Polybridge Season 3: Ecosystem effects of polychaete-assisted sand filters

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Executive summary

This study is an extension of research undertaken in the first two seasons of the Polybridge Project (2013-2016: for results see Palmer et al., 2016), which sought to investigate operational aspects of polychaete-assisted sand filters (PASF) when deployed for scaled prawn farm recirculation at the Bribie Island Research Centre (BIRC). The aims of the present work were to assess its functionality with increased organic loading rates provided by higher prawn stocking densities than previously trialled, and to assess the ecological effects on this integrated farming system when using PASF to initially fill ponds for a range of biosecurity purposes.

Using prawn postlarval stocking densities in excess of 44 m^{-2}, prawn production of up to 12 tonnes ha^{-1} was achieved without discharge of any wastewater during the production season (2015-16). However, the average production for the two ponds was 9.4 tonnes ha^{-1}, which was lower than in the previous season (9.9 tonnes ha^{-1} in 2014/15) which used a lower prawn stocking density (37.5 postlarvae m^{-2}). The prawns and worms produced were again healthy and of high commercial quality and value, but slower prawn growth (particularly after 140 d) and lower worm survival limited overall production in the fully-recirculated system. There were also several concerning aspects to this closed-system approach that need to be highlighted. Firstly, nutrient levels in the pond waters rose to particularly high levels, and some of the more toxic parameters, such as ammonia, reached critical levels that could be considered dangerous for routine prawn culture operations. Worm production in the PASF beds also suffered from the very rich wastewaters in the integrated system, and the capacity of PASF to filter water via percolation was limited by excessive sand clogging and a build-up of organic matter on the upper surface of the sand beds. The resultant excessive anoxic conditions created in the sand beds appeared to reduce worm productivity which, in turn, reduced their sand cleansing actions, for an overall lower functionality in terms of nutrient (and particularly nitrogen) removal rates.

Alternatively, within the confines of the overall study, there were no significant deleterious effects on worm production or nutrient removal efficacies from using the PASF beds to initially fill the prawn production pond. The apparent effect of this on pond plankton communities was: 1) to slow the development of copepod populations; and 2) change the assemblage of algal species in the first few weeks after filling. This slower development of natural feed organisms in the PASF-filled pond may have provided lower survival of the particularly-young (PL 13) prawn seedstock used to stock the pond. Importantly however, there may be several potential remedies to this issue. These include management for a longer period for bloom development after fill and before stocking, and assuming a greater reliance on artificial feeds more suited to small prawns. As expected, this pond-fill strategy appeared to beneficially help exclude some problematic algal species, and greatly reduced barnacle fouling, though tube worm fouling did not appear overly affected.

The project successfully demonstrated a third successive season of zero-water discharge from an integrated prawn/ worm production system, though ultimately, the water in ponds with some residual nutrients were discharged. The expansion of prawn farming in Australia is limited by nutrient discharge issues, and biosecurity measures are also of increasing interest to this industry. In this legacy project, polychaete-assisted sand filters are further demonstrated to hold potential for biosecurity controls whilst minimising nutrient discharge.

Introduction and background

Since 2005, the Queensland Department of Agriculture and Fisheries (DAF) has been engaged in the development of a novel water treatment system called polychaete-assisted sand filters (PASF). Results thus far have demonstrated excellent capacity for farm integration to cost-effectively treat mariculture wastewater, whilst also producing marine polychaete worms from waste nutrients. This innovation has sustainable resource management at its core, specifically through its potential to reduce nutrient discharge from land-based mariculture farms, but also to help avoid natural habitat disturbances associated with the collection of wild polychaetes. Ongoing research (outside the scope of this report) is also investigating if it can provide a biosecurity tool for farmers in terms of reducing viral loads during wastewater treatment for recirculation, and be capable of providing disease-free supplies of worms despite potential contaminations from the open pond environments where it can be deployed.

Experimentation focused on nutrient abatement and documenting several beneficial aspects of PASF have been the basis of our previous work at increasing scales at the Bribie Island Research Centre (BIRC) and participating prawn farms over the last decade (Palmer, 2008; 2010; 2011; Palmer et al., 2014). The PASF system has also been used to routinely treat wastewater from commercially relevant pond-based programs at BIRC, and most recently has involved the deployment of a refined prototype and its testing for nutrient removal efficacies and prawn and worm production in a fully recirculated culture system (see Palmer et al., 2016). Increasing levels of prawn stocking (from 31 up to 38 postlarvae m^{-2}) have been applied, where the expected resultant increases in nutrients and organic loading have provided challenging conditions for this prototype system. Potential to produce up to 10 tonnes of prawns ha^{-1} with 100% recirculation and no water or nutrient discharge during the grow-out phase has been demonstrated. This approach appears to have reduced the overall nutrient discharge to the lowest levels so far reported for prawn aquaculture (i.e. 5.9 kg of nitrogen and 0.6 kg of phosphorus per tonne of prawns produced). However, even higher stocking densities are of interest to industry seeking somewhat higher production levels that are alternatively possible with flow-through water exchange methodologies.

Following on from these developments, DAF has also identified additional ways that PASF could provide industrial benefits. Given its demonstrated utility to remove fine particles (e.g. phytoplankton) from large volumes of pond wastewater, its further use to help control algal blooms, and/or serve as a selective filter for seawater used to fill ponds is of interest. In this regard, the nutritional suitability for prawns of resultant pond-based communities of aquatic organisms (e.g. phytoplankton and zooplankton) are of particular interest. These considerations extend further to the potential avoidance of problematic species which may not be suitable for this particular recirculating culture system, or which can cause problems at various stages in the cropping cycle. Examples include the relatively thick chain-forming diatoms (e.g. *Helicotheca,* formerly known as *Streptotheca* sp.) which can overload and clog PASF beds if flow rates and organic loading during their proliferation in ponds are not closely controlled; or filamentous blue-green species such as *Oscillatoria* sp. which produce toxins known to adversely affect crustaceans (Smith, 1996). In these cases, pre-screening with the PASF sand filter beds could reduce or prevent the influx of these larger or filamentous species, and restrict the seed organisms entering ponds to those smaller species which can more easily pass through the sand filter matrix.

Zooplankton species could also be trapped by the sand filters in such pre-screening activities, and so those pond zooplankton populations which rely to an extent on the initial seed (e.g. copepods) could also be affected. Furthermore, the continuous recirculation of pond waters through this type of filter may also have a mediating effect on blooms of aquatic organisms, particularly if clearance rates can be higher than their rates of in-pond reproduction and proliferation. Other examples of problematic organisms that could be reduced with this approach are the range of encrusting organisms, like tube worms and barnacles, which often uncontrollably infest broad-scale brackish-water ponds throughout the world. Such organisms can enter culture ponds as larvae with intake waters, where they grow to maturity and reproduce to cause several problems. In the early stages of the prawn production cycle they compete with stock for natural planktonic feeds, and in later stages their effects on water alkalinity, through sequestration of calcium carbonate for their shell matter, can lead to water quality problems (reduced pH) and additional costs to farmers through the need for increased liming rates. Furthermore, there is considerable cost associated with defouling encrusted pond equipment like paddlewheels during and after each crop. This latter issue alone has been estimated to cost over $1,000 per hectare in Australian prawn farms (APFA, 2010).

Prior to the development of PASF, this type of sand-filter pre-screening of pond-fill seawater was not practically achievable because of the necessary scale of the filter to handle the large volumes of water. Whilst farm designs that incorporate PASF can make this possible, the effects of this on initial and subsequent pond plankton communities and on survival and growth of the prawn stock are yet unknown. In support of ongoing investigations into the potential virtues of PASF for the remediation and reuse of wastewater at broad-scale marine prawn farms, a third successive season of experimentation was conducted during the 2015/16 summer season in the purpose-built facilities at BIRC. This work builds on the results generated by Palmer et al. (2016) in 2013-15, and similarly was undertaken within a scaled fully-recirculated prawn/ worm production system with replication of experimental units for robust scientific results. This present research was designed to further confirm the nutrient removal capacities of PASF when placed under higher-than-previous organic loads, whilst also investigating in more detail the effects that PASF may have on outdoor culture pond ecosystems. In particular, the effects of PASF on pond plankton and biofouling communities were specifically investigated.

Materials and methods

Experimental system

The same experimental system and most of the same management approaches[1] used in previous Polybridge research at BIRC (2013-2015) were deployed. In short, this incorporated ten operationally identical 54 m^2 PASF beds supplied with water from two identical 1,600 m^2 outdoor HDPE lined ponds (Figure 1). To close the loop and provide 100% recirculation, PASF-treated pond water was recirculated back to the ponds from a submersible pump in a common sump. Importantly, this meant that PASF-treated pond water from one pond mixed with PASF-treated water from the other pond prior to returning equally to both ponds.

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[1] Unless otherwise stated, methods applied were the same as documented for Season 2 in Palmer et al., 2016.

Figure 1 The polychaete-assisted sand filter testing facility at the Bribie Island Research Centre.

Prawn pond management

Residual organic matter remaining in the middle of ponds (as sludge) from the previous prawn crops was sun-dried during the winter dry out period and spread around the bottom of each pond just before filling. These residual nutrients prompted a reduction of the initial dose of fertilisers to one quarter of the standardised approach used in previous years. Dolomite (3 x 21 kg bags pond^{-1} = 394 kg ha^{-1}) was also spread around the pond bottoms before filling.

Filling began for both prawn culture ponds on 25/9/15. Pond G2 was filled with raw seawater filtered through five of the PASF beds (Beds 6, 7, 8, 9 and 10), whereas Pond G1 was filled at a similar rate with raw seawater filtered through the normal 300-μm sock as used in previous years. Due to the slower potential passage of water through the PASF beds, both ponds took about five days to fill. Blue dye (one satchel of Aqua Blue per pond) was initially (29/9/15 and 8/10/15) applied to both ponds to help prevent the growth of macrophytes.

Both ponds were stocked with *Penaeus monodon* postlarvae (70,000 x PL13 from TPF Management Co) at 44 m^{-2} on 2/10/15 (day 0). Feeding and management was guided by the Australian Prawn Farming Manual (2006), and feeds used were again from the Ridley Aquafeed MR range. One feed tray in each pond guided the feeding rates applied up to four times per day. As in Seasons 1 and 2, automatic belt feeders aided by air lifts (4 per pond) delivered the midnight ration. Standardised twice-daily (at 6 am and 3-4 pm) measurements of pond water pH, dissolved oxygen, temperature and salinity (using YSI Pro Plus multiprobe), and daily Secchi depth readings guided water quality and pond management.

To help establish a strong phytoplankton bloom, follow-up doses of our standardised $^{\text{(see footnote 1)}}$ inorganic fertilisers were applied to both ponds on 6/10/15, 9/10/15, 16/10/15 and 23/10/16. Molasses (1-2 L pond^{-1} d^{-1}) was also added from 1/10/15 to 3/10/15 to help prevent excessive pH increases due to the developing algal blooms, and later if total ammonia levels became excessive. Hydrated lime (3 kg pond^{-1} or 18.8 kg ha^{-1}) was dissolved in a bucket and slowly added to ponds on a daily basis when alkalinities dropped below 80 ppm. To ensure sufficient supplies of magnesium were present for hard prawn shells, one bag of dolomite (21 kg) was also routinely added to each pond at least on a fortnightly basis from day 83 (23/12/15). Up to two paddlewheels per pond (25 hp ha^{-1}) were applied to maintain dissolved oxygen levels above 3 ppm. After the last partial (trap) harvest this was reduced to one paddlewheel per pond.

Partial trap-harvests began for pond G1 on day 139 (17/2/16) and for pond G2 on day 146 (24/2/16), and the last partial harvests for both ponds occurred on day 165 (14/3/2016). During the last partial harvest, approximately 7 kg of prawns from each pond's harvest, comprising about 70 of the largest males and females from each pond were released back into their respective grow-out ponds. This was undertaken to on-grow some stock for maturation trials (reported separately). The low densities of prawns remaining in the ponds after the last trap harvest were managed assuming that naturally available feeds and a low artificial feed ration (1.0–2.5 kg per pond once per day) would be sufficient to maintain healthy growth. Feed tray checks every second day confirmed this approach during an extended period of further culture (approximately 2 months), which was applied to encourage the run-down of nutrients in pond waters prior to final discharge.

Ponds were drain harvested on day 231 (19/5/16 for pond G1) and day 238 (26/5/16 for pond G2). The bulk of harvested prawns from the trial were supplied as live product to Truloff Prawn Farms (TPF Management Co) for quality appraisals and costs recovery.

PASF bed management

On the basis that deeper PASF beds had previously provided more reliable operation and less sand clogging than shallower beds (see results from Palmer et al., 2016), prior to the start of the present experiment, clean sand of similar grade (GTS2000 from Southern Pacific Sands Pty Ltd) was added to the previously shallow beds so that all 10 sand beds had depths of about 22 cm. When the prawn ponds were full (30/9/15) those PASF beds used to screen water for filling pond G2 (see above) were allowed to completely dry, with no further manipulations until stocking with worm juveniles and renewed wastewater treatment and recirculation several weeks later.

Pond water was arranged to automatically pump to the PASF beds from the day before stocking worm juveniles (Figure 2). Daily water-flow patterns reflected the same approach used in previous years with three simulated tidal flows (at 4 pm, 10 pm and 4 am) and routine sun drying of surface sands each afternoon. As in previous years, flow meters measured daily supplies to each PASF bed, and this allowed fine valve-adjustments to equalise flows and resultant organic loading rates among the beds.

Regular manipulation of drainage pipes with different-sized discharge holes (14, 16, 18, 22, 25, 35, or 50 mm) again allowed similar drain-down times to be achieved, and the records of hole sizes used in this endeavour provided a good overall measure of progressive bed clogging or clearing. In addition to this filtered-water release strategy, different-sized short circuits (see Palmer et al., 2016) effectively increased the release of unfiltered pond water from the beds. These short circuits (8, 10, 13, 16 or 19 mm) were only put in place when the unrestricted 50 mm drainage pipe did not allow sufficient release of filtered water for sun drying of beds in the afternoons, and they were removed or reduced if percolation rates improved.

Water treatment rates were initially set in the order of 5% of pond volume each day (Figure 2), whereby the PASF beds routinely treated pond water at rates of about 450 L m^{-2} d^{-1}. This was gradually reduced during the season to about 4% each day (350 L m^{-2} d^{-1}) towards the end of the prawn cropping cycle. After drain-harvesting the prawn ponds, seawater from one smaller (200 m^2) pond (N2) was used to continue daily water supplies to unharvested PASF beds. Discharge from the beds during that time was recirculated back to this smaller pond until all PASF beds were harvested.

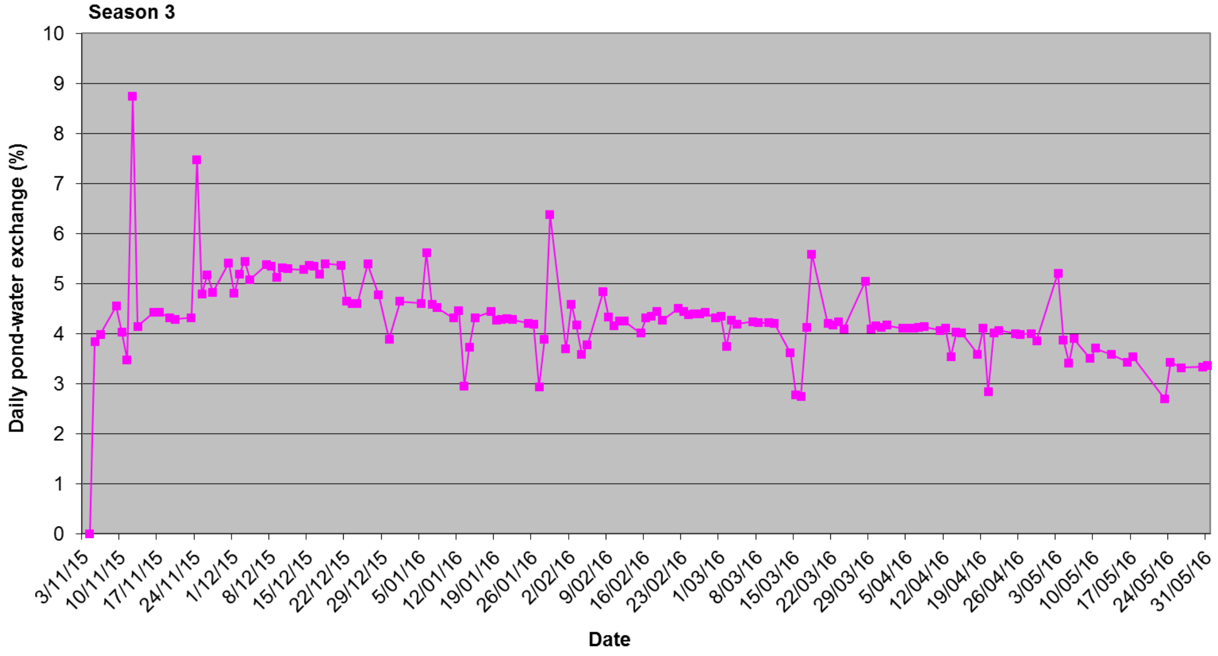

Figure 2 Water exchange rates applied in the PASF integrated system in Season 3.

One-month-old *Perinereis helleri* juveniles were stocked into the PASF beds at approximately 3,000 m^{-2} on 3/11/15 (beds 2, 3, 7 & 8), 4/11/15 (beds 4, 5, 9 & 10) and 27/11/15 (beds 1 & 6). These were from tenth-generation domesticated stocks supplied under contract by P&C Palmer Consolidated Pty Ltd. Stocking involved evenly planting small aliquots of juvenile-carrying-sand from nursery tubs into the surface of the recently drained PASF beds. Soon after stocking and for 1-2 days thereafter, beds were managed under the wet-weather operational mode (see Palmer et al. 2016), which involved an outer standpipe for each bed that maintained saturated sand bed conditions. After this, beds were operated under the dry-weather operational mode (sun-drying each afternoon) unless the threat of significant rainfall forced a change to the standardised wet-weather operational mode[2]. No artificial feeds were applied to PASF beds in Season 3.

The PASF beds were progressively harvested specifically to supply commercial prawn hatcheries with live or frozen worms for their broodstock. In attempts to maximise overall worm biomass production, beds with the lowest numbers of worms shown from controlled subsamples were harvested first; due to density-dependent production shown in previous work (Palmer, 2010) it was assumed that worms in lower density beds would grow faster and reach their potential maximum biomass earlier than higher density beds. Worms were generally harvested on Wednesdays and/or Thursdays, and supplied (or frozen) on Fridays each week. This approach allowed at least 20 h of purging in clean seawater before packing and despatch. On most occasions, individual beds were harvested with two separate harvests on different days.

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[2] Wet-weather operational mode for PASF beds allows ongoing water filtration but maintains a permanent lens of brackish water over the sand beds at all times. This protects the marine worms from the lethal effects of freshwater.

Experimental design

Prawn pond comparisons

The application in this experiment of a higher prawn stocking density (i.e. 44 m^{-2}) than has previously been used in trials with this fully recirculated prawn/worm culture system (e.g. 31-38 m^{-2}), was expected to generate higher nutrient levels in pond waters and higher organic loading in the PASF beds. Whilst this prawn density increase was not studied with experimental treatments, since the same integrated system and most of the production and assessment methods were similar between seasons, it was considered reasonable to draw direct comparisons in this regard between the present and previous seasons.

Stock growth, feed conversion and the overall productivity of prawn ponds were therefore compared with those of Seasons 1 (2013-14) and 2 (2014-15) (see Palmer et al. 2016) as well as industry standards (see Australian Prawn Farming Manual, 2006), to identify the apparent effects of these higher prawn densities in such a system. Prawn size sampling was undertaken on a weekly basis from day 49 (19/11/15) onwards. Prawns (12) caught on feed trays were used for size estimates up to day 84 (24/12/15) and thereafter a 10-mm-mesh cast net provided the samples. Total harvest weights for each pond were tallied, and overall production and survival for each pond included samples removed for size estimates and future broodstock. Samples of 50-100 harvested prawns per pond were weighed and counted to provide estimates for the numbers of prawns removed with each harvest.

Along with prawn production comparisons between the two ponds, other pond-based factors studied included phytoplankton and zooplankton abundance, sludge accumulation, barnacle density and the overall nutrient levels in the two ponds.

Plankton samples (500 mL) were taken from each pond at regular intervals on 33 occasions between 2/10/15 and 11/5/16. These were preserved with Lugol's solution so that the final concentration of iodine was approximately 0.2% (giving a weak tea colour in preserved samples). Preserved samples were stored at 4 °C until assessed in terms of zooplankton and phytoplankton abundance to provide measured pond densities. This involved undiluted samples for larger organisms (e.g. copepods and rotifers), and dilutions with cross-sectional counts using a 1 mL Sedgewick Rafter cell for smaller organisms (phytoplankton). In the early stages of the cropping cycle when plankton communities were still establishing, a plankton net (62 μm) was also used to sample (short oblique tow) larger volumes of pond water, although results from this approach only provided qualitative data. Zooplankters were surveyed for two predominant taxonomic groups (i.e. Rotifera and Copepoda) and phytoplankton species were identified down to the genus level.

The sludge that accumulated in the centre of each pond was assessed and sampled the day after the drain harvest of each pond. Measurements were taken for averaged diameters (calculated from a measured north-south transect through the centre and a measured east-west transect through the centre) and four evenly spaced depths across the four constructed radii for each pond. These stratified mound measurements were converted to flat stacked circular discs to allow volume calculations from a stacked cylinder approach. Using an Excel spreadsheet[3] this systematic approach fitted a third-order polynomial equation to averaged depths across the mounds radii, and then used incremental 1-mm height measures to precisely integrate the cross-sectional areas of the mounds to calculate total mound volumes. Incremental 10-mm height measures were also applied to assess volume estimate sensitivity to the height resolution used in the stacked circular disc method.

Sludge samples (3 per pond) were stored at 4 °C for 3-10 d before submitting to the UnityWater and ALS laboratories for NATA-approved analyses of total solids, total volatile solids, total nitrogen and total phosphorus contents. Weight-to-volume assessments from Season 2 provided the conversion factors for mass balance nutrient estimates.

Initially, tube worm and barnacle shells from the previous crop were removed from the sides of ponds before filling. This was undertaken to allow valid density comparisons for encrusted organisms that developed in the two ponds following their different pond-fill strategies. Barnacle densities were assessed the day after drain harvests, using the same stratified sampling method used in Season 2 (12 x 0.075 m^2 vertical quadrats per pond). Tube worm densities were qualitatively assessed.

PASF bed comparisons

The main experimental worm bed treatment studied (with replication $n = 5$) in Season 3 was the use of PASF beds to selectively screen pond-fill seawater. Statistical comparisons between “pond-fill beds” (i.e. Beds 6, 7. 8, 9 & 10) and “normal beds” (not used for pond filling) (i.e. Beds 1, 2, 3, 4 and 5) were made for a range of PASF-treated-water parameters, including total suspended solids (TSS), turbidity, total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (Chl*a*), total ammonia (TAN), nitrite, nitrate, dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), phosphate and total alkalinity (TA). Parameter values for samples taken from the two ponds were averaged for comparison. As in previous years, water samples were taken at fortnightly intervals and in the mornings (around 7 am) during mid-tidal flows while the beds were still fully covered with pond water. Samples were stored on ice and submitted to UnityWater laboratories on the same day.

To evaluate (and possibly validate) the potential to simplify future water sampling programs and integrate the tidal fluctuations that are known to occur for some nutrients flowing from PASF beds (e.g. ammonia: see Palmer et al., 2016), pooled samples of recirculating water were also regularly taken and similarly analysed for comparisons with the PASF bed means. These “integrated pool” samples encompassed a representative subsample of recirculated water returning to the ponds over a full 24-h period. The afternoon before each sampling day, a small and consistent flow of water from the recirculation pump was pooled in a tank to provide a volume of about 3,000 L at the end of the following morning’s tidal cycle. This was homogenously mixed with paddles prior to taking two samples for nutrient analyses.

Drainage rates for normal ($n = 5$) and pond-fill PASF beds ($n = 5$) were assessed by comparing the mean sizes of drainage holes needed to equalise percolation rates during the season. The size of short circuits was also built into this analysis by adding their size to the open-pipe size (i.e. +50 mm).

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[3] Example spreadsheet available on request from David Mann, Bribie Island Research Centre.

Worm populations were also assessed in a replicated fashion with pond-filling method as the treatment. On 15/3/16 and 16/3/16, random samples of worms occurring in 0.5 m^2 rectangular quadrats of each PASF bed were harvested. The day after this experimental harvest, worm samples from each bed were weighed *en masse* and counted.

Statistical analyses

In cases where nutrient results were lower than the laboratory's minimum detection limits, arbitrary levels of half their detection limit were assigned in order to avoid un-natural skewness in the data towards zero. Results were analysed with GenStat (2015) using one- or two-way ANOVA and LSD pairwise comparison of means. The time-series nature of the data was taken into account by an analysis of variance of repeated measures (Rowell and Walters 1976), via the AREPMEASURES procedure of GenStat (2015). This forms an approximate split-plot analysis of variance (split for time). The Greenhouse-Geisser epsilon estimates the degree of temporal autocorrelation, and adjusts the probability levels for this.

Results

Prawn production

Prawn growth in Season 3 fell well short of that predicted (Figure 3). On average, prawns grew slower in pond G2 than in pond G1, recording mean sizes of >20 g on sample days 126 and 147, respectively. Prawns also exhibited slower growth and larger size variations compared with the previous season. For example, when prawns were first recorded to have reached this average 20 g size in the present season (see above), standard errors (*n* = 12) were ±2.01 g for pond G1 and ±2.22 g for pond G2; this shows a greater spread of sizes compared with ±1.92 and ±1.69 g, respectively, on day 98 in the previous Season 2.

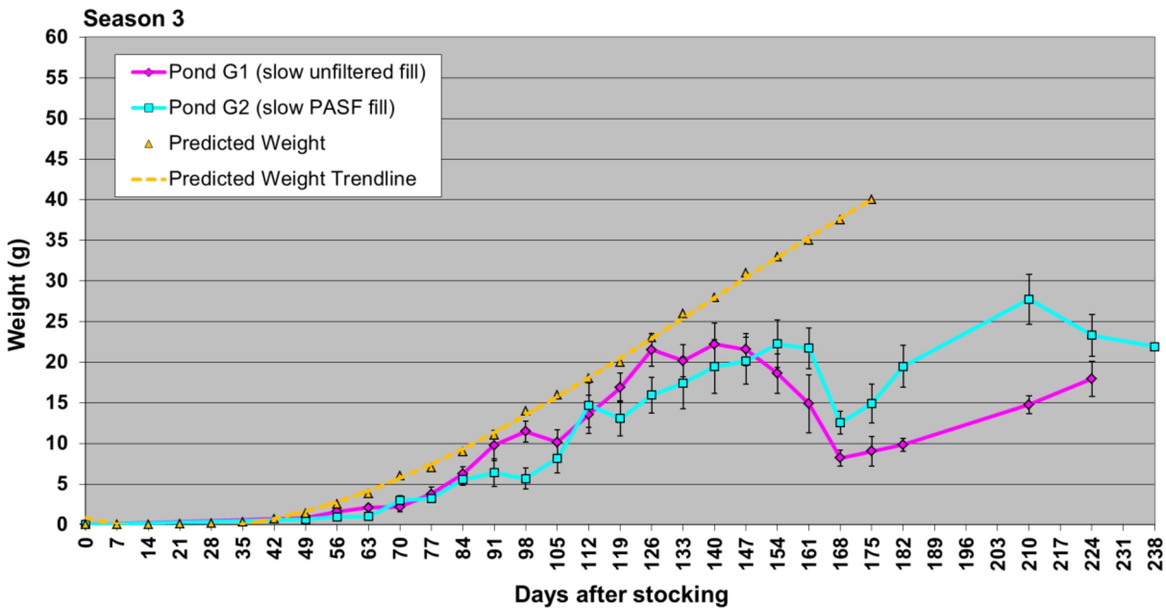


Figure 3 Growth of black tiger prawns in Season 3 in two PASF-recirculated ponds and expected growth according to the Australian Prawn Farming Manual (2006).

Although weekly growth increments based on the data collected with cast net samples were acceptable up until day 140 (where they followed somewhat similar trajectories to that predicted), a significant underclass of small prawns developed in both ponds, and particularly in pond G1. These smaller prawns became increasingly evident as partial harvests proceeded. Partial (selective-trap) harvests, which gradually removed the larger prawns from pond populations, resulted in much lower averages by the end of partial harvests. Despite the longer grow-out period, mean final-harvest prawn sizes were smaller than in previous years, and this was again an artefact of the larger number of stunted animals present. These smaller prawns appeared to have a pronounced impact on mean size data from day 147 in pond G1 and day 161 in pond G2.

Table 1 provides a summary of prawn production statistics for Season 3. Over three tonnes of prawns (averaging 9.4 tonnes ha^{-1}) were collectively harvested from the integrated system. Pond G1 produced the highest amount so far demonstrated in this fully recirculated initiative (i.e. almost 12 tonnes ha^{-1}). Total artificial feed applied was 875 kg higher for pond G1 (2,564 kg) than G2 (1,689 kg). However, production from pond G1 was only about 700 kg (i.e. 4.4 tonne ha^{-1}) higher than G2.

Table 1 Prawn production statistics for Polybridge Season 3.

Parameter	Season 3 2015/16	
	Pond G1	Pond G2
Number of postlarvae (PL13) stocked*	70,000	70,000
Stocking density (PLs m^{-2})	44	44
Number harvested**	78,265	47,877
Calculated** survival (%)	111.8	68.4
Total harvest (kg pond^{-1})	1,855.5	1,158.7
Total harvest (tonnes ha^{-1})	11.6	7.2
Age*** at first trap harvest (d)	139	146
Mean prawn weight in the first trap harvest (g)	23.5	23.4
Age*** at final drain harvest (d)	231	238
Mean prawn weight in drain harvest (g)	18	21.9
Harvested amounts at drain harvest (kg)	174	113.5
Feed conversion ratio	1.38	1.46

*Estimate supplied by commercial hatchery. **Calculated from weights harvested divided by harvest-size estimates. ***Number of days after stocking as PL13.

Assuming that both ponds received 70,000 postlarvae, survival was lower in pond G2 (68%), compared with pond G1 where it was calculated to be higher than 100%. Apparently, more postlarval prawns than expected had been stocked into pond G1, and this can be expected to have created considerable inaccuracies in predicted feed requirements during the season. Despite these difficulties, feed conversion was still reasonable at about 1.4 (Table 1), although this was somewhat lower than in previous seasons.

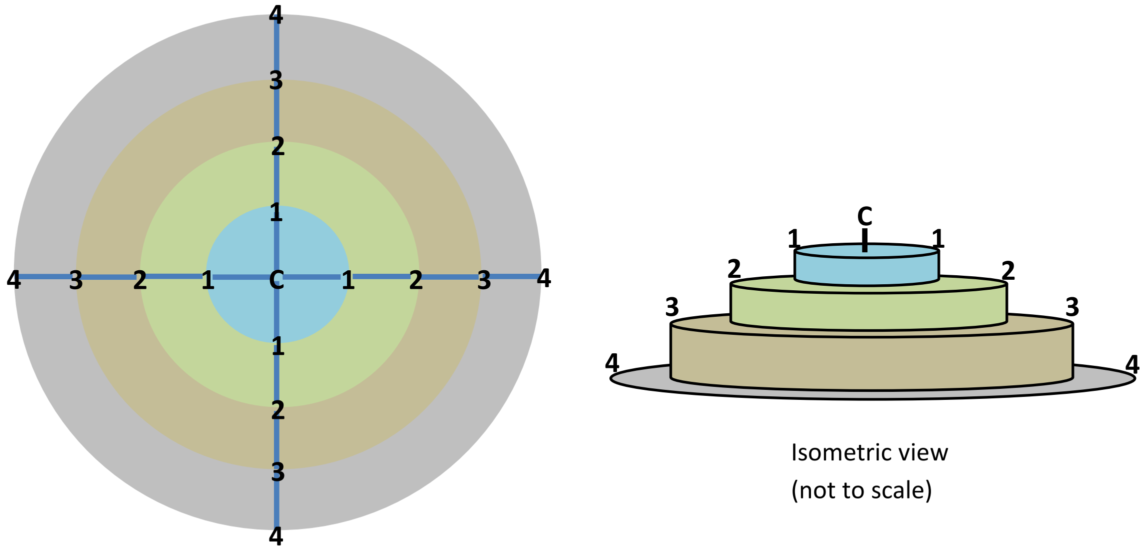
Prawn quality was again rated highly by the commercial receivers, though the prawn’s sizes were generally noted to be marginally smaller than in previous years. There was no evidence of tail bite or shell necrosis, and shell colour and hardness were both considered acceptable and similar to the receiver's own farm product.

Sludge production

The radii lengths and depths (heights) of the sludge mounds at specified points on each radius are provided (directly from the calculator spreadsheet) in Figure 4, along with stylised representations of this approach to volumes estimation.

Pond G1		Sludge height (mm) at point from centre [C]			
radii	**length (m)**	**Centre**	**1**	**2**	**3**
North	7.5	120	85	70	50
East	7.5	120	185	120	115
South	7.5	120	110	95	70
West	7.5	120	85	70	60
Average	**7.5**	**120**	**116.25**	**88.75**	**73.75**

Pond G2		Sludge height (mm) at point from centre [C]			
radii	**length (m)**	**Centre**	**1**	**2**	**3**
North	7	115	110	100	65
East	7.5	115	140	100	75
South	7	115	105	90	30
West	7.5	115	95	80	60
Average	**7.25**	**115**	**112.5**	**92.5**	**57.5**



Plan view

Isometric view
(not to scale)

Figure 4 Sludge radii lengths and heights of sludge mounds at specified points (above) and schematic representations of the stacked cylinder approach to volume estimates (below). Notes: All measurements in mm unless otherwise stated; sludge heights at all 4th points shown in plan view equal zero mm.

Results from the 1-mm height resolution calculation (i.e. 1-mm high stacked flat circular discs) approach to sludge volume estimation yielded accumulated volumes in ponds G1 and G2 of 13.72 m^3 and 9.41 m^3, respectively. By comparison, calculation using 10-mm height resolution provided a slightly lower estimate of 12.91 and 8.71 m^3 for pond G1 and G2 respectively. Similar ($P>0.05$) levels of moisture (from total solids) and volatile solids were measured in sludge samples from the two ponds (Table 2). Marginally higher levels of nitrogen occurred in sludge from pond G1, but this difference was not statistically significant ($P=0.56$). Significantly higher levels of phosphorus occurred in sludge from pond G1 compared with sludge from pond G2 ($P=0.04$).

Table 2 Contents (mean ± se, *n* = 3) of accumulated sludge in ponds after the drain harvest in Season 3. Within rows, means with similar letters are not significantly different ($P > 0.05$).

Parameter	Pond G1	Pond G2
Moisture (%)*	89.8 ± 0.26^a	90.2 ± 0.81^a
Total volatile solids (% total solids)**	32.8 ± 0.31^a	32 ± 0.74^a
Total nitrogen as N in dry matter (g kg^{-1})***	193.4 ± 13.81^a	179.5 ± 17.63^a
Total phosphorus as P in dry matter (g kg^{-1})***	168 ± 1.48^a	124.8 ± 14.21^b

*By calculation from total solids; **Tested by UnityWater Laboratories; ***Tested by ALS Laboratories

Mass balance calculations for the estimated volumes of sludge in each pond, using the 1-mm increment sludge volume estimates are provided in Table 3. These data show how pond G1, which produced the highest quantity of prawns (and which also received the most feed), had the highest amount of accumulated nutrients as sludge. Using the (above) estimated volumes of sludge left in each pond, and the nutrient calculations shown in Table 3 (below), for every respective kilogram of prawns produced in ponds G1 and G2, 154 g and 151 g of nitrogen, and 134 g and 105 g of phosphorus accumulated in sludge.

Table 3 Mass balance calculations using mean values provided in Table 2 for sludge left in the middle of each pond after the drain harvest in Season 3.

Parameter	Pond G1	Pond G2
Average density of wet sludge (kg L^{-1})*	1.057	1.057
Wet weight of sludge (tonnes pond^{-1}) (= volume x average density)	14.5	9.9
Wet weight of sludge (tonnes ha^{-1})**	90.6	62.2
Dry weight of sludge (kg pond^{-1}) (minus moisture)	1,479.2	974.7
Dry weight of sludge (tonnes ha^{-1})**	9.2	6.1
Total nitrogen in sludge (kg pond^{-1})	286.1	175.0
Total nitrogen in sludge (tonnes ha^{-1})**	1.8	1.1
Total phosphorus in sludge (kg pond^{-1})	248.5	121.6
Total phosphorus in sludge (tonnes ha^{-1})**	1.6	0.8

*A conversion factor of 1.057 was used from mean weight-to-volume estimates made for sludge in Season 2.
**Using multiplier of 6.25 for per hectare extrapolations.

Worm production

A total of 132 kg of worm biomass was harvested in Season 3 (details provided in Table 4). This was considerably less than was harvested from the PASF complex in each of the first two seasons (i.e. 570 kg and 353 kg respectively, in Seasons 1 and 2). On average, only 0.24 kg m^{-2} was harvested, and this included those worms removed with sample harvests.

Operational harvests for prawn hatchery supplies began with bed 5 on 27/4/16, 177 days after stocking (Table 4). The last worm harvest occurred on 29/9/16, 332 days after stocking, when bed 1 was completely harvested on that day. Trends for daily production were compromised by the need to progressively harvest some beds much earlier than others, as well as the non-random approach taken to harvest apparently-lower-density beds first. As a result, worm biomass production data was not significantly affected by age at harvest (Table 4) where the R^2 value for production rate (g m^{-2} d^{-1}) was 0.0007.

Table 4 Worm harvest weights for polychaete-assisted sand filters in Season 3.

PASF bed	Date of final harvest	Harvest total* (kg)	Harvest total (kg m^{-2})	Age after stocking** (d)	Polychaete production (g m^{-2} d^{-1})
5	28/04/2016	5	0.093	178	0.522
2	5/05/2016	12.4	0.23	185	1.243
7	12/05/2016	11.6	0.214	192	1.115
10	19/05/2016	11.6	0.215	199	1.080
8	1/06/2016	14.8	0.274	212	1.292
4	8/06/2016	11	0.204	219	0.932
9	11/08/2016	9.1	0.169	283	0.597
3	31/08/2016	19	0.351	303	1.158
6	14/09/2016	21.3	0.394	317	1.243
1	29/09/2016	16.4	0.303	332	0.913

*Includes biomass harvested in controlled samples. **Time from first stocking to date of last harvest.

Sample harvest estimates on 15/3/16 and 16/3/16 (Table 5) provided more valid assessments of the potential effect of using PASF beds to fill ponds. These assessments were undertaken 133-135 days after stocking most beds (note this was 110-111 days after stocking beds 1 and 6, which were stocked about 1 month later than other beds - see methods above). No significant differences ($P>0.05$) were found between normal and pond-fill beds in terms of worm densities, survival, individual worm weights or total worm biomass. Worm densities measured in this third season were about half that measured in the same way (0.5 m^2 quadrates) and at about the same age (126-128 d) as in Season 2. Survival was less than one third of that found in Season 2, and both worm size and overall worm biomass were also comparatively lower in Season 3.

Table 5 Production variables from controlled samples on 15/3/16 and 16/3/16 for normal and pond-fill PASF beds in Season 3. Within rows, means (n = 5) with similar letters are not significantly different ($P > 0.05$).

Production variables	Normal PASF beds		Pond-fill PASF beds	
	mean	±se	mean	±se
Worm density (number m^{-2})	243.6^a	39.725	228^a	48.707
Worm survival (%)	7.1^a	1.04	7.27^a	1.576
Individual worm weight (g)	0.63^a	0.039	0.61^a	0.039
Worm biomass (g m^{-2})	151.44^a	24.622	145.6^a	35.512

Wastewater treatment

Volumes and drainage rates

The daily wastewater treatment rates of 4-5% of pond volume (see Figure 2 above) yielded the cumulative volumes of treated water shown in Figure 5. Beds 1 and 6 showed a separated pattern of accumulating water volumes due to their later start in the season. A total of 43.37 megalitres of pond water was collectively treated by the 10 PASF beds during Season 3 up to 31/5/16. A pump timer malfunction on 12/11/15 (where the timer failed to turn the pump off overnight) provided an abnormally high average PASF treatment rate of 778 L m^{-2} d^{-1} for that day, however during normal operations this was in the order of 400-500 L m^{-2} d^{-1} for the first half of Season 3, and 300-400 L m^{-2} d^{-1} for the second half of Season 3.

Bed clogging became pronounced in mid-to-late December and early January for most PASF beds (Figure 6). The first bed discharges needing short circuiting (Beds 1, 4, 5 and 9) occurred on 21/12/17, which was 81 days after stocking prawn postlarvae into the ponds. Interestingly, Bed 10 continued its unhindered function for much longer than the other beds and without significant clogging or need for a short circuit (until 2/2/16: 43 days later); notably, on several occasions, discharge pH and dissolved oxygen readings that are routinely used to check for water tracking past the sand matrix[4] confirmed that it (Bed 10) was, in fact, functioning correctly.

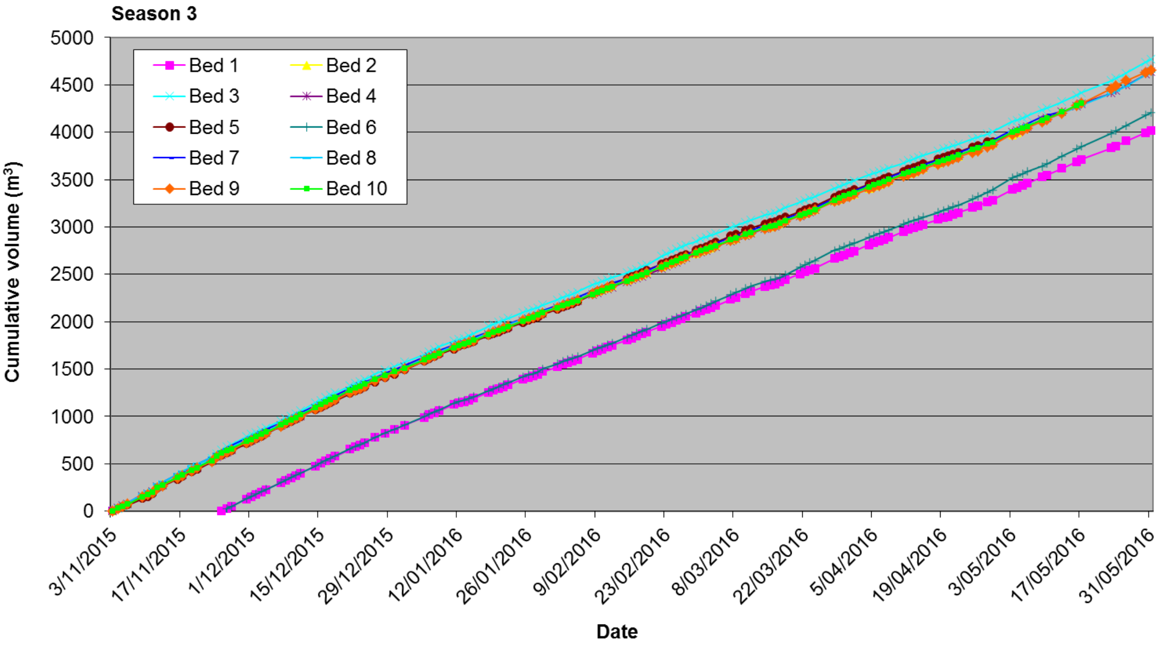


Figure 5 Cumulative water volumes treated by different PASF beds in Season 3.

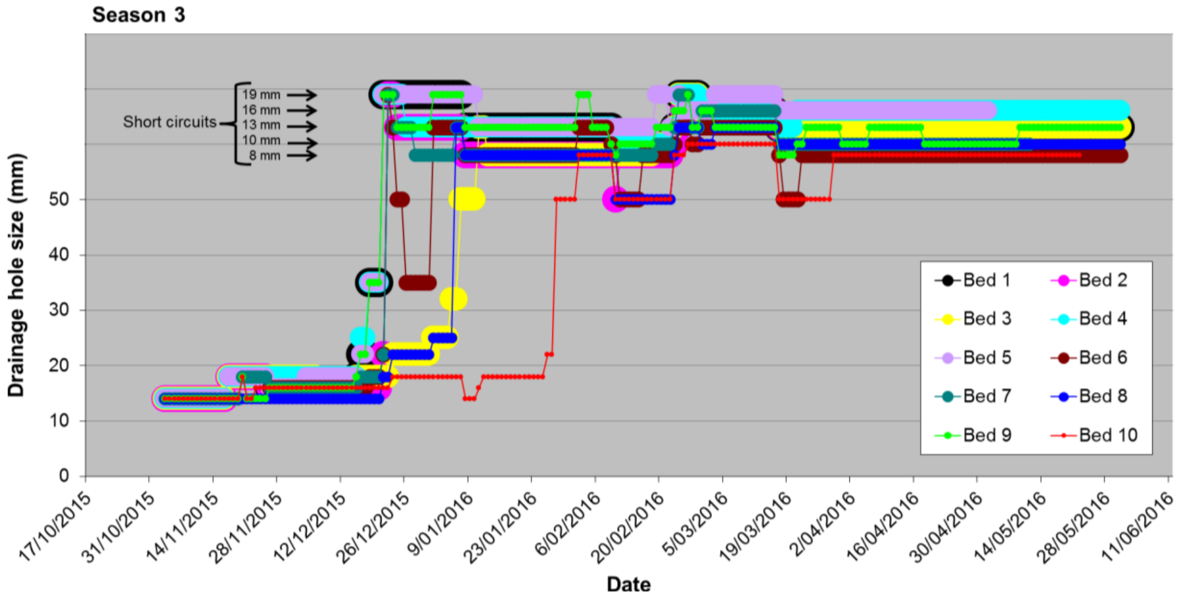


Figure 6 Drainage-hole sizes used to equalise PASF bed discharge rates in Season 3.

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[4] Treated water discharging from PASF beds normally has depressed levels of pH and dissolved oxygen.

Figure 7 provides the averaged discharge-hole sizes used for the five beds that were used to fill pond G2, and for the other five beds that were not. This data suggests that the beds used to fill the pond were marginally less predisposed to clogging, and hence on average required marginally smaller drainage-hole sizes to equalise flows.

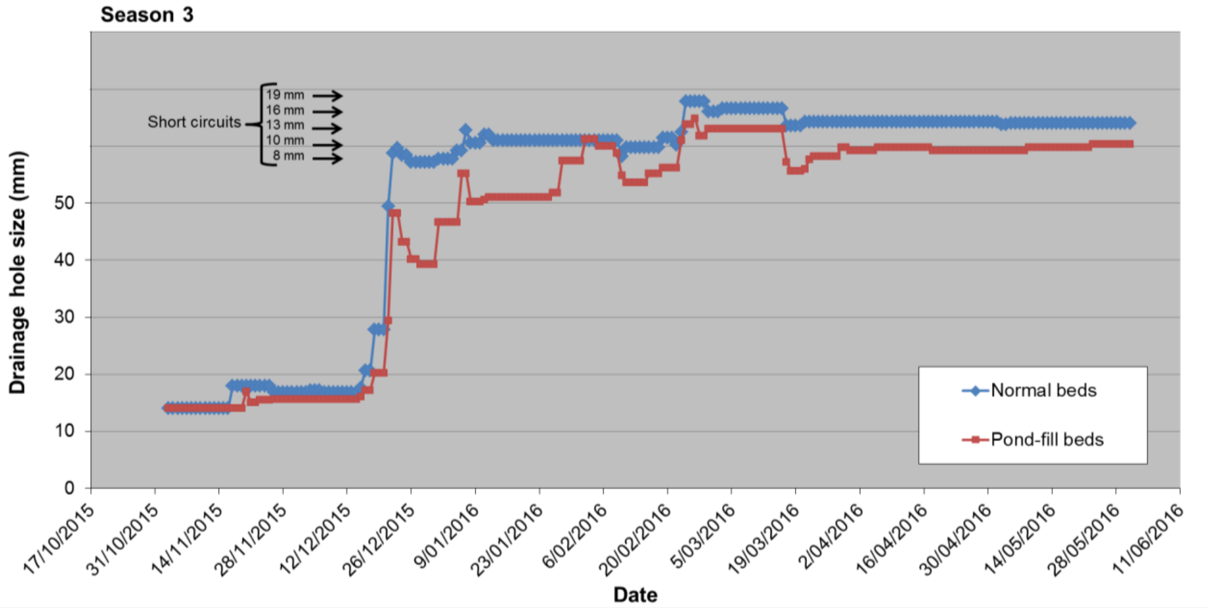

Figure 7 Mean drainage-hole sizes used to equalise drainage rates for normal PASF beds (n = 5) and those beds used to fill Pond G2 (n = 5).

Statistical analyses revealed no significant difference (P=0.068) between the normal and pond-fill treatments in this regard, but the consistently smaller discharge–hole sizes for pond-fill beds evident in Figure 7 does indicate a possible trend. Time had a highly significant overall effect (P<0.001) on drainage-hole sizes for both treatments, but the interaction of time and treatment was not significant (P=0.417) suggesting that both bed treatments were similarly affected during the season.

Water qualities

Prevailing levels of pH, dissolved oxygen, temperature, salinity and Secchi depths for waters in the two recirculated prawn ponds in Season 3 are presented in Figures 8, 9, 10 and 11, respectively.

Water pH (Figure 8) followed a typical pattern during the season with a gradual decline associated with the increasing accumulation of organic matter. Compared with the first two seasons, pH values through the summer months generally fell below the minima of Season 1, but remained slightly higher than those experienced in Season 2.

Patterns for dissolved oxygen (Figure 9) were similar to the previous two seasons of pond data, which is not surprising since this is closely managed. Levels typically ranged from 4 mg L^{-1} in the early mornings to 8-9 mg L^{-1} in the afternoon.

Water temperatures also followed typical daily and seasonal patterns and were very similar between ponds (Figure 10). Compared with the average temperatures of previous growing seasons (i.e. between mid-November and the end of March), Season 3 had similar morning (am) and afternoon (pm) averages (26.8 and 28.5 °C, respectively) to that of Season 1, which on average was slightly cooler than Season 2.

Low rainfall during Season 3 caused a slow increase in pond salinities, where by the end of March it had climbed to 44.3 ppt. (Figure 11).

Secchi readings (Figure 11) were deeper on average in pond G2 (34 cm) compared with pond G1 (28 cm) between mid-November and the end of March, suggesting that the algal blooms were stronger in pond G1 during this high-prawn-growth period.

Total suspended solids (Figure 12) climbed to the highest levels in ponds so far seen in this series of recirculation experiments with PASF (see also Palmer et al., 2016 for Season 1 and 2 results). On 12/1/16, pond G1 provided the maximum reading of 111 mg L^{-1} during a strong green algal bloom. By comparison, PASF system outflow remained well below the BIRC discharge licence maximum for TSS (50 mg L^{-1}) throughout Season 3, as it did in the previous two seasons. Statistical analyses revealed no differences ($P>0.05$) in TSS between normal and pond-fill beds, where both treatments provided highly significant reductions[5] in TSS compared with the levels in the ponds.

The overall average TSS reduction rate (only using mid-tidal samples taken directly from individual bed discharges) was 64.9%, with a maximum of 83.7% late in the season on 3/5/16 and minimum of 40.5% on 8/3/16. The integrated pools closely reflected the mean TSS levels provided by the PASF beds ($P>0.05$), and the last pooled samples taken suggests that TSS levels from the PASF system continued to fall during the last fortnight of the experiment.

Turbidity levels in the ponds increased and remained high (though not as high as in Season 2) until partial prawn harvests began in late February (Figure 13). Both bed treatments provided significant reductions in turbidity though pond-fill beds provided marginally higher ($P<0.05$) mean turbidity levels compared with normal beds (Figure 13).

The overall average turbidity reduction rate (only using samples taken directly from individual bed discharges) was 34%, with a maximum of 69.1% early in the experiment on 17/11/15 and minimum of 4.1% late in the experiment on 3/5/16. The integrated pools' turbidity results were similar ($P>0.05$) to those mid-tide results taken directly from individual bed outlets.

Total nitrogen also climbed to the highest levels so far seen in a recirculated prawn culture system using PASF (Figure 14). On 23/2/16 Pond G1 returned a TN level of 15.8 mg L^{-1}, and this approximately coincided with (five days after) the highest levels of feeding just before partial harvests began. No differences ($P>0.05$) were found between mean TN levels from pond-fill and normal beds. The PASF beds consistently provided significant ($P<0.05$) TN reductions throughout the season.

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[5] To clarify, reduction means the drop in level after a single pass through the bed.

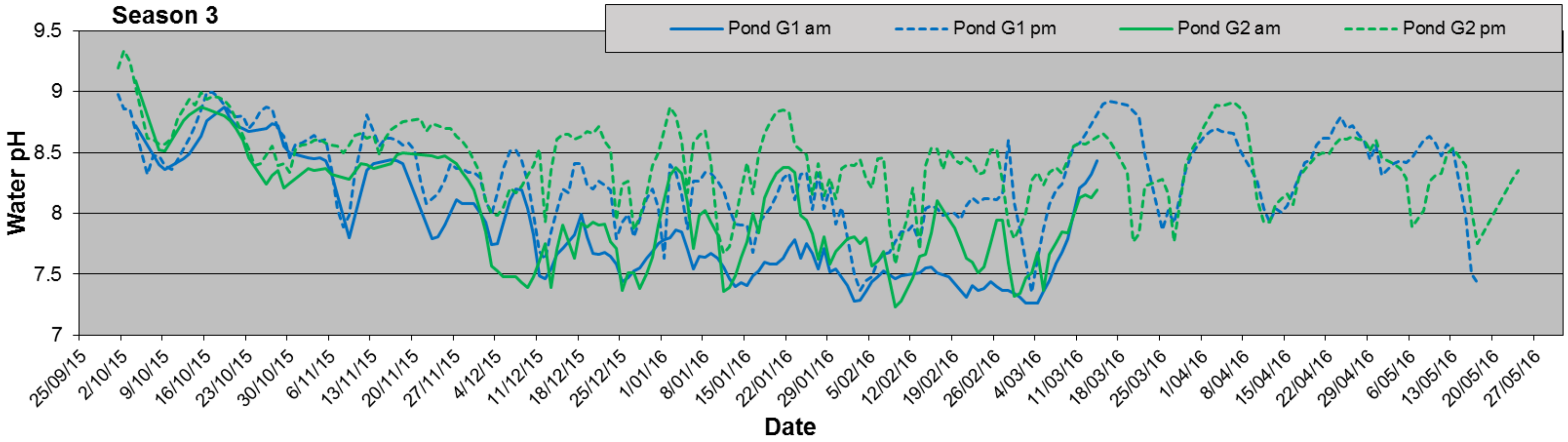

Figure 8 Water pH levels in two recirculated prawn ponds (G1 and G2) in Season 3.

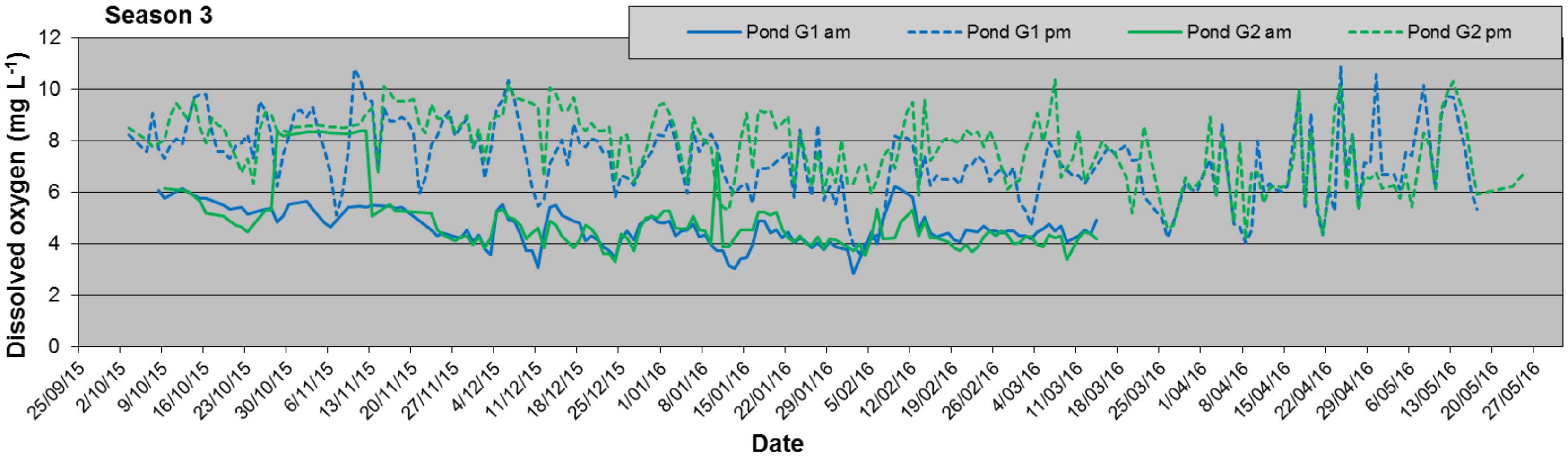

Figure 9 Dissolved oxygen levels in two recirculated prawn ponds (G1 and G2) in Season 3.

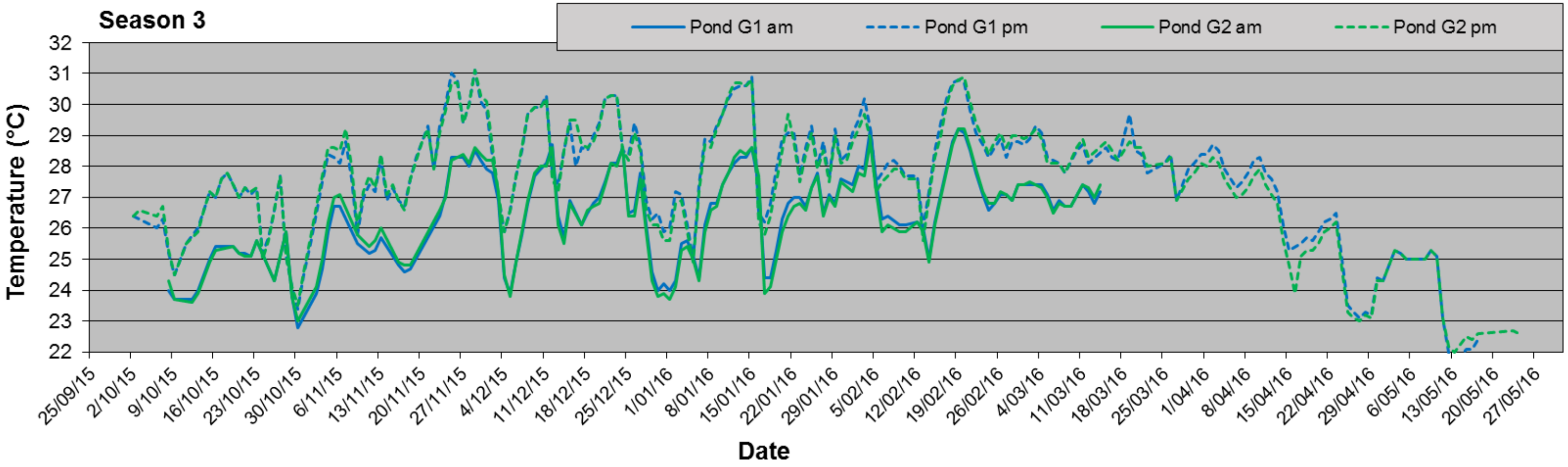

Figure 10 Water temperatures in two recirculated prawn ponds (G1 and G2) in Season 3.

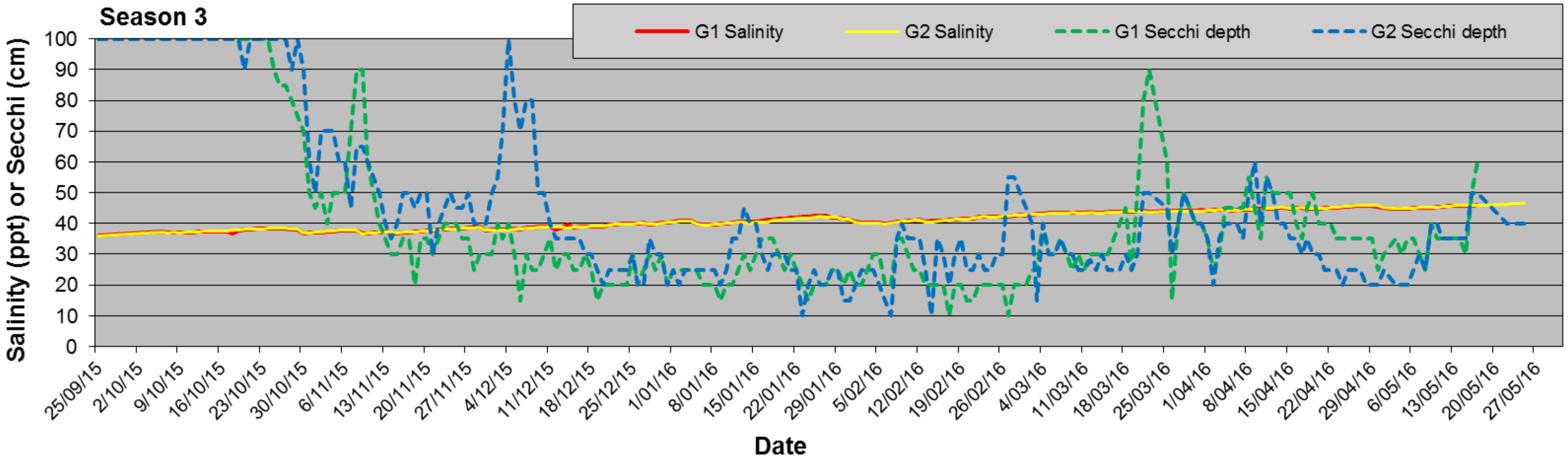

Figure 11 Salinities and Secchi depths in two recirculated prawn ponds (G1 and G2) in Season 3.

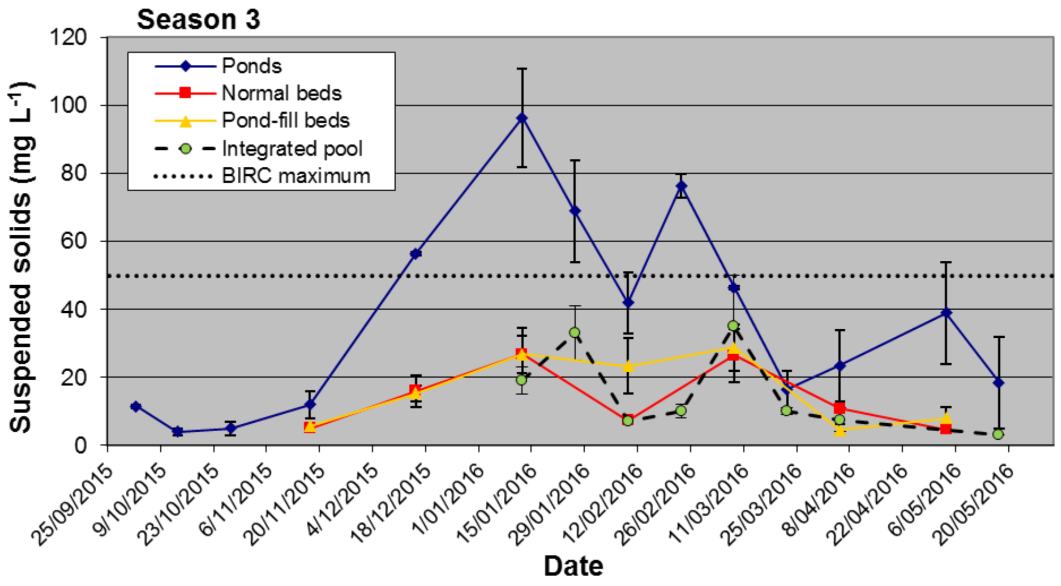

Figure 12 Mean (± se) total suspended solids levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3. The licensed discharge maximum for BIRC is also provided.

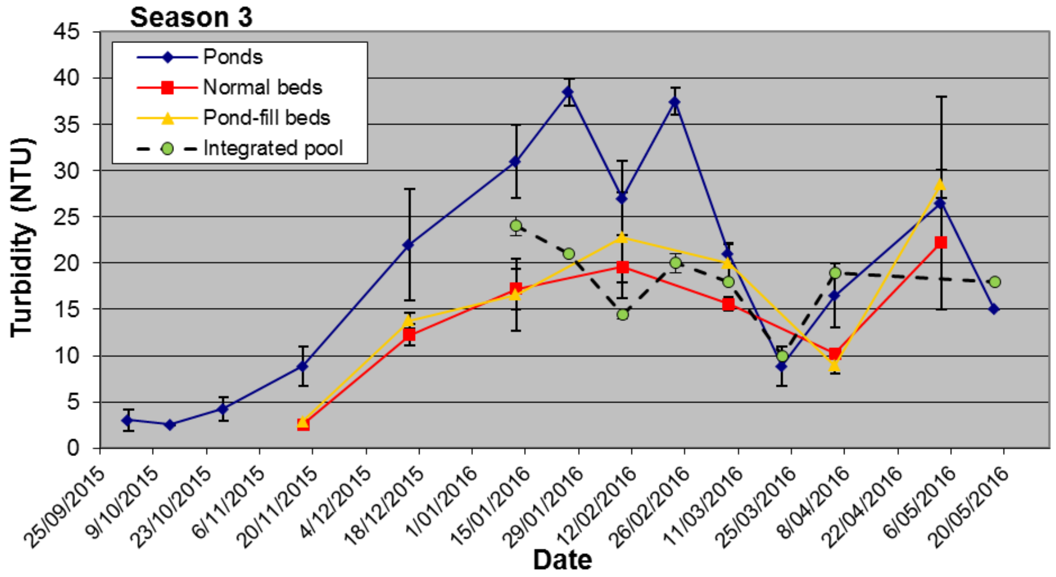

Figure 13 Mean (± se) turbidity levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

The overall average TN reduction rate (only using samples taken directly from individual bed discharges) was 26%, with a maximum of 46.1% on 15/12/15 and minimum of 12.7% late in the experiment on 3/5/16. The integrated pools' TN results were similar ($P>0.05$) to those from mid-tide samples taken directly from PASF bed outlets. Importantly, at no time during the experiment did TN levels in PASF discharge fall below the required BIRC discharge water maximum of 1 mg L^{-1}, though this did not affect BIRC discharge at the time because all of this was recirculated back to the ponds.

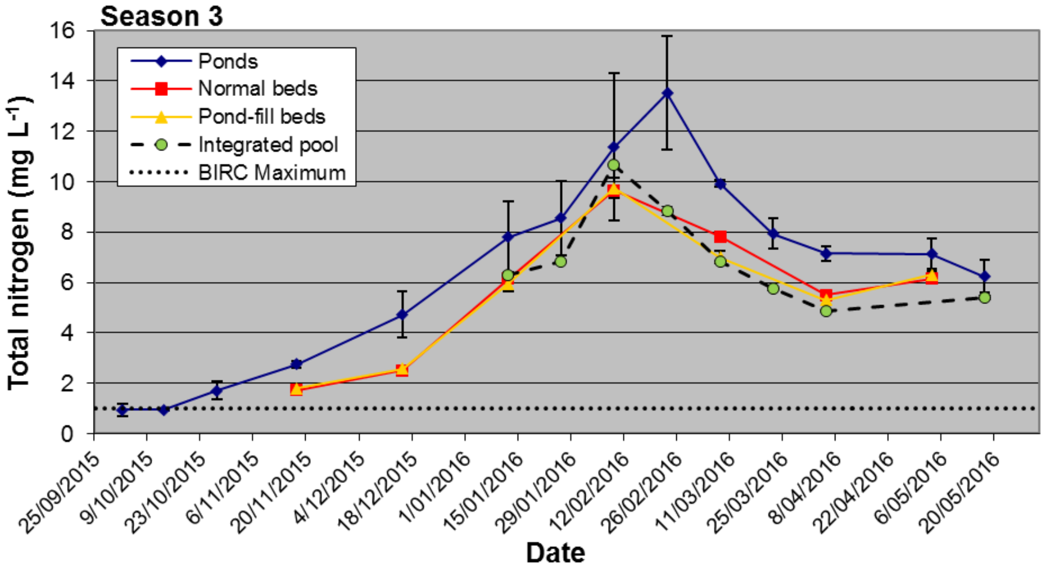

Figure 14 Mean (± se) total nitrogen levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3. The licensed discharge maximum for BIRC is also provided.

Total phosphorus levels in ponds also showed a steady increase up to the time when partial harvests began (Figure 15), and it also reached the highest levels so far seen using PASF recirculation (up to 1.34 mg L^{-1} in Pond G1 on 26/1/16). No differences ($P>0.05$) were found between TP levels from normal and pond-fill beds, and both bed treatments provided significant ($P<0.05$) reductions compared with mean pond levels.

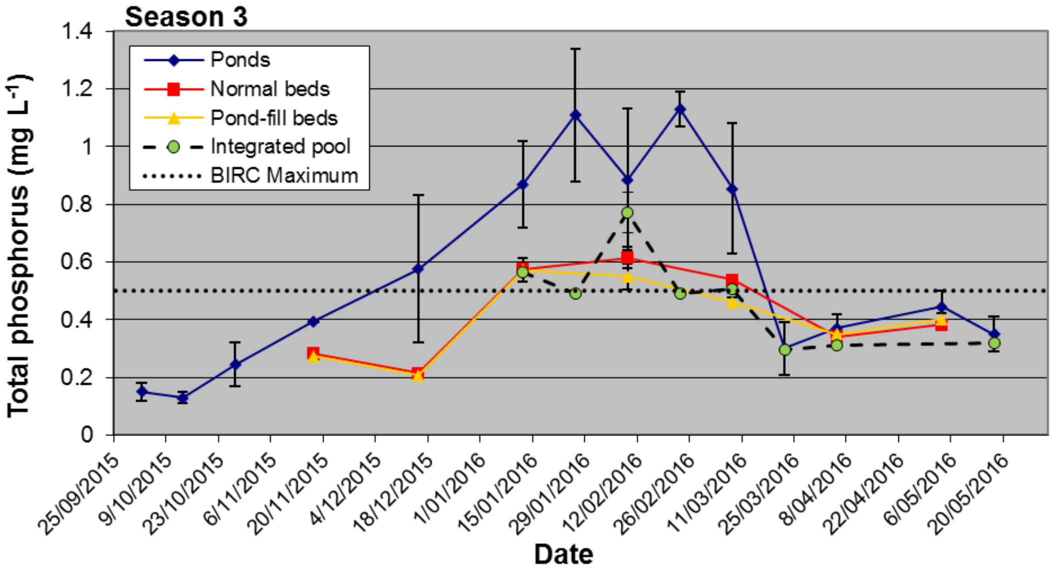

Figure 15 Mean (± se) total phosphorus levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3. The licensed discharge maximum for BIRC is also provided.

The overall average TP reduction was 31.6%, where the maximum was 63.3% on 15/12/15 and minimum was 6.5% on 5/4/16. Mean TP levels in integrated pool samples were not significantly different (P>0.05) to results provided by individual beds. Through the mid-crop period (January–March), outflow from the PASF beds exceeded the TP discharge maximum for BIRC (0.5 mg L^{-1}); this was the first time in three sequential seasons that this had occurred for TP, though again, full recirculation meant that during these times the actual discharge from the prawn/ worm culture system was zero.

Chlorophyll *a* levels in the ponds typically showed considerable fluctuations during the season and large differences between the two indirectly-connected ponds (Figure 16). Differences in Chl*a* between ponds did not follow consistent trends where lesser levels were found in pond G1 on six sample days and in pond G2 on eight sample days. In general, Chl*a* levels in (this) Season 3 had similar magnitude to levels found in previous seasons, except for an exceptional sample from pond G2 which recorded a high of 930 μg L^{-1} on 23/2/16. No differences (P>0.05) were found between Chl*a* levels from normal and pond-fill bed outflows.

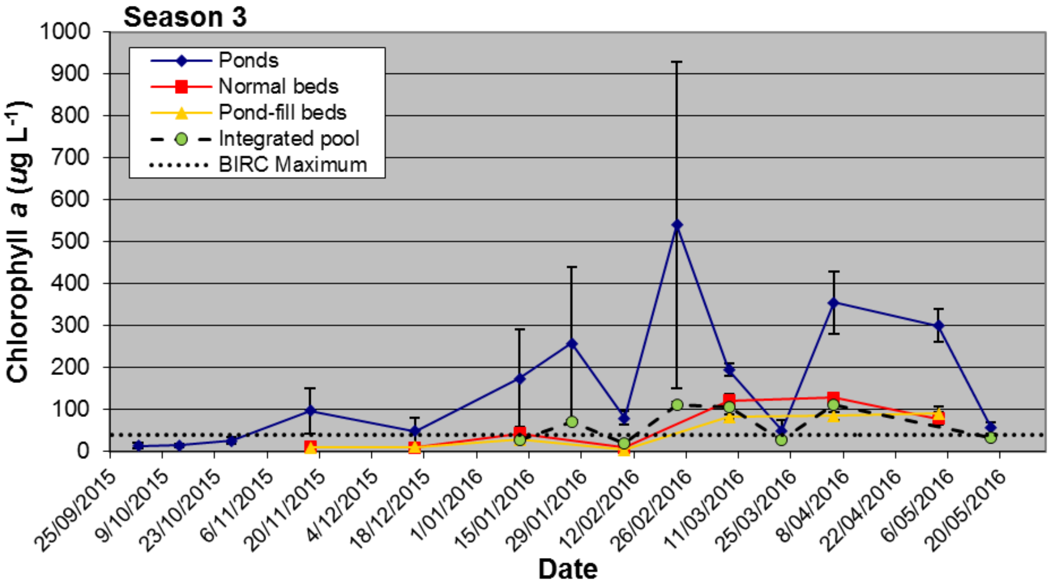

Figure 16 Mean (± se) chlorophyll *a* levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3. The licensed discharge maximum for BIRC is also provided.

Both bed treatments provided significant (P<0.05) reductions compared with mean levels in ponds, although on several occasions these still exceeded BIRC's Chl*a* discharge levels (40 mg L^{-1}). The overall average Chl*a* reduction was 75.8%, where the maximum was 91.9% on 9/2/16 and minimum was 47.8% on 8/3/16. Mean Chl*a* levels in the integrated pool samples were not significantly different (P>0.05) to results provided by individual beds.

Total ammonia levels showed a very strong peak in February 2016 (Figure 17). Pond G1 was the main cause of this averaged pond peak, where it returned a NATA-approved result of 9.88 mg L^{-1} on 9/2/16. It should be noted here that in-house-testing using Palintest reagents was stepped up during this time, and that these results were added to the NATA-approved laboratory results shown in Figure 17. Follow-up molasses additions to both ponds (as documented in Materials and Methods) during this peak quickly arrested this potentially damaging situation. No differences (P>0.05) were found between TAN levels from normal and pond-fill beds.

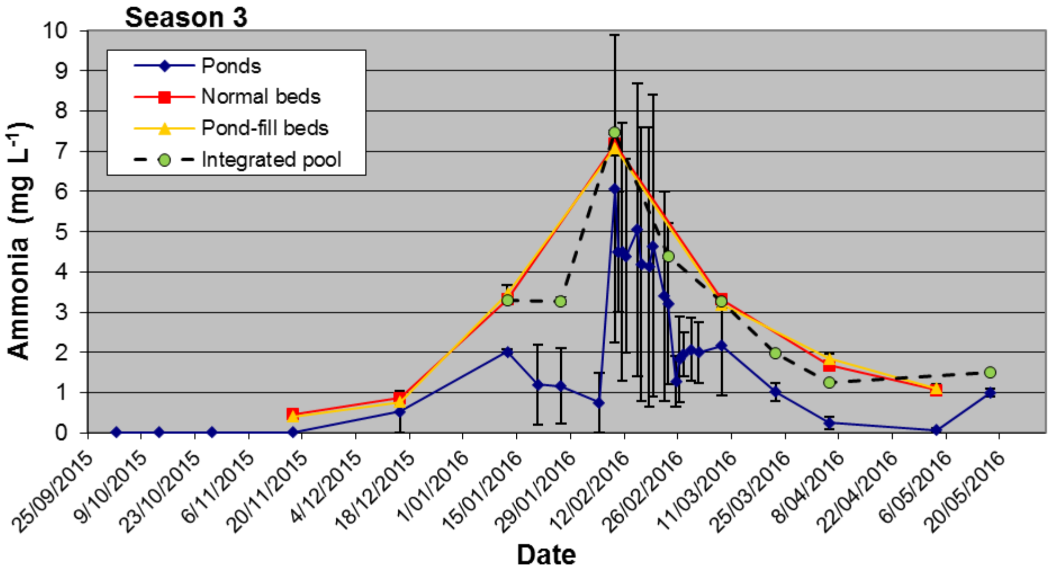

Figure 17 Mean (± se) total ammonia levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

Both bed treatments provided significant (P<0.05) TAN increases compared with mean levels in ponds. The overall average TAN increase was 1,064%; this average was greatly affected by a maximum of 4,974% on 17/11/15 when pond levels were very low. A minimum increase of 18% occurred on 9/2/16. Mean TAN levels in the integrated pool samples were not significantly different (P>0.05) to results provided by individual beds.

Nitrite levels in ponds peaked between late-February and mid-March (Figure 18), as it did in Season 2 and to a lesser extent in Season 1 (see also Palmer et al., 2016). This peak in Season 3 provided the highest nitrite levels so far seen in PASF-recirculated ponds, where the sample taken on 8/3/16 from Pond G1 contained a cross-seasonal maximum of 4.34 mg L^{-1}. Mid-tide discharge from normal and pond-fill beds provided similar (P>0.05) levels of nitrite, and both bed treatments provided significant (P<0.05) reductions in nitrite compared with levels in the ponds.

The average nitrite reduction for the experiment was 51.9%, which included a minimum of zero reduction when pond levels were very low (e.g. 0.005 mg L^{-1}), and up to 86.7% reduction when pond levels were higher (on 3/5/16). In general, results from the integrated pool samples closely reflected nitrite trends from individual beds; however, where these results were similar (P>0.05) to those from pond-fill beds, they were found to be statistically different (P<0.05) to those from normal beds.

Nitrate levels in ponds followed similar trends to those of nitrite, with very low levels early in the season and a peak in early March 2016 (Figure 19). A cross-seasonal maximum for nitrate levels in ponds occurred in Season 3 in pond G1 on 8/3/16 with 1.59 mg L^{-1}. Normal and pond-fill beds provided similar (P>0.05) levels of nitrate, and significant (P<0.05) reductions compared with pond levels.

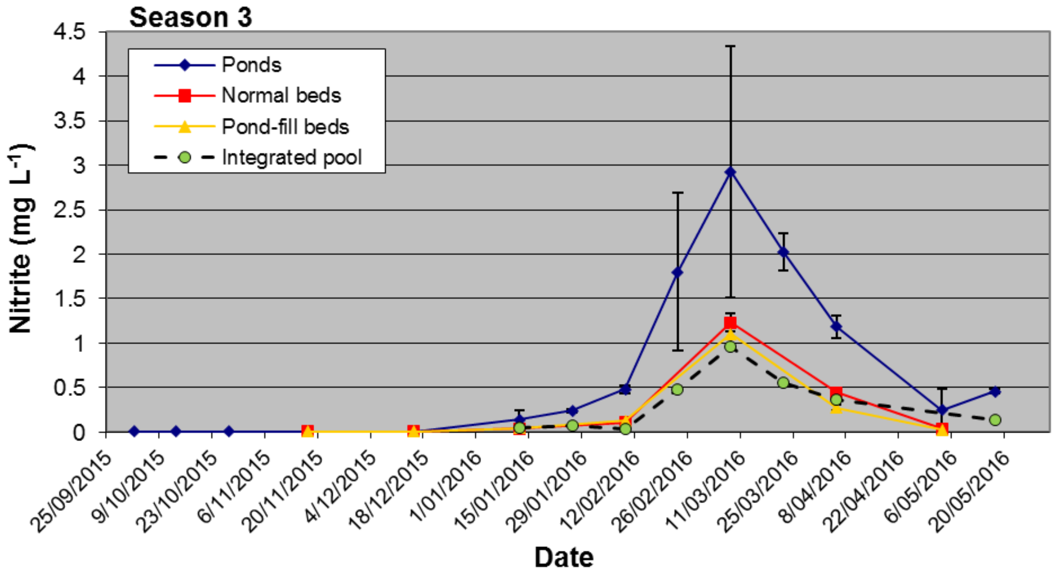

Figure 18 Mean (± se) nitrite levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

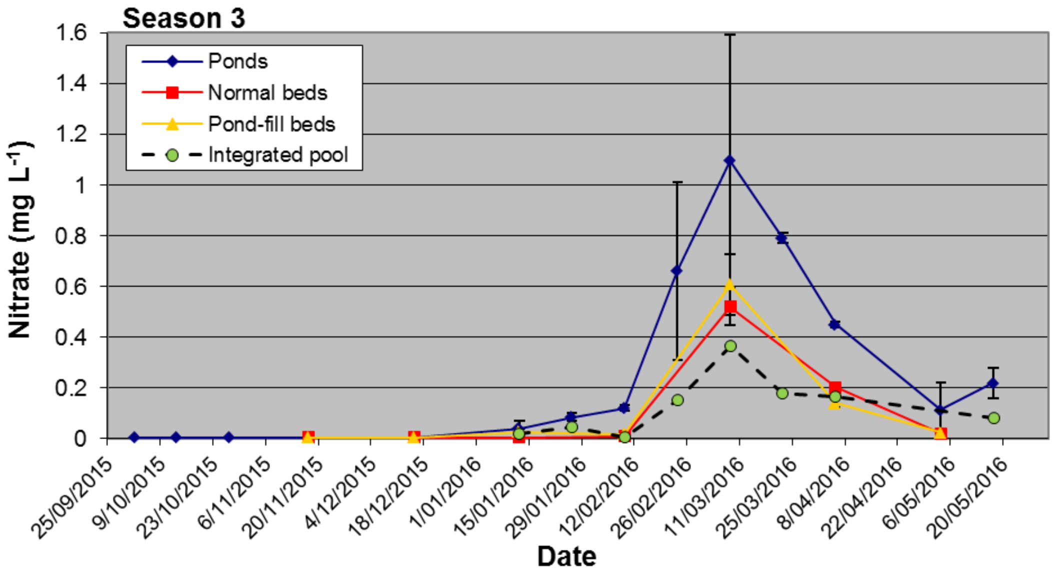

Figure 19 Mean (± se) nitrate levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

The average nitrate reduction provided by PASF beds was 48.6%, and again this was influenced by zero reduction early in the season when nitrate levels in the ponds were low. PASF beds provided the maximum reduction of 88.3% on 9/2/16. Whilst nitrate trends in the integrated pools followed the mid-tidal results of samples taken from individual beds reasonably well, on average they were found to be significantly lower ($P<0.05$), meaning that the pooled approach to nitrate assessments may underestimate nitrate levels in PASF discharge.

Dissolved organic nitrogen levels generally increased gradually in the prawn ponds during the season (Figure 20), but did not show the decline seen in other nutrient categories when prawn feeding was reduced or ceased late in the season. In late-February and early-March levels in pond G1 appeared to become temporarily reduced, and on the last sample day (17/5/16) pond G2 returned the highest cross-seasonal DON level so far seen for PASF-recirculated prawn ponds of 3.14 mg L^{-1}. No differences (P>0.05) were found between DON levels from normal and pond-fill beds.

Whilst DON levels in PASF discharge were less than levels in ponds on most sampling occasions, these differences were not found to be significant (P>0.05). The wide variability between ponds on 23/2/16 and 8/3/16 are likely to have greatly influenced this result. On average PASF reduced DON levels by 20.2%, with a maximum reduction of 35.8% on 9/2/16 and minimum reduction (-2.6%) or increase of 2.6% on 8/3/16. The integrated pools were on average found to provide similar (P>0.05) DON results to samples taken at mid-tide from individual beds.

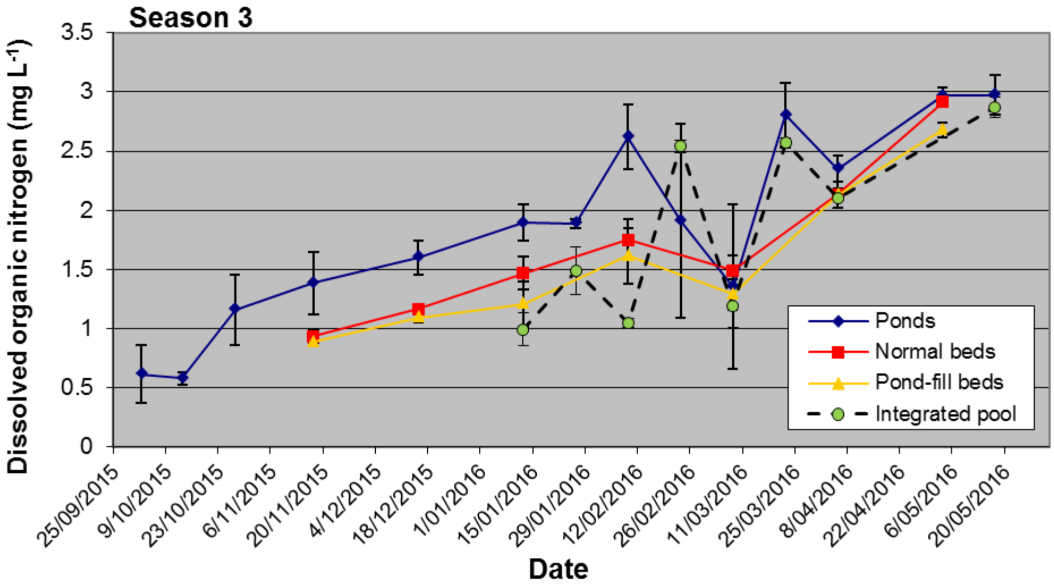

Figure 20 Mean (± se) dissolved organic nitrogen levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

Dissolved organic phosphorus levels in ponds increased over the first two months, but then varied without clear trends for the remainder of the experiment (Figure 21). The overall levels of DOP in ponds in (this) Season 3 were generally not very different from those seen in previous seasons. Levels in discharge from normal PASF beds were similar (P>0.05) to those from pond-fill beds, and both of these bed treatments provided significant (P<0.05) reductions of DOP compared with mean pond levels.

PASF beds provided an overall average DOP reduction of 53.7% during the experiment, with a maximum reduction of 75% on 17/11/15 and minimum reduction of 14.2% on 3/5/16. No differences (P>0.05) were found between DOP levels in the integrated pool samples and the mid-tidal discharge samples routinely taken from individual beds.

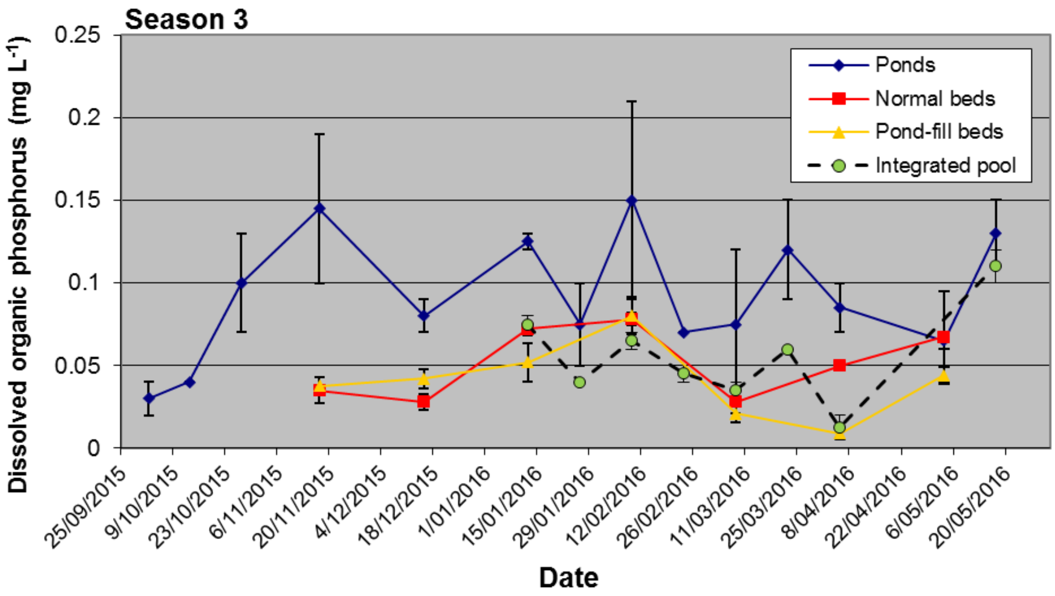

Figure 21 Mean (± se) dissolved organic phosphorus levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

Unlike other nutrient categories, phosphate levels in ponds did not show increasing trends during the latter part of the season (Figure 22), and levels seen in Season 3 were also quite similar to those seen in previous seasons. Discharge from normal and pond-fill beds provided similar ($P>0.05$) levels of phosphate, and both of these bed treatments provided significantly higher ($P<0.05$) levels compared with mean pond levels.

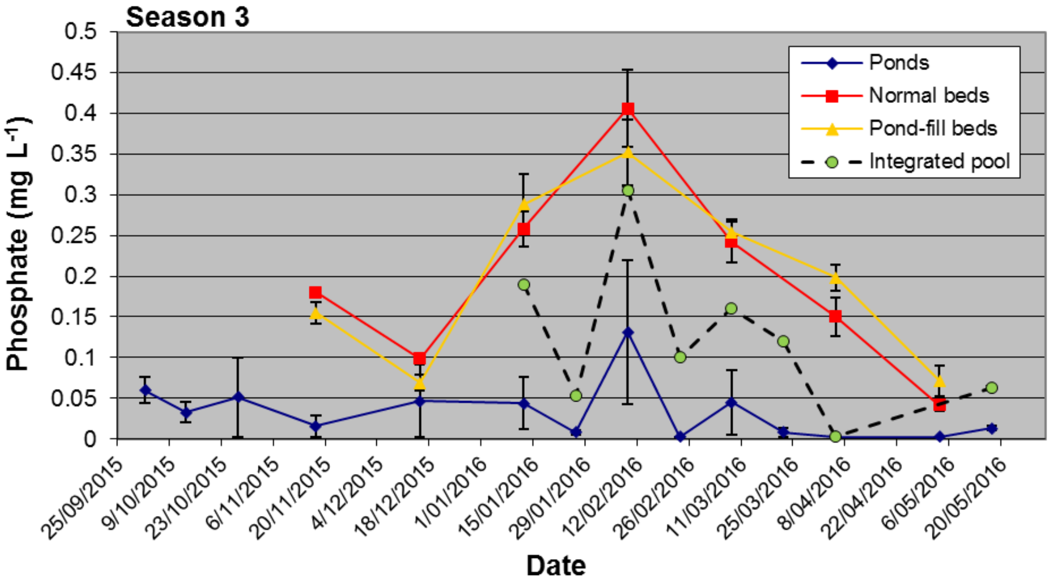

Figure 22 Mean (± se) phosphate levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

PASF beds provided an overall average increase in phosphate of 1,604%, and this was greatly influenced by the maximum increase of 6,680% on 5/4/16 when pond phosphate levels were very low; the minimum increase of 79.5% occurred on 15/12/15. Phosphate levels in the integrated pool samples were found to be significantly lower ($P<0.05$) than in mid-tidal discharge samples from individual beds, meaning that this pooled approach could significantly underestimate levels in PASF discharge (particularly during mid-tide periods).

Total alkalinity levels in ponds remained relatively stable for the first 2 months of the experiment (Figure 23). During this time no lime or dolomite (other than that applied when filling ponds) was added to either pond. After this initial period, strong downward pressure on TA developed. In mid-December, pond G2 particularly needed regular hydrated lime additions to keep TA levels above preferable levels (e.g. >80 mg L^{-1}); an unexpected low measurement of 56 mg L^{-1} in pond G2 on 15/12/15 prompted daily additions of hydrated lime (3 kg d^{-1}) until 23/12/15, with further similar applications on 28/12/15 and 1/1/16. (Note that intermediate Palintest alkalinity measurements used for pond management during this period are not provided in Figure 23). Thereafter, despite the regular additions of dolomite (21 kg per week or fortnight pond^{-1}), TA levels generally fell in both ponds from 23/3/16.

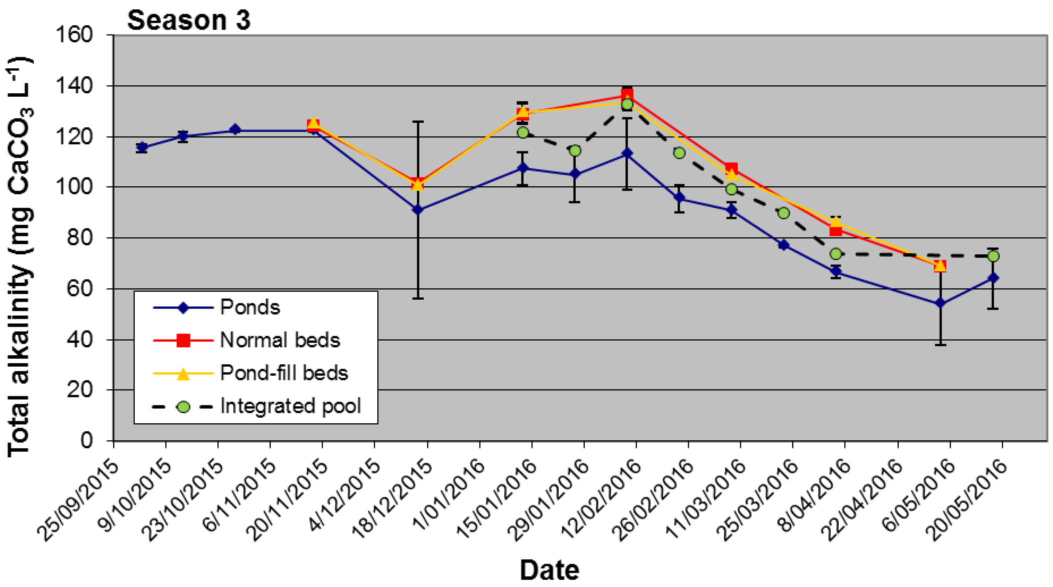

Figure 23 Mean (± se) total alkalinity levels in two recirculated prawn ponds, discharge from five normal and five pond-fill PASF beds, and the integrated pool (*n* = 2) of discharge from all PASF beds in Season 3.

Total alkalinity levels in discharge from normal and pond-fill beds were not significantly different ($P>0.05$). Discharge from both bed treatments provided significantly higher ($P<0.05$) TA levels compared with pond levels. PASF beds provided an overall average increase of 17.8%, with a maximum increase of 27.8% late in the season on 3/5/16, and minimum increase of 1.7% early in the season on 17/11/15. No differences ($P>0.05$) were found between TA levels in the integrated pool samples and mid-tidal samples from individual beds.

Plankton blooms

Qualitative plankton assessments revealed distinct divergence of algal and zooplankton bloom types in the two ponds soon after fill. Six days after both ponds began filling (30/9/15) pond G1 (filled with unfiltered seawater) developed a light brown bloom consisting mainly of chain-forming diatoms such as *Melosira* sp. This initial bloom persisted until after prawn postlarvae were stocked. By contrast, very little phytoplankton developed during this first week in pond G2 (filled through the PASF sand bed).

Plankton net samples from each pond the day after stocking postlarvae (3/10/15) revealed the presence of populations of rotifers, copepods and *Melosira* sp. (90% of diatom cells in surveyed samples) in pond G1, but only rotifers, protozoans, and the pennate diatom *Nitzschia* sp. (90% of diatom cells in surveyed samples) in pond G2. Ten days later (13/10/15), plankton net samples from pond G1 had good quantities of copepods, rotifers and large quantities of chain-forming diatoms represented in the concentrate, whereas that from pond G2 had similar entities but comparatively much less of each than in pond G1. By this stage pond G1 was showing a distinctly brown colour and pond G2 had a light green tinge *en masse*.

In the third week after pond filling began (16/10/15) pond G1 also developed a strong bloom of the large, ribbon-forming diatom, *Helicotheca* sp. This bloom was very short-lived (2 days), so it did not feature highly in data collected from preserved plankton samples that were taken for specific enumeration. Interestingly, *Helicotheca* sp. did not proliferate in pond G2. Alternatively, at the same time in pond G2, a small green Euglenoid flagellate became evident, in conjunction with comparatively low densities of rotifers, copepods and nematodes. Background populations of Chlorophytes also developed in both ponds but these were not studied in detail.

Figure 24 provides the measured densities of diatoms in ponds in Season 3. The chain-forming *Melosira* sp. was often the dominant diatom in samples taken from both ponds during the first month. This species of centric diatom was also well represented in many samples surveyed between January and March from both ponds.

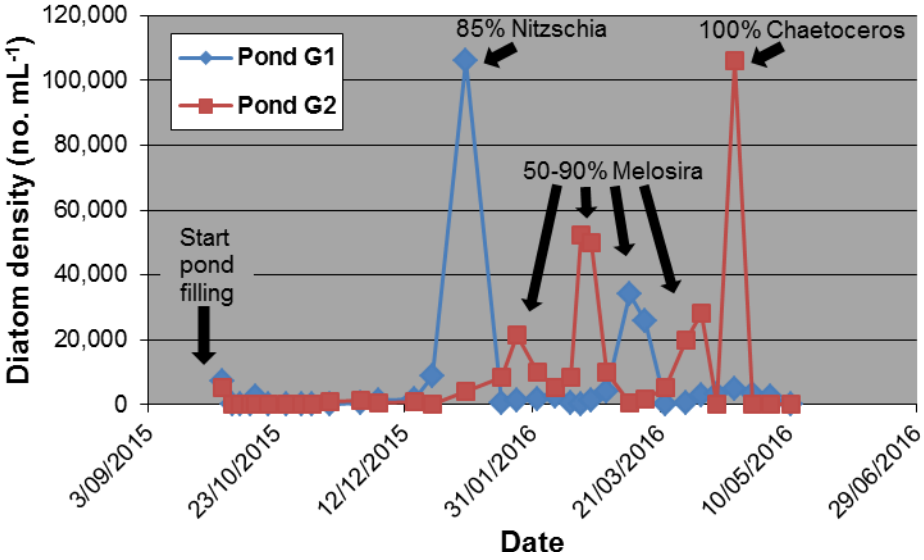

Figure 24 Diatom densities in two fully recirculated prawn ponds using PASF as water source.

Two other diatom species notably dominated when very strong blooms prevailed. These were *Nitzschia* sp. in pond G1 on 5/1/16 (85% of diatom cells present), and *Chaetoceros* sp. in pond G2 on 19/4/16 (100% of diatom cells in the sample). Overall there were few other consistent differences between ponds in terms of the prevalence of diatom species during the season. Table 6 provides an overall summary of the occurrence of all diatom species in the two ponds during the experiment.

Table 6 Diatom occurrence in the two fully recirculated prawn ponds using PASF as water source.

Diatom Genus	**Pond G1 (normal fill)**		**Pond G2 (PASF fill)**	
	Prevalence in 33 samples	Levels of occurrence*	Prevalence in 33 samples	Levels of occurrence*
Melosira	26	10-100%	26	50-100%
Chaetoceros	9	5-100%	9	5-100%
Bellerochea	7	5-20%	13	1-100%
Nitzschia	3	5-85%	2	90%
Leptocylindricus	3	1-10%	5	1-30%
Thalassiosira	6	1-10%	4	1-20%
Coscinodiscus	9	1-10%	3	1%
Skeletonema	1	70%	0	-
Hemiaulus	3	5-20%	2	10-30%
Cyclotella	3	1-5%	4	1-5%
Lauderia	1	5%	0	-
Climacodium	0	-	1	5%
Helicotheca	1	high**	0	-
Cocconeis	1	2%	0	-
Dactyliosolen	1	1%	0	-
Navicula	1	1%	0	-

*Range of occurrence levels amongst diatoms cells when species were present in sample. **Not measured

Melosira sp. was by far the most common diatom species noted in both ponds, occurring in 79% of the 33 samples taken, and often dominating the diatom communities throughout the culture cycle. It made up 50% or more of the diatom cells identified in 70% and 79% of samples from ponds G1 and G2, respectively. *Chaetoceros* sp. and *Bellerochea* sp. were the next most prevalent diatoms, noted in 27% and 30% of samples from both ponds, respectively. All three of these chain-forming species demonstrated capacity to almost completely dominate the diatom communities at different times in the ponds. There were also six low-prevalence diatom species that occurred in pond G1 but not in pond G2, suggesting that there was somewhat lower species diversity in the pond (G2) filled through the PASF beds.

Dinoflagellates were evident in the first preserved samples taken from both ponds on 2/10/15 (8 days after start of pond fill). At this early stage densities were relatively low, where pond G1 had 2,400 dinoflagellate cells mL^{-1} made up of three species, namely *Protoperidinium* sp., *Scrippsiella* sp. and *Gymnodinium* sp., and pond G2 had 1,300 mL^{-1} which was mainly *Gymnodinium* sp. Figure 25 summarises the proliferation of dinoflagellates (other than *Noctiluca* sp.[6]) in both ponds in Season 3. *Gymnodinium* sp. was the most prevalent dinoflagellate, occurring in 79% of samples from both ponds, and often making up a high percentage (80-100%) of the dinoflagellate communities when strong blooms prevailed (see Figure 25).

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[6] *Noctiluca* sp. was treated separately from other dinoflagellates in all summaries and analyses.

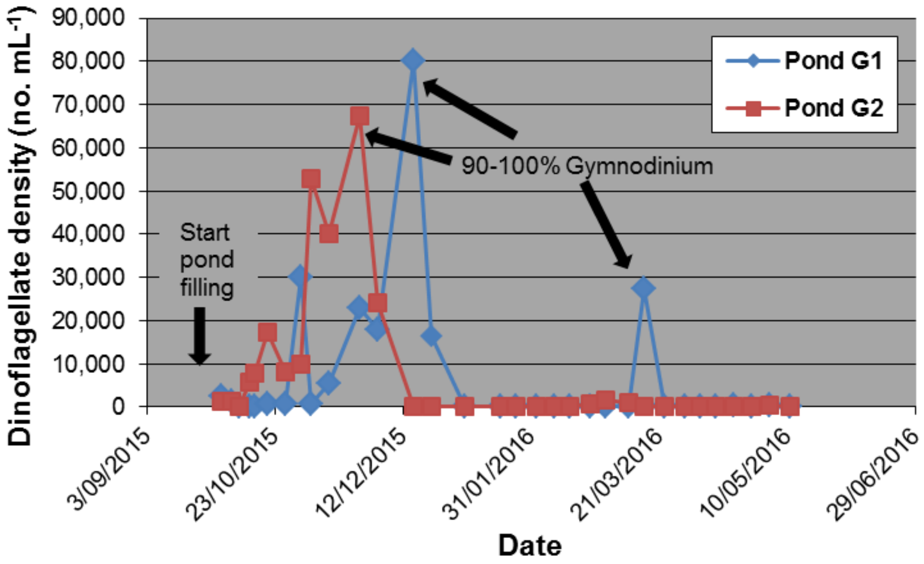

Figure 25 Dinoflagellate densities in two fully recirculated prawn ponds using PASF as water source.

The next most prevalent dinoflagellate was *Gyrodinium* sp. which occurred in 21-24% of samples at 10-80% occurrence, followed by *Scrippsiella* sp. which occurred in 6-12% of samples at 2-30% occurrence levels, and *Protoperidinium* sp. which occurred in 9% of the 33 samples taken from both ponds at 2-40% levels of occurrence. *Prorocentrum* sp. was the dominant species of dinoflagellate (50-100% occurrence) for a short period in mid-February when it occurred in one sample (3% of samples) from pond G1 (i.e. on 15/2/16) and three (9% of) samples from pond G2 (between 2/2/16 and 19/2/16). Other dinoflagellate species that occurred at low levels were *Diplopsalis* sp. and *Polykrikos* sp. which each occurred in one sample from pond G2 at about the 5% occurrence level. *Noctiluca* sp. also proliferated in pond G2 in late February, and in mid-March in pond G1 (Figure 26); in both ponds it exhibited strong bioluminescence when disturbed (at night) in the water column.

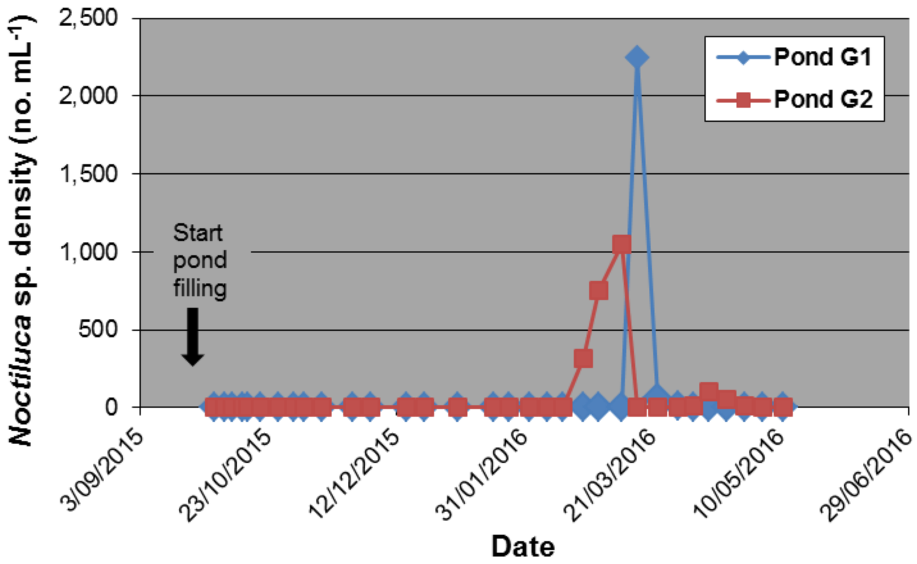

Figure 26 *Noctiluca* sp. densities in two fully recirculated prawn ponds using PASF as water source.

Bluegreen algae typically became more dominant in the plankton as the pond cultures matured (Figure 27). *Gloeocapsa* sp. and *Aphanothece* sp. were the most prevalent blue-green species in Season 3. Each demonstrated capacity to dominate the blue-green community at different times. Other blue-green species identified in preserved samples at very low rates of occurrence (<1% of blue-green cells present) included *Oscillatoria* sp., which occurred in 7 (21%) of the 33 samples from pond G1 and 5 (15% of the) samples from pond G2, and a few other species (e.g. *Synechocystis* sp., *Spirulina* sp., *Pseudanabaena* sp.) which only occurred in one or two samples from either pond.

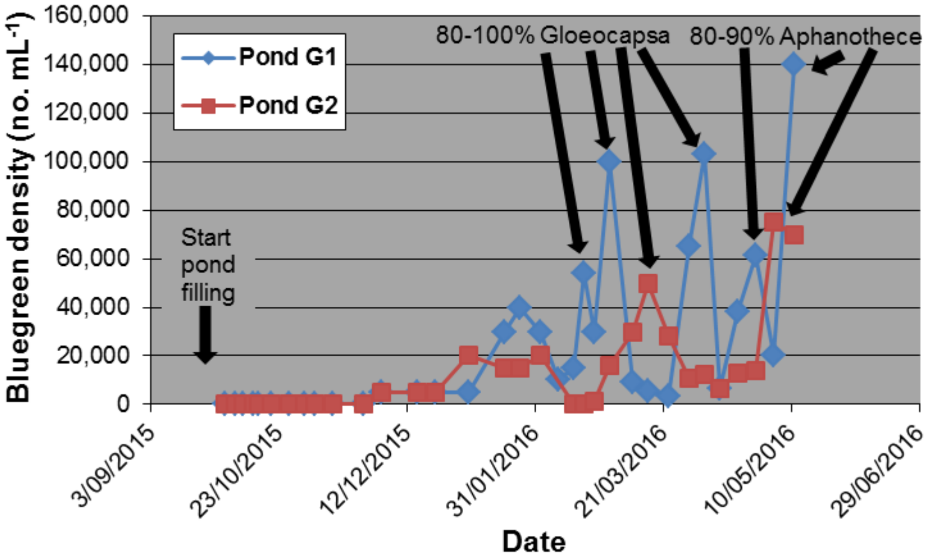

Figure 27 Blue-green algae densities in two fully recirculated prawn ponds using PASF as water source.

Apart from the qualitative population differences noted above during the first week after fill, copepod densities from preserved samples were similar between ponds up to mid-December (Figure 28). However, samples taken on 23/12/15 displayed several-fold higher copepod densities in pond G2 compared with pond G1, and this population difference persisted until the beginning of February.

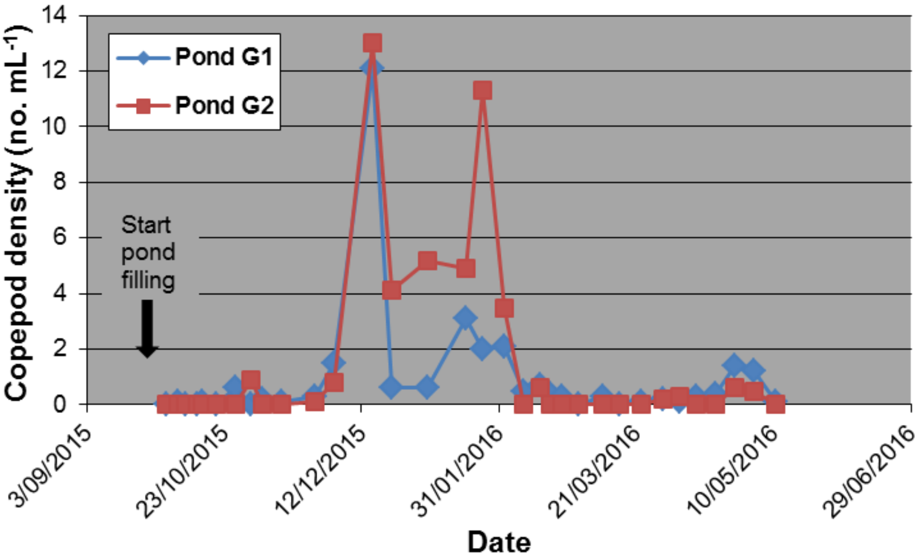

Figure 28 Copepod densities in two fully recirculated prawn ponds using PASF as water source.

Rotifer densities were also quite similar between ponds (Figure 29), except for a few sample days when relatively strong blooms had apparently prevailed (2/12/15 in pond G1; 23/12/15 in pond G2; 2/2/16 in pond G1). The main species of rotifer that occurred in both ponds was from the Genus *Brachionus*.

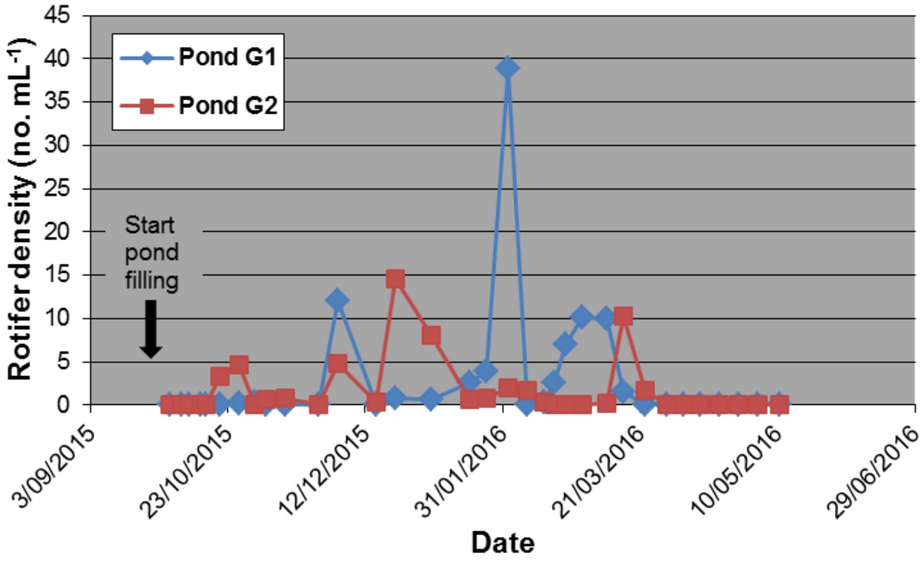

Figure 29 Rotifer densities in two fully recirculated prawn ponds using PASF as water source.

Surface-fouling organisms

There were marked differences between the two ponds in terms of the numbers of barnacles on the side walls in experimental quadrates after the drain harvests. Mean (± se) barnacle densities were 5,635 ± 1,070 m^{-2} (*n* = 12) in the pond filled with unfiltered seawater (pond G1) and 110 ± 31 m^{-2} (*n* = 12) in the pond filled through the PASF sand beds (pond G2). The smaller acorn barnacle *Amphibalanus variegatus* was predominant in both ponds. Whilst there was a very low prevalence (approximately 0.5%) of the larger royal barnacle *Austromegabalanus nigrescens* amongst this census of the unfiltered-seawater pond (G1), none of these were detected in quadrates in the pond filled through PASF (G2).

Tube worms (*Hydroides sp*.) started to become evident on the sides of both ponds in mid-December (3 months after filling). This was initially most prevalent on the airlifts being used to help distribute feed from auto feeders (Figure 30). At this stage there were no barnacles at all on the sides of pond G2 (PASF fill) and only very limited coverage (e.g. a dozen-or-so barnacles m^{-2}) on the sides of the normal-fill pond (G1). Whilst no density estimates were made for encrusting tube worms in the study, it was apparent by the end of December that higher levels of tube worms were present in the pond filled through the PASF beds (pond G2) (Figure 30).

Figure 30 Lower sections of air lifts used to help distribute feed from automatic belt feeders showing higher levels of encrusting tube worms in pond G2 on left compared with pond G1 on right.

Discussion

Prawn and worm production

Slower prawn growth was expected in this third season given the higher stocking density that was applied, so it was not surprising that at no time did the growth rates exceed those expected from the standardised growth curves provided in the Australian Prawn Farming Manual (2006), as they did in previous seasons with lower stocking densities. No mortalities were noticed during the season, either on feed trays or on the pond bottom during routine monthly inspections, so the lower survival noted for the pond filled through the PASF beds was probably due to lower early survival, and perhaps mediated by the slower development of its plankton community.

The selective nature of sampling prawn populations with a cast net, which allows some of the smallest prawns to escape through the mesh, may have obscured the true scale of stunting that developed in Season 3. The higher stocking density used in this third season, and a degree of imprecision associated with postlarval counts, coupled with some inconsistencies noted in the pellet sizes of the feeds, could well have contributed to these results. The unusually high prawn size variation that prevailed made it particularly difficult to efficiently feed the ponds. Unlike in the previous season when feed rates were predictable and consistent, on many occasions during Season 3 the prawns' appetites (apparent from the feed trays) appeared to vary on a daily basis without any logical dependence on environmental factors that normally affect appetites (e.g. weather, temperature). The size-selective nature of the prawn traps used for partial harvests further demonstrated the large number of stunted prawns that were present; as the larger prawns were progressively removed with partial harvests, the average sizes of prawns left in the ponds were considerably reduced.

The excessive sand bed clogging that occurred in this third Polybridge season of study is thought to be the main reason for the lower worm production compared with previous seasons. Considerable anaerobic sediments were created by the increased organic loading, and whilst this is tolerated to a certain degree by *P. helleri*, there seems to be a limit to its ability to thrive under such conditions. This excessive organic clogging of beds was also exemplified by the higher amounts of sludge (compared with season 1 and 2) that accumulated elsewhere in the system (on pond bottoms), which seems to have been driven by the higher prawn stocking densities and associated higher levels of artificial feeds applied.

Despite the use of deeper sand beds which had shown promise to reduce clogging rates in the previous two seasons, all PASF beds inevitably became clogged with organic matter. Unfortunately, less surface slope was also applied during bed construction in this third season, and despite the remedial use of short-circuits which otherwise helped previously to clear water from surface depressions (when clogging of the sand matrix became pronounced), the beds' surfaces could not completely dry in the sun. Accumulated organic matter on the beds' surfaces could therefore not dry, crack, and lift to facilitate oxygen transfer. As a consequence, large parts of the beds became increasingly anoxic leading to lower worm survival.

Nutrient mitigation and water treatment

Compared with previous seasons, in the present study the PASF system performed with reasonably similar levels of efficiency in terms of nutrient removal and associated water treatment rates. This was surprising given that greater sand clogging reduced percolation of water through the sand matrix, which was previously thought to offer the main water treatment action. Wastewater supply rates to the PASF system in this third season (4-5% of pond volume replacement per day – see methods) were lower than applied in previous seasons (3-10% in Season 1; 5-7% in Season 2), because it was anticipated that despite the use of deeper beds, the higher organic loading could well still cause sand bed clogging. And, as expected, percolation rates were generally affected to the point of needing short circuits by mid-December, approximately 81 days after stocking prawn postlarvae into ponds.

By comparison, this significant clogging did not occur until mid-February in Seasons 1 and 2 (138 and 116 days after stocking prawn postlarvae into ponds, respectively). But interestingly, the more extended use of short circuits in Season 3 did not seem to diminish the efficacy of water treatment as much as would be expected, suggesting that settlement or other processes above the sand matrix still affords a significant degree of nutrient removal. Furthermore, the PASF beds used to screen and fill the pond appeared to be marginally less-predisposed to clogging, and on average required slightly smaller drainage-hole sizes to equalise flows (although these differences were found to be non-significant).

Nutrient clearance rates achieved with a single pass of pond water through PASF were up to 84% for TSS, up to 46% for TN, up to 63% for TP, and up to 92% for Chl*a*. Table 7 provides a more standardised comparison of the average removal efficiencies for these water parameters across the three successive seasons. Removal rates for TSS and Chl*a* appeared to increase with each season, whilst TP and TN demonstrated lesser removal efficiencies in this third season compared with Season 1. Again, the higher organic clogging of beds in this third season was the likely cause of this, and particularly for TN whereby this reduced the percolation rates that aid in denitrification processes.

Table 7 Comparisons of average removal efficiencies provided by polychaete-assisted sand filters for water quality parameters across three successive experimental seasons given average prawn production statistics.

Prawn production statistics and water quality parameters	**Season 1***	**Season 2***	**Season 3****
Prawn stocking density (postlarvae m^{-2})***	31.3	37.5	> 44
Prawn harvest rate (tonnes ha^{-1})***	7.1	9.9	9.4
Prawn feed conversion	1.3	1.1	1.4 - 1.5
Total suspended solids (% reduction)	40.1	62.8	64.9
Total nitrogen (% reduction)	30.6	35.1	26
Total phosphorus (% reduction)	39.8	30.9	31.6
Chlorophyll *a* (% reduction)	68.7	71.9	75.8

*Data presented in Palmer et al. 2016. **Present study. ***Average for the 2 recirculated ponds.

These water quality results (across three successive seasons), were all using 100% recirculation of PASF-treated wastewater, and were generated under increasing prawn stocking densities where the most recent third season demonstrated the highest prawn production thus far (up to 11.6 tonnes ha^{-1}). Accordingly, nutrient levels in the ponds in this third season reached the highest levels so far seen with this initiative, and were up to 111 mg L^{-1} for TSS, up to 15.8 mg L^{-1} for TN, up to 1.34 mg L^{-1} for TP, and up to 930 μg L^{-1} for Chl*a*. Whilst these higher levels of nutrients and particulates in the water column were the subject of some concern, the prawns again remained in an apparent state of very good health, and again presented favourably in market assessments after harvest.

Of more importance and concern, however, were the more toxic forms of nitrogen (e.g. un-ionised ammonia) that under such conditions were given greater potential to unexpectedly emerge from the pool of available base organics, as driven by biological and decomposition processes in the ponds. Total ammonia initially appeared to be well under control in this third season, but unexpectedly reached 9.88 mg L^{-1} in one pond on day 131. Given a salinity at that time of 40.7 ppt, and afternoon temperature of 28 °C and maximum daily pH of 7.8, the percentage of un-ionised ammonia at that time would have been in the order of 3.5% of total (EIFAC, 1986), giving an un-ionised ammonia level of 0.35 mg L^{-1}. Whilst this is only marginally above that normally recommended for prawns (e.g. <0.25 mg L^{-1} by Australian Prawn Farming Manual, 2006) this cannot be ruled out in terms of its possible detrimental effect on the growth of prawns in that particular pond (pond G1). This is especially so because it took a further week or so of molasses additions to bring levels in that pond down to more acceptable levels. However, ammonia concentration was not the only factor affecting growth rates, which were also relatively slow in the second pond, despite low ammonia levels.

Plankton and ecosystem effects

The development of nutritionally suitable phytoplankton and zooplankton communities is widely considered a critical aspect for stocking commercial prawn ponds in Australia. For several weeks after stocking, prawn postlarvae feed directly on some types of algae and zooplankton as well as the resultant detritus in ponds, so the suitability of these natural feeds can be very important for high survival. Alternatively, there are many brands of early-starter artificial feeds that could be utilised if natural feeds are lacking. However, economics and efficiencies across wide areas in broad-scale culture operations encourage managers to rely on natural plankton to provide adequate nutrition for young postlarvae.

Furthermore, the types of phytoplankton and zooplankton that enter ponds during the initial fill can be expected to strongly affect the subsequent succession of species and blooms in the pond community, and this is particularly important in fully recirculated systems that may only receive this initial "first-fill" group of seed organisms. Our working hypothesis in the present work was that the ability to screen out undesirable phytoplankton or biofouling organisms could improve farm biosecurity and lead to greater algal bloom stability in order to provide more predictable culture conditions. However, screening out large proportions of seed organisms could just as easily be detrimental by limiting diversity or by slowing plankton community development and resulting in much less intense blooms.

Passive (gravity driven) sand filters have not been widely investigated for screening prawn farm pond water in the past, primarily because of the large scale of filters that would be needed to handle the very large volumes of seawater. However, this would become achievable with the integration of PASF into farm designs. If, for example, PASF integration was scaled to achieve operational treatment and recirculation rates of 10% of pond water each day, ponds should only take 10 days to fill (10% each day). Alternatively, with capabilities of 5% daily treatment, pond filling would take 20 days. Whilst this is somewhat slower than normal practice using micron-rated (or shade cloth) socks, because the sand filters place greater restrictions on flow rates, this slower fill strategy may not be unmanageable if sensibly built into routine production strategies.

Notwithstanding this, and despite the absence of pond replication in the present study, the demonstrated effect of this approach in the present study was, as could logically be expected: 1) to slow ecosystem development where phytoplankton and zooplankton communities took longer to develop, and 2) to change the resultant plankton composition with less incidence of coarser species. Sensible production strategies could therefore allow for greater reliance on artificial feeds for stocked postlarvae, longer periods between pond filling and stocking to allow natural feed organisms to build to significant levels, and/or artificial enhancement of planktonic seed organisms with managed cultures.

The Australian Prawn Farming Manual (2006) recommends using screen sizes as small as 120 μm to avoid the influx of fish and crustacean eggs and larvae which can affect the survival of the prawns, and/or compete with them for artificial feeds. Slightly larger (300 μm) nylon mesh socks have routinely been used in the past at BIRC to screen seawater used to fill ponds, and without this, species such as sand whiting (*Sillago ciliata*), sea mullet *(Mugil cephalus*), eastern king prawn (*Penaeus plebejus*) and blue swimmer sand crabs (*Portunus armatus*) are known to enter and take up residence in the ponds. At some commercial sites, this screen size has necessarily been further relaxed (e.g. shade cloth) due to the shear volumes of debris that can be present in intake waters. However, with the recent incursion of white spot syndrome virus (WSSV) into Queensland, many farms are looking to upgrade their water intakes to provide increased biosecurity by limiting the influx of potentially contaminated organisms (e.g. copepods and other crustaceans).

Biofouling and calcareous encrustation is also a significant problem in contemporary prawn farming systems, and much smaller screens are necessary to potentially prevent the entry of barnacle and tube worm larvae which lead to resident colonies and procreation within these broad-scale culture systems.

Barnacle larvae are smaller than most fish and prawn larvae but have a very similar body form to that of larval prawns. They are free-living in the plankton once released as nauplii from their hermaphrodite parent. They are recognised to have six naupliar stages, the first of which is the smallest which could be expected to be screened out by mesh sizes of 100 μm. For example, Thiyagarajan et al. (1997) described the larval stages of *Balanus reticulatus* (commonly found in the Indian Ocean) and compared their sizes to several species within this Genus, where carapace widths of Stage 1 nauplii ranged from 120-147 μm. Alternatively, Egan and Anderson (1988) described the larval stages of three coronuloid barnacles (*Austrobalanus imperator*, *Tetraclitella purpurascens* and *Tesseropora rosea*) that occur in eastern Australia, where carapace widths of Stage 1 nauplii ranged from 143-147 μm. Interestingly, the density of barnacles on the walls of ponds can have a marked influence on their capacity to reproduce in the ponds. If their densities are very low and they settle at distances greater than their penile length (i.e. several body lengths), they cannot fertilise adjacent barnacles rendering the population infertile (although some species have been found capable of secondarily reverting to self-fertilisation e.g. *Balanus improvisus*: Furman and Yule, 1990).

Encrusting tube worm larvae are also relatively small, but due to their nature may need even smaller screen sizes for exclusion. For example, *Hydroides elegans*, which is likely (though not confirmed) the species we experienced in the present study, has a larval body width of about 100 μm (see Shikuma et al., 2014). However, by their nature, polychaete larvae can contort into much smaller sizes to potentially squeeze through micron-rated screen meshes without physical damage. They also tend to grovel in the substrate as they seek appropriate settlement surfaces. Whilst they are unlikely to easily penetrate the PASF sand filter matrix which successfully filters out very small phytoplankton species in the 1-5 μm particle size range, it is not unfeasible that unlike barnacle larvae, tube worm larvae could progressively work their way through the sand matrix, somehow following the percolating water to clear the filter and settle and proliferate on suitable pond surfaces. Their metamorphosis prior to settlement is mediated by certain bacteria and the structured biofilms they produce (Shikuma et al., 2014; 2016), and given this and the anecdotal evidence from farmers regarding the often high occurrence of either barnacles or tube worms in ponds, it seems likely that the prior presence of one may influence the potential of the other to colonise.

Copepods have even smaller nauplii than prawns and barnacles, and their eggs, which can be dislodged from adults during the water screening process, are of further consideration in terms of mesh sizes for inclusion or exclusion. For example, the collection and concentration of cultured *Parvocalanus crassirostris* nauplii at BIRC has required a screen size of 44 μm (pers. comm. M. Hutchison). This is one of the smallest copepod species available for culture and controlled feeding of small-mouthed marine fish larvae, so this appears to be a good indicator regarding relative screen sizes needed. However, Kline and Laidley (2015) needed to use a 38 μm screen to collect nauplii (Stage 1 being 49 μm wide) and eggs (being 60 μm in diameter) in an intensive production system, probably because the eggs may become somewhat plastic under pressure. By comparison, other species of Calanoid copepods, such as *Temora stylifera* commonly found in the Atlantic Ocean and Mediterranean Sea, have slightly larger Stage 1 nauplius body widths of 64 μm (Carotenuto, 1999), and many other species (also including Cyclopoids and Harpacticoids) that have also been studied for marine fish nutrition have nauplii body widths or lengths of no less than 40 μm (e.g. Klein Breteler et al., 1990; Yang and Hur, 2014).

For many years in the past, ponds at BIRC filled at the same time have tended to initially react with similar yellow-brown blooms of mixed diatoms followed then by an often-strong brown bloom comprising mainly *Helicotheca* sp. However, in the present season's experiment, this only occurred in the pond filled through the 300 μm sock. This coarse ribbon-forming species has previously regularly over-bloomed in BIRC ponds, requiring water exchanges, and has also clogged PASF beds with a significant layer of organic matter on sand bed surfaces that takes a week or two to decompose and/or be consumed by the worms. But this typical *Helicotheca* bloom did not occur in the pond filled through the PASF beds, and we speculate (without strong evidence because of lack of pond replication) that this demonstrates a direct beneficial effect on phytoplankton species diversity.

The copepod blooms were also quite different between the two ponds, despite apparent similarities from the quantitative data. Larger-scale sampling would have been necessary to confirm the qualitative evidence, which suggested higher densities of copepods in the pond filled through the 300 μm sock compared with that from PASF. Since only 1 litre samples were taken, mainly to study the phytoplankton communities, larger sample volumes such as those undertaken in earlier pond studies at BIRC (e.g. 100 L: Palmer et al., 2010) would have been better to also demonstrate this in quantitative terms. The copepod population differences that were later quantitatively found between ponds, as identified from samples taken from the end of December to the beginning of February were, however, unlikely to have been due to these different methods of pond filling, and probably more due to the higher rate of predation by prawns in pond G1.

The slower development of a copepod bloom in pond G2 following its fill through the PASF beds could well have been to the detriment of prawn survival in that pond. Copepods are known to be one of the most important natural feeds for prawn postlarvae stocked into culture ponds, and the lack of other important natural feeds in the pond at that time (e.g. chain-forming diatoms) may well have compounded this effect. Of course, a strategy of allowing the pond that was filled through the PASF more time to develop its plankton community before stocking prawn postlarvae could have given a better prawn survival result, and this could therefore be recommended in the future if this approach was desired.

Conclusion and future needs

This study has demonstrated the application of PASF in a fully recirculated prawn production system for a third successive season at BIRC. Whilst there was no pond replication of the initial filling method within this trial, other elements of the study adhered to more robust scientific methodologies. Additionally, considering the current results in combination with those of the previous two seasons of investigation provides a further level of confidence in the validity of results and their interpretation. In particular, the nutrient mitigation results were well replicated providing robust data that compared PASF beds used for pond filling with those that were not. The system was also tested under the most extreme organic loading conditions so far trialled, and the study effectively highlighted some concerns and limits for the application of PASF in this form for complete recirculation.

This scaled demonstration of PASF provides further knowledge that would be useful in commercial application at prawn farms. Not unlike other mechanical and biological systems, there are certainly limits to the organic loading that PASF can handle. However, unlike the present work, which placed the unmitigated pressures of the prawn culture environment directly on the PASF system, the deployment of PASF at commercial farms would not likely be in isolation from a range of other methods that could be used to relieve loading rates during critical times. Examples may include water transfer channels that simultaneously store and treat wastewater thereby reducing loading rates, and active management that helps to avoid overloads during algal bloom peaks. As such, the integration of PASF with other farm management techniques would likely aid in its utility and help avoid issues such as excessive clogging of the sand matrix which renders it less productive.

With focus on this approach to organic waste management, future work could further seek to accelerate decomposition processes, and augment denitrification processes that offer solutions to the build-up of nitrogen in such closed systems. Additives such as probiotics may provide a useful tool to help clear clogged beds, and some bacterial modifications within sand matrices may even assist in the conversion of available nitrogen into nitrogen gas. However, this later approach would make less use of this nutrient resource, whereby alternatively linking PASF with seaweed reactors (as suggested by Palmer, 2010) would be a further way to remove and convert this waste nutrient into more useful forms.

The recirculation of treated wastewater is also of some concern from a farm biosecurity point of view. Disease outbreaks in particular ponds need to be contained, rather than spread around the farm, and wastewater flowing from different ponds into a common storage/ recirculation pond creates concerns unless the treatment process disinfects prior to redistribution. PASF may offer a degree of disinfection because it removes particulate matter and organisms like copepods which may act as disease vectors. PASF may also offer a degree of chemical treatment due to the low levels of dissolved oxygen, high levels of ammonia, and high levels of sulphide that it routinely emits with its discharge. If these attributes were realised as disinfection agents in future research, the potential long-term benefits of PASF to contemporary prawn farming operations would surely outweigh the short-term capital costs of its implementation.

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