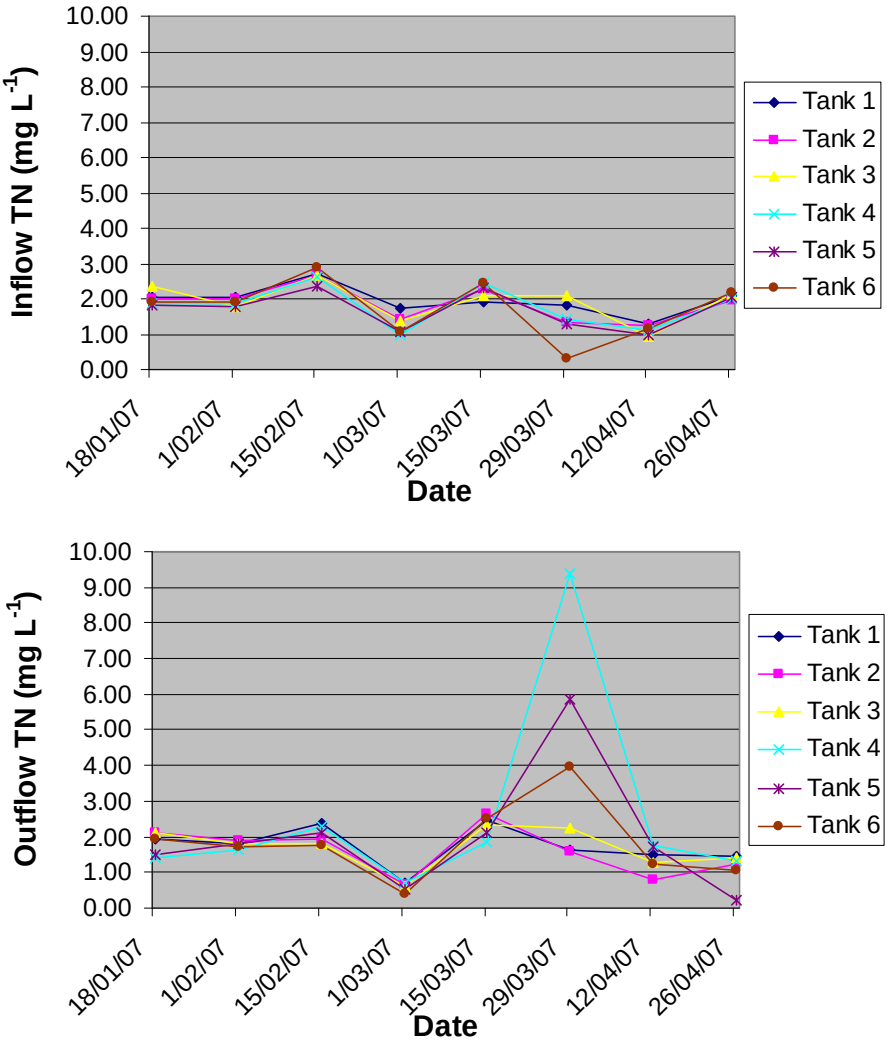

Figure A 11 Total nitrogen (TN) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

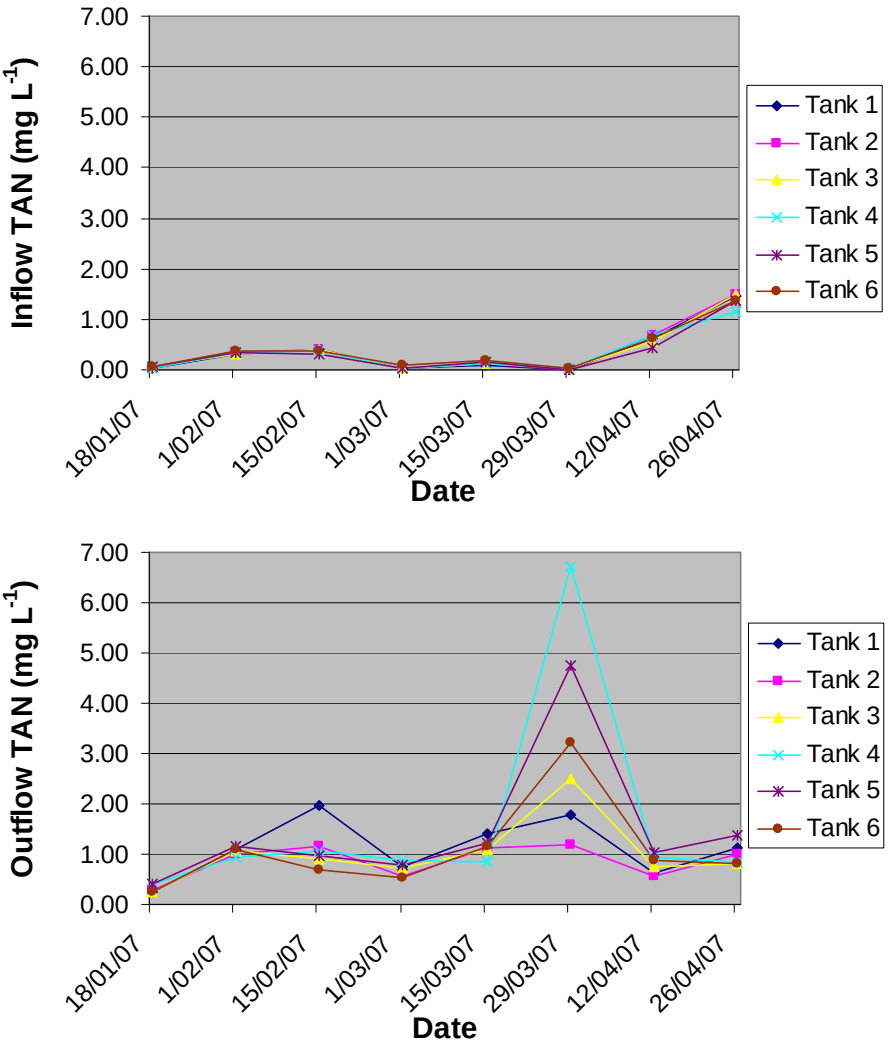

Figure A 12 Total ammonia (TAN) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

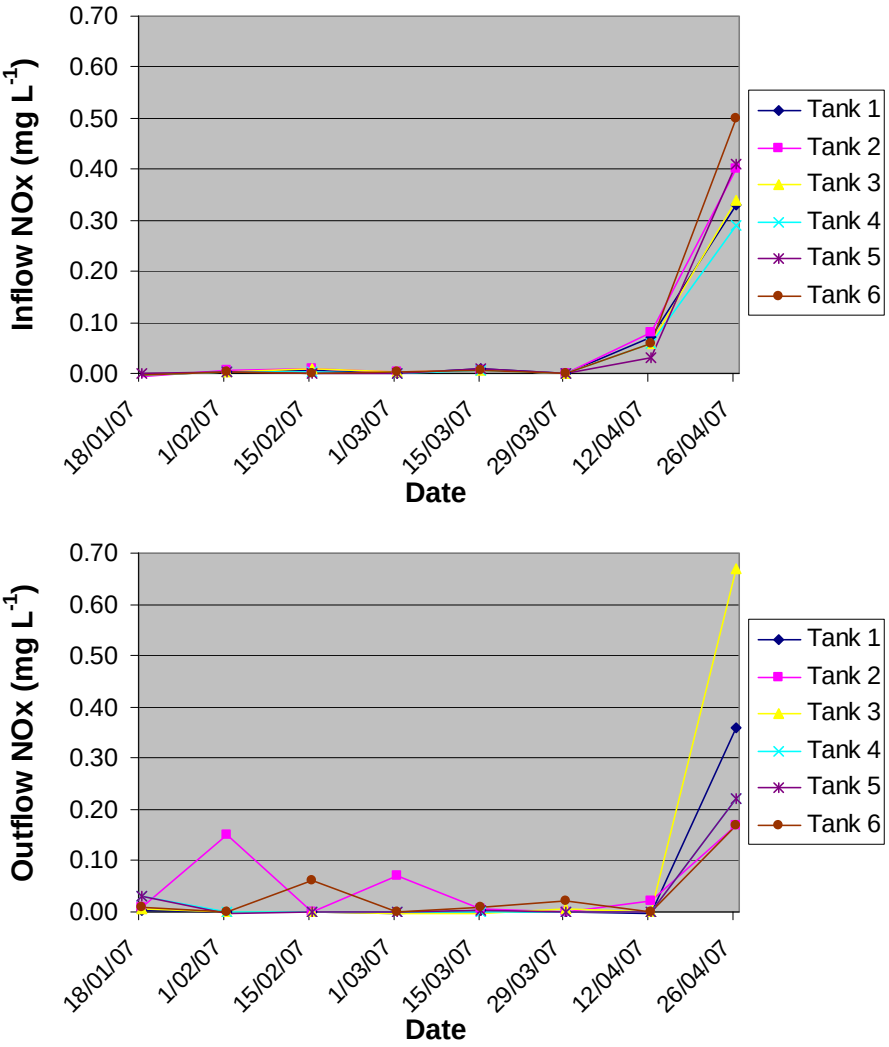

Figure A 13 Nitrate + Nitrite (NOx) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

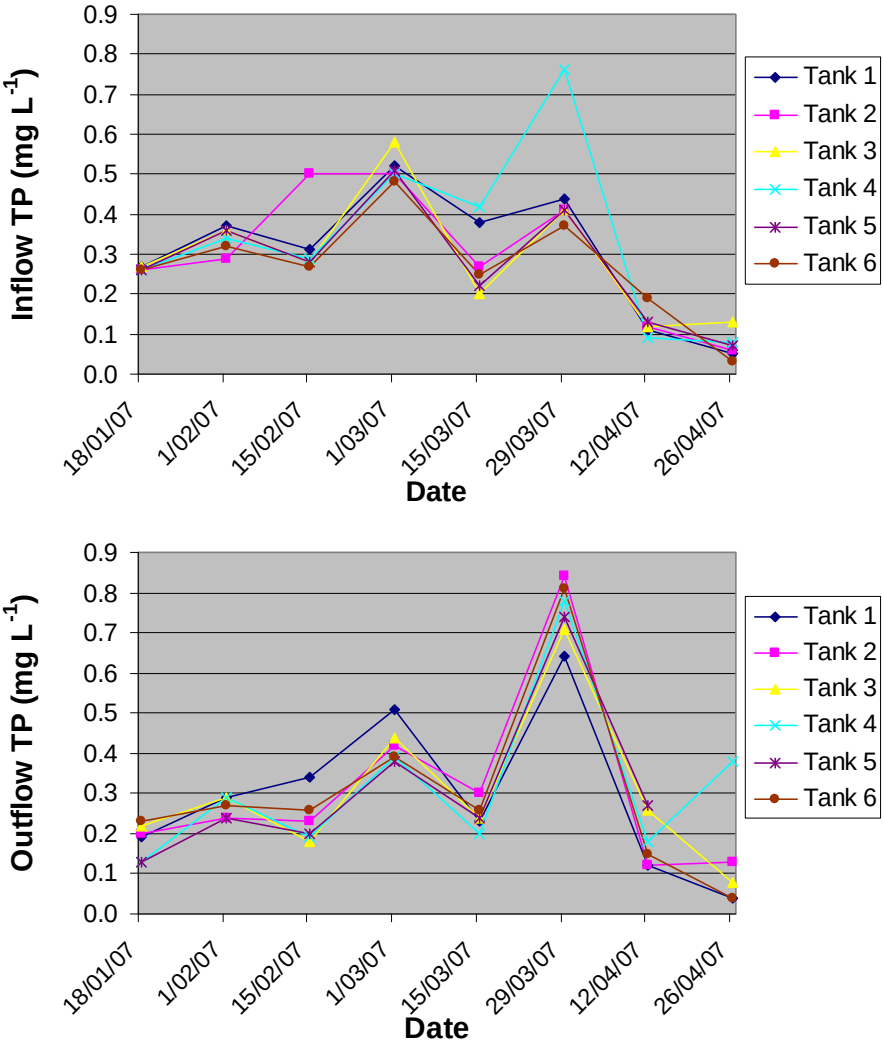

Figure A 14 Total phosphorus (TP) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.

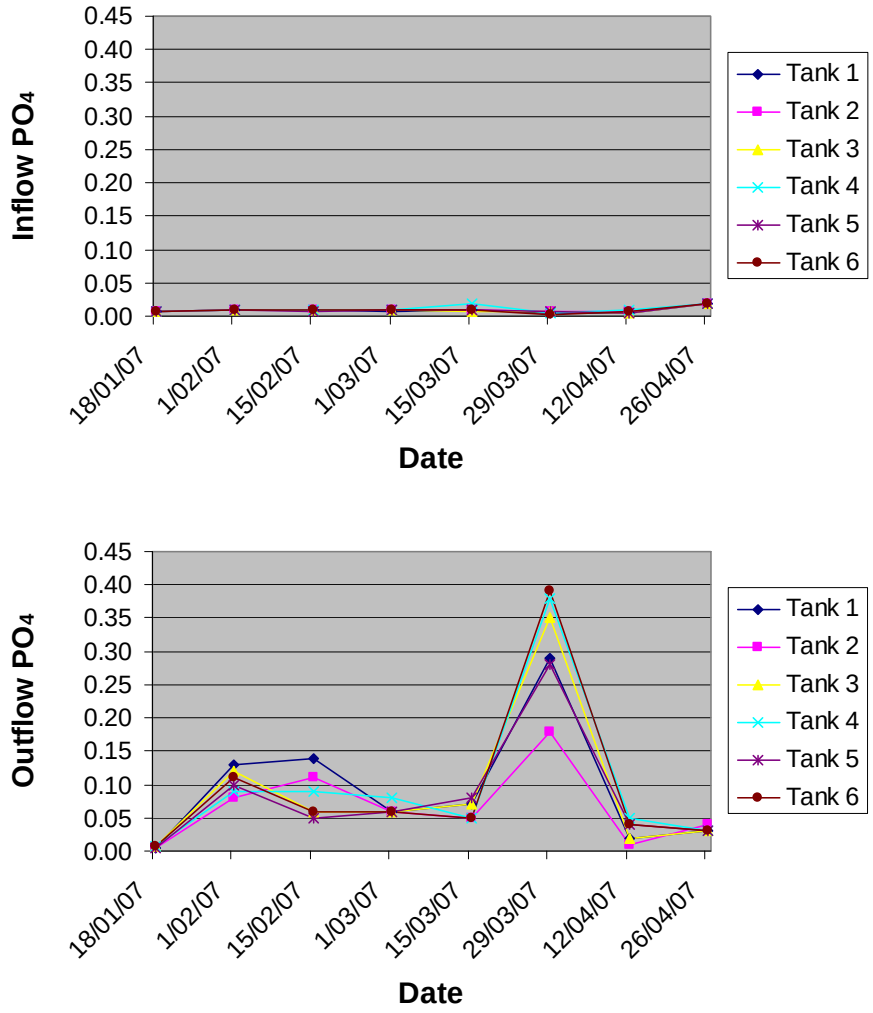

Figure A 15 Orthophosphate (PO_4) levels in inflow and outflow (filtered) waters for each sand filter during fortnightly sampling at 12 noon.