Polybridge: Bridging a path for industrialisation of polychaete-assisted sand filters

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Executive summary

This study examined the physical and chemical properties of a novel, fully-recirculated prawn and polychaete production system that incorporated polychaete-assisted sand filters (PASF). The aims were to assess and demonstrate the potential of this system for industrialisation, and to provide optimisations for wastewater treatment by PASF. Two successive seasons were studied at commercially-relevant scales in a prototype system constructed at the Bribie Island Research Centre in Southeast Queensland. The project produced over 5.4 tonnes of high quality black tiger prawns at rates up to 9.9 tonnes per hectare, with feed conversion of up to 1.1. Additionally, the project produced about 930 kg of high value polychaete biomass at rates up to 1.5 kg per square metre of PASF, with the worms feeding predominantly on waste nutrients. Importantly, this closed production system demonstrated rapid growth of healthy prawns at commercially relevant production levels, using methods that appear feasible for application at large scale.

Deeper (23 cm) PASF beds provided similar but more reliable wastewater treatment efficacies compared with shallower (13 cm) beds, but did not demonstrate significantly greater polychaete productivity than (easier to harvest) shallow beds. The nutrient dynamics associated with seasonal and tidal operations of the system were studied in detail, providing technical and practical insights into how PASF could be optimised for the mitigation of nutrient discharge. The study also highlighted some of the other important advantages of this integrated system, including low sludge production, no water discharge during the culture phase, high ecosystem health, good prospects for biosecurity controls, and the sustainable production of a fishery-limited resource (polychaetes) that may be essential for the expansion of prawn farming industries throughout the world.

Regarding nutrient discharge from this prototype mariculture system, when PASF was operating correctly it proved feasible to have no water (or nutrient) discharge during the entire prawn growing season. However, the final drain harvest and emptying of ponds that is necessary at the end of the prawn farming season released 58.4 kg ha^{-1} of nitrogen and 6 kg ha^{-1} of phosphorus (in Season 2). Whilst this is well below (i.e., one-third to one-half of) the current load-based licencing conditions for many prawn farms in Australia, the levels of nitrogen and chlorophyll *a* in the ponds remained higher than the more-stringent maximum limits at the Bribie Island study site. Zero-net-nutrient discharge was not achieved, but waste nutrients were low where 5.91 kg of nitrogen and 0.61 kg of phosphorus was discharged per tonne of prawns produced. This was from a system that deployed PASF at 14.4% of total ponded farm area which treated an average of 5.8% of pond water daily and did not use settlement ponds or other natural or artificial water remediation systems.

Four supplemental appendices complement this research by studying several additional aspects that are central to the industrialisation of PASF. The first details an economic model and decision tool which allows potential users to interactively assess construction and operational variables of PASF at different scales. The second provides the qualitative results of a prawn maturation trial conducted collaboratively with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) to assess dietary inclusions of PASF-produced worms. The third provides the reproductive results from industry-based assessments of prawn broodstock produced using PASF. And the fourth appendix provides detailed elemental and nutritional analyses of bacterial biofilm produced by PASF and assesses its potential to improve the growth of prawns in recirculated culture systems.

Introduction and background

Remediation and reuse of wastewater holds many benefits for intensive land-based aquaculture. On-farm water treatment and recirculation takes positive control of the most important element in an aquaculture operation: the water supply. With 100% recirculation, water and nutrient discharge during the growing season can be minimised, and reliance on natural waterways to replace water is reduced. In some places – for example, where water quality may be poor during or following heavy rainfall – water recirculation can offer much better surety of supply and quality than adjacent rivers and waterways. And for biosecurity, it can provide better protection from endemic diseases by greatly reducing a key vector for transmission (viz., inflow water).

In Australia, large-scale settlement ponds have provided water treatment services for prawn farms for many years (Preston et al., 2000). Upwards of 30% of farm ponded area is often committed to settlement ponds, and whilst this generally takes the place of otherwise productive pondage, farmers opt for this approach for nutrient mitigation because it is technically feasible and because it is accepted best practice. Alternative water treatment methods that use lower farm footprints and potentially create valuable commodities from waste nutrients have more recently been developed. Examples include sand beds and algal reactors by MBD Energy (2015) at the Pacific Reef Fisheries prawn farm in northern Queensland. Of similar utility and particular interest in this study are the polychaete-assisted sand filters (PASF) described by Palmer (2010), which have shown potential for water treatment (Palmer, 2008; 2011) and economical production of nutritious polychaete biomass (Palmer et al., 2014).

The intensification of prawn farming practices in Australia is also driving the investigation of better water treatment mechanisms. At the lower prawn stocking densities that were initially used in the 1980s and 1990s (e.g., 20 m^{-2}) prawns were successfully farmed in ponds (typical area 0.5-1 ha) without any appreciable water exchange. This was made possible because the algal blooms that proliferate in these large-scale outdoor systems absorb dissolved nutrients and in turn provide an environment that facilitates high health and vigour in prawns. More recently however, farms in Australia are stocking ponds at higher rates (30-40 m^{-2}, or higher), which invariably increases the levels of waste nutrients produced. As a result of these intensifications waste products need to be continually removed for ecosystem health. Licenced water discharge levels can also tend to be lower than operational levels in grow-out ponds, so farms need to more effectively treat their discharge.

Prawn domestication is also seen as a high priority for farming practices in Australia. This is known to hold promising productivity gains through selection and specialised breeding practices (CSIRO, 2015), and also offer better surety of seed stock supplies and more control over disease within vertically integrated farms. Biosecure broodstock production systems will be needed to support such industrial endeavours in the future, as will increasing supplies of suitable polychaetes that are widely considered to be indispensable for prawn maturation diets (Harrison, 1990; Kawahigashi, 1998). Several operational imperatives will be necessary in these prawn broodstock culture systems, including well balanced nutrition, good water qualities, maintenance of adequate water temperatures, and minimised disease vectors. Low water exchange can be important in achieving many of these goals, so recirculation and water treatment will likely be integral in the design and management of specialised culture systems for broodstock.

In considering PASF as an industrial option, there are several important considerations including: 1) water volumes able to be treated, 2) percentage of farm area needed, 3) capacity to adhere to licensed discharge levels, 4) suitability for water recirculation, 5) complexity for on-farm management, 6) risk factors and surety of critical supplies, 7) investment and operational costs, 8) depreciation of equipment and long-term economic feasibility, 9) functional advantages the mechanism can provide.

The present study assesses PASF on these industrially relevant terms, and standardises the results for direct comparison with other potential methods. Since sand bed depth is an important aspect of PASF design, being likely to affect both water treatment efficacies and worm productivities, this was specifically investigated within a replicated prototype system and through robust water chemistry and seasonal system productivity assessments. Other factors investigated help to assess its industrial suitability and highlight novel aspects of this innovation for consideration by the mariculture industry. The overall aim was to provide insight into how the PASF system can integrate with, and provide value for commercial prawn farming systems.

Materials and methods

PASF construction

In 2013, a 10-bed PASF system was constructed at the Bribie Island Research Centre (BIRC) funded by a Queensland Government Research Facilities Infrastructure grant. The system was designed to treat wastewater from any of the four larger (each 1600 m^2) outdoor ponds on the site (Figure 1).

Figure 1 Aerial view of BIRC showing position of PASF development.

During the experimental period two of these ponds were stocked with prawns. Each PASF bed in the system was constructed to be operationally identical providing a 10-module configuration that could support replicated trials. System design provided similar operational conditions for each bed (e.g., surface areas, plumbing, inflow from ponds, head and suction pressures) and was based on the best prototype identified in previous work. Site preparation involved clearing vegetation, compaction and finishing the ground surface with a very slight slope (i.e., 50 mm in 10 m), and provision for heavy vehicle access (e.g., trucks carrying sand). Water supply and discharge installations involved pipe trenching for correct flows, and backfilling and re-levelling for smooth, flat ground surfaces.

After this, the PASF system was constructed. Pipes and materials most-commonly used in local rural activities were used, with considerations for their durability in the harsh (sun and seawater) environment. High-density polyethylene (HDPE) pond liners (2 mm thickness) were used to form impervious bottoms and sides for the PASF beds. The corners of each bed were folded and welded to provide water-tight seals. Each bed was 9.3 m long and 5.8 m wide giving a surface area of 53.94 m^2 (nominally 54 m^2). This provided maximum width for beds constructed from rolls of plastic that were 7 m wide. Lengths of corrugated slotted ag-drain (50 mm diameter) were positioned across the width of beds at 900 mm centres to provide under-sand drainage, and this was connected to one main drainage line fashioned from 50 mm rural poly pipe. In the first season of the project, a heavy-duty geotextile sock (RTS grade) was used to cover the slotted drainage pipes. However in the second season, this was replaced with a slightly coarser (standard) grade to potentially help reduce the sock clogging that was apparent in Season 1.

PASF integrated design

Pumps were installed to draw water from inside a coarse (6 mm) screen next to the monk drain of two lined 1600 m^2 ponds (Figure 2). These pumps were controlled with timers and synchronised to pump at specified times and for variable periods to deliver the desired daily volumes and flows.

A manifold was installed to mix the water being supplied from the two ponds, so that each PASF bed was supplied with the same quality of wastewater. Flow meters (BIL DN 40 mm) measured the volumes of water supplied to each PASF bed, and daily adjustments to flows provided approximately similar total volumes (where possible) to each bed so that similar organic loading rates were applied. A 2,000 L sump with submersible pump and float switch was installed to collect all PASF-filtered water, and this was recirculated back to both ponds at equal rates.

Figure 3 provides a schematic diagram of the recirculated prawn/worm system. The total volume of the system (when the ponds were full) was 4.8 megalitres. The surface area ratio of PASF-treatment area (540 m^2) to ponds (3200 m^2) was 1 : 5.9. Not including the collection sump, water distribution pipes and manifold, the PASF beds used 14.4 % of total ponded farm area (ponds + PASF beds).

The external standpipe arrangement (Figure 4) allowed the beds to be operated during wet weather[1] by retaining a pool of pond water over the sand mass whilst potentially still filtering water.

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[1] Whilst *Perinereis helleri* is tolerant of brackish water, it cannot survive for long in freshwater and therefore PASF beds need to be protected from heavy rain using the standpipe configuration (see below).

Alternatively, during dry weather the drainage holes were used to continuously release filtered water from the PASF beds at controlled rates.

During dry weather the PASF beds were managed with three simulated tides each day, and preferably so that the surfaces of beds could drain and dry in the sun each afternoon[2]. An example of the timing of pumps to achieve approximately 5% exchange with the ponds per day is given in Table 1 below. During wet operations, similar total daily pumping times (and volumes) were applied, but this was split into more pumping periods, 1) to provide more regular oxygenation of surface waters at night, and 2) to provide more regular supply for a more continuous percolation through the sand beds when head differentials were smaller.

Figure 2 Construction of the PASF recirculation system at BIRC showing one of the pond water supply pumps (top left), the pond water mixing manifold (top right), the filtered-water collection sump (bottom left) and the completed PASF beds (bottom right).

Internal overflow pipes installed on all beds were not generally used during normal operations so that all water pumped into the beds (according to flow meter readings) was treated via percolation

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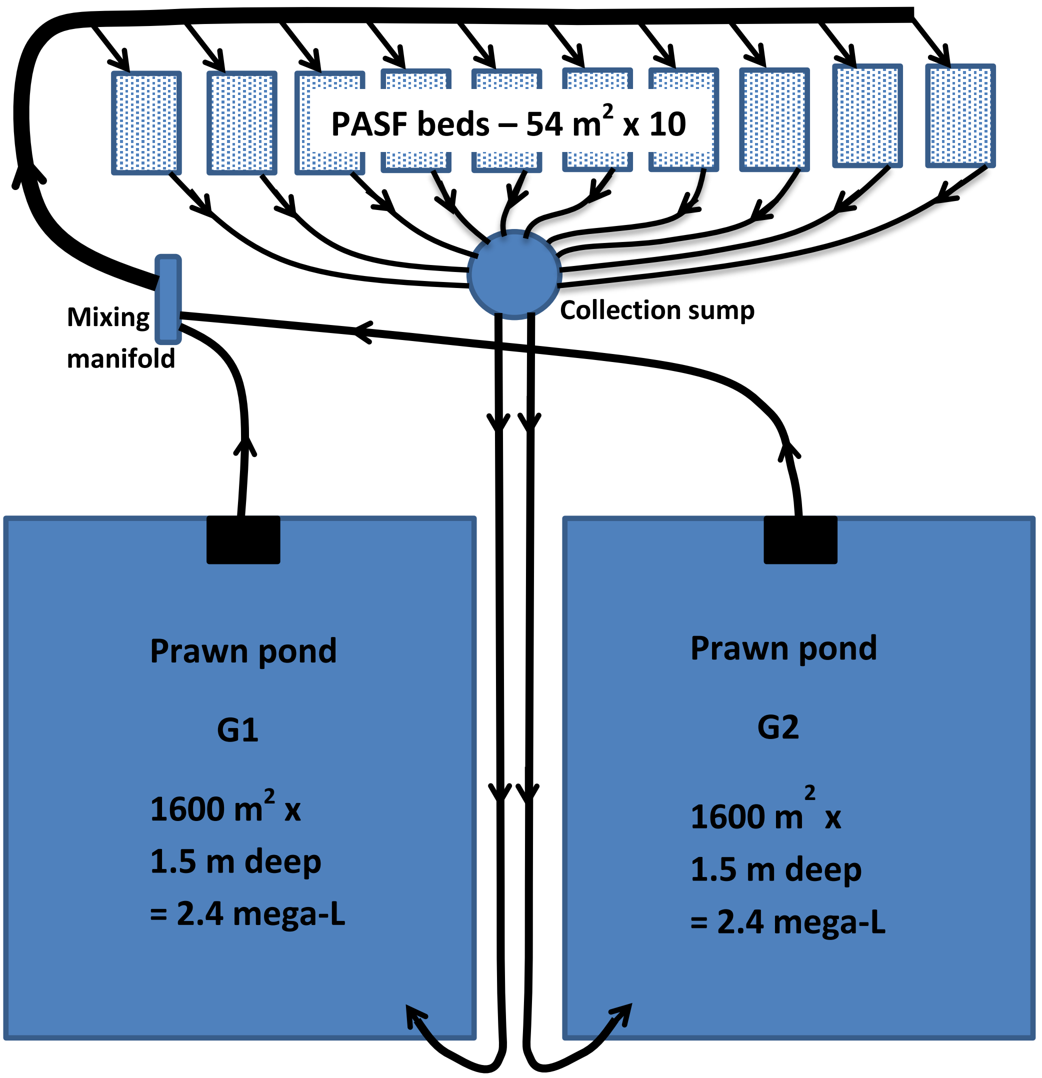
[2] Drying beds in the sun each afternoon helps to prevent the build-up of benthic algae and the proliferation of other intertidal organisms (e.g., sea squirts and sponges) that compete with the worms for available nutrients.

through the mass of sand. These overflows were a necessary design factor for use, 1) in case of oversupply of pond water, and 2) potentially during heavy rainfall when they would allow the lens of freshwater to be skimmed from the water surface.



(Not to scale)

Figure 3 Schematic diagram of the experimental integrated PASF recirculation system.

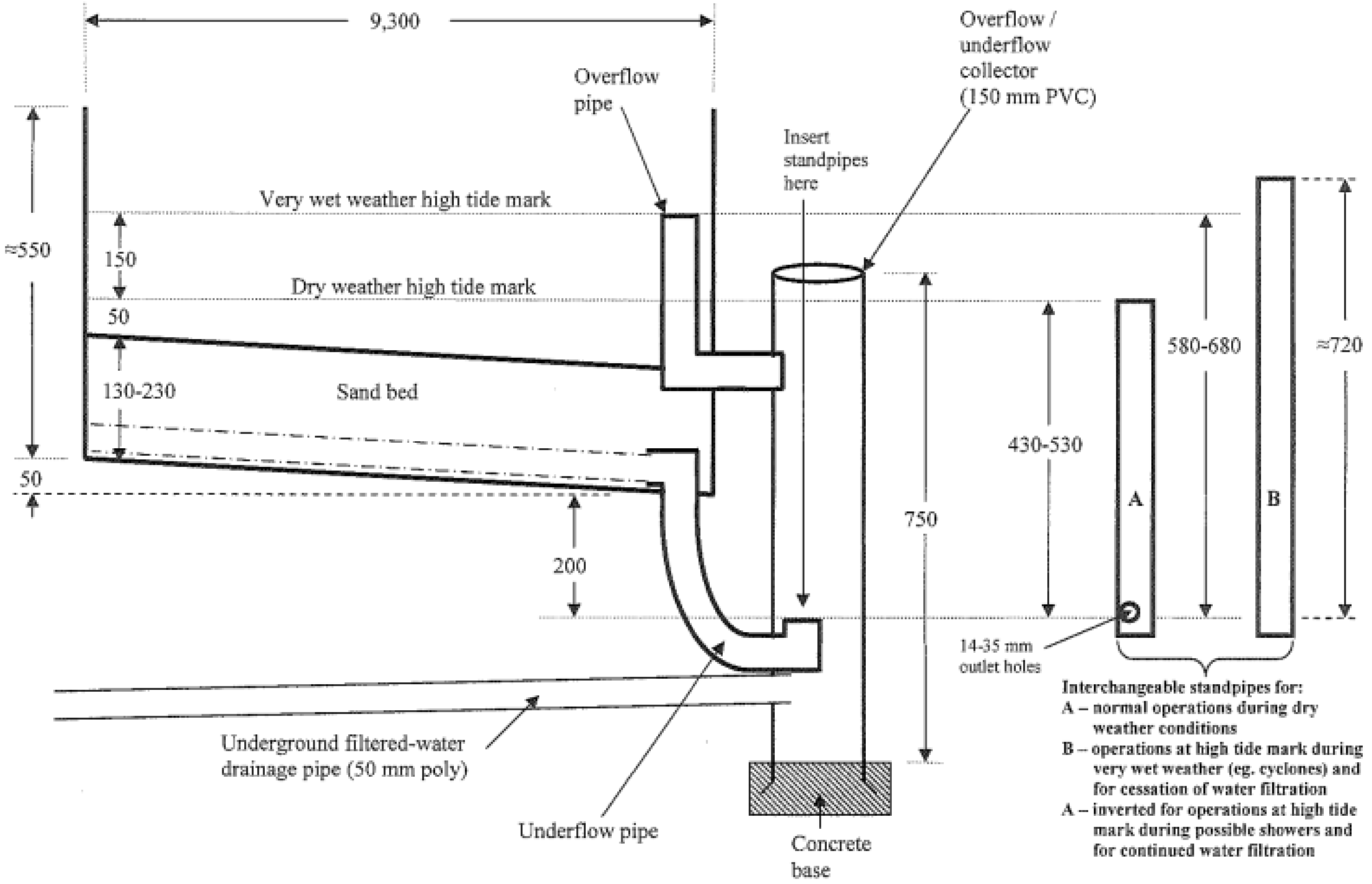


Figure 4 Schematic view of PASF prototype (all measurements in mm).

Table 1 Example of pond-water-supply pump timing for dry and wet weather operations of PASF.

Dry weather operations			**Wet weather operations**		
Pump on	Pump off	Pumping time	Pump on	Pump off	Pumping time
4 pm	6 pm	2 hr	4 pm	5 pm	1 hr
10 pm	12 am	2 hr	8 pm	9 pm	1 hr
4 am	6 am	2 hr	12 am	1 am	1 hr
			4 am	5 am	1 hr
			8 am	9 am	1 hr
			12 pm	1 pm	1 hr
Total daily pump time		**6 hr**	**Total daily pump time**		**6 hr**

Filtration sand

Bulk sand purchased from a local sand mine (Southern Pacific Sands at Godwin Beach near Bribie Island) was used in the PASF system. The grade of sand used was GTS2000 (see Table 2) which had a hydraulic conductivity of 2,243 mm hr^{-1}. This offered slightly higher percolation rates compared with a similar product that was successfully used in previous work (BMS2000 – 1,727 mm hr^{-1}).

Table 2 Particle size distribution of sand used in the PASF beds.

SAMPLE	**Sieves (mm)**	2.00	1.00	0.50	0.25	0.15	0.53	Pan
GTS2000	**% Retained**	0.8	6.4	27.3	60.0	5.0	0.2	0.4

After delivery to the site by single body trucks, a front-end loader tractor was used to initially load the sand into each bed. When loading the sand the bucket on the front of the tractor was used as a relative measure for sand volume. All deep beds (5) received 35 buckets and all shallow beds (5) received 22 buckets of sand. A small (5 tonne) excavator was used to roughly spread the sand, and this was finished to an even thickness and flat surface by hand rakes after drainage pipes were installed (via hand trenching with shovels). The finished average thickness after wetting and settling was 130 mm for shallow beds and 230 mm for deep beds.

Prawn pond operations

The two prawn culture ponds used in the study were an average of 1.5 m deep, had surface areas of 1600 m^2, and therefore each pond held a maximum volume of 2.4 megalitres. They were fully lined and sealed with welded 1.5-mm high-density polyethylene plastic, which was replaced with new liner at the end of Season 1. One monk drain was positioned on one side of each pond, which provided a screened (6 mm) discharge/overflow point. All inflow seawater was screened with a 300-μm nylon sock. Pond filling began on 30/09/13 and 16/10/14 in Seasons 1 and 2, respectively.

A standard pond fertilisation protocol (after Palmer et al., 2010) consisting of dolomite (300 kg ha^{-1} spread evenly over pond bottoms before fill) and N and P fertilisers[3] was applied during filling to

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[3] Lucern chaff: 62.5 kg ha^{-1}; rice pollard: 62.5 kg ha^{-1}; monoammonium phosphate (9.5%): 7 kg ha^{-1}; urea (47.5%): 36 kg ha^{-1}; potassium nitrate (43%): 32 kg ha^{-1}.

encourage an algal bloom. Follow-up doses of inorganic fertilisers[4] were then added to the ponds on a biweekly basis until Secchi depths[5] reached 50 cm; this continued until about two weeks after stocking when enough nutrients to sustain the phytoplankton bloom was being added as formulated prawn feed. Blue dye (Aqua Blue – 2 satchels) was added to each pond when the initial algal bloom typically waned, and molasses was also added to each pond (1 L per day for 3-4 days) while the bloom was stabilising to help prevent the pH from climbing too high due to photosynthesis.

Ponds were stocked with *Penaeus monodon* postlarvae nine days after filling began in Season 1 and seven days after filling began in Season 2. In Season 1, prawn ponds were stocked with approximately 50,000 postlarvae (PL15) on 8 October 2013[6], and in Season 2 both ponds (G1 and G2) were stocked with 60,000 PL15 on 23 October 2014. This provided prawn stocking densities of about 31 m^{-2} in Season 1 and 38 m^{-2} in Season 2. In each year prawn seedstock were sourced from Truloff Prawn Farms at Alberton in southeast Queensland. They were progeny from wild broodstock captured in Joseph Bonaparte Gulf (14°06'S 128°50'E) in the Northern Territory.

Water quality measurements were undertaken in both ponds twice daily using a multi-probe meter (YSI Pro Plus) that simultaneously measured pH, dissolved oxygen, temperature and salinity. Measurements were taken in the morning (about 6 am) when dissolved oxygen levels were at their lowest (before any appreciable daily increase had occurred), and in the afternoon (about 3-4 pm) when pond pH values were near their maximum due to photosynthesis of the algal bloom.

Management of the prawn ponds was guided by the Australian Prawn Farming Manual (2006). This involved up to four feeds per day, with total daily feed amounts given by standardised percentages of total stock biomass; this was estimated from the average body weights of prawns multiplied by an assumed survival of those stocked (80% survival was assumed in both seasons). The feeding program was based on amounts recommended in the manual for subtropical regions (average daytime temperature <28°C). Ridley Aquafeed's formulated prawn feeds were used in both years: the "Enhance" range was used in Season 1, and the "MR" range was used in Season 2. Feed trays (one per pond) were deployed each season from day 45 (45 days since stocking) to provide daily measures of the prawns' appetite and fine-tune feeding according to standard practices. Automatic belt feeders (four per pond) were also deployed at this time in association with airlifts to deliver and partially spread the mid-night ration.

Towards the end of each crop, and particularly in Season 2 when pond carrying capacities were particularly high and pH values started to decline, dolomite[7] (21 kg per week = 131 kg ha^{-1}) was applied to each pond to help stabilise pH and ensure adequate calcium and magnesium supplies for hard prawn shells. This was added by broadcasting the dry product onto the water surfaces around the entire periphery of the ponds. Also towards the end of each crop, when morning pH values

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[4] Monoammonium phosphate (9.5%): 1.2 kg ha^{-1}; urea (47.5%): 6 kg ha^{-1}; potassium nitrate (43%): 5.4 kg ha^{-1}.

[5] Secchi depth is a convenient measure of the turbidity of pond water.

[6] In Season 1 pond G2 was stocked with juveniles on the 15th November 2013 after its PLs had alternatively been reared in a smaller pond for 6 weeks due to pond availability issues.

[7] Dolomite: 21 kg bags from Flinders Trading Pty Ltd – 35% as calcium carbonate, 33% as magnesium carbonate.

declined below 7, hydrated lime[8] was also added to each pond (1-3 kg per day for 3-4 days in a row) by placing it in a 20-L bucket, mixing with pond water at high concentration in the bucket, and then partially submerging the bucket by suspending it in the flow of the aerator to produce a slow release of dissolving material. Care was taken during these later stages not to add too much lime due to low pH, so that afternoon pH values remained below 8.4[9]; at this pH and with relatively high salinities and temperatures, total ammonia levels of over 2 ppm would yield increasingly dangerous levels of un-ionised ammonia (recommended to stay below 0.25 ppm by the Australian Prawn Farming Manual, 2006).

Both prawn culture ponds were continuously aerated with paddle wheels (2 hp; Chenta) and/or Force 7.2 submersible aspirators (1.5 hp; Acqua & Co.). Each pond was initially equipped with one operating paddlewheel. When morning dissolved oxygen (DO) levels started falling below 3 ppm (day 100), more aeration horsepower was added by installing an aspirator on the opposite side of each pond. Towards the end of Season 2 when high prawn carrying capacities were evident from low morning DO measurements, it was necessary to replace this aspirator with another paddlewheel (giving two paddlewheels per pond), which provided up to 25 hp of aeration ha^{-1}. In Season 2 after almost all prawns had been harvested using traps, aeration was reduced to a minimum of one aspirator per pond.

Weekly prawn harvests (partial harvests from both ponds) commenced when they reached an average size of 30 g (on 19 February in 2014 and 2015). Up to four traps per pond were deployed and all prawns captured were transported live (Figure 5) to Truloff Prawn Farms at Alberton where they were processed using their standard methods. Quality assessments were made by comparing several physical attributes (e.g., apparent health and condition, shell hardness, and general appearance including colour and any superficial necrosis) with those of the farm's own product.

Figure 5 Harvest of prawns using traps (left) and live transport (right).

As is typical of most prawn farming operations, no water exchange was applied to ponds for the first one to two months after fill. This approach generally conserves the nutrients added as fertilisers and allows a strong phytoplankton bloom to develop. Typically in the third month of culture, increasing

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[8] Hydrated lime: LIMIL from Sibelco, Aust.

[9] At pH 8.4, temperature of 30°C, and salinity of 32-40 ppt, 14% of total ammonia is un-ionised (EIFAC. 1986)

amounts of pond water are discharged to waste, and this is normally replaced with new intake water so that phytoplankton densities and nutrients are regularly diluted (up to 10% exchange per day is often used where necessary). In the present study, instead of discharging this water to waste, it was treated by the PASF beds and fully recirculated back to the ponds. In Season 1, all wastewater was treated and recirculated up until the first prawn harvest (day 135 after stocking); after that, wastewater was replaced with new intake water to ensure that no stock losses occurred[10]. However in Season 2, due to improvements made to the PASF system, all wastewater was treated by the PASF beds and recirculated back to the prawn culture ponds. This meant that in Season 2, no water was discharged from the integrated prawn/worm system until the final drain harvest of prawns (day 204 after stocking).

In Season 2, a prolonged period (50-51 d) between the last partial trap harvest (24/03/15) and final drain harvests (13 & 14/05/15) was implemented in an attempt to run the nutrient levels in pond waters down below BIRC's discharge limits with PASF treatment. During this final water treatment period, no prawn feed was added to the ponds and aeration was reduced to one aspirator per pond.

PASF operations

All PASF beds were fully dried and screeded to provide a smooth sand surface before wetting with pond water. An even depth of sand was provided throughout each bed. Since the bottom liners had a 50 mm fall towards the underflow outlet (Figure 4), the surfaces of sand also had about 50 mm fall. This slight fall in the sand bed surface aids in maintaining a smooth progression of the advancing artificial tides, and also helps to prevent excess organic loading in depressions that inevitably form when the sand settles.

As a general rule, pond water supplies to the PASF beds in the past have been minimised before stocking polychaete seedstock, so that populations of potential predators (e.g., nematodes) that may exist in pond water are also minimised in the beds prior to stocking. After stocking polychaete seedstock, a three-day settling-in period has also been applied where wet-weather operations (see "PASF integrated design" Section above) maintains a pool of water above the sand beds, before normal dry-weather drain-down operations are implemented. Accordingly in the present study, pond water supplies to the PASF beds began just a few days prior to initially stocking the PASF beds with polychaetes each season, which was on 22/11/13 (46 days after stocking prawns into ponds in Season 1), and on 19/11/14 (28 days after stocking prawns into ponds in Season 2).

Polychaete seedstock used in the study were eighth generation (in 2013) or ninth generation (in 2014) domesticated *Perinereis helleri* supplied under contract by P&C Palmer Consolidated Pty Ltd. Nectochaete (three-day-old larvae) estimates were undertaken volumetrically in seawater and juvenile estimates were undertaken volumetrically in nursery sand. Nectochaetes were stocked into the pool of pond water over the PASF beds after a 50:50 dilution and a five-minute acclimation period. Juveniles were stocked into recently drained beds by shallow-planting small quantities (e.g., 400-500 mL) of nursery sand containing juveniles evenly across the entire beds' surfaces, with pond water supplies resuming soon after.

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[10] It was a high priority that prawn production statistics were not compromised, and given the rich nature of pond water at that stage it was decided to err on the side of caution.

Several approaches to seedstock supplies for the PASF beds were investigated in the study. In the first season higher densities and a more varied approach to stocking age was implemented (see below) to potentially demonstrate high production levels from various methods, and with a focus mainly on maximum polychaete biomass production. In the second season lower stocking densities were used with greater focus on live supplies and production of larger worms that are more suitable for bait markets.

Specifically in Season 1, 399,250 *P. helleri* nectochaetes were stocked into Beds 7, 8, 9, and 10 on the 25/11/13 and 26/11/13. This provided nectochaete stocking densities of 7,400 m^{-2} for these four beds. Beds 7 and 8 had been previously filled with water from Pond G1 containing a strong (50 cm Secchi depth) green chlorophyte bloom supporting a healthy copepod population; Beds 9 and 10 had alternatively been filled with water from Pond G2 containing a moderate (100 cm Secchi depth) brown diatom bloom supporting healthy populations of rotifers and copepods. To provide a pelagic environment for settlement of nectochaetes, beds were given three days of static operations (water pooled over sand beds with no water percolation) followed by three days of wet-weather operational mode (pooled water over sand with percolation), before normal drain-down operations commenced. In addition to these nectochaetes, Beds 7, 8, 9 and 10 were also later stocked with a small number (20,000 per bed = 370 m^{-2}) of two-month-old juveniles (on 24/01/14) when it appeared that the previously stocked nectochaetes had had low survival. The remaining six beds (Beds 1, 2, 3, 4, 5 and 6) were each stocked exclusively with 324,000 one-month-old juveniles on 28/11/13 and 29/11/13, giving stocking densities of 6,000 m^{-2}.

In Season 2, all 10 beds were stocked with 108,000 one-month-old juvenile *P. helleri* on the 20/11/14 and 21/11/14, giving stocking densities of 2,000 m^{-2}. Again, a minimised one-to-two day operational period was applied before stocking, and a three-to-four day settling-in period (percolation with pooled water over the beds) was applied prior to commencement of normal drain-down operations.

After stocking and settling in periods, all beds were managed in exactly the same way regarding water volumes supplied and operational modes depending on the prevailing weather conditions (wet or dry weather operations – see "PASF integrated design" section above). Water supply rates to beds were managed and equalised on a daily basis by monitoring flow meter readings[11]. Dry-weather operations were routinely applied unless the beds needed protection from rain with the wet weather operational mode. In Season 1, water treatment rates began with about 3% exchange of pond water per day, then after about one month rising gradually to a maximum of 9-10% exchange per day; whereas in Season 2, treatment rates began at a slightly higher rate with 4-5% exchange per day, rising to a maximum of 6-7% per day several weeks later (Figure 6). During the experimental periods the PASF beds treated an average of 6.6% of pond water daily in Season 1 and 5.8% in Season 2. Total volumes pumped from the ponds to the PASF beds each day were governed by how long the pumps operated each day. After the prawn ponds were drain harvested, clear water from one or two recently filled ponds (no fertilisers applied and no appreciable algal bloom) was used to continue this daily water supply to the PASF beds until they were also harvested.

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[11] Water supply rates to some beds were necessarily reduced from the beginning of February in Season 1 due to bed clogging; however rates were applied uniformly throughout Season 2.

One factor that was identified with potential to confound studies of nutrient concentrations flowing from the PASF beds was drainage rate. For example, higher drainage rates would tend to dilute dissolved nutrients being produced through the mineralisation of organic matter within the sand beds. To validly assess such differences between beds of different depths it was necessary to regularly equalise drainage rates during the experimental operations. This was made possible in the present work by the interchangeable size of drainage holes in the external standpipes (refer to Figure 4 above). Interchangeable standpipes with different sized holes ranging from 14 to 35 mm (diameters) were constructed for each bed, and removing the standpipe effectively provided the largest possible (50 mm) outlet. Daily observations on bed drain-down times guided the equalisation of bed drainage rates by changes to the size of drainage holes. As a general rule, if beds had not drained by noon each day, drainage hole sizes were increased to the next graduation, or if a bed appeared to be draining more rapidly than the rest, the hole size was reduced.

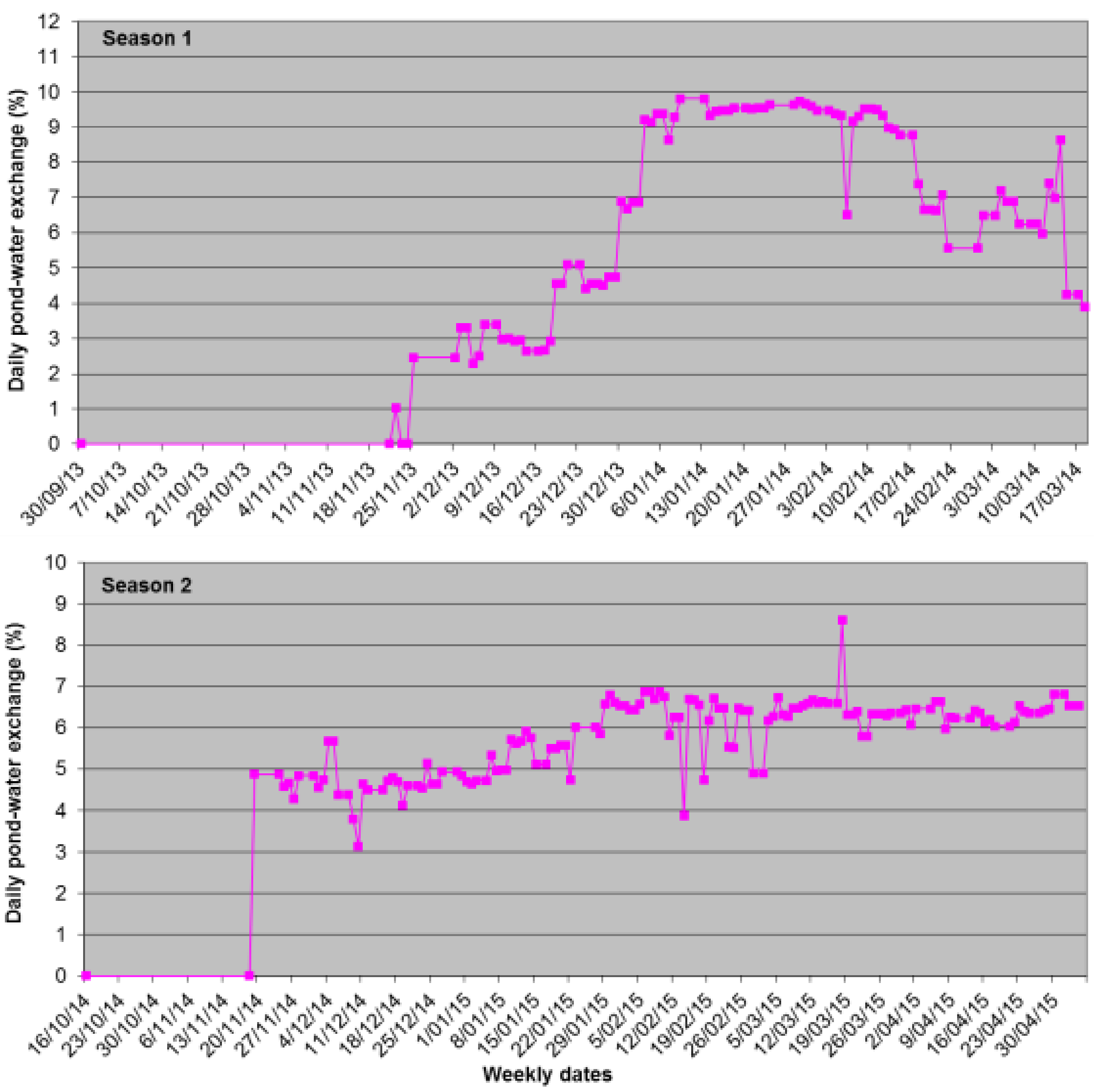

Figure 6 Water treatment rates applied in the PASF integrated system in Seasons 1 and 2.

Beds that clogged to the point where their upper surfaces were not fully drying in the afternoon sun were short circuited by digging a hole in the sand close to the bottom outlet, disconnecting the first 19-mm drainage line from the main collection pipe and alternatively installing a 19-mm poly elbow (inside diameter of 16 mm) (Figure 7). This allowed a continuous slow (16 mm) discharge of unfiltered water from above the sand mass. Whilst this was expected to reduce the efficacy of sand filtration, it still encouraged water to percolate down through the sand bed to filter some water and provide oxygen to worms within the mass of sand. This backup plan for clogged beds was developed towards the end of Season 1, but was standardised and routinely applied in Season 2 when the maximum outlet size (50 mm) could not provide sufficient water release for afternoon sun drying.

Artificial feeding of the worms was initiated each season when the green-water supply from the ponds ceased. This was on the 3/04/14 in Season 1 and 15/05/15 in Season 2. Up to 400 g of prawn feed starter (Ridley Starter 1) was evenly spread on each bed when fully drained every one or two days. This was undertaken for two to four days per week and continued until the harvest of each bed began. Generally, no feeding was undertaken on weekends to allow uneaten feed to be fully consumed, and no feeding was applied during wet weather operations when benthic algae tended to develop on the beds providing an alternative feed source for the worms.

Worms were harvested progressively from beds in Season 1 from 29/5/14 till 1/10/14, and in Season 2 from 1/04/15 till 14/10/15 using a modified dredge system that separated the worms from sand (Figure 7). Harvested worms were immediately placed on coarse sieves to remove sand and silt-laden mucus, then transferred to a 500 L raceway (6.5 m long x 30 cm wide x 25 cm deep) supplied with moderate aeration (6 x 30 mm airstones) and clean seawater exchange (10 L min^{-1}) to allow purging of gut contents. The next day purged worms were weighed (after 10 s drip time in a fine net) to provide harvest weights from the previous day. Purged worms were then sorted so that only large worms (>0.6 g) were supplied to bait markets; large and small worms were supplied to prawn hatchery markets (live or frozen).

Figure 7 PASF bed showing short-circuit where water could routinely be released from the bed to allow sun drying (left) and the worm harvester (right).

One half of each bed was harvested weekly in Season 1, with small worms and those large worms not sold as live product each week frozen in 250 g flat packs. Demand from live worm markets guided the amounts harvested in Season 2, where at times only one-quarter of a bed was harvested each week. Weights of worm biomass harvested from each bed were tallied, but importantly, comparisons of total harvests between beds were confounded by different culture periods; only quadrat samples (described below) provided valid worm production comparisons between treatments (i.e., between shallow and deep beds).

Experimental design

Sand bed depth was the main treatment investigated. It was studied both in terms of efficacies of water treatment and worm productivities. Two depths were each implemented in five beds during the initial loading of sand prior to Season 1. After wetting and settlement this provided five replicates of either 230 mm (Beds 1, 3, 5, 7, 9 = deep beds) or 130 mm (Beds 2, 4, 6, 8, 10 = shallow beds). Using experience gained from several previous years of development, these depths were at the perceived extremes that could be practically managed and which would still offer a reasonable degree of water filtration. For example, overly deep sand beds present more difficulties in the harvest of worms using the current mechanical harvesting equipment, and overly shallow beds do not provide sufficient filtration media over drainage pipes. These two depths studied were on either side of the nominal 200-mm depth originally reported for PASF by Palmer (2010).

The maximum possible drainage rates of shallow and deep beds were experimentally compared at around the same stage in both seasons after a significant period of operation and at a time following the heaviest organic loading. In Season 1 this was undertaken on 25/02/14 after 94 days of operation, and in Season 2 on 23/02/15 after 97 days of operation. These comparisons were made with the maximum hole size (50 mm – i.e., with stand pipes removed) in two ways: 1) using equal maximum head pressure with the same total depth of water (60 cm) over the discharge point (see "underflow pipe" in Figure 4) for all beds (note that over flow pipes were extended in shallow beds to facilitate this test), and 2) using the operational heads that the PASF system normally used, namely 43- or 53-cm depth over the discharge point for shallow or deep beds , respectively, which gave a 50 mm water depth over the highest surface of the sand beds at the highest part of the artificial tide.

Worm populations in the PASF beds were experimentally assessed at two times in Season 1, and once in Season 2. All worms occurring in a 0.5 m^2 rectangular quadrat (2.5 m x 20 cm) per bed (Figure 8) were collected by manually feeding the harvester with excised sand. All worms in each sample were counted and weighed to provide the total number (for survival estimates) and total production (per square metre), as well as the derived average sizes of worms. The first assessment in Season 1 was undertaken on the 1/04/14 (Beds 1-5) and 2/04/14 (Beds 6-10). This was 125-126 days after stocking worms into beds, after the final drain harvest of prawns, and prior to any artificial feeding of the worms. The second assessment in Season 1 was undertaken on the 21/05/14 (Beds 1-5) and 22/05/14 (Beds 6-10), 175-176 days after worm stocking, and after 50 days of artificial feeding. Quadrat sampling in Season 2 was undertaken before any artificial feeding on the 25/03/15 and 27/03/15, which was just after the last partial trap harvest of prawns and 126-128 days after stocking the beds with juvenile worms.

Figure 8 Quadrat sampling from PASF beds showing worm holes/burrows and substrate structure with mottling of aerobic and anaerobic sediments.

To assess prawn growth, weekly samples of 10-12 prawns were randomly taken from each pond and individually weighed. Initially these were captured with fine nets or taken from feed trays, but when prawns reached an average size of 8-9 g a cast net was used to obtain each sample. Mean sizes in each pond were compared with the growth expected under sub-tropical conditions as provided by feeding charts in the Australian Prawn Farming Manual (2006). Total prawn production was assessed by adding partial harvest amounts with final drain harvest amounts and weights of progressive size check samples.

The replacement of pond liners at BIRC between Seasons 1 and 2 allowed some other interesting assessments (e.g., accumulation of pond sludge and barnacle growth) that otherwise would have been more difficult in previously used ponds. To estimate the volumes, solids and organic contents of settled materials in each pond, the circular sludge piles that accumulated on the middle bottom of each pond in Season 2 were sampled and measured one or two days after draining Pond G2 or G1, respectively. An approximate diameter and four evenly-spaced depths across the radii of the piles provided a rudimentary way of estimating volumes, using the CAD computer graphics program (see Results) which converted the piles to flat circular discs and calculated volumes as a cylinders. Three samples (300 mL) of sludge were taken across radii of the piles in each pond on the same days that volume estimates were made. These were submitted to Unitywater Laboratories and ALS Environmental Laboratories for NATA-approved analyses for total solids and total volatile solids (Unitywater – Test method CM17), and total nitrogen and total phosphorus contents (ALS – Test methods EA055, EK059G, EK061G, EK062, EK067G). Three wet sludge samples (300-340 mL) from each pond were also measured and weighed to provide weight-to-volume assessments for mass balance nutrient estimates.

Densities of barnacles on the walls of each pond were also assessed after draining ponds in Season 2. This was undertaken by counting the number of barnacles within 12 stratified rectangular quadrats for each pond. A frame fashioned from aluminium angle was used to standardise quadrat samples at 50 mm wide and 1500 mm long (0.075 m^2). Quadrats were vertically arranged from the water level down, with one sample positioned in each pond corner (4 per pond), and two samples (10 m from corners) on each wall.

Water quality and nutrient measurements

To facilitate routine prawn pond management decisions, twice-daily water quality measurements of dissolved oxygen (DO), pH, temperature and salinity were undertaken by submerging a multi-probe system (YSI Pro Plus) in one corner of each pond. Daily measurements were generally timed to coincide with the lowest daily DO levels (about 6 am) and the highest daily pH levels (around 4 pm). These water qualities were also studied in the PASF discharge through weekly or fortnightly measurements (around 8 am) taken by submerging the probe inside the discharge pipe of each PASF bed.

Notably in Season 1, pond availability at BIRC prevented the study of two identical ponds prior to the start of water recirculation through the PASF beds. As a proxy for pond G2 during this time, data collected from the smaller covered nursery pond (N1) holding the prawn juveniles (that were later transferred to pond G2) is presented. This caused several water quality differences that are apparent in the data between the ponds before the prawns were transferred on 15/11/13, including higher water temperatures and different Secchi depths.

To study nutrients and other water chemistry, weekly or fortnightly water samples were taken from the discharge of each bed and from the mid-water column near the pump intakes in each pond. Samples from PASF beds reflected the same water being recirculated back to the ponds. This seasonal sampling was routinely undertaken in the mornings during mid-tidal flows, which tended to occur between 7 and 8 am. Water samples were immediately placed on ice and submitted to Unitywater Laboratories for NATA-accredited analyses on the same day that samples were collected. Parameters studied included total suspended solids (TSS), turbidity, chlorophyll *a*, total nitrogen (TN), total ammonia (TAN), nitrite, nitrate, dissolved organic nitrogen (DON), total phosphorus (TP), phosphate, dissolved organic phosphorus (DOP), total sulphide and total alkalinity[12].

In Season 1, water nutrient sampling was suspended after the short circuiting of beds (that was necessary due to sand bed clogging) because this confounded the PASF filtration effect by allowing water to track past the sand filtration process. However in Season 2 water quality studies extended through a bed-clogging (e.g., overload) event, and then well past the last trap-harvest of prawns to study the potential for PASF to recover from clogging and to continue operating. This later interest in the ability of PASF to reduce nutrient levels in the ponds after pond feeding had ceased, when prawn stocks were at a minimum, relates to the disposal of pond water with the drain harvest. The effect of wet-weather operational mode on PASF efficacies was studied on two occasions when scheduled sample days coincided with wet weather. This occurred on 7/01/14 in Season 1 and on 12/02/15 in Season 2. All other water samples were taken during routine dry-weather operations.

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[12] In Season 1, total alkalinity was only studied from 21/1/14.

In addition to these routine weekly/fortnightly water samples, two time-series experiments were performed in Season 2 as the organic loading built to a maximum (i.e., on 19/01/15 and 16/02/15). This was undertaken to evaluate the changes in nitrogen, phosphorus and dissolved nutrients present at different stages of the tidal outflow from PASF. The experiments simulated normal afternoon tidal flows with an inflow duration of 2.5 hr, with samples taken as pond water slowly covered the sand beds and then began to recede (e.g., approx. 10% covered at 5 min, 50% at 15 min, 75% at 30 min, 100% at 60 min, 3-4 cm deep over highest part of bed at 120 min, and 1-2 cm deep at 240 min). They involved samples from two shallow and two deep beds at 5, 15, 30, 60, 120 and 240 min after the inflow began at 4 pm. Beds 5 and 9 (deep) and 8 and 10 (shallow) were studied on 19/01/15, and beds 7 and 9 (deep) and 8 and 10 (shallow) were studied on 16/02/15. Parameters measured included TN, TP, nitrite, nitrate, TAN, phosphate, DOP, DON, total sulphide and total alkalinity.

Statistical analyses

In cases where laboratory results were lower than their 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 seasonal and tidal water quality 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 and discussion

Prawn production

Prawn production during the study was very successful in the PASF-recirculated system. Compared with the “Sub-tropical Growth” projections provided in the Australian Prawn Farming Manual (2006), above-average prawn growth was achieved in both seasons (Figure 9). Growth was particularly good in Season 2, but this may have been due to several factors unrelated to the recirculation of wastewater, including higher prevailing temperatures (see the section below on Seasonal water qualities) and the change to the more-advanced MR range of feeds offered by Ridley Aquafeeds.

Table 3 provides a summary of other prawn production statistics for the two seasons. In Season 1, higher survival was apparent in Pond G1 than in Pond G2, which was very likely due to the need to grow and transfer the prawns between two ponds during the grow-out cycle. This also likely caused the lower prawn production and higher feed conversion ratio in pond G2.

In Season 2, survival was slightly higher than in Season 1, and was more similar between the two replicate ponds. Excellent feed conversion was demonstrated in Season 2 (i.e., 1.1 kg of feed for 1 kg of prawns), and prawns reached a harvestable size of about 28 g some 15 days earlier than in Season 1. Particularly strong growth was exhibited in pond G2 after the last trap harvest, in the extended time leading up to the drain harvest during which time the prawns were not fed with artificial feed. This was likely due to the last trap harvest in Season 2 being particularly efficient at clearing prawns from that pond; sufficient natural feed (including detritus and pond biota) may have allowed the remaining lowest-density prawns to maintain a reasonable growth trajectory despite the lack of a high-protein pellet diet.

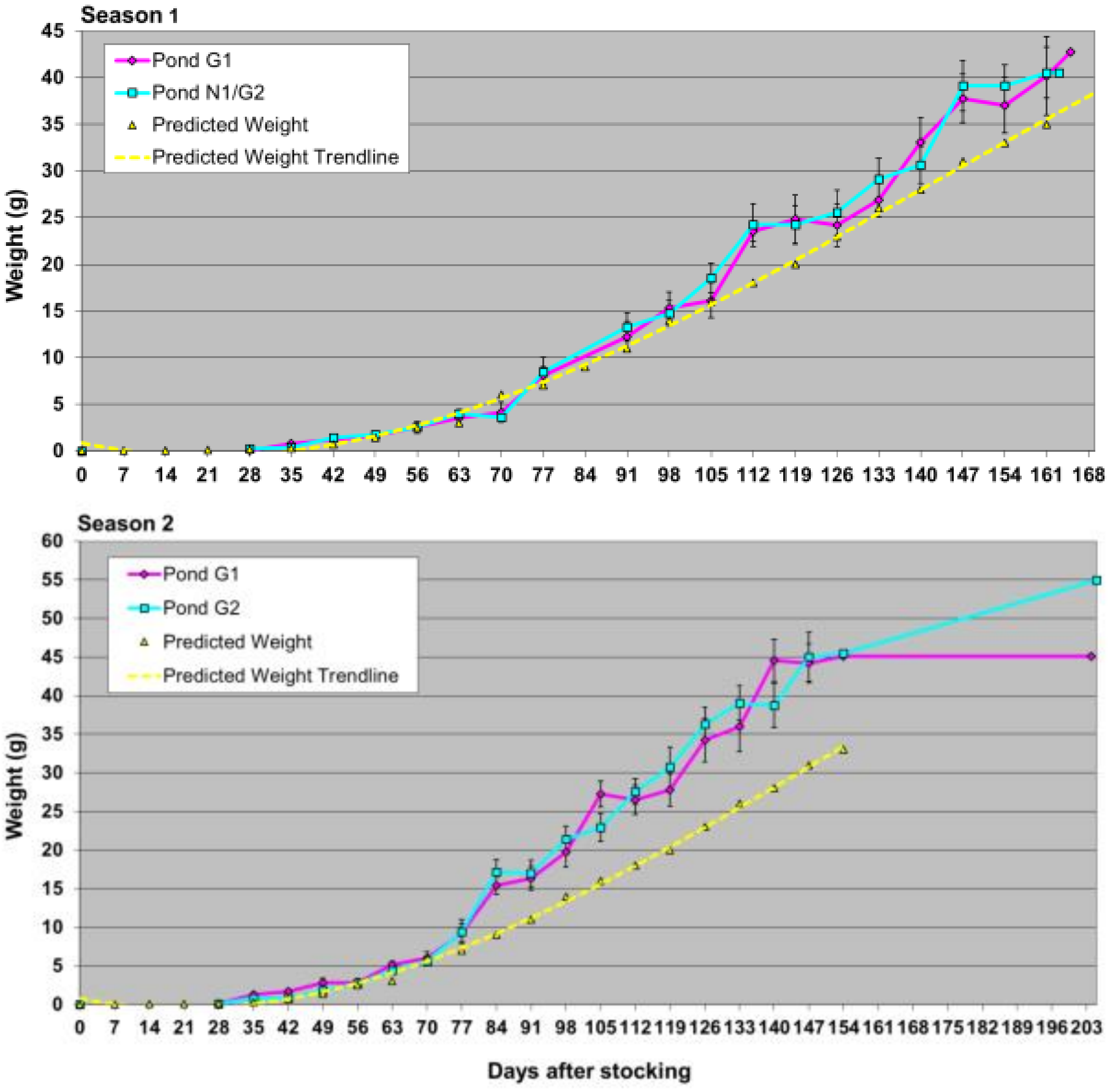


Figure 9 Growth of black tiger prawns over 2 successive seasons (2013/14 and 2014/15) in 2 PASF-recirculated ponds. Mean (± se) weights achieved after stocking, relative to expected growth according to the Australian Prawn Farming Manual (2006).

Prawn quality was assessed as outstanding in the study. In both seasons harvested prawns that were supplied live to one of the major prawn producing companies in Australia were visually no different from that farm's own produce. Being supplied live to the farmers meant that there was no deterioration of the product due to chilling or storage prior to cooking. Factors taken into consideration were overall size, evenness of size, colour, and general health. There was no appreciable tail bite, no damage, necrosis or discolouration of shells, the shells were hard, and the prawns were particularly lively when delivered to the farm's processing facilities after the two-hour transport time. In the farm's view the prawns were as good as their own farm produce, and they were processed through their processing plant in a similar manner to their own product.

Table 3 Prawn production statistics for Seasons 1 and 2.

Parameter	**Season 1 2013/14**		**Season 2 2014/15**	
	Pond G1	**Pond N1/G2**	**Pond G1**	**Pond G2**
Number of postlarvae (PL15) stocked	50,000	50,000	60,000	60,000
Stocking density (PLs m^{-2})	31.3	31.3	37.5	37.5
Calculated* survival (%)	67.3	61.6	71.1	68.9
Total harvest (kg pond^{-1})	1164.8	1095.1	1579.9	1580.5
Total harvest (tonnes ha^{-1})	7.28	6.84	9.87	9.88
Age** at first trap harvest (d)	135	135	120	120
Mean prawn weight at first trap harvest (g)	26.9[a]	29.1[a]	27.8[b]	30.8[b]
Age** at final drain harvest (d)	165	163	204	205
Mean prawn weight at drain harvest (g)	42.7	40.5[a]	45.1	54.9
Harvested amounts at drain harvest (kg)	265.2	191.4	38	21.6
Feed conversion ratio	1.3	1.32	1.12	1.14

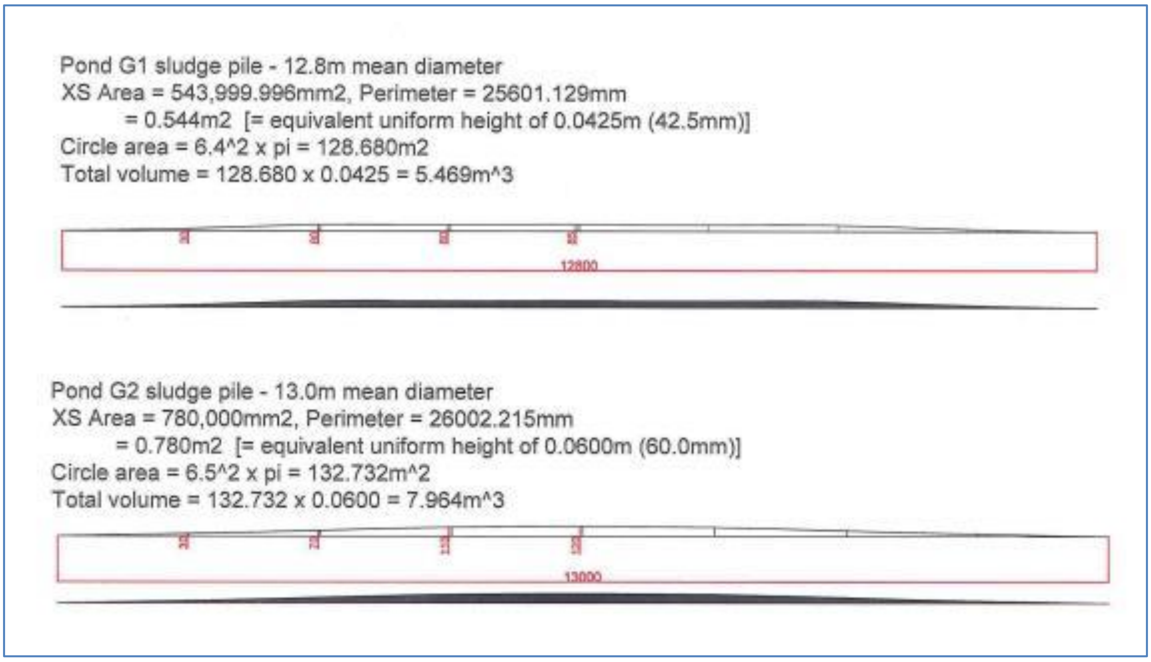
*Based on weights harvested divided by previous size estimates. **Number of days after stocking as PL15.
[ab]Estimates made 2 days[a] or 1 day[b] before harvest.

Sludge production

The sludge piles that accumulated in each pond in Season 2 (Figure 10) had approximate volumes of 5.47 m^3 in pond G1 and 7.96 m^3 in pond G2 (see Figure 11 for volume calculations). Levels of moisture, volatile solids and macronutrients of the sludge piles (Table 4) were not significantly different between the two ponds ($P>0.05$).

Table 5 provides some mass balance calculations for the estimated volumes of sludge in each pond at the end of Season 2, including wet weight estimates, dry weight calculations, and calculated total nitrogen and total phosphorus.

Figure 10 Pictures of recently drained ponds G1 (left) and G2 (right) showing accumulated piles of sludge.



Pond G1 sludge pile - 12.8m mean diameter
XS Area = 543,999.996mm2, Perimeter = 25601.129mm
= 0.544m2 [= equivalent uniform height of 0.0425m (42.5mm)]
Circle area = 6.4^2 x pi = 128.680m2
Total volume = 128.680 x 0.0425 = 5.469m^3

Pond G2 sludge pile - 13.0m mean diameter
XS Area = 780,000mm2, Perimeter = 26002.215mm
= 0.780m2 [= equivalent uniform height of 0.0600m (60.0mm)]
Circle area = 6.5^2 x pi = 132.732m^2
Total volume = 132.732 x 0.0600 = 7.964m^3

Figure 11 Sludge volume estimates given by CAD program output on cross-sectional (XS) area and standard cylinder volume calculations.

Table 4 Contents (mean ± se, *n* = 3) of accumulated sludge in ponds after the drain harvest in Season 2. Within rows, means with similar letters are not significantly different ($P > 0.05$).

Parameter	**Pond G1**	**Pond G2**
Moisture (%)*	90 ± 1.54^a	91.6 ± 0.64^a
Total volatile solids (% total solids)**	32.7 ± 1.92^a	34.9 ± 0.49^a
Total nitrogen as N in dry matter (g kg^{-1})*	213.2 ± 60.43^a	259.2 ± 36.76^a
Total phosphorus as P in dry matter (g kg^{-1})*	111.4 ± 39.72^a	180.3 ± 7.03^a

*Tested by ALS Laboratories; **Tested by Unitywater Laboratories

Table 5 Mass balance calculations using mean values provided in Table 4 for sludge left in the middle of each pond after the drain harvest in Season 2.

Parameter	**Pond G1**	**Pond G2**
Average weight (mean ± se, *n* = 3) of wet sludge (kg L^{-1})	1.048 ± 0.007	1.065 ± 0.021
Wet weight of sludge (tonnes) (= volume x average weight)	5.732	8.482
Wet weight of sludge (tonnes ha^{-1}) (x 6.25 multiplier)	35.825	53.01
Dry weight of sludge (tonnes ha^{-1}) (minus moisture)	3.583	4.983
Dry weight of sludge (kg pond^{-1})	573.28	797.28
Total nitrogen in sludge (kg ha^{-1})	763.9	1,291.6
Total nitrogen in sludge (kg pond^{-1})	122.2	206.7
Total phosphorus in sludge (kg ha^{-1})	399.1	898.4
Total phosphorus in sludge (kg pond^{-1})	63.9	143.7

Whilst these data were considered somewhat imprecise[13], they do provide a standardised way to evaluate and compare sludge piles in future work with PASF and in other prawn farming systems. For example, Robertson et al. (2003) compared the traditional flow-through prawn farming model with a recirculated design using vertical artificial substrate within a settlement pond. Although our methods of calculating sludge volumes were different from those used by Robertson et al., the amounts of sludge that accumulated in ponds in the present study were much lower. In Robertson et al., dry weight sludge volumes were estimated to be 96.9 tonnes ha^{-1} for recirculated ponds and 47.38 tonnes ha^{-1} for flow-through ponds, which are both many fold higher than estimates made in the present study (less than 5 tonne ha^{-1}: Table 5).

Robertson et al. (2003) considered methods of sludge removal during the cropping cycle for their recirculated approach. In the present study, by contrast, all sludge was left in the pond at the end of Season 2, and spread around the bottom of the pond before refilling (Figure 12) so that it could usefully contribute to nutrients needed for bloom development in the next crop. About 33% of the solids which accumulated in the centre of ponds in Season 2 was organic matter (volatile solids), and this is also quite low compared with previous key nutrient-budget studies for flow-through prawn farming systems with 5-10% water exchange per day (e.g., Funge-Smith and Briggs, 1998: 63% organic matter).

Figure 12 Pond G2 prior to refilling after Season 2. Sludge was allowed to dry and crack in the sun and was then spread away from the centre of the pond (as shown) to provide nutrients for the next pond bloom.

This is an important finding, since sludge production and its removal from ponds and disposal can be significant problems for farmers. Standard practice is to allow it to de-water after the drain harvest and dry until it cracks in the sun before physical removal with heavy machinery. It is generally placed into land fill sites that are well away from natural water flows. However, this accumulation of sludge is greatly increased when the suspended solids contents of intake waters at flow-through farms are

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[13] Imprecision is inherent in measuring the exact width and depth of the pile when still saturated.

continually added to ponds with regular (daily) water exchanges. On some farms this can be excessive requiring removal each year.

Essentially, prawn farmers using flow-through systems are providing two settlement zones for suspended solids that are captured from adjacent natural waters; namely, the centres of their prawn grow-out ponds, and (in Australia) their dedicated settlement ponds, which capture further settleable solids prior to release of their wastewater. Many farmers have lined the sides of their grow-out ponds with HDPE plastic to minimise the erosion of pond embankments caused by circulating pond waters, since bank erosion can contribute significantly to settleable solids in the farming system (Burford et al., 1998). Full recirculation eliminates these regular additions of silts (with exchange water) to the farming system. And, in turn, PASF appears to further reduce this accumulation of settled solids and organic matter sludge of prawn ponds, probably because of sediment capture in the worm beds.

Worm production

A total of 570 kg of worm biomass was harvested in Season 1. This provided a production average of 1.056 kg m^{-2} over a 307-day culture period. This total was a combination of the smaller controlled-harvest samples taken during the culture phase and larger operational partial harvests. The total amount of supplemental feed added to the worm beds in Season 1 was 263 kg providing a 1:2.17 feed-to-worm conversion ratio. This artificial-feed conversion ratio does not take into account the algae-based feed and includes the considerable growth of worms during the water filtration phase of the experiment during which no supplemental feed was added (up to day 126).

In Season 2, a total of 353 kg of worm biomass was harvested providing a production average of 0.654 kg m^{-2} over a 328-day culture period. Again this included both controlled samples (to estimate survival and compare production across treatments) and operational harvests[14]. Most of this polychaete biomass was sold at critical times each season into fishing bait and prawn broodstock feed markets, either as live or frozen products. Supplemental feed totalling 92 kg was used in Season 2 (from day 177), which was much less than in Season 1 because of the longer availability of green water which alternatively fed the worms for longer in Season 2. This gave an artificial-feed conversion of 1:3.85. Pictures of live harvested worms in the storage raceway and frozen flat packs of worms are provided in Figure 13, and the harvested amounts and relative dates of final harvests for each bed with their respective ages at final harvest are provided in Table 6.

Operational worm harvests began on 29/05/14, 184 days after stocking in Season 1, but earlier in the second season (1/04/15) at 134 days after stocking. This earlier first harvest date in Season 2 was stimulated by market interest and larger prevailing worm sizes due to the relatively low stocking density. Although age (or culture period) had a strong influence on quantities harvested, which gradually increased during each season, this was unlikely to have been the main reason for the higher production in Season 1. This was presumably more due to the lower stocking densities applied in Season 2 (2,000 m^{-2}), compared with Season 1 (>6,000 m^{-2}). Although compromised by

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[14] Progressive harvesting prevented the use of final harvest figures for formal statistical analyses of the effects of the various experimental treatments (e.g., bed depth, etc.), because beds were of different ages when harvested. However, progressive harvest was necessary in the project to engage industry with this product at critical demand times, and experimental harvests provided robust comparisons regardless.

different ages and culture periods, in Season 1 higher mean (± se) total harvests were obtained from PASF beds that were stocked with one-month-old juveniles (1.24 ± 0.07 kg m^{-2}) than beds that were predominantly stocked with nectochaetes (0.78 ± 0.14 kg m^{-2}). The highest production in both seasons was a deep bed (Bed 3) in Season 1 which produced over 79 kg (1.474 kg m^{-2}) harvested 293 days after stocking 6,000 m^{-2} one-month-old juveniles.

Figure 13 Harvested live worms in raceway (left) and frozen flat packs of worms (right).

Harvest strategies each season took into account the densities and sizes of worms that were apparent from the controlled quadrat sampling. Beds with more smaller worms were harvested last because they were assumed to have more potential to grow to larger sizes for higher overall polychaete biomass production. Conversely, beds that appeared to have lower densities and larger worms were harvested first because they were assumed to have reached closer to their production potential, and because they could supply markets wanting larger worms earlier. Of course, sequential harvests were necessary to some degree because all beds could not be harvested at once, and as discussed previously the commercial orientation of this strategy prevented total harvest data being used in thorough statistical analyses. However, this is partially circumvented by using daily growth rates which took into account their relative ages (see last column in Table 6). Deep beds produced an average (± se) of 4.33 ± 0.25 and 2.47 ± 0.1 g m^{-2} d^{-1}, and shallow beds produced an average of 3.82 ± 0.44 and 2.8 ± 0.2 g m^{-2} d^{-1}, respectively in Seasons 1 and 2. In most cases it appeared that the longer the worms grew in the beds, the greater the amounts of polychaete biomass that could be harvested; but these data also show that daily growth rates generally increased during each season, which is likely due to the onset of summer and warmer waters for faster metabolism and growth later in each season, coupled with the exponential growth (as is seen in other animals) that is facilitated by increasing feed intake as the they grow to larger sizes.

Experimental harvests using quadrat samples provided more robust assessments of the polychaete populations in different beds in Season 1, and better comparisons of treatment effects including methods of stocking and bed depth. Densities of worms were not significantly (P>0.05) affected by sample date in Season 1, where means (± se) of 1,350 ± 231 and 1,461 ± 231 worms m^{-2} occurred in successive samples (Figure 14). Worm densities were also not significantly (P>0.05) affected by sand bed depth. However, much higher (P<0.05) densities did prevail in beds stocked with one-month-old juveniles, compared with beds predominantly stocked with worm larvae (nectochaetes) (Figure 14).

Table 6 Worm harvest weights for polychaete-assisted sand filters in Seasons 1 (2014, grey) and 2 (2015, blue).

Sequence and dates of final harvests	PASF bed	Harvest total (kg)	Harvest total (kg m^{-2})	Age after stocking* (d)	Polychaete biomass production (g m^{-2} d^{-1})
4/06/2014	8	22.9	0.425	191	2.22
18/06/2014	7	43.6	0.808	205	3.94
2/07/2014	9	42.6	0.788	219	3.60
15/07/2014	10	58.8	1.088	232	4.69
8/08/2014	6	58.5	1.083	253	4.28
20/08/2014	5	64.8	1.2	265	4.53
3/09/2014	4	54	0.999	279	3.58
17/09/2014	3	79.6	1.474	293	5.03
25/09/2014	1	73.8	1.368	301	4.54
1/10/2014	2	71.7	1.328	307	4.33
8/04/2015	9	14.8	0.274	138	1.98
6/05/2015	10	24.9	0.461	166	2.78
27/05/2015	8	28.3	0.523	187	2.80
1/07/2015	6	30.3	0.56	222	2.52
5/08/2015	4	48.9	0.905	258	3.51
19/08/2015	2	34.8	0.644	272	2.37
1/09/2015	7	50.8	0.94	284	3.31
16/09/2015	5	38.2	0.708	300	2.36
7/10/15	1	42.5	0.787	321	2.45
14/10/15	3	39.5	0.732	328	2.23

*Time from first stocking to date of last harvest.

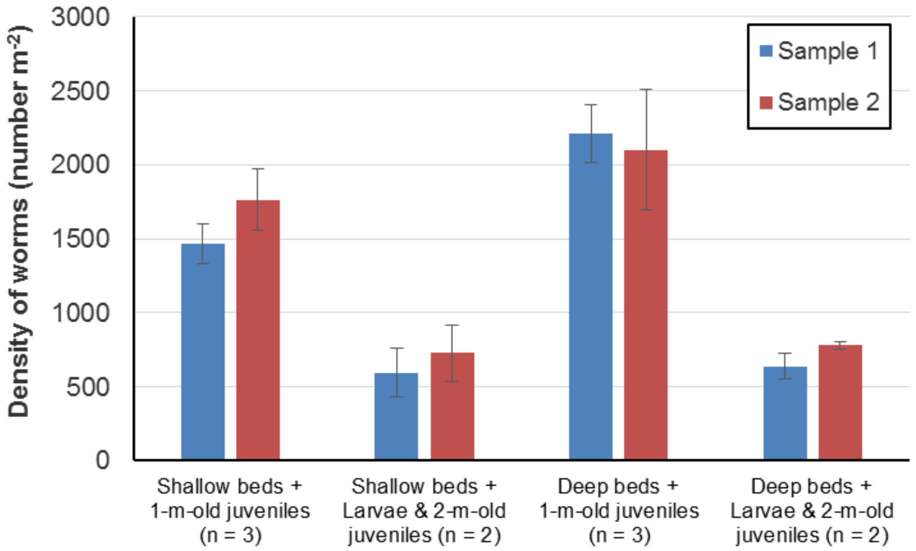

Figure 14 Mean (± se) densities of worms sampled from beds before (Sample 1) and after (Sample 2) supplemental feeding for seven weeks in Season 1.

Taking into consideration the total number of larvae and/or juveniles that were stocked in Season 1, survival was also not significantly affected by sample date or sand bed depth (Figure 15), but again, one-month-old juveniles provided much higher ($P<0.05$) mean rates of survival (32.2 ± 3.62 %) compared with larvae (9.7 ± 1.04 %). Furthermore, the mean (± se, n=2) survival of larvae stocked into PASF beds that had an underlying base of chlorophytes and copepods from one source pond (as estimated on the second sample date of Season 2) was 8.6 ± 1.75 %, which is similar to the result of 10.8 ± 1.08 % given in two different beds supplied with an underlying base of diatoms and rotifers from the other pond. These subtle changes in the ecology of PASF beds did not seem to affect the survival of larvae.

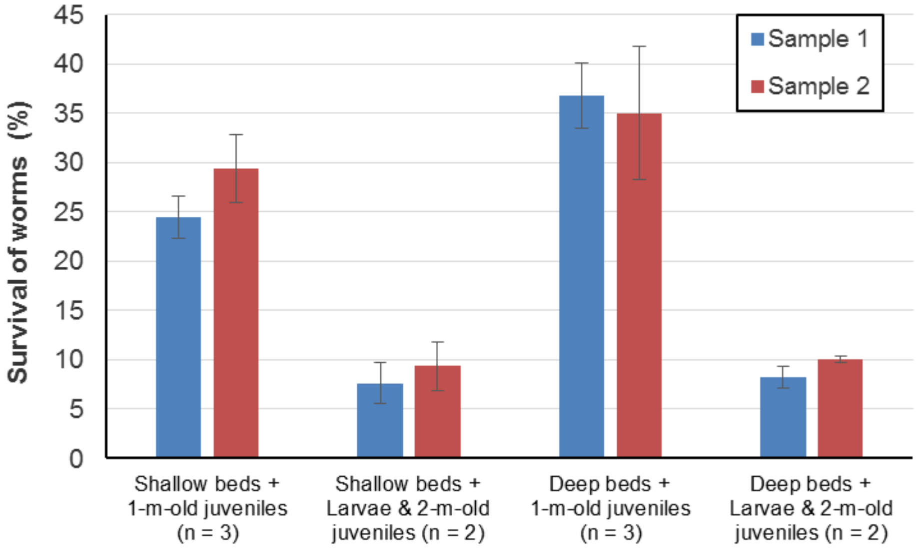

Figure 15 Mean (± se) survival of worms sampled from beds before (Sample 1) and after (Sample 2) supplemental feeding for seven weeks in Season 1.

Worm sizes (Figure 16) and worm biomass production (Figure 17) in Season 1 were again not significantly affected by sand bed depth, but both worm size and worm biomass were much higher ($P<0.001$) in older worms (in Sample 2). Worm sizes were larger ($P<0.05$) in PASF beds stocked predominantly with larvae, and we speculate that this is due to the lower densities and lesser competition that prevailed. Worm biomass was higher ($P<0.05$) for beds stocked with one-month-old juveniles.

Quadrat sampling of the worm beds in Season 2 revealed no differences ($P>0.05$) in these worm population statistics between shallow and deep beds (Table 7). As in the first samples taken in Season 1, this was undertaken in the middle of the season (126-128 days after stocking) and before any artificial feeding in Season 2. Biomass estimates had particularly large standard errors which demonstrated the gregarious nature of these animals and their spatial patchiness, particularly when stocked at lower densities. Compared with data generated at a similar stage in Season 1 (first samples taken 125-126 days after stocking), survival in Season 2 was marginally lower, the growth of worms was faster, biomass production was similar, and as previously mentioned, densities were much lower.

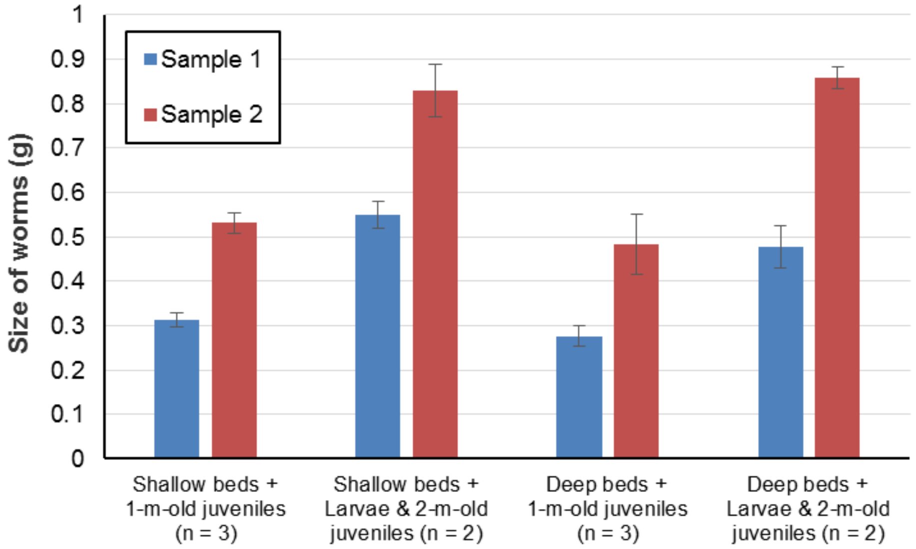

Figure 16 Mean (± se) size of worms sampled from beds before (Sample 1) and after (Sample 2) supplemental feeding for seven weeks in Season 1.

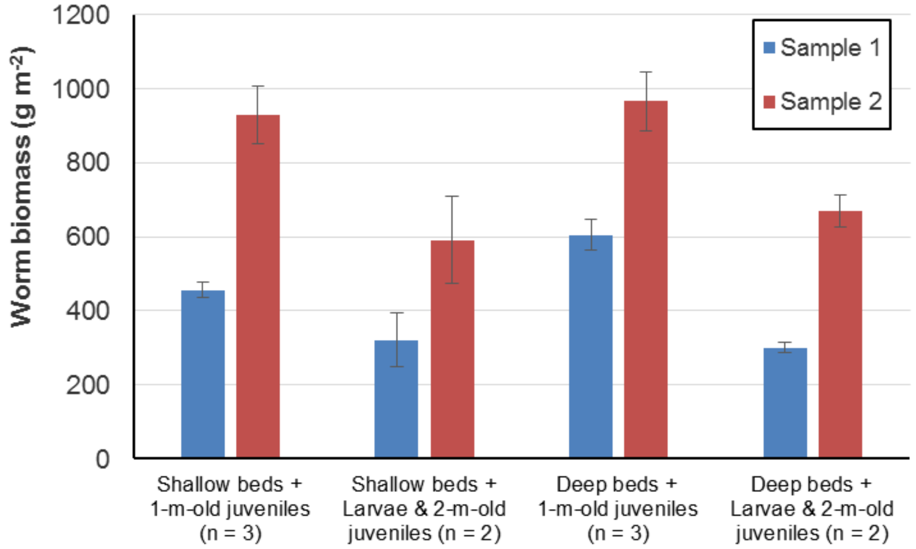

Figure 17 Mean (± se) biomass of worms sampled from beds before (Sample 1) and after (Sample 2) supplemental feeding for seven weeks in Season 1.

Table 7 Mean (±se) worm population assessments for 5 shallow and 5 deep beds, 126-128 days after stocking 2,000 m^{-2} one-month-old juveniles in Season 2. Within rows, means with similar letters are not significantly different (P>0.05).

Parameter	Deep beds	Shallow beds
Density (number m^{-2})	486.8 ± 96.4^{a}	488 ± 63.2^{a}
Survival (%)	24.34 ± 4.82^{a}	24.4 ± 3.18^{a}
Size (g)	0.82 ± 0.08^{a}	0.89 ± 0.02^{a}
Biomass (g m^{-2})	371.8 ± 42.2^{a}	437 ± 58.6^{a}

Wastewater treatment rates

The pond water exchange rates applied to the PASF beds during both seasons are given in the Materials and Methods/PASF operations section above (Figure 6). As previously discussed, the initial water treatment rates in Season 1 (3% of ponds volume d^{-1}) were lower than in Season 2 (4-5% d^{-1}), but later rose to higher levels (9-10% d^{-1}) than the maximum applied several weeks later in Season 2 (6-7% d^{-1}). The cumulative volumes processed by each bed are provided in Figure 18. In Season 1, a total of 36.18 megalitres of pond water was treated by the 10 PASF beds up to the 18/03/14, whereas in Season 2 a total of 42.36 megalitres of pond water was treated by the PASF beds up to 5/05/15.

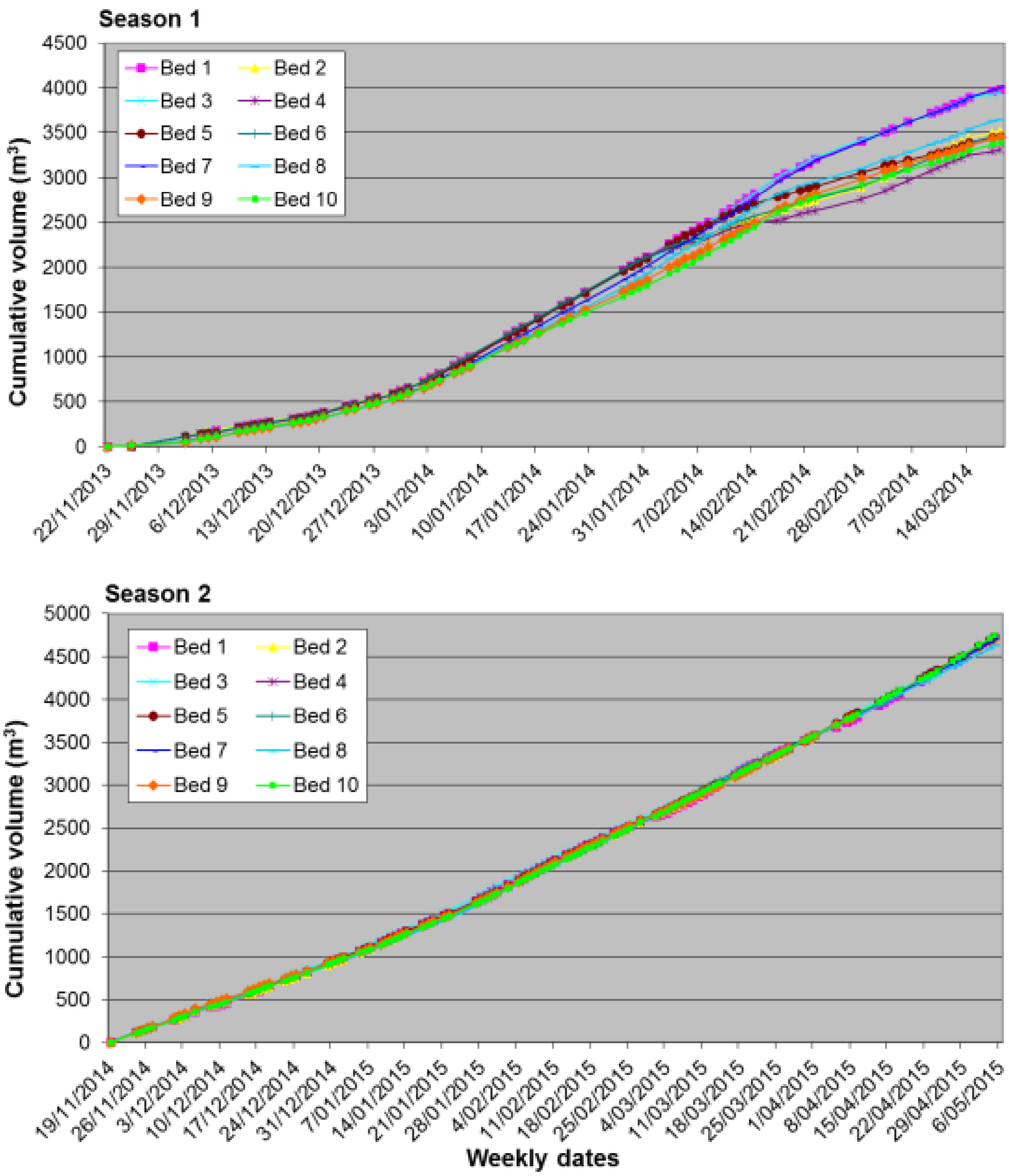

Figure 18 Cumulative water volumes treated by the different PASF beds in Seasons 1 and 2.

The maximum average pond water treatment rate for PASF beds in Season 1 was 872 L m^{-2} d^{-1} applied on 13/01/14, and slightly lower average rates of about 850 L m^{-2} d^{-1} were routinely applied in January and the first part of February in 2014. As also discussed previously, bed clogging limited the duration and water treatment rates of many beds in Season 1. To compensate for this in Season 1, water deliver rates were reduced for beds that were showing signs of clogging, and this reduced cumulative volumes treated for some beds from mid-February 2014. However, in Season 2, the water delivery rates to all beds were maintained at similar levels with a greater reliance on the short circuiting of beds if they started to clog. In Season 2, the maximum average treatment rate applied was 765 L m^{-2} d^{-1} on 18/03/15 but routinely treatment rates were less in the range 530-600 L m^{-2} d^{-1}. Figure 19 provides the drainage hole sizes used to equalise discharge rates for different beds.

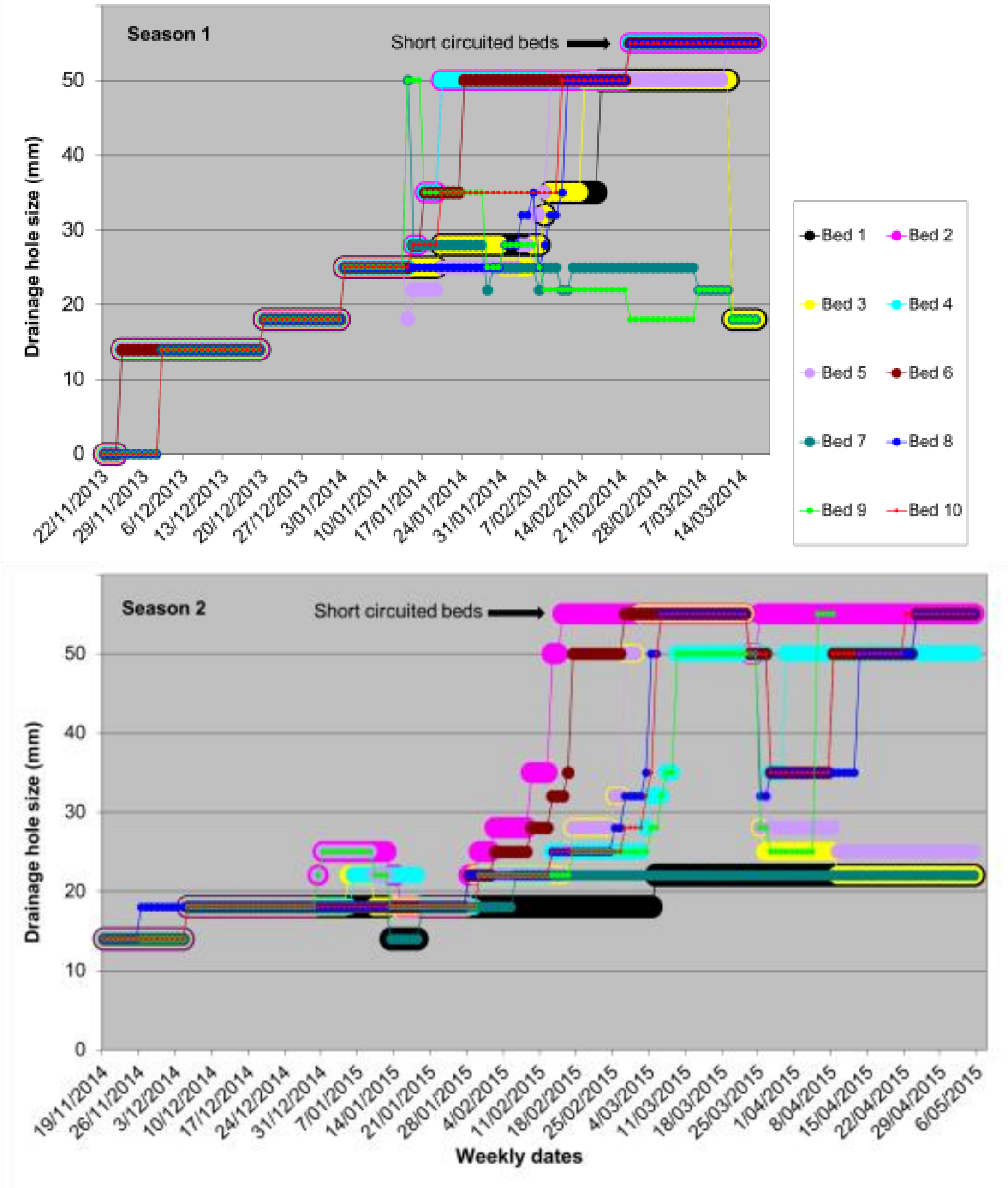

Figure 19 Drainage-hole sizes used to equalise PASF bed discharge rates in Seasons 1 and 2.

This analysis (presented in Figure 19) provides an appreciation of the close management that was applied to beds to equalise flows. Initially in both seasons, 14-mm drainage holes were used for all beds. Holes were then increased to 18 mm after three to four weeks. Thereafter, hole sizes were periodically increased (up to an open-pipe maximum of 50 mm) as the PASF beds took longer to fully drain. If drainage rates were too slow when the maximum hole size was installed, the beds were given a 19-mm short circuit. If beds drained more quickly than expected, hole sizes were reduced to the next size down.

Figure 20 provides the mean sizes of drainage holes for shallow and deep beds in each season (standard errors not shown for better figure clarity). Clear differences were apparent in both seasons, where shallow PASF beds required larger drainage-hole sizes to provide adequate discharge rates. All shallow beds towards the end of Season 1 needed to be short circuited so they could dry each afternoon. In Season 2, there was a two-week period in mid-March when hole sizes reached a maximum, and when shallow beds again needed to be short-circuited, but thereafter the beds recovered and hole sizes could be reduced. This recovery was sustained in deep beds through to the end of the experiment; however, shallow beds eventually again needed to be short circuited.

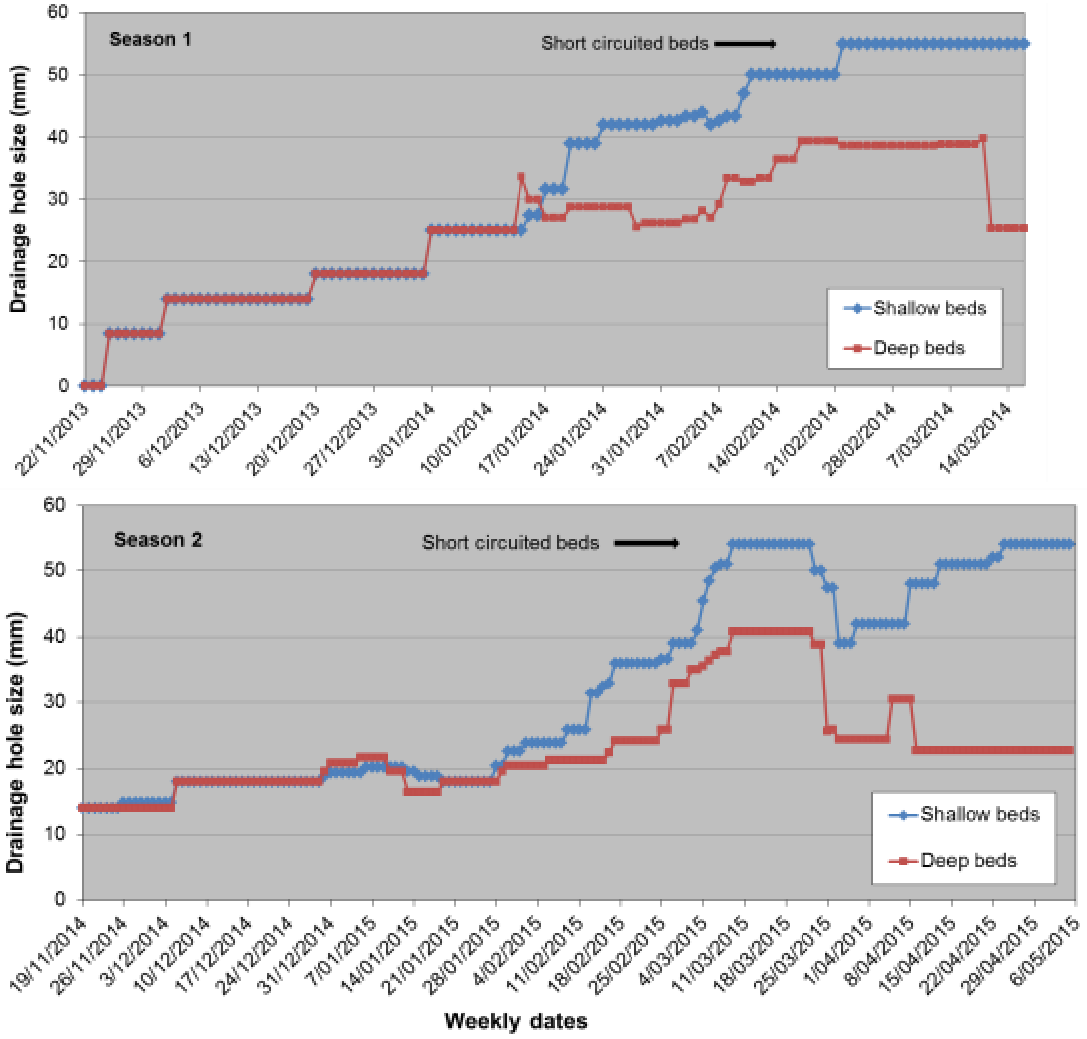

Figure 20 Mean drainage-hole sizes used to equalise drainage rates for shallow and deep PASF beds in Seasons 1 and 2.

The maximum possible drainage rates that could be provided by shallow and deep PASF beds after 94-97 days of operation are described below. When equal maximum head pressure was applied (Figure 21), deep beds provided marginally higher drainage rates that were not significantly different (P>0.05) from those of shallow beds. When operational heads of water were applied (Figure 22), differences caused by sand bed depth were more prominent, with deep beds providing significantly higher (P=0.01) discharge rates. Under both experimental conditions, drainage rates were much higher (P<0.001) in Season 2 than in Season 1 (Figures 21 and 22), which we attribute to the coarser ag-pipe sock that was used in Season 2. These data clearly show that deeper PASF beds can generally provide higher rates of water treatment than shallow PASF beds, perhaps because of their greater sediment-holding capacity.

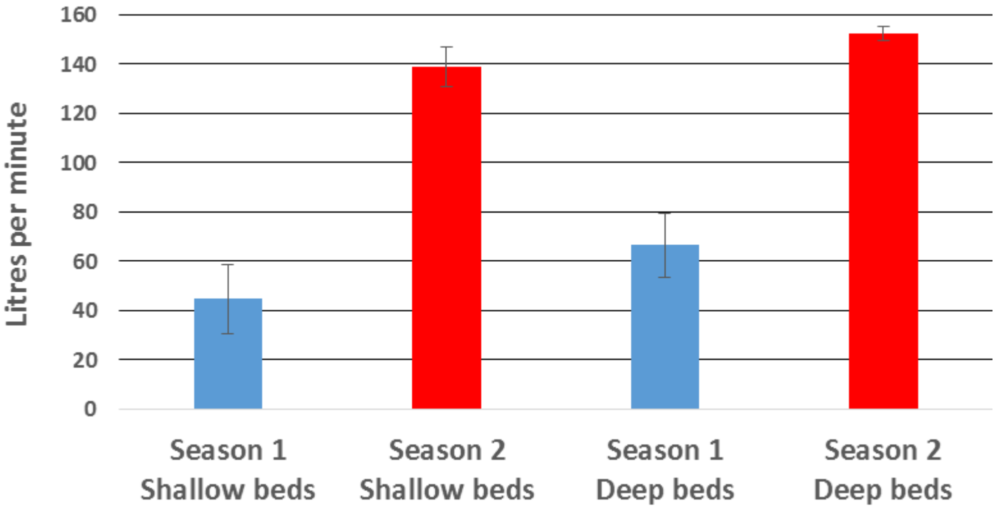

Figure 21 Mean (± se, *n* = 5) drainage rates from PASF beds after 94 days in Season 1 and 97 days in Season 2 with equal maximum heads of 60 cm for shallow and deep beds.

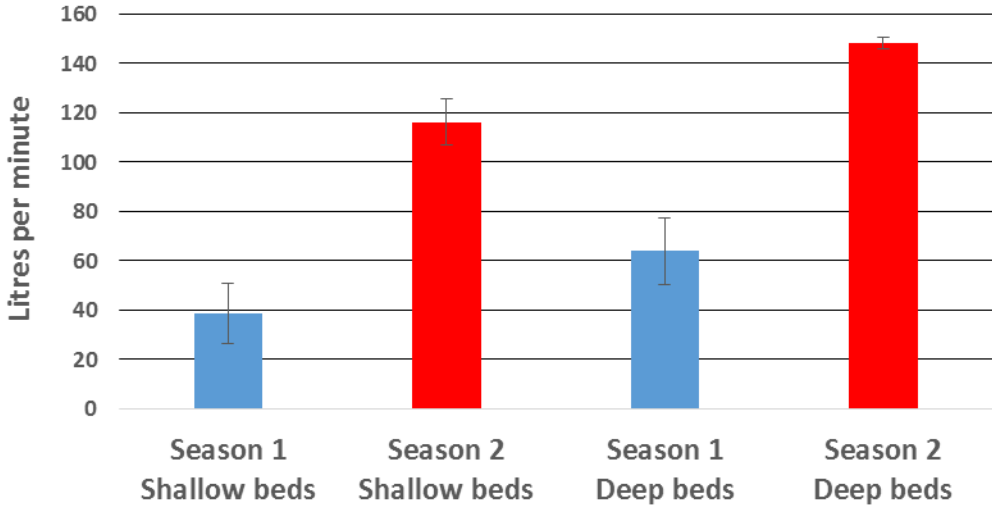

Figure 22 Mean (± se, *n* = 5) drainage rates from PASF beds after 94 days in Season 1 and 97 days in Season 2 with typical operational heads of 43 cm for shallow and 53 cm for deep beds.

Algal blooms

Compared with un-recirculated (flow-through) pond dynamics observed in previous years at BIRC (P.J.Palmer, S. Wang, qualitative-unpublished data), the recirculated ponds in this study appeared to have particularly strong and consistent phytoplankton blooms. When water was being recirculated back from the PASF beds there seemed to be less algal species shifts and no significant clear-water periods in between different blooms. Secchi depths demonstrated typical patterns as blooms developed. The blue dye added to both ponds to reduce light penetration when prawn postlarvae were stocked persisted for an extended period due to the lack of system water discharge/ replacement. There was a tendency for one pond to develop a particular algal change and then a few days later the other pond would tend to follow suit. However, the two ponds in our recirculation system never had exactly the same planktonic compositions, suggesting a degree of independence for bloom developments that was not overwhelmed by the water filtration being applied by PASF.

Blooms had a wide range of phytoplankton species, presenting the PASF beds with a typical range of filtration challenges during the study. This varied from large-celled diatoms (Figure 23) which settled quickly and matted readily on the sand bed surfaces, to small chlorophytes which had a low tendency to settle and appeared to penetrate deeper into the pore space of the sand.

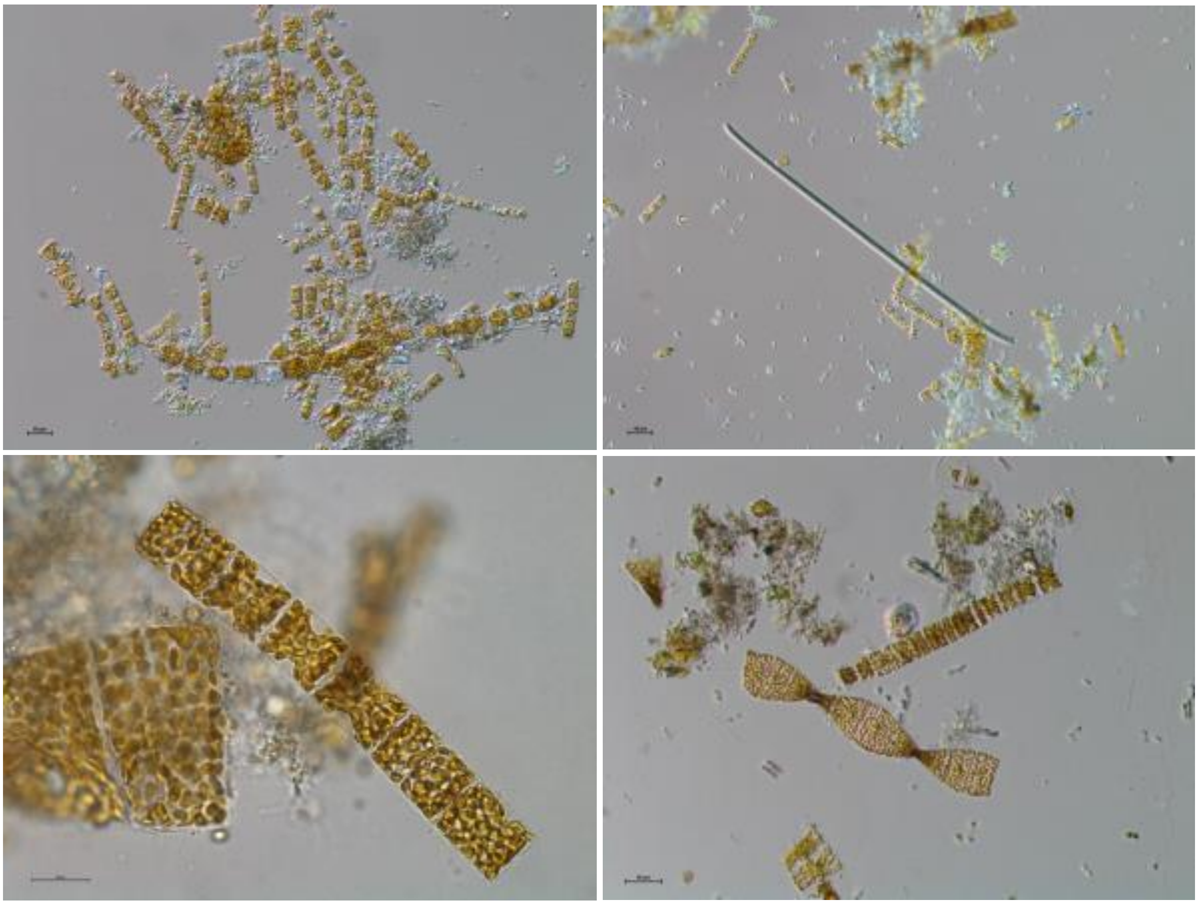

Figure 23 Typical chain-forming diatom species proliferating in the recirculated ponds towards the end of the 2nd Season (mid-February 2015): Pond G1 with smaller-celled species (top left), and very low prevalence of *Oscillatoria* sp. (top right); Pond G2 with larger-celled species *Bellerochea* sp. (bottom left) and *Streptotheca* sp. (bottom right). Scale bars are 50 μm except bottom left (20 μm).

The ponds followed normal initial trends for yellow-green blooms soon after fill, followed by green, then yellow-brown blooms several weeks later. In Season 2 an unusual pale colourless bloom (unidentifiable cells with low-powered microscopy) developed and prevailed for a few weeks in both ponds early in the season, just before the PASF beds began filtering water in the system. This turned the PASF beds a powdery off-white colour, but did not appear to dramatically affect bed percolation rates, and it was not possible to determine if this affected the survival or behaviour of the stocked worm juveniles. *Oscillatoria* sp. was noted with very low prevalence in one pond late in Season 2 (February) (Figure 23), but neither this nor other potentially problematic algal species proliferated. The filamentous configuration of that species could be expected to be well-filtered by the sand beds, and this could be expected to offer some degree of active control for many other species with larger cell sizes and strand-like morphology.

In both seasons strong blooms of chain-forming diatoms developed late in the season (Figure 23), seemingly on cue when pond carrying capacities for prawns and feeding rates were at their maximum. This coincided with prawns attaining an average size of about 30 g and the start of partial harvests. These large-celled chain-forming diatoms tend to grow very quickly and build to high loading levels with significant algal scum developing on the surface of ponds each day. Since they are very readily removed from water with sand filtration, their rapidly accumulating bulk on the sand surface represents a particularly difficult situation for the PASF beds. Ideally, they would occur when the PASF beds have been in operation for at least a few months, when the worms have grown to an appreciable size so their higher appetite can go some way towards matching the relatively fast organic accumulation rates on the surfaces of the sand-worm filters. In both seasons during these more difficult-to-manage blooms, we applied consistent rates of water treatment through the PASF beds, and as expected they struggled with significant levels of blockage. At this point in Season 1 we reverted to water exchange in the prawn ponds using clean seawater, so that the prawn and worm crop were not potentially compromised and could be properly assessed for the purposes of the study. However, in Season 2 we continued with normal PASF recirculation, partially short-circuiting (see Materials and Methods section) those beds which blocked, and this in turn led to successful harvest of all products in the fully-recirculated system. It is particularly pertinent here that in this later case some of the deeper beds continued to operate very effectively until the large-celled blooms gave way to more manageable unicellular chlorophyte blooms. The reason that these particular beds continued to operate under such high organic loadings is not clear at this stage, but their extra depth is likely a factor.

Blooms of bulky larger-celled species, such as *Streptotheca* sp. (Figure 23), are common in ponds at BIRC, and we accept that they do appear to represent a problematic situation for PASF beds in their current configuration. In the past, these diatom blooms have tended to persist for only short periods (1 week) before giving way to other more easily managed species. This may be due to their relative size and associated larger nutrient or micronutrient demands. Given that it will always be possible to overload PASF beds with unrealistic levels of organic matter, and that these bulky blooms do tend to naturally run a relatively rapid boom-and-bust pattern of occurrence, one solution to this would be to reduce PASF treatment rates during their high prevalence in recirculated systems in the future; for example, by varying the operational flow pattern to only one tidal flow per day instead of the normal three. In other words, rather than directing a large portion of the bloom through to the PASF beds, it may be better to allow the majority of it to settle in the middle of the grow-out ponds where organic matter naturally accumulates regardless. This approach could avoid the potential for organic overload and blocking of the PASF beds, and accelerate the demise of the bloom that could otherwise be sustained for longer periods by higher available nutrients returning to the ponds with the PASF-treated waters.

Zooplankton, incidental non-target species and surface-fouling organisms

A range of planktonic animals also proliferated in the ponds' ecosystems after seed-organisms entered in the initial-fill seawater (through the 300-μm-rated screen). Strong mixed pelagic and benthic copepod blooms prevailed in both ponds in both seasons, whilst the prevalence of various species of rotifers rose and fell away periodically. Tube worms and barnacles (which have planktonic larvae) settled and grew in typical fashion on the sides and bottoms of ponds, on paddle wheels and on other pond furniture, but not in the PASF beds.

Filamentous algae (e.g., *Enteromorpha* sp., which also have planktonic gametes) also typically settled and grew on the paddle wheels and securing ropes during the final two months of the prawn cultures, but again it did not proliferate in the worm beds. Zooplankton and macro-algal cells that were routinely pumped with water from the ponds to the PASF beds would have added significantly to the organic matter being routinely processed by the PASF beds. Although the free-water above the worm bed surfaces would have provided a temporary aquatic refuge for short-term accumulation of these pond-based organisms, the periodic complete draining and daily drying of sand surfaces in the sun rendered them unviable, and provided an additional mix of nutritious foods for the polychaetes. Since intertidal polychaetes are one of the few animals that can survive under these relatively harsh conditions, those stocked into the PASF beds became the dominant consumers of this pelagic-based detritus.

However, it is important to note that in previous work at different sites over the last several years, other benthic species have co-occurred at low levels with *P. helleri* in the PASF beds. At one prawn farm sited in a mangrove environment, significant amounts of a small unidentified burrowing bivalve were harvested with the worms (Figure 24), and there have also been low numbers of very small surface-dwelling gastropods that inhabit the benthic algae which can develop on the beds.

Figure 24 Bivalve by-product harvested with worms from PASF beds at another prawn farming site.

Additionally, at another farm where the PASF beds were allowed to operate for several weeks without drying in the sun (during a particularly wet season when beds were necessarily protected with a lens of brackish water), barnacles settled on sand grains and some persisted through to the worm harvest regardless of later more regular sun-drying episodes. It therefore follows that the source of water supplying the beds will govern the potential mix of other species that can co-occur with the target polychaetes, and that the regularity of surface drying so that their larval settlement is impeded will govern their degree of successful recruitment.

Barnacle and tube worm densities appeared to vary spatially on the walls of ponds (Figure 25), but on average were not significantly different (P= 0.07) in the two ponds at the end of Season 2. The average (± se) densities on the walls of the ponds after drain harvest were 4,775 ± 772 m^{-2} in pond G1 and 6,914 ± 808 m^{-2} in pond G2. Although not investigated in the present study, the potential to screen inflow seawater with the PASF beds has been suggested as one way to exclude (or reduce) these problematic encrusting organisms from broad-scale prawn culture systems. Whilst this would require changes to normal pond-filling rates and several modifications to our standardised PASF bed operations, the advantages offer greatly reduced cleaning and maintenance of pond equipment, and possibly lower lime inputs needed to maintain appropriate alkalinity levels in pond waters, which are otherwise reduced through incorporation of calcium carbonate in barnacle shells. Potential problems with this approach could be reduced diversities of algal species which could be selected by the broad-scale sand filtration processes, and reduced seed populations of copepods which provide natural feed for the postlarval prawns when initially stocked.

Figure 25 Recently drained ponds G1 (left) and G2 (right) showing encrusted barnacles and tube worms on the sides and bottoms at the end of Season 2 after only one prawn crop in newly-lined ponds.

Seasonal water qualities

Many water qualities are of vital importance in prawn culture systems. Their levels and functional interactions have direct bearing on the health and growth of prawns, and many are regulated in licenced discharge arrangements. This section details our specific studies in this area over two successive seasons, and provides some relevant background for each water quality parameter in terms of the improvements (or otherwise) that PASF can provide. It is important to consider here that PASF-treated water was fully recirculated, even though BIRC discharge limits are provided.

Water pH

Pondus Hydrogenium (pH) is a measure of the balance between positively charged hydrogen (H+) ions (which form acids when mixed with neutral water) and negatively charged hydroxide (OH-) ions (which form bases when mixed with neutral water). When these are equal the water is neutral (pH = 7), but preferably in a shrimp pond there will be more hydroxide ions so that the pH remains between 7.5 and 9.0 (Australian Prawn Farming Manual, 2006). Diurnal pH fluctuations occur naturally in eutrophic (nutrient rich) waters because of chemical reactions associated with the consumption of carbon dioxide (during the day) from photosynthesis by the algal bloom, and its production by the respiration of algae (at night) and other microorganisms and animals in the pond (during night and day). Hence there are natural patterns of pH rises during the day and declines at night, and it is recommended that this diurnal fluctuation be kept to a minimum in prawn ponds (e.g., ideally < 0.5 change per day: Australian Prawn Farming Manual, 2006).

Other factors such as the percentage of un-ionised ammonia are also important when considering the pH of pond water at different times in the crop. For example, lower pH fortuitously tends to occur later in the crop when organic matter levels are highest in the pond. The resultant lower pH values work in the favour of minimised toxicity when ammonia from prawn excretions are at their highest.

The diurnal fluctuations of pH that are generally typical of phytoplankton-rich outdoor ponds occurred as expected in both ponds in both seasons (Figure 26). Overall, pH levels in Season 2 were somewhat lower than in Season 1, and this was probably mainly due to the higher organic loading associated with higher stocking densities and feeding in Season 2. In particular, greater daily fluctuations in pH appeared to occur from February in Season 2, and this was caused by the slowly reducing alkalinities of ponds (see below) which reduced the buffering capacity limiting pH changes of pond water. However, during both seasons, PASF discharge remained well within the BIRC discharge limits of 6.5-9, and pond pH levels generally remained within the recommended range outlined above.

In Season 1, pH levels in discharge from deep and shallow beds were similar ($P>0.05$) for all sampling occasions except those on the 3/12/13 and 17/12/13 when deep bed pH levels were significantly lower. Similarly in Season 2, differences ($P<0.05$) in pH only occurred on 4 of the 20 sampling occasions (25/11/14, 17/03/15, 21/04/15, 5/50/15), when deep bed pH levels were again significantly lower. Wet weather operational mode for the PASF beds (on 7/01/14 and 12/02/14, see Figure 26) did not appear to affect the pH of water; the difference between dry-operational mode on 10/02/14 and wet-operational mode two days later was not significant ($P>0.05$).

In both seasons the pH of PASF discharge tended to closely reflect trends in morning pH readings in ponds. Using morning (am) pH measurements in ponds for detailed comparisons, mean PASF pH levels in Season 1 were significantly lower ($P<0.05$) than ponds on all but two sampling occasions (17/12/13 and 7/01/14). In Season 2, mean PASF pH levels were only different ($P<0.05$) from mean morning pond pH measurements on 6 of the 20 sampling occasions for deep beds (25/11/14, 16/12/14, 23/12/14, 7/04/15, 21/04/15, 5/05/15), and on 4 of the 20 sampling occasions for shallow beds (17/03/15, 7/04/15, 21/04/15, 5/05/15). For all but one of these sampling occasions (17/03/15) the PASF pH was lower ($P<0.05$) than the comparative pond pH.

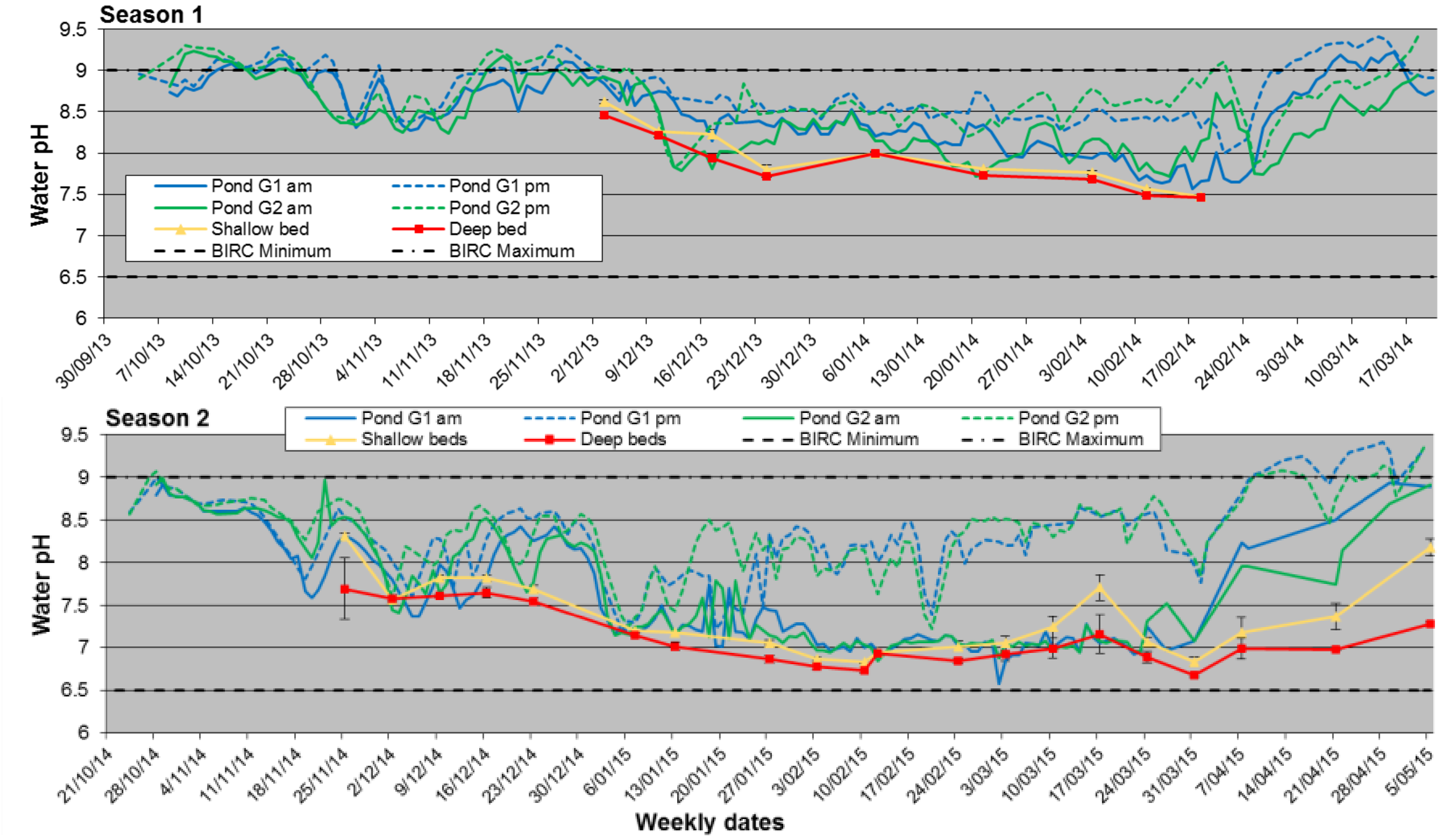

Figure 26 Water pH levels in two recirculated prawn ponds (G1 and G2) and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge minimum and maximum for BIRC are also provided.

Dissolved oxygen

Dissolved oxygen (DO) levels of >5 mg L^{-1} are generally recommended for optimal survival and growth of prawns in ponds (Australian Prawn Farming Manual, 2006). In the current study (as in commercial practice) levels of 4 mg L^{-1} were considered acceptable but when levels started approaching 3 mg L^{-1} supplemental aeration was applied.

Diurnal fluctuations in dissolved oxygen that are typical of phytoplankton-rich outdoor ponds occurred as expected in both Seasons (Figure 27). In Season 1, the mean DO levels in filtered water from deep and shallow beds were similar ($P>0.05$) on all sample days except 10/12/13 and 23/12/13 when deep beds had higher levels. In contrast, in Season 2 deep beds mostly had lower levels of DO than shallow beds. The overall mean level for deep beds (0.67 mg L^{-1}) was found to be significantly lower ($P<0.05$) than that of shallow beds (1.7 mg L^{-1}). Since new (previously unused) sand was necessarily used in Season 1, differences between seasons may be partially attributed to chemical changes and/or residual levels of organic matter left in the re-used sand in Season 2.

Wet operations mode for the PASF beds (on 7/01/14 and 12/02/15) when compared with the samples before and after, did not affect ($P>0.05$) the DO of PASF discharge.

As with pH, early morning DO measurements in the ponds were used for comparison with the DO levels in PASF bed discharge. The PASF filtration process routinely reduced DO to levels well under the minimum morning readings taken in the ponds, and often to very low levels close to zero. Compared with Season 1, this depression of DO seemed to occur earlier in the cycle in Season 2, again potentially because residual organic matter in the sand would have enhanced bacterial activity in the beds in the second year of the study. However, there was a distinct increase in PASF DO from 24/02/15 in Season 2 when levels generally increased, and on 10/03/15 and 17/03/15 levels from shallow beds appeared to mirror levels in the morning (am) in ponds. This occurred at a time when the PASF beds were facing their heaviest challenges from the proliferation of dense brown diatom blooms in both ponds (Secchi depths of 25 cm). At this point the PASF beds began clogging and needed increased drainage hole sizes or short circuits (see Figures 19 and 20 above) so they could drain in time for daily sun drying. This necessary short circuiting of beds would have certainly increased the DO levels in PASF discharge, as would also the tracking of water through the beds (more evident in shallow beds) whereby water bypasses the sand filtration process and avoids the DO depression caused by biological activity in the sand.

This DO depression (or lack thereof) appears to help confirm that the PASF beds are in fact operating correctly. For example, during water quality sampling on the morning of 13/01/15 in Season 2, it was noticed that discharge from Bed 2 (one of the shallow-bed replicates) had a much higher DO level of 3.75 mg L^{-1}, compared with the other beds which were in the order of 0.2 mg L^{-1}. Upon closer inspection it was discovered that part of the under-sand drainage pipe system had given way to the head pressure so that sand and unfiltered water were entering the network of discharge pipes. This was easily rectified by digging up the damaged pipe, repairing it and re-burying it for continued normal operations. Whilst all water quality data collected from that bed on that sample day was omitted from the study, this instance helps to demonstrate one simple way that PASF beds can potentially be checked for their individual filtration efficiencies.

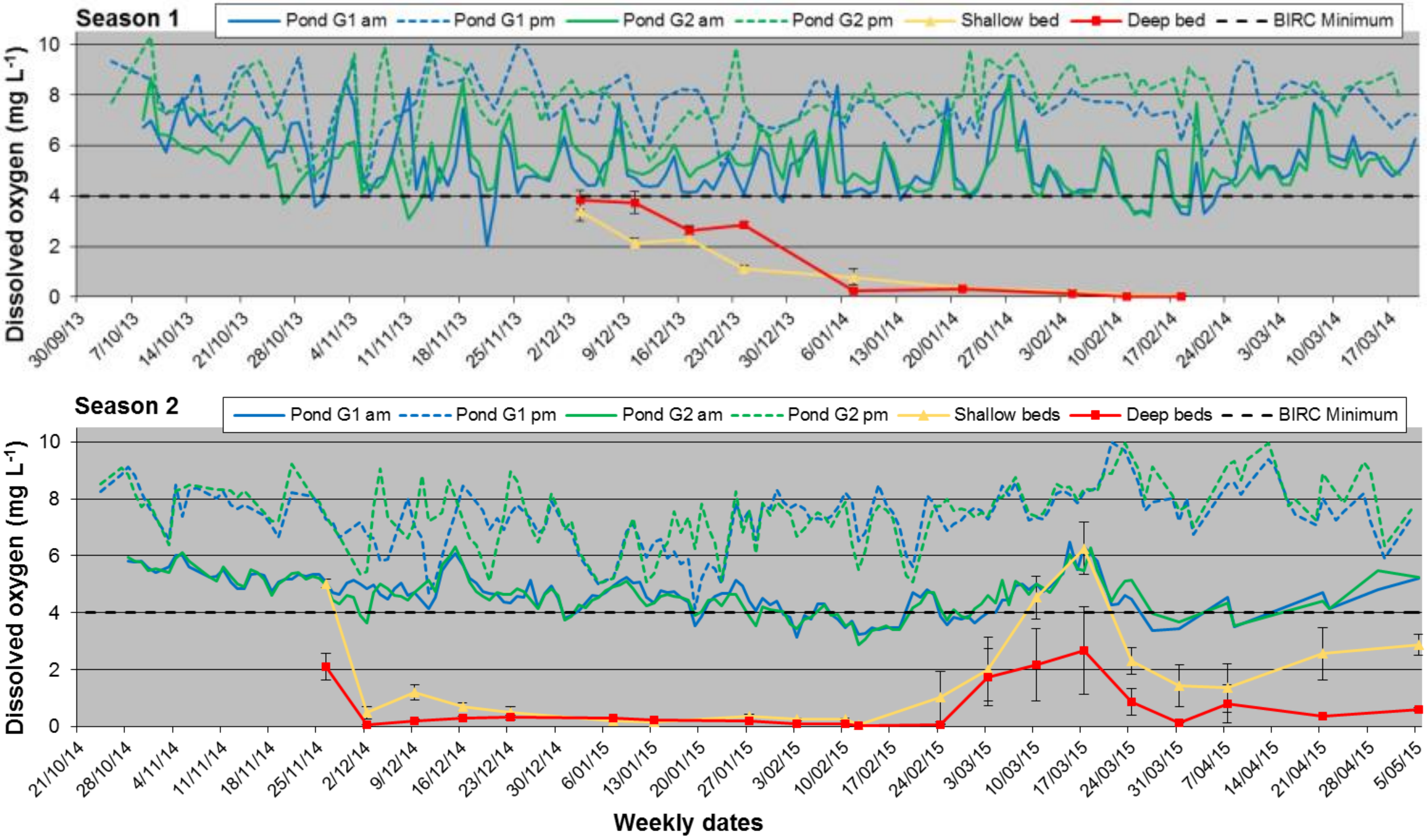

Figure 27 Dissolved oxygen levels in two recirculated prawn ponds (G1 and G2) and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge minimum for BIRC is also provided.

Temperature

The apparent water temperature differences between the two ponds during the first six weeks of prawn culture in Season 1 (Figure 28) are attributable to the elevated temperatures in the covered nursery pond (N1) where pond G2 prawns were initially held (before 15/11/13). Nursery pond N1 had better solar collection properties and hence was warmer. Fortuitously, this higher water temperature helped to accelerate the growth of those prawns and compensate for the lower relative abundance of natural food (plankton), such that overall growth of the two replicate prawn groups during this initial period was similar.

After the 15/11/13 (when prawn juveniles were transferred from the proxy pond) water temperatures in the two (now similar) grow-out ponds appeared to track in unison with about 2°C daily fluctuations, and with three cooler periods in the remainder of the season (early-December, early-February, and mid-March) when morning (am) temperatures reaching as low as 23-24°C, and several warmer periods when afternoon (pm) water temperatures rose above 30°C.

In Season 2, water temperatures in both ponds reacted similarly to environmental conditions, again with about 2°C daily fluctuations, although with only one notable period in mid-December when morning water temperatures fell below 24°C, and two particularly warm periods (early- and mid-January) when afternoon water temperatures rose above 32°C. Although am and pm water temperatures were monitored for an overall longer period in Season 2, during the main part of grow-out (mid-November to end of March) available data suggest that Season 1 (2013/14 with an average of 26.6°C for am and 28.2°C for pm readings) was a somewhat cooler year than Season 2 (2014/15 with an average of 27.3°C for am and 29°C for pm readings). These apparently warmer conditions in Season 2 may have contributed to faster growth and larger final harvests compared with that of Season 1.

In both seasons there was a tendency for deep beds to discharge water with lower temperatures than shallow beds. In Season 1, the discharge temperature from deep beds was significantly lower ($P<0.05$) than that of shallow beds for all but two sampling occasions (21/01/14 and 18/02/14), and in Season 2 this occurred on the first five sampling occasions and later on the 27/01/15, 10/03/15 and 17/03/15.

In general there were no clear trends of statistical significance when the morning water temperatures of ponds were compared with the bed discharge temperatures. This is largely because PASF discharge temperatures are affected by the weather whereby on fine days the sun can be expected to quickly heat the shallow body of water over the beds, and hence the percolating water. The observed water temperature differences between deep and shallow beds described above are evidence of this weather effect where the temperature of deeper sand beds would take longer to be affected by the temperature of percolating water given the similar percolation rates of deep and shallow beds as set within the experimental and systems designs of the study. However, early in Season 2 (first four sampling occasions) the temperatures of discharge from shallow beds was higher ($P<0.05$) than morning pond temperatures, and thereafter (except 17/03/15) deep- and/or shallow-bed discharges had lower ($P<0.05$) temperatures than pond waters in the early mornings.

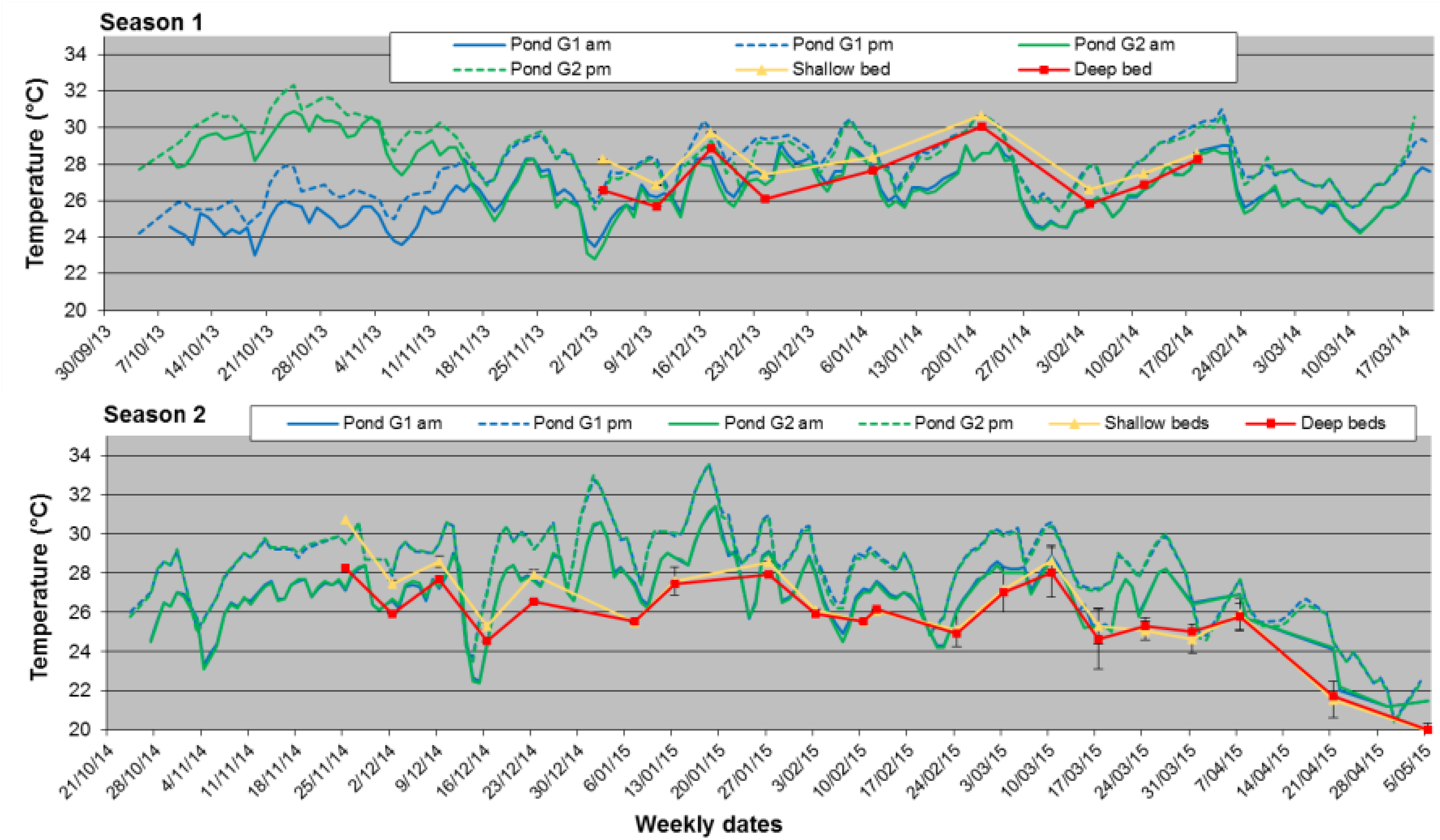

Figure 28 Water temperatures in two recirculated prawn ponds (G1 and G2) and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Salinity

Prawn pond salinity tends to be site dependent: farms in northern Queensland are more regularly affected by monsoonal rainfall than more southern farms. Although black tiger prawns and the marine polychaete used in this study (*Perinereis helleri*) are both very tolerant of low salinities (both known to survive down to 10 ppt), growth optima are higher (in the order of 15-25 ppt for *Penaeus monodon* - Australian Prawn Farming Manual, 2006). Farmers therefore need to watch the weather closely and apply specific management protocols to the culture systems during times of heavy rainfall to minimise rapid declines in pond salinities, which can cause stock stress and less stable pond plankton conditions. Conversely, during relatively dry conditions, salinities in fully recirculated systems can be expected to slowly rise due to evaporation.

Relatively high salinities prevailed in ponds during the study in both seasons (Figure 29). In both seasons there was enough rainfall to largely compensate for evaporation, although salinities did rise above those of seawater used to initially fill the ponds. In Season 1 the average salinities during recirculation were 39.7 ppt for pond G1 (max of 41.9; min of 37.3) and 38.9 ppt for pond G2 (max of 42.1; min of 34.2)[15]. The small differences here between the two ponds in Season 1 were mainly due to the earlier fill of pond G1 and accumulated salinity from evaporation prior to filling pond G2. In Season 2, the average salinities during recirculation were 36.3 ppt for pond G1 (max of 41.4; min of 26.7) and 36.3 ppt for pond G2 (max of 41.4; min of 26.8).

Salinity was not directly affected by the PASF filtration process in the study. However, it is important to highlight that the inclusion of a PASF system in the farm design would provide additional area for water evaporation or collection of rainfall within the culture system. Whilst these effects would likely be overwhelmed by each year’s prevailing weather conditions, they could accentuate salinity extremes in years when less favourable weather conditions prevail.

Secchi depth

As described in the Methods, Secchi depth is a convenient measure of the turbidity of pond water. High turbidity in prawn ponds is normally due to the prevailing phytoplankton bloom. Relatively high turbidity is necessary to provide a low-stress environment for optimal survival and growth of prawns. This also stops sunlight from penetrating too deep into the pond to limit the growth of benthic algae on the ponds’ sides and bottoms. Secchi depths of 20-30 cm are considered optimal for prawn pond management (Australian Prawn Farming Manual, 2006).

Figure 29 shows the prevailing Secchi depths for ponds in both seasons. In Season 1, the average Secchi depth (after 15/11/13) for pond G1 was 43 cm (max of 90; min of 20), and for pond G2 was 50 cm (max of 100; min of 20). Whilst the two ponds had relatively similar Secchi depth trends from mid-December 2013, before that pond G2 demonstrated less stable turbidity and more periods when there were higher levels of light penetrating into the water column (likely the result of the later start to prawn feeding in that pond). In Season 2, both ponds had very similar Secchi depth patterns, where the average for pond G1 was 37 cm (max of 100; min of 20), and for pond G2 was 40 cm (max of 100; min of 20).

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[15] These summary data do not include salinities in either pond before the 15/11/13 when the stock destined for pond G2 were grown in the previously described proxy pond (N1). Data shown in Figure 28 include the salinities of the proxy pond before prawns were transferred to pond G2.

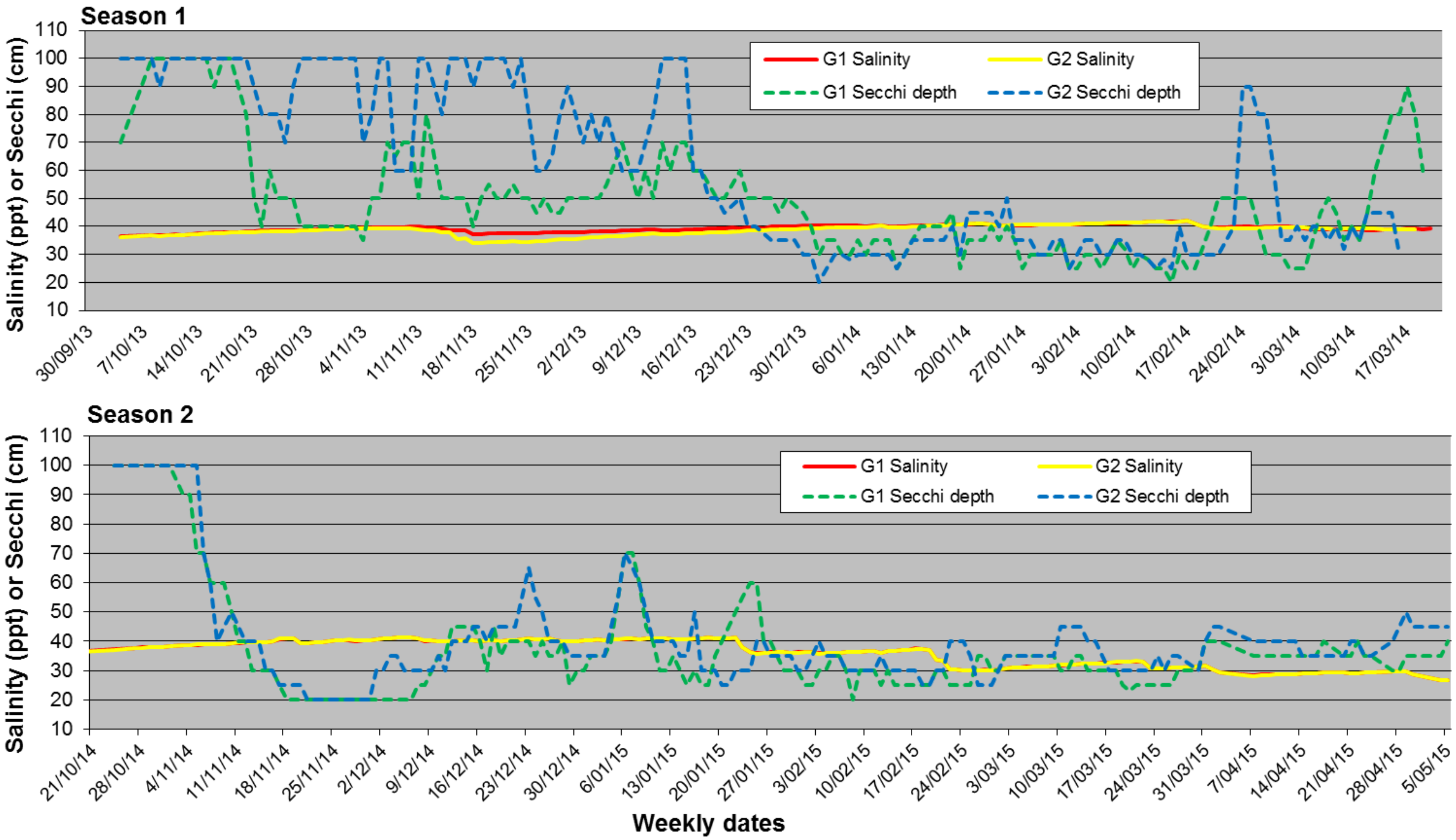

Figure 29 Salinities and Secchi depths in two recirculated prawn ponds (G1 and G2) in Seasons 1 and 2.

Total suspended solids

Total suspended solids (TSS) is a weight-based measure of inorganic and organic particles that are held in the water column. It can generally be described as any material in water that will not pass through a filter[16]. In prawn culture ponds this can include silt and detritus that is continually re-suspended by stock movements, flocculated bacterial particles that develop when organic matter and suitable nutrients are available in an aerated environment, and a multitude of phytoplankton species which naturally occur and proliferate with the availability of dissolved nutrients.

In both seasons there were no significant differences ($P>0.05$) between TSS levels from shallow and deep beds. In Season 1 there was some evidence ($P=0.053$) of an interaction between time and PASF treatment (bed depth). Whist filtration through the PASF beds provided TSS reductions at most time points, statistically significant ($P<0.05$) reductions compared to the ponds were only apparent for samples taken on the 11/02/14 and 18/02/14 (Figure 30). In Season 2 however, the interaction between time and treatment (bed depth) was not significant ($P=0.087$), and highly significant ($P<0.001$) TSS reductions occurred throughout the trial.

Unlike many other parameters that were studied, there did appear to be a marginal effect on TSS of the wet-weather operations mode. Removal efficacies of PASF were somewhat lower on 7/01/14 compared with samples immediately before and after this (high-rainfall date) in Season 1, and in Season 2 this is evident given the increase in TSS levels discharging from PASF from 10/02/15 (on dry ops) to 12/02/15 (on wet ops). However, given the variability over time and the similar trends of TSS in the pond water at these times, these effects were not significant ($P>0.05$).

Reductions in TSS provided by PASF in Season 1 averaged 40.1% and ranged from 2.2% (for first samples taken on 3/12/13) to 82.3% (on 18/02/14), and in Season 2 averaged 62.8% and ranged from 18.3% (average of first samples taken) to 91.5% (on 24/02/15). Collectively, PASF therefore appears to have provided very effective and continuous TSS removal during this study, and successfully provided levels that were well below the BIRC discharge limit of 50 mg L^{-1}. The lower efficacy for TSS removal demonstrated each season soon after the PASF beds began operations can likely be attributed to the initial flushing of residual particulate matter as the sand mass settles. And importantly, despite the short-circuiting of some sand beds (operationally necessary for beds that were clogging in Season 2) PASF continued to provide significant TSS reductions.

Turbidity

Turbidity is another measure of water clarity that is most often applied to potable water[17]. It is greatly affected by suspended solids but importantly also includes very small particles (e.g., bacteria) that are not visible to the naked eye but nevertheless cause the water to appear cloudy. As opposed to TSS, turbidity is not based on the weight of suspended material, but rather provides a measure of the scattering of light (as does a Secchi disk). Turbidity is measured with an instrument called a nephelometer and uses units called Nephelometric Turbidity Units (NTU).

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[16] TSS analyses in the present study used standard glass fibre filters with a pore size of 1-1.2 μm.

[17] Municipal water treatment plants strive for turbidity levels of 0.1 NTU in drinking water, whereby WHO recommends <5 NTU.

In Season 1 turbidity was significantly (P<0.05) reduced by PASF filtration on all but the last sampling occasion (Figure 31). Turbidity reductions (P<0.05) were also apparent during the first part of Season 2 (up to samples taken on 16/12/14); however, after mid-January there was a tendency for PASF discharge to have similar (or somewhat higher; P<0.05 on 31/03/15) turbidity compared with pond water.

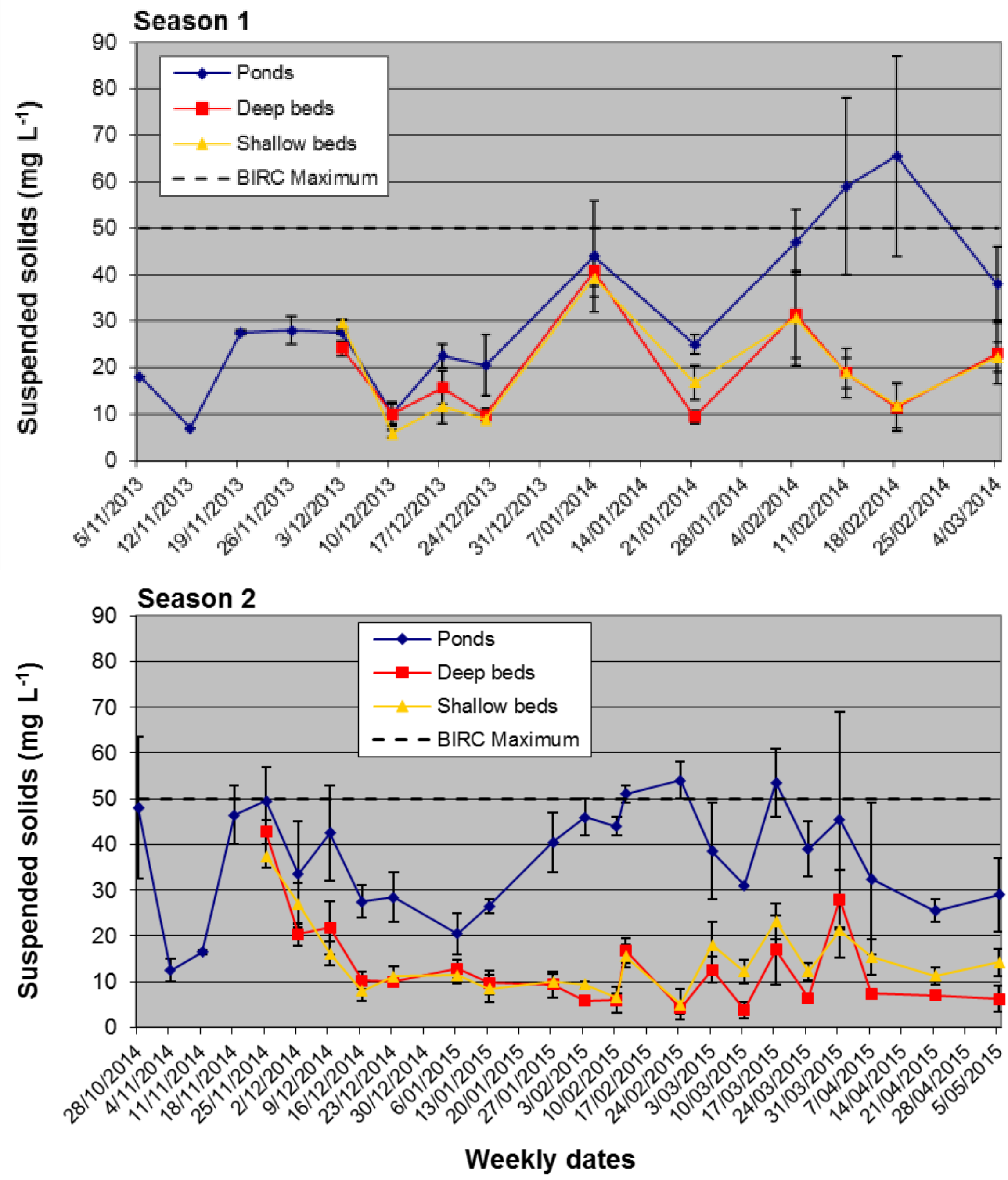

Figure 30 Total suspended solids levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge maximum for BIRC is also provided.

Turbidity was not significantly (P>0.05) affected by sand bed depth in Season 1. On most sampling occasions in Season 2, shallow and deep beds also had similar (P>0.05) levels of turbidity; exceptions were early in the season (25/11/14) when deep beds provided lower (P<0.05) levels, and later in the season (24/02/15) when deep beds provided higher levels (P<0.05) than water in the ponds or that from shallow beds. There was a notable significant (P<0.05) reduction of turbidity in PASF-treated water from 10/02/15 to 12/02/15 in Season 2, suggesting that wet ops improved turbidity reduction.

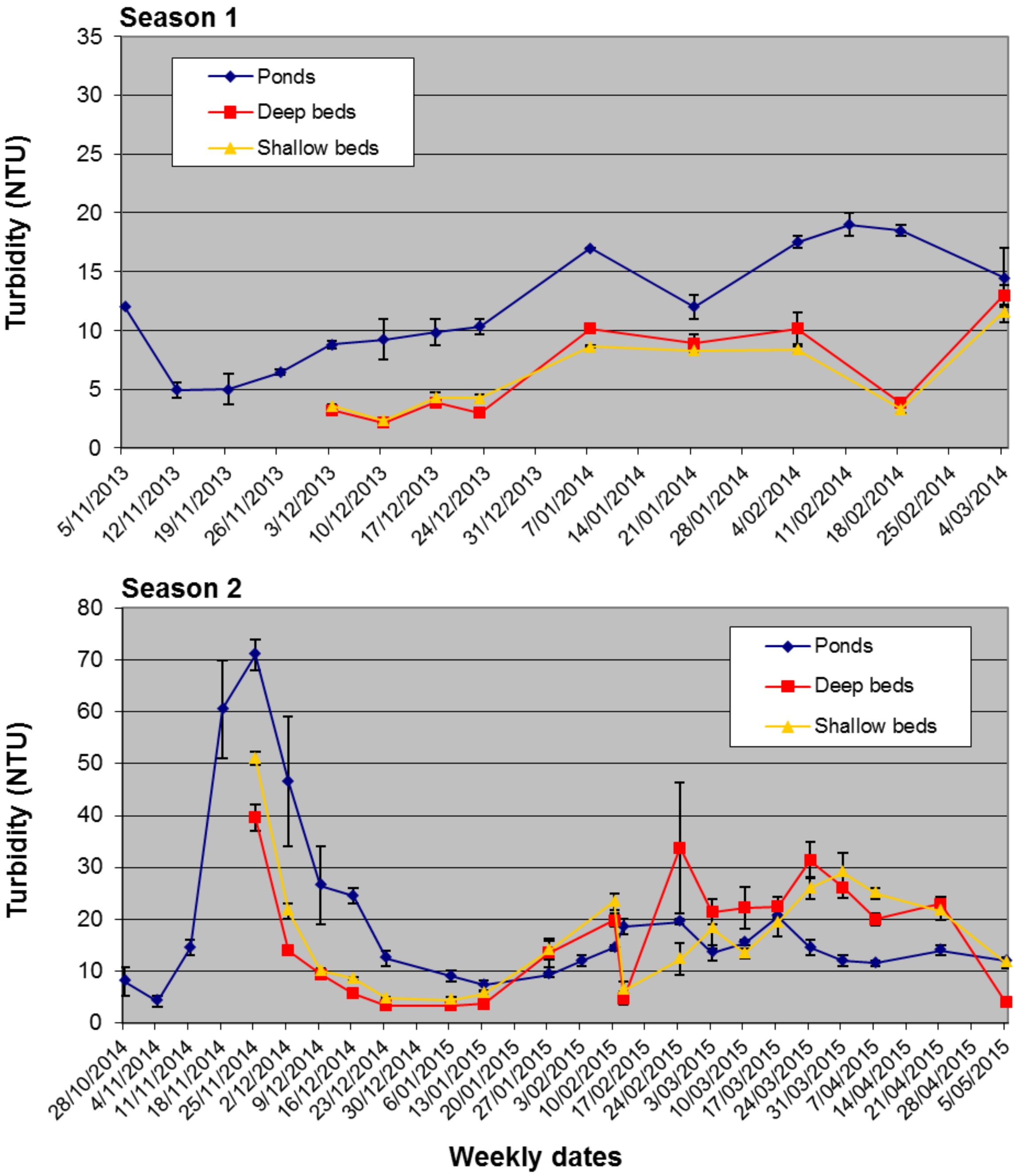

Figure 31 Turbidity levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

PASF therefore appears to have been effective at reducing turbidity levels early in each season, but this efficacy diminished and was much less effective late in Season 2. Whilst the short-circuits applied to some beds in this study would have affected results in this way, it is likely that this diminished ability for PASF to provide turbidity reductions during the season could be caused by the significant development of bacterial populations in the sand beds and the associated presence of bacterial floc in the PASF discharge (see Appendix 4). This assertion is supported by the simultaneous continuous efficacy for TSS removal (described above); note that bacterial components contribute to turbidity, but not TSS, measurements. The higher organic loading of the reused sand in Season 2 may have also reduced the turbidity-clearing capacity at a much earlier stage than it did in Season 1.

Total nitrogen

Nitrogen is the most abundant element on earth (78% of atmosphere) and its cycling in the environment involves many complex processes which include its conversion to a gas. As the name suggests, total nitrogen (TN) is the sum of all forms of nitrogen that can be contained within water. It includes ammonia (NH_3), nitrate (NO_3), nitrite (NO_2) as well as organically-bound nitrogen such as in suspended organic particulates and dissolved organic matter. This should not be confused with total Kjeldahl nitrogen (TKN) which is the sum of ammonia and organically bound nitrogen without the inclusion of nitrate or nitrite.

The PASF beds provided continuous reductions of TN during Season 1 (Figure 32); these reductions were significant ($P<0.05$) for deep beds from 17/12/13, and for shallow beds from 7/01/14. On most occasions deep beds had greater TN reductions than shallow beds, but these differences were only significant ($P<0.05$) on the last sampling occasion (4/03/14).

Similar trends were apparent in Season 2, although the overall TN levels were higher than in Season 1 due to the higher prawn stocking densities and associated higher amounts of feed added to ponds. Again, PASF provided continuous reductions of TN. This was at significant ($P<0.05$) levels for all sampling occasions except 25/11/14, 9/12/14 and 5/05/15. And again, deep beds tended to provide marginally better TN removal compared with shallow beds; significant differences were only apparent between shallow and deep beds on 24/2/15 and 24/3/15 when deep beds provided significantly lower ($P<0.05$) TN levels compared with shallow beds. The wet-weather operational mode applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect trends in TN.

Reductions in TN provided by PASF in the first season averaged 30.6% and ranged from 21.4% (on 21/01/14) to 36% (on 7/01/14). In the second season reductions averaged 35.1% and ranged from 13.8% (on 24/02/15) to 49.9% (on 23/12/14). Whilst these TN removal efficacies were not sufficient to prevent the slow increase of TN in ponds during maximum prawn feeding, they did appear to help provide slow reductions after feed inputs slowed with the beginning of prawn harvest activities (on 19/02/14 and 19/02/15). Nitrogen reduction efficiencies were also insufficient to bring pond TN levels down below BIRC's licenced discharge level of 1 mg L^{-1} during the timeframe of the study.

Total phosphorus

Phosphorus is an essential nutrient for plants and animals because of its incorporation into basic cellular structures. Unlike nitrogen, phosphorus does not have an atmospheric form, and it is particularly prone to adsorb to sediments. This means that unlike nitrogen, the strategy to drive off waste nutrient as a gas through manipulations of the system does not exist for phosphorus. Total phosphorus (TP) is the sum of all forms of phosphorus that can be contained within water. In aquatic systems it can occur as organic or inorganic forms. In aquaculture systems this includes dissolved inorganic phosphate (PO_4; often also referred to as orthophosphate) which is readily taken up by plants (algae), suspended organic phosphorus that is bound within particulates and plant matter (e.g., phytoplankton), and dissolved organic phosphorus produced by animal excretions and through decomposition and breakdown of organic matter.

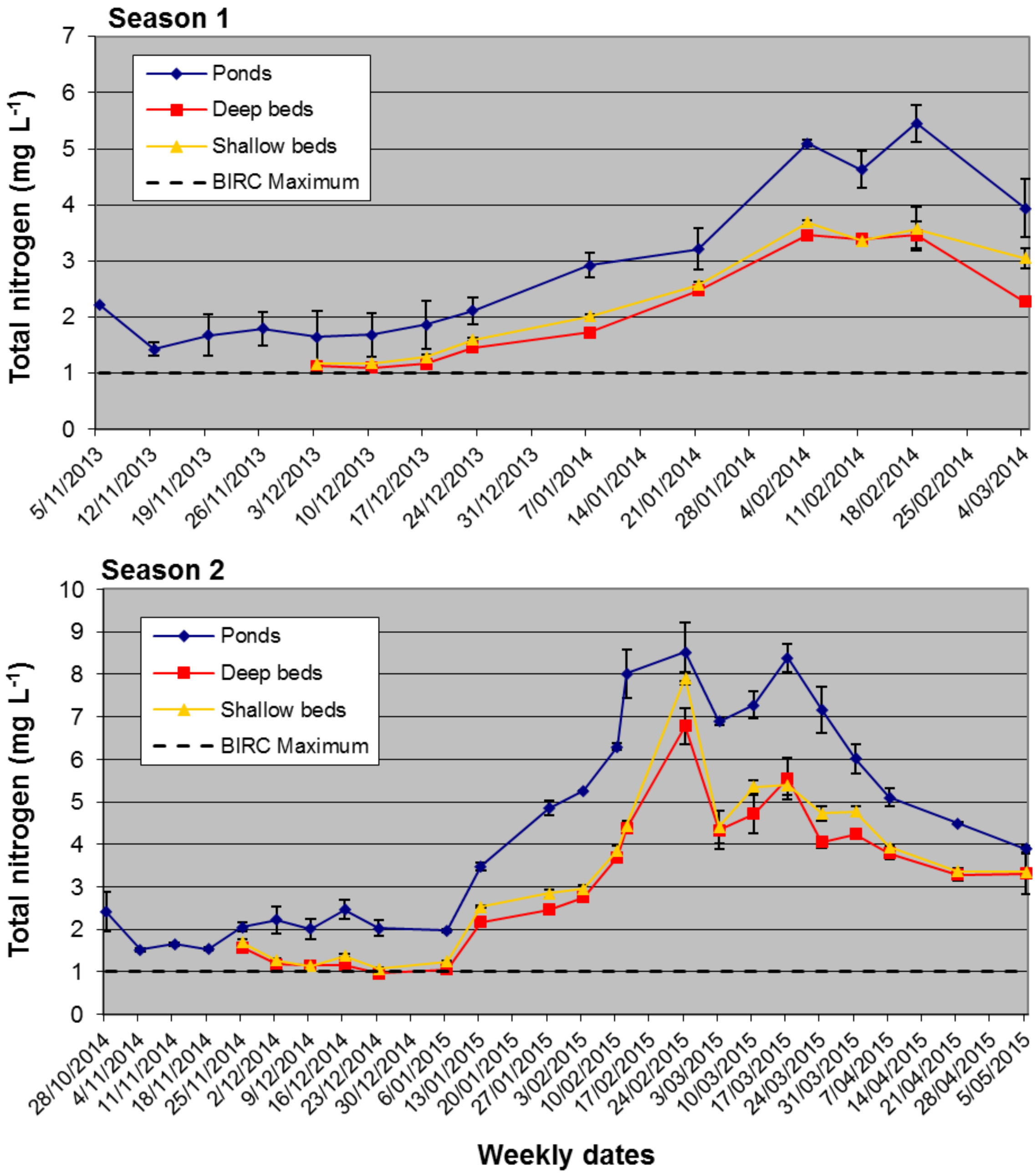

Figure 32 Total nitrogen levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge maximum for BIRC is also provided.

In the present study the PASF beds provided continuous reductions of TP during Season 1 (Figure 33); these reductions were significant ($P<0.05$) for deep beds for all samples except 21/01/14, and for shallow beds for all samples except 10/12/13, 17/12/13 and 21/01/14. Similarly in Season 2, PASF provided continuous phosphorus reductions after the first sample. Compared with pond water in Season 2, deep beds provided significantly lower (P<0.05) TP levels for the main part of the prawn grow-out season (i.e. from 16/12/14 to 24/03/15), whilst shallow beds only provided significant (P<0.05) reductions from 23/12/14 till 24/03/15 with exceptions (P>0.05) for 6/01/15 and 10/03/15.

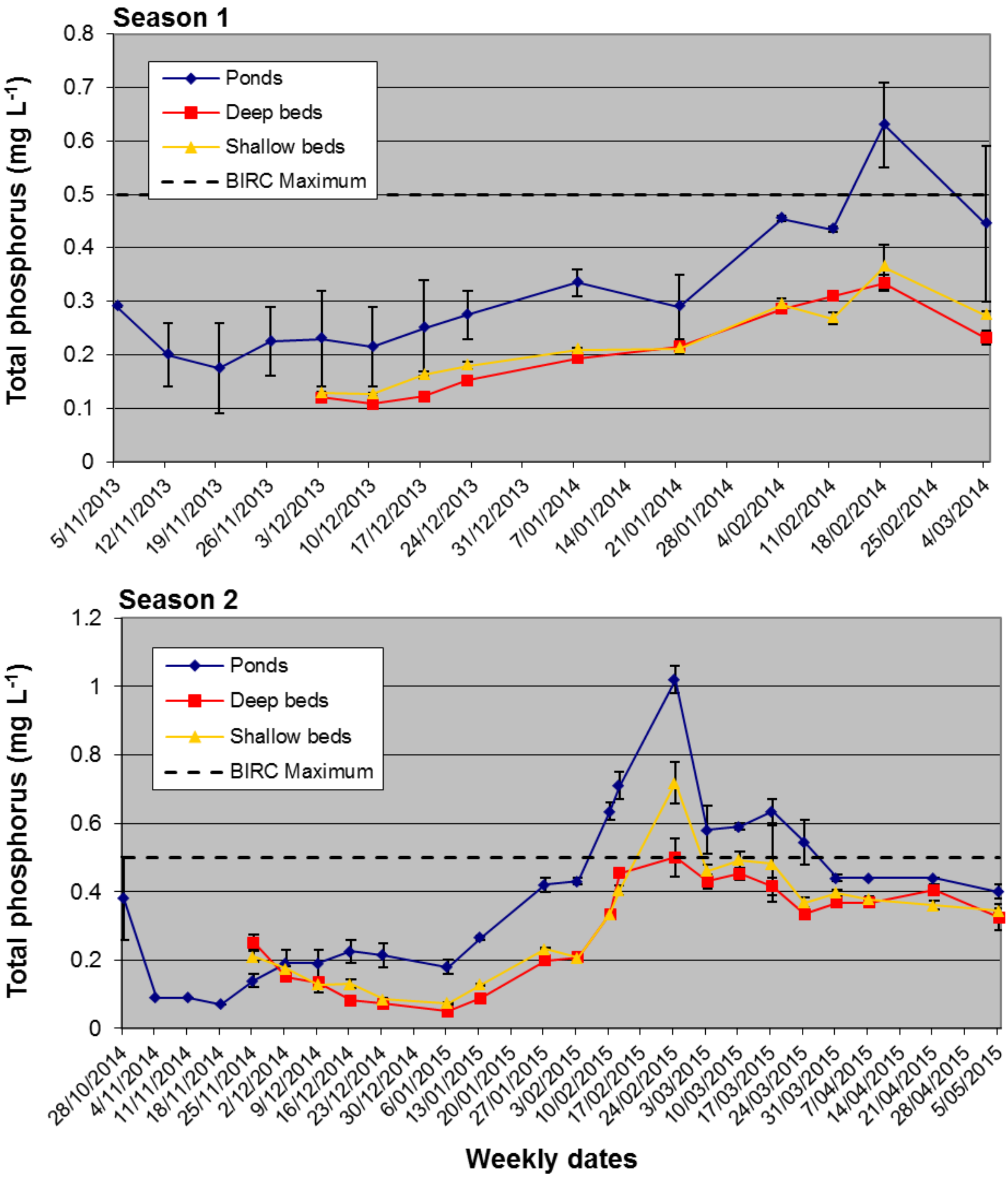

Figure 33 Total phosphorus levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge maximum for BIRC is also provided.

There were no significant differences (P>0.05) in TP between PASF-treated waters from shallow and deep beds in Season 1. Similarly in Season 2, there were no TP differences between shallow and deep beds except on 24/02/15 when shallow beds were higher (P<0.05). The wet-weather operational mode applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect the levels and trends in TP.

Given that the TP levels from PASF were somewhat elevated on the first sampling occasion in Season 2 (25/11/14) (even though differences were not statistically significant, $P>0.05$), it is likely there was residual phosphorus in the reused sand in Season 2 that was flushed from the sand masses early in the cycle. This is supported by slightly elevated TP (and phosphate, see below) levels from deep beds compared with shallow beds, whereby the larger volumes of sand in the deep beds would naturally hold more residual nutrient if it were homogenously distributed within the sand mass (as expected after the harvest process which fully mixes the sand prior to reuse).

The average TP removal in Season 1 was 39.8%, and this ranged from a minimum of 26.2% on 21/01/14 to a maximum of 46.1% on 3/12/13. By comparison in Season 2, TP reductions averaged 30.9%, which included the 65% increase on the first sampling occasion (25/11/14), and a maximum proportional reduction of 65.6% midway through the grow-out cycle on 6/01/15.

Chlorophyll *a*

Chlorophyll is a green pigment found in most algae and plants. It has a specific molecular structure that allows plants to capture energy from light through photosynthesis. Whilst there are several forms of chlorophyll that broaden the spectrum of light absorbed in photosynthesis (a, b, c, d, e, f), Chlorophyll *a* is central to the process of light excitation and the most common and dominant form of chlorophyll found in plants.

The PASF beds provided clear reductions of chlorophyll *a*, relative to the ponds, throughout Season 1 (Figure 34); however, due to the relatively large differences between the two ponds (causing large standard errors for the pond means), these reductions only became statistically significant ($P<0.05$) from 21/01/14. In Season 2, PASF also provided excellent chlorophyll *a* removal efficacies, with significant ($P<0.05$) removal on 23/12/14 and from 3/02/15. On most occasions levels were reduced to below (or close to) the BIRC limit of 40 μg L^{-1}.

The average chlorophyll *a* removal in Season 1 was 68.7%, and this ranged from a minimum of 44.1% on 3/12/13 to a maximum of 84.7% on 18/2/14. In Season 2 the average was 71.9% with a minimum of 10.6% on 25/11/14 and maximum of 98.5% on 23/12/14. For both seasons, minimum removal efficacies were associated with the first sampling occasions, and like other parameters previously mentioned this was also likely due to the settling of sand soon after PASF operations began.

There were no chlorophyll *a* differences ($P>0.05$) between the discharge from shallow and deep beds in Season 1, except on the last sample day where the data suggested much greater reductions by deep beds. In Season 2, sand bed depth also did not significantly ($P<0.05$) affect chlorophyll *a* levels, although on several occasions deep beds appeared to again offer slightly better removal efficacies. Wet-weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect the levels and trends in chlorophyll *a*.

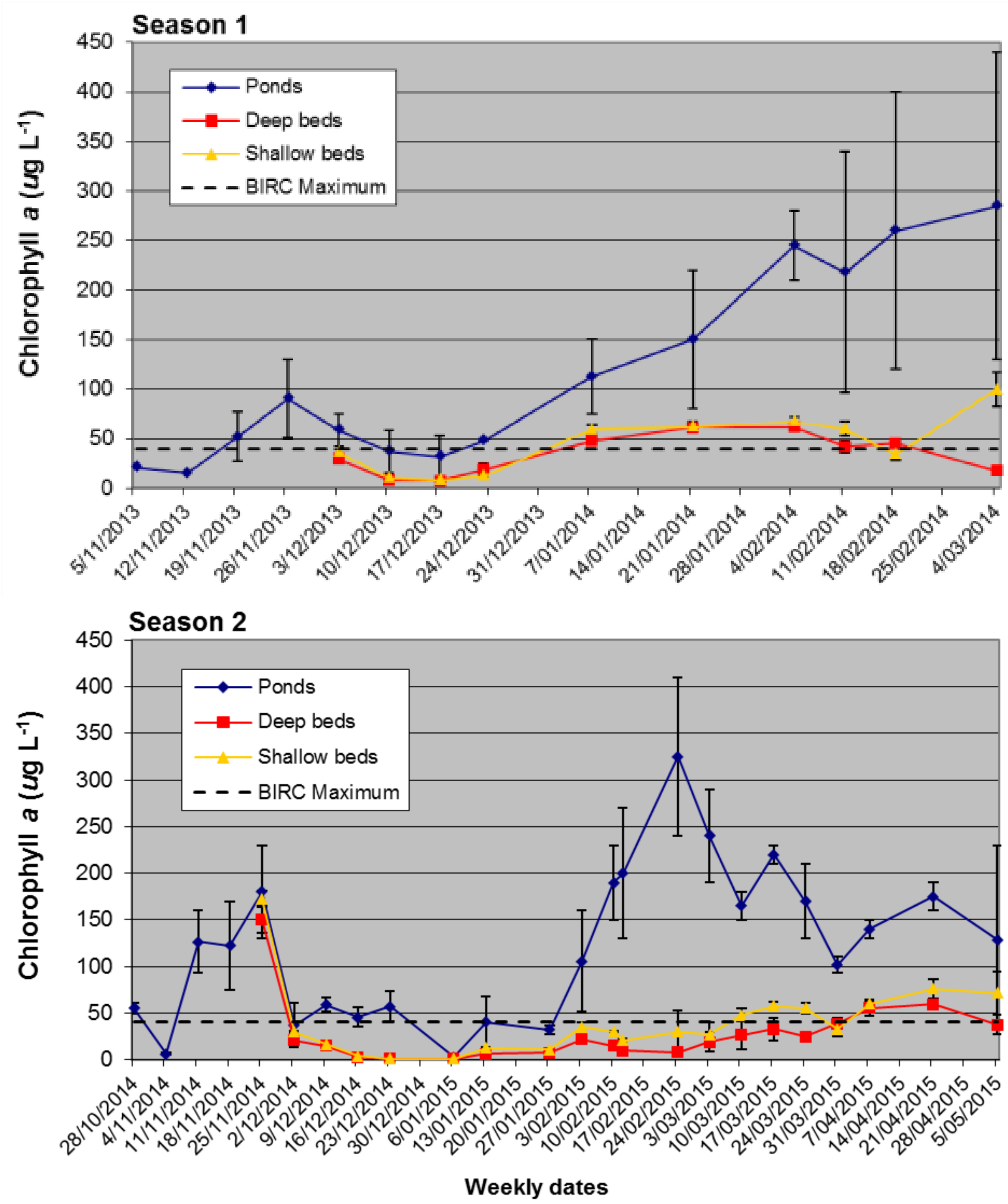

Figure 34 Chlorophyll *a* levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2. The licenced discharge maximum for BIRC is also provided.

Total ammonia

Ammonia is an important source of nitrogen for growing plants and is a key ingredient in most fertilisers used today. Total ammonia nitrogen (TAN) is the sum of un-ionised ammonia (NH_3) and the ammonium ion (NH_4^+). Un-ionised ammonia can be particularly toxic to aquatic life whilst the ammonium ion is relatively non-toxic. Water pH and temperature strongly influence the proportion of each of these forms; as pH and temperature increase so too does the percentage of TAN that is in the toxic un-ionised form. Although different aquatic species can have quite different sensitivities to unionised ammonia, levels of less than 0.2 mg L^{-1} are tolerated by many fish species, whilst levels in excess of 1 mg L^{-1} are considered dangerous and would be likely to cause long-term physiological problems. The Australian Prawn Farming Manual (2006) recommends that levels of un-ionised ammonia remain below 0.25 ppm (0.25 mg L^{-1}) in prawn culture ponds; however, other authors have suggested much lower safe levels for adolescent *P. monodon* (e.g., 0.08 mg L^{-1}: Chen et al., 1990).

There was a strong increase in levels of TAN in PASF-filtered water during both seasons (Figure 35). Compared with the water in ponds, significant increases began in Season 1 on 21/01/14, and in Season 2 on 3/02/15. Maximum TAN production by the PASF beds occurred towards the end of each prawn crop and in mid-to-late February in both seasons.

In all but the last water sample taken during Season 1 there were no differences ($P>0.05$) between shallow and deep beds in levels of ammonia discharged. Similarly in Season 2, on most occasions shallow and deep beds produced similar ($P>0.05$) levels of TAN (exceptions were 24/02/15 and 5/05/15 where $P<0.05$). The wet-weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect the levels and trends in TAN.

Nitrite

Nitrite (NO_2) is an intermediate form of nitrogen between ammonia and nitrate within the nitrification cycle. Like ammonia its toxicity is also dependent on several factors including pH, temperature and salinity. In freshwater fish levels over 10 mg L^{-1} can be lethal and prolonged exposure to even very low levels (e.g., 0.5 mg L^{-1}) can cause nervous and circulatory system damage. However, in the marine environment toxicity is somewhat lower because the abundance of chloride out-competes nitrite in the various uptake mechanisms (e.g., <10.6 mg L^{-1} nitrite is recommended for adolescent *P. monodon* at salinity of 20 ppt, pH 7.57, and temperature of 24.5°C: Chen et al., 1990). The Australian Prawn Farmers Manual (2006) recommends <10 mg L^{-1} NO_2 for salinities >15 ppt, and <5 mg L^{-1} for salinities <15 ppt.

Nitrite levels in pond waters increased during each season and displayed somewhat elevated levels in February when maximum prawn feeding rates were being applied each year. Like ammonia, nitrite levels in the ponds declined to low levels in the later stages of Season 2 when prawn stock and feeding were reduced.

Nitrite levels in PASF-filtered water tended to remain low throughout Season 1 (Figure 36), and for most samples were below the detection limits of the laboratory (<0.01 mg L^{-1}). No differences ($P>0.05$) in nitrite levels were found between shallow and deep beds in Season 1; and the PASF beds provided significant reductions ($P<0.05$) when levels in the pond waters increased from the 4/02/14.

Results were similar for Season 2 with predominantly low levels produced by PASF except for the period between 3/03/15 and 24/03/15; at this time shallow beds tended to provide higher levels of NO_2 than deep beds ($P<0.05$ for 10/03/15 and 17/03/15), and this was likely to have been due to the greater amounts of biological filtration and nitrification in deeper sand beds. Wet-weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect nitrite trends.

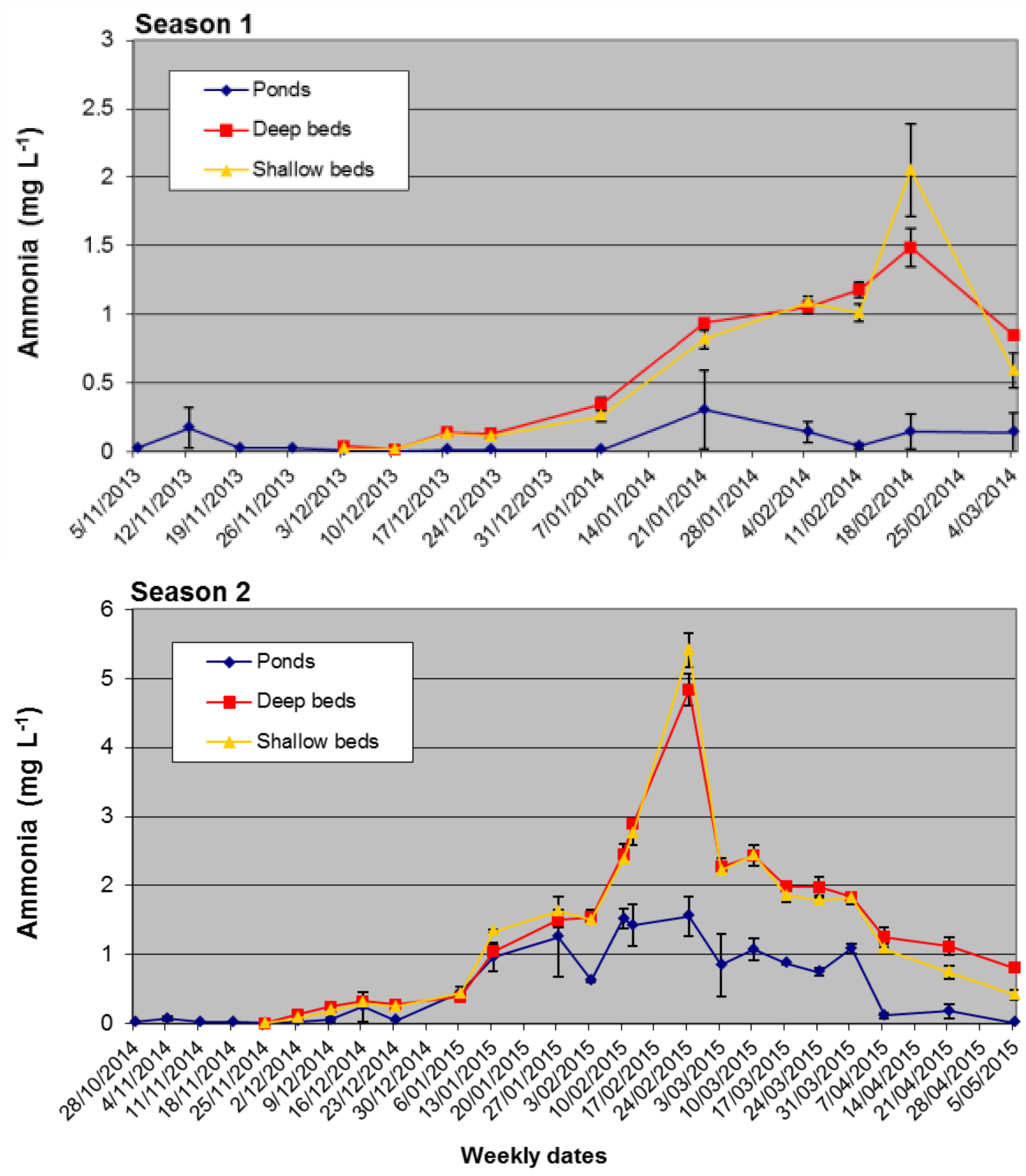

Figure 35 Total ammonia levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Nitrate

Nitrate (NO_3) is another important source of nitrogen for growing plants. It is the final product of nitrification so it can accumulate to higher levels if ammonia is in constant supply and there is no plant or bacterial uptake. Nitrate is also the main form of nitrogen that is used by a large group of naturally occurring heterotrophic facultative anaerobic bacteria in denitrification. This process of nitrate reduction (denitrification) occurs in low-oxygen environments when organic carbon is readily available. It is widely considered a very useful process in wastewater treatment because it acts to drive off nitrogen as a gas.

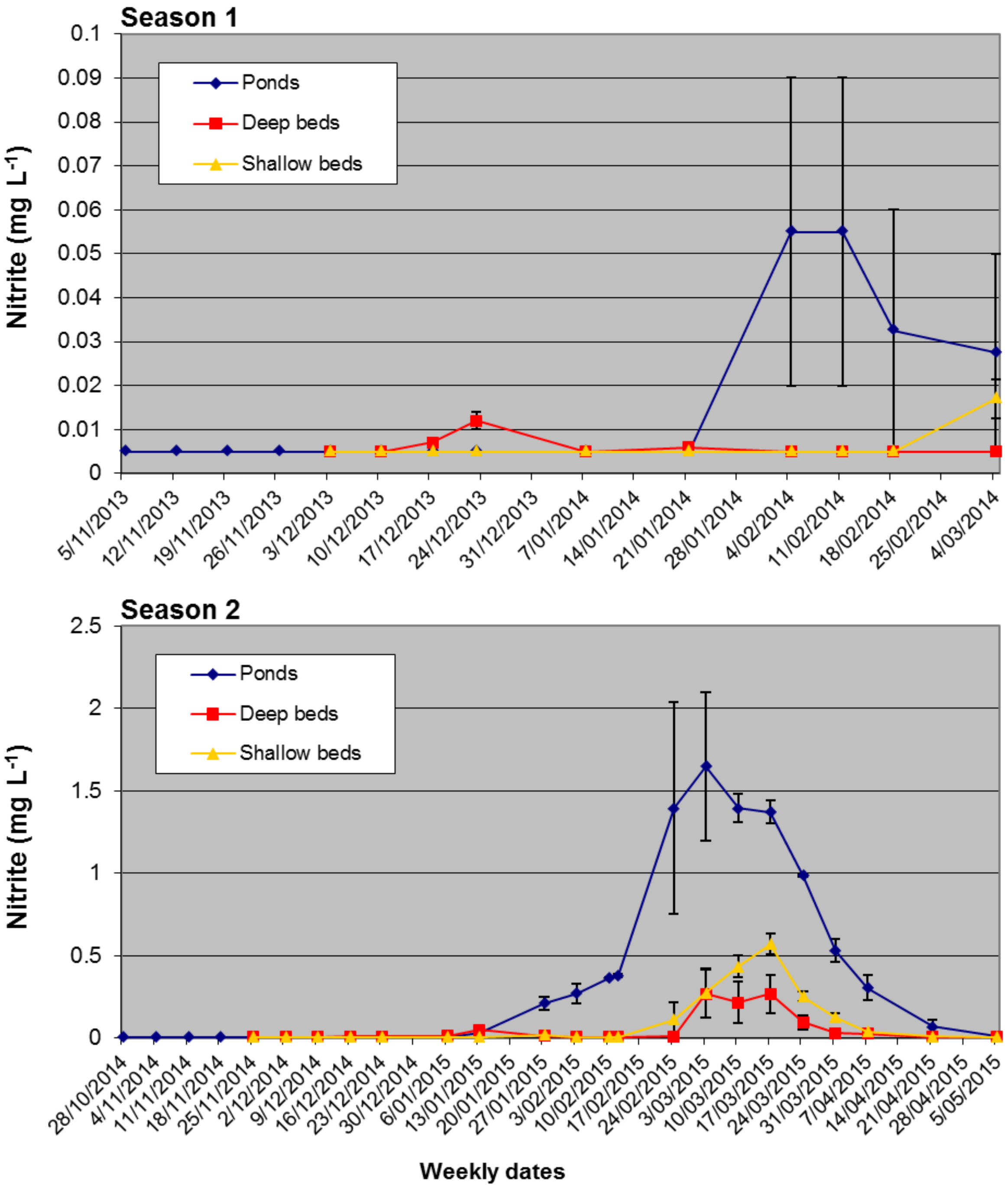

Figure 36 Nitrite levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Whilst nitrate has much lower toxicity than ammonia and nitrite it can cause chronic health issues for prawns at very high levels, particularly at reduced salinities. For example, the survival and growth of *Litopenaeus vannamei* have been shown to be adversely affected in one-third ocean salinity with

220 mg L^{-1} NO_3 (Kuhn et al., 2011). However, in normal aquaculture systems nitrate would seldom build to such high levels.

Nitrate levels in the prawn ponds of the present study remained at comparatively low levels in both seasons (Figure 37). In Season 1 there was an early spike recorded in one pond prior to the start of PASF recirculation, but nitrate levels in the ponds then remained very low for the remainder of the cropping cycle. In Season 2, pond waters had elevated nitrate levels from 12/02/15 until the last sample taken on 5/05/2015. During this time, PASF significantly ($P<0.05$) reduced pond nitrate levels between 12/02/15 and 7/04/15. Between the 3/02/15 and 21/04/15 when the highest feeding rates and organic loading were applied to ponds and when nitrate levels were detectable, PASF on average removed 83.6%.

In Season 1 levels of nitrate in PASF-filtered water were mostly below minimum levels of detection (i.e., <0.01 mg L^{-1}) and were not significantly different ($P>0.05$) for shallow and deep beds (with the exception of the last sample assumed to be anomalous). Similarly in Season 2, there were no significant differences ($P>0.05$) between the nitrate levels from shallow and deep beds except on 17/03/15 when shallow beds were higher ($P<0.05$) (evidence of greater denitrification in deep beds). Wet- weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect nitrate trends.

Dissolved organic nitrogen

Dissolved organic nitrogen (DON) is defined and measured as the fraction of organic nitrogen that passes through the filter (not including ammonia, nitrite and nitrate). It can be a mixture of simple and complex compounds including amino acids, urea and humic substances (e.g., tannins) and generally comes from prawn feeds and faeces. Although DON tends to comprise small molecular weight compounds it is reported to be refractory in prawn ponds and only slowly used by pond microbes (Burford et al., 2001).

Throughout both seasons there were slow increases in DON in pond waters (Figure 38), which is normal in prawn grow-out ponds (Burford et al., 2001). Interestingly, DON did not display the decrease that other forms of nitrogen showed after the reduction of feed inputs towards the end of Season 2. This was likely due to the liberation of DON from decomposing organic matter that was trapped in the PASF beds and in the sludge pile in the middle of each pond.

There were no differences ($P>0.05$) between the DON levels of water discharged from shallow and deep PASF beds in either season. In Season 1, although the PASF beds provided continuous reductions in DON, these only became statistically significant ($P<0.05$) in samples taken from 21/01/14. In Season 2, levels of DON were reduced by PASF on most sampling occasions ($P<0.05$ on 23/12/14, 13/01/15, 3/02/15 to 12/02/15, 17/03/15) except on 24/02/15 when it was higher ($P<0.05$). The averaged reductions of DON were 23.6% in Season 1 and 13.5% in Season 2.

As with many other parameters assessed, trends in DON did not appear affected by the wet-weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14.

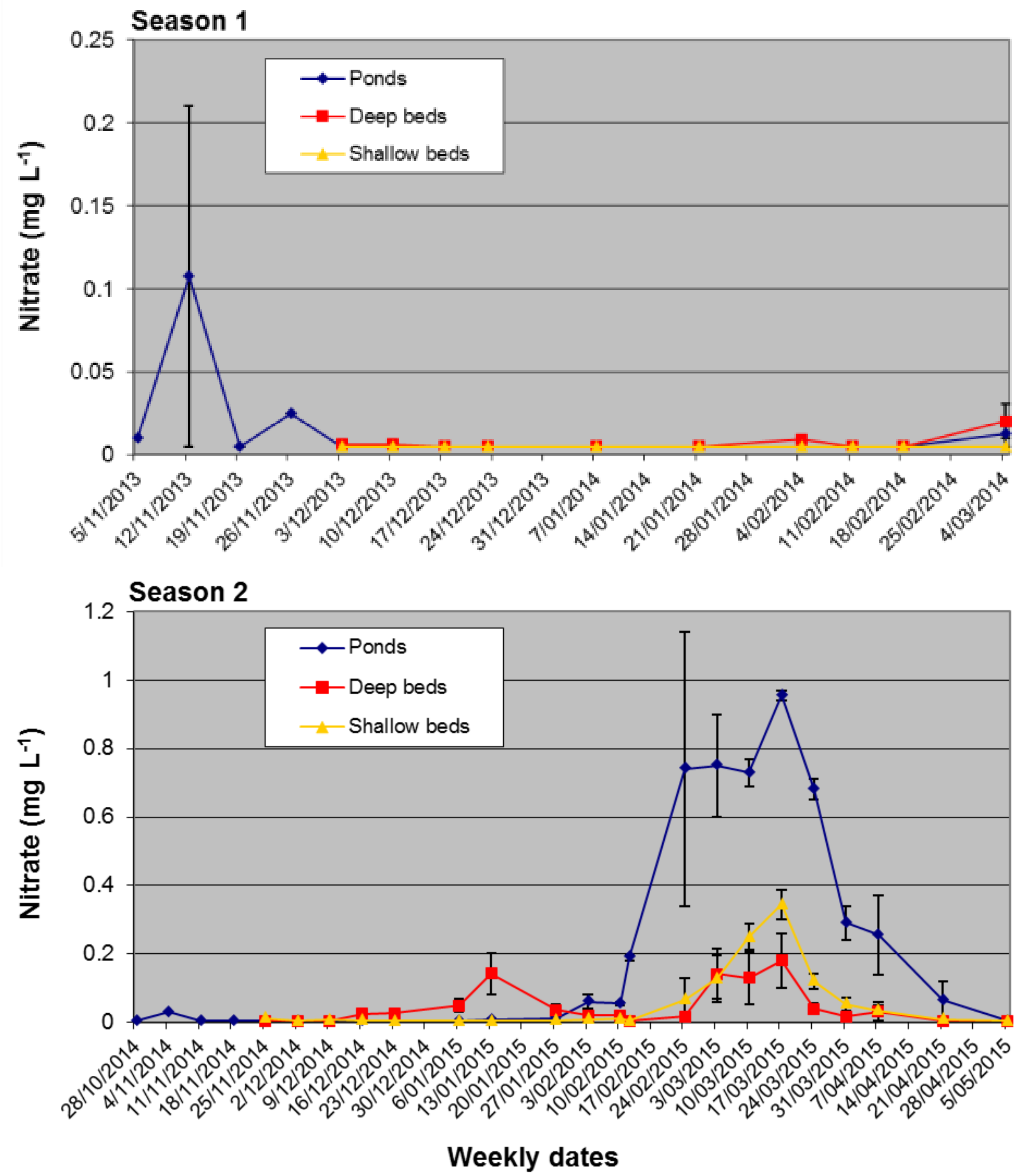

Figure 37 Nitrate levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

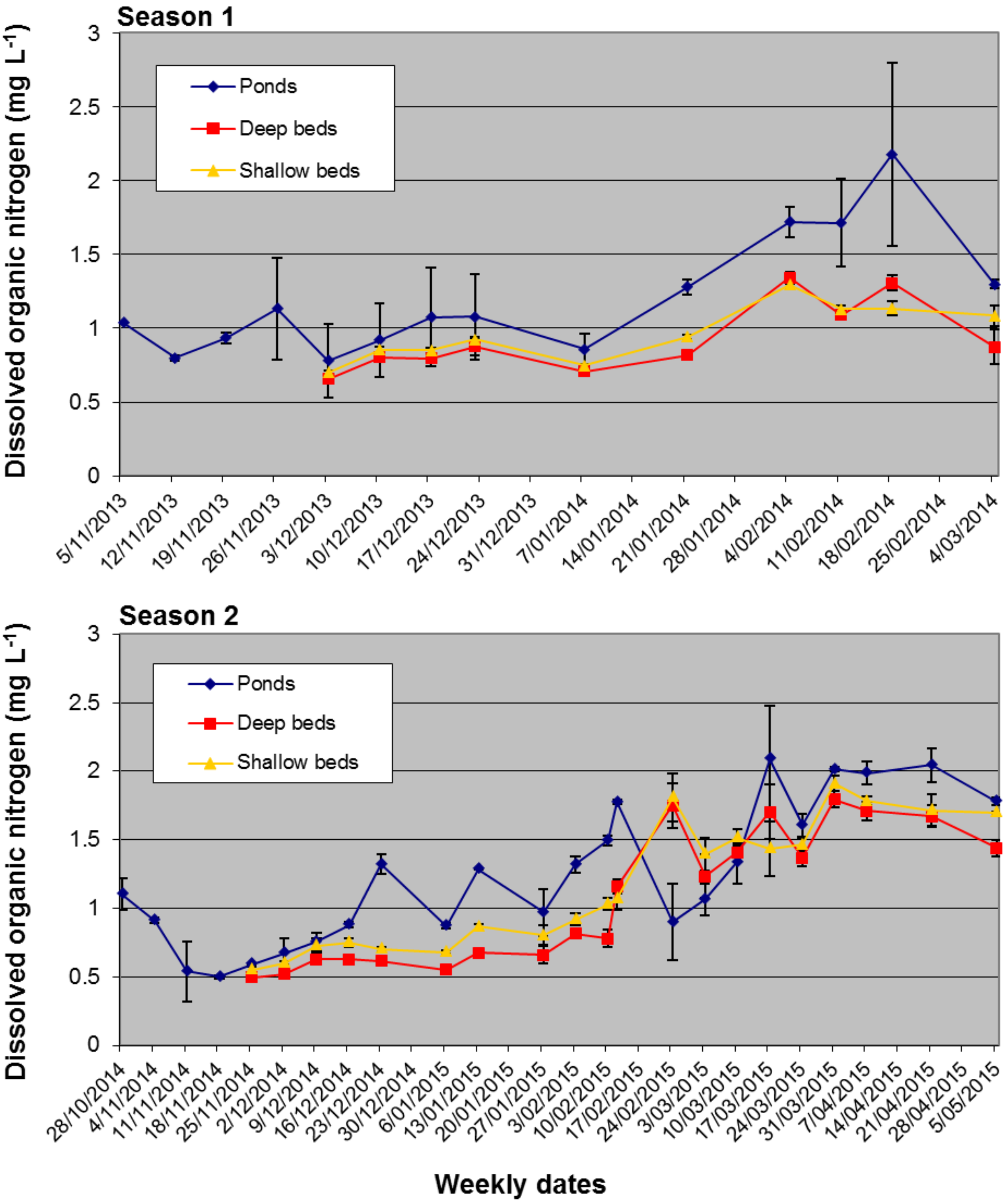

Figure 38 Dissolved organic nitrogen levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Dissolved organic phosphorus

Dissolved organic phosphorus (DOP) is defined and measured as the fraction of organic phosphorus that passes through the filter (not including phosphate). It comprises organic molecules (e.g., nucleic acids) which have dissolved in water following the decomposition of organic matter.

A slight build-up of DOP in the pond waters was evident in both seasons; however, this was not as clear as it was for DON. Like DON, there was little evidence for reduced levels of DOP in ponds after prawn feeding ceased late in Season 2. Also like DON, wet-weather operational mode as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect trends in DOP.

In Season 1 there were no significant differences ($P>0.05$) between the DOP levels of water discharge from shallow and deep PASF beds. Although the PASF beds provided continuous reductions in DOP (Figure 39), these reductions were only at statistically significant levels ($P<0.05$) on four occasions (17/12/13, 11/02/14, 18/02/14 and 4/03/14) for deep beds, and on one occasion (18/02/14) for shallow beds.

In Season 2, continuous significant ($P<0.05$) reductions of DOP were apparent. However, sand bed depth had a highly significant ($P<0.001$) effect on DOP, where the average level from deep beds (0.027 mg L^{-1}) was lower than that from shallow beds (0.039 mg L^{-1}). The averaged reductions of DOP were 32% in Season 1 and 48.9% in Season 2.

Phosphate

Phosphate (PO_4) (also known as orthophosphate) is readily taken up by plant growth. It is often in short supply in the natural freshwater environments and excess availabilities often lead to eutrophication. It is produced from the breakdown and mineralisation of organic matter and in itself is not toxic at levels normally experienced in aquaculture systems.

Phosphate levels in pond waters remained low throughout both seasons (Figure 40); the highest mean level (0.12 mg L^{-1}) occurred early in Season 1, presumably due to fertilisers (mono-ammonium phosphate) added to initially stimulate the algal blooms. Wet-weather operations as applied to the PASF beds on 7/01/14 and 12/02/14 did not appear to affect phosphate levels and trends.

Significant phosphate production in PASF began around the beginning of February each season. Early in Season 1 there were no significant differences ($P>0.05$) between the phosphate levels of ponds and PASF bed discharges, but from mid-January levels in PASF discharge began rising to become significantly higher ($P<0.05$) from 4/02/14 (except shallow beds on 4/03/14). In Season 2 deep beds began producing significantly higher ($P<0.05$) levels than ponds from 27/01/15, and shallow beds reciprocated a little later from 10/02/15.

There were only two occasions when shallow and deep beds provided statistically different ($P<0.05$) phosphate results in Season 1 (11/02/14 and 4/03/14), and on both occasions deep beds were higher. In Season 2, there were four occasions when deep beds had higher ($P<0.05$) levels than shallow beds (10/02/15, 12/02/15, 21/04/15, 5/05/15), and one occasion when shallow beds were higher ($P<0.05$) than deep beds (24/02/15).

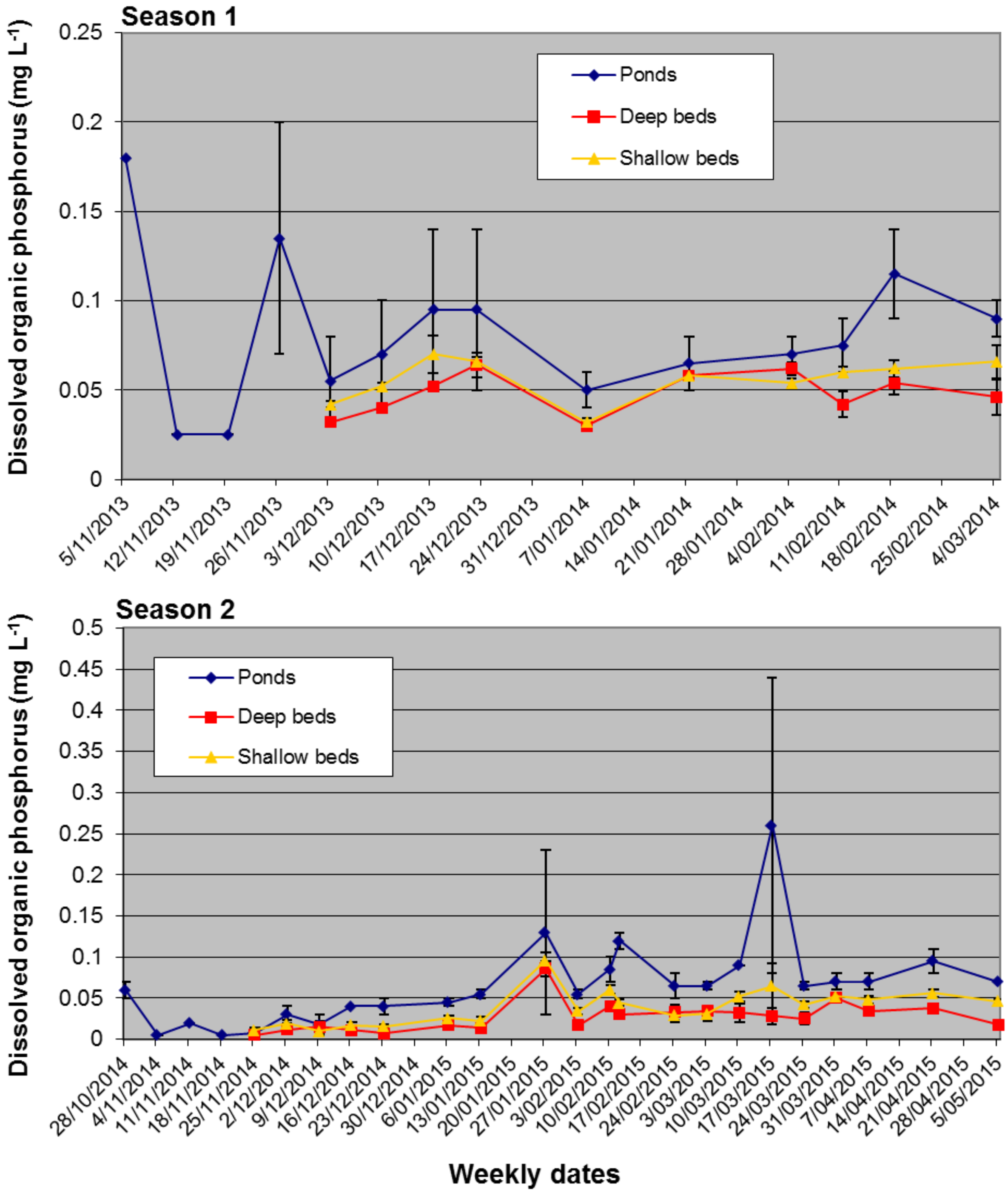

Figure 39 Dissolved organic phosphorus levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

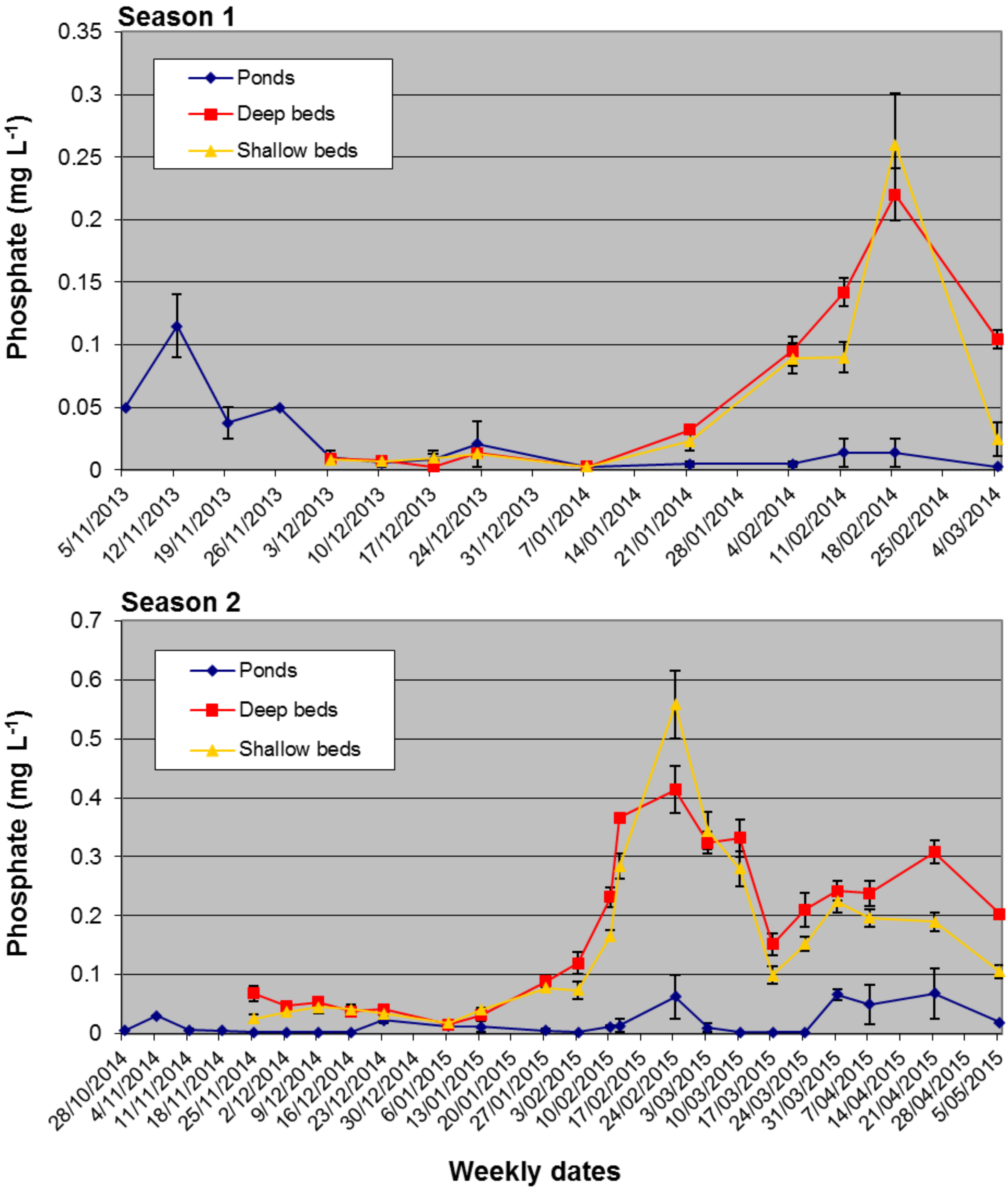

Figure 40 Phosphate levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Total sulphide

Sulphur is an essential element for all life due to its central role in metabolism and many biochemical processes. Its oxidised form, sulphate (SO_4), is not toxic at levels normally found in aquatic systems, and occurs widely in seawater, and in sediments where there is decomposing organic matter. Complex aerobic and anaerobic bacterial processes drive the reduction of sulphate to the more toxic sulphide (S^{2-}) form, and the cycling of sulphur in the environment. These processes are of particular interest in this study because of the nature of some polychaetes (including *P. helleri*) to live in sulphide-rich sediments and potentially utilise the associated bacterial biomass present in the marine benthos (Palmer, 2010).

Sulphide is often easily detected in sediments due to the black colour generated and by the presence of the strong odour of hydrogen sulphide gas (H_2S) that it releases. In toxicity studies its concentrations are often reported as H_2S, but since the molecular weight of hydrogen is very low, in practice[18] there is little difference between concentrations reported as sulphide or H_2S. Since sulphide very readily oxidises into sulphate, sampling techniques must use stabilisers or testing procedures that quickly evaluate collected samples with a minimum of agitation or aeration prior to adding reagents. This factor also greatly lessens the potential for sulphide to be a problem in aerated ponds, since any sulphide that is liberated into an oxygen-rich environment very quickly changes into non-toxic sulphate.

There is a large body of information available regarding the toxicity of sulphide. In aquatic systems its toxicity (relating to the percentage of un-ionised sulphide) is pH- (and to a lesser degree temperature-) dependant, becoming more toxic at lower pH values (and at lower temperatures). Levels of 0.1 to 2 mg L^{-1} have been shown to have sub-lethal effects on shrimp whilst levels of 4 mg L^{-1} are lethal (Shigueno, 1975). The Australian Prawn Farming Manual (2006) recommends that H_2S levels in pond water should remain below 0.1 mg L^{-1}. However Boyd (1979) recommends that any detectable level of sulphide in fish ponds can be problematic and should be investigated. Interestingly its toxicity is tempered by the presence of iron oxide, which is important given the abundance of iron in seawater and in many Australian coastal marine sediments.

Despite the production of significant sulphide levels in PASF (Figure 41), and its regular addition to ponds through the routine recirculation of PASF-treated water, levels in ponds during the study were only detected (above the detection limit of 0.1 mg L^{-1}) on one occasion in Season 2 on 31/03/15 when it was measured at 0.3 mg L^{-1} in one pond (G2).

In both seasons, sulphide levels in PASF-filtered water displayed rising trends as the crops proceeded. In Season 1 levels rose to significantly higher ($P<0.05$) levels than in ponds from 4/02/14, and in Season 2 this occurred around the same time of year from 27/01/15. On most occasions each season there were no significant differences ($P>0.05$) in sulphide levels from shallow and deep beds; exceptions ($P<0.05$) included 18/02/14 in Season 1 when particularly high mean levels were measured from shallow beds (e.g., up to 10.7 mg L^{-1} from Bed 4), and from 7/04/15 in Season 2 when deep beds were higher.

Although there was no apparent effect of the wet-operations mode on sulphide levels (applied to PASF beds on 7/01/14) in Season 1, in Season 2 there was a significant ($P<0.05$) increase from 10/02/15 (when beds were on dry ops) to 12/02/14 (when beds were on wet ops). This increase fell away significantly again on the next sampling occasion (24/02/15; which was again dry ops) suggesting that sulphide levels could be increased significantly with a prolonged wet-weather operational mode.

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[18] To convert from sulphide to H_2S multiply by 1.06.

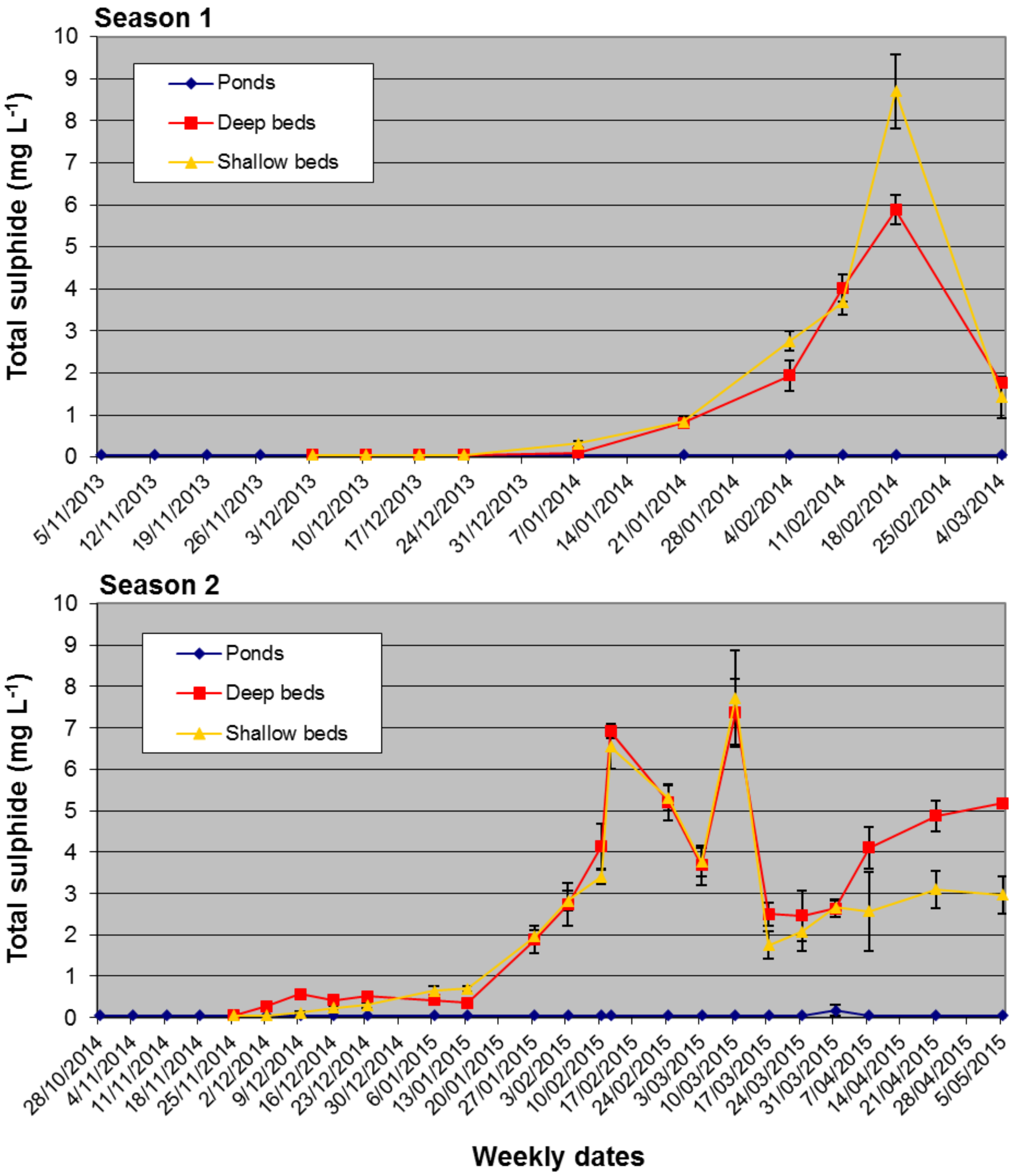

Figure 41 Total sulphide levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Total alkalinity

Total alkalinity is the measure of all the ions that are dissolved in water. It is affected by the source water, the soil type, additions of ion-rich materials (such as lime) and biochemical processes that occur in water and sediments in contact with the water. It needs to be monitored closely in closed aquaculture systems such as the present recirculated system. Without the regular inflow of ions that occurs in flow-through aquaculture, in an intensive culture environment alkalinity can fall to low levels (see below) which reduces the buffering capacity of water to pH change. It is generally recommended that the alkalinity of shrimp pond water remains in the range 50-200 mg L^{-1}, but for optimal pond management it should remain above 80 mg L^{-1} (Australian Prawn Farming Manual, 2006). This is so that a more stable stress-free environment is provided, which can otherwise upset the vigour and health status of the prawns.

Alkalinity is also known to be generated in the oceans from anaerobic degradation of organic matter. Levels in oceans receiving significant anthropogenic nutrient inputs, such as the North Sea adjacent to Europe, have been shown to vary seasonally (Thomas et al. 2009) with average levels in the order of 2,300 μmol kg^{-1} (≈230 ppm as $CaCO_3$). By comparison, at BIRC when the salinity of its intake seawater is high (36 ppt) its pH will be 8.1-8.2 and its alkalinity will be around 108 mg L^{-1} (as $CaCO_3$). So, regular additions of new seawater to a pond, as in mariculture flow-through systems, can help keep eutrophic pond waters at mildly basic levels with alkalinities in the order of 80-100 mg L^{-1}.

In the present study, total alkalinity was only studied in detail from mid-January 2014 (Figure 42). Although a limited data set is therefore available for Season 1, by mid-February a downward trend was apparent for the ponds. From this point water returning from the PASF beds generally had higher alkalinity than the ponds, and this was significantly higher ($P<0.05$) for shallow beds on 18/02/14 and for deep beds on 11/02/14 and 18/02/14. Deep and shallow beds had similar ($P>0.05$) alkalinity except on 18/02/14 when shallow beds were higher ($P<0.05$).

No additional lime (or other buffering agent e.g., bicarbonate) was added to the system during the last few months of culture in Season 1, when alkalinity levels remained at acceptable levels (down to about 80 mg $CaCO_3$ L^{-1}). However, in Season 2, the higher densities of prawns appear to have placed additional demand on available calcium carbonate in the system. This was also at a time when the pool of calcium carbonate in the sediment was low due to the previous removal of all sediment and shell material when pond liners were replaced prior to the start of Season 2. Calcareous growths of barnacles and tube worms on the walls of the new pond liners in Season 2 contributed to the extraction of carbonate from the recirculating sea water. This meant that despite regular (weekly and daily) additions of dolomite and hydrated lime to the ponds, alkalinity levels still fell well below levels recommended above (down to 27-28 mg L^{-1} in mid-March).

With a focus on alkalinity results in Season 2, levels from PASF early in the season were lower than in the ponds ($P<0.05$ on 25/11/14 and 9/12/14), but from 6/01/15 PASF generated higher ($P<0.05$) alkalinity than in the ponds (except on 5/05/15 when shallow beds were similar with $P>0.05$). Statistically significant differences ($P<0.05$) between shallow and deep beds only occurred on three occasions (23/12/14, 6/01/15 and 5/05/15) and in each case deep beds produced higher alkalinity compared with shallow beds.

Consistent trends in alkalinity levels from PASF around the 12/02/15 (wet ops) in Season 2 suggest that this water quality parameter is not greatly affected by the wet-weather operational mode.

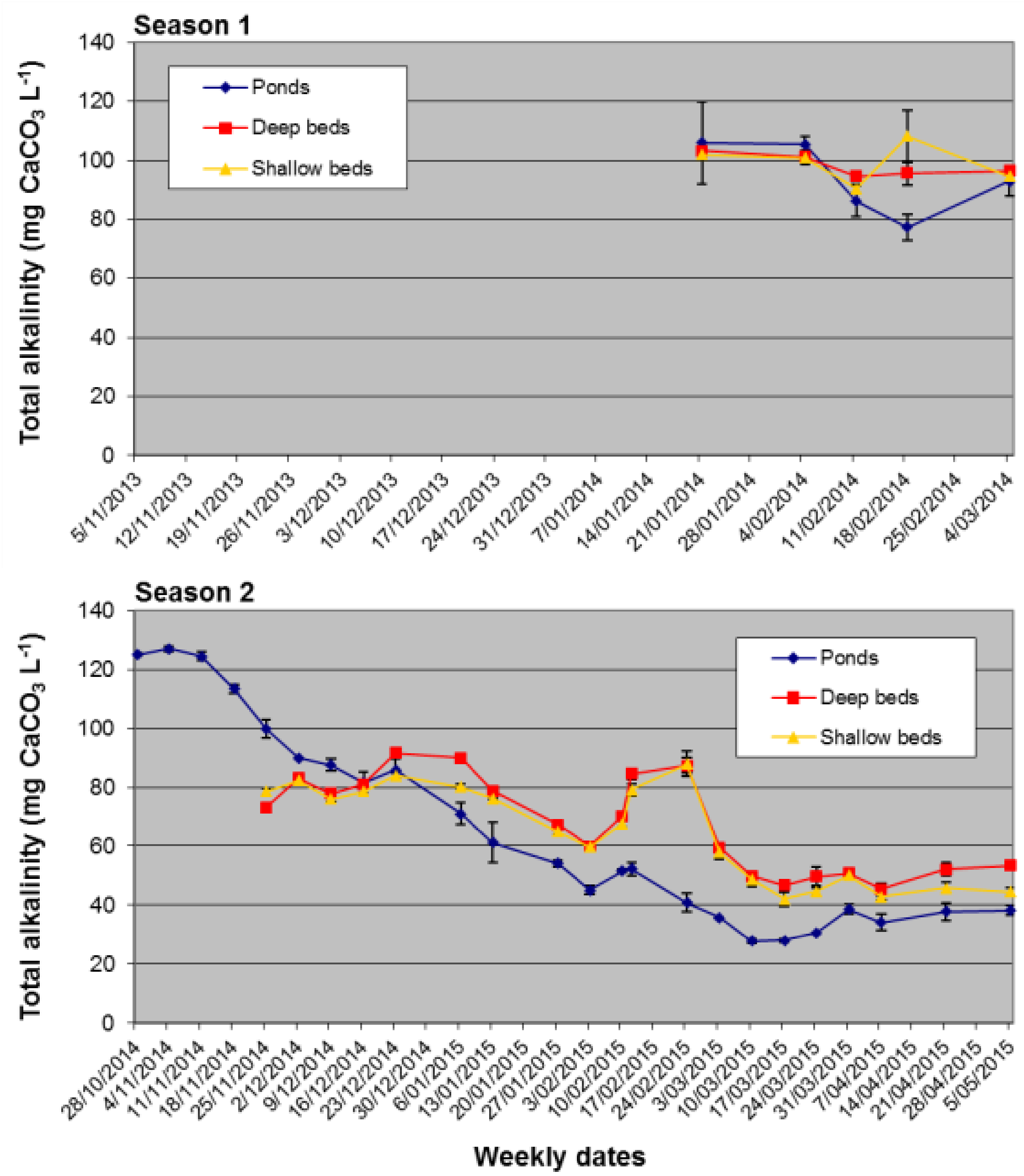

Figure 42 Total alkalinity levels in two recirculated prawn ponds and discharge from five deep and five shallow PASF beds (mean ± se) in Seasons 1 and 2.

Tidal water qualities

This section deals with patterns in water qualities that occur during the daily operations of PASF.

Total nitrogen

Levels of TN in PASF discharge were significantly affected (P=0.001) by the different stages of the artificial tide (Figure 43) where there were steady declines during both experiments. The overall mean declines (both dates inclusive) were not significant (P>0.05) from 5 to 30 min after the start of the tide, but were significant for different times after 60 min. Higher (P=0.001) mean TN levels occurred later in Season 2 (6.5 mg L^{-1} on 16/02/15) than earlier in the season (3.79 mg L^{-1} on 19/01/15).

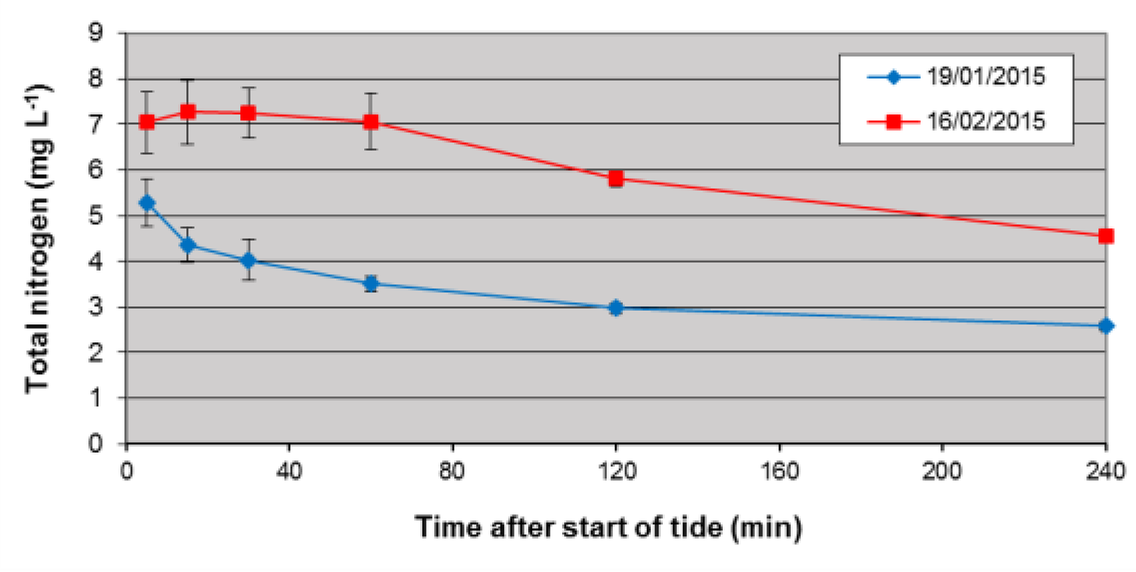

Figure 43 Total nitrogen levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Total phosphorus

Levels of TP in PASF discharge were also significantly affected (P<0.001) by the different stages of the artificial tide (Figure 44) where again there were steady declines during both experiments. The overall mean declines (both dates inclusive) were not significant (P>0.05) from 5 to 30 min after the start of the tide, and TP levels at the last two time points (120 and 240 min) were also not significantly different (P>0.05). Higher (P<0.001) mean TP levels occurred later in Season 2 (0.53 mg L^{-1} on 16/02/15) than earlier in the season (0.26 mg L^{-1} on 19/01/15).

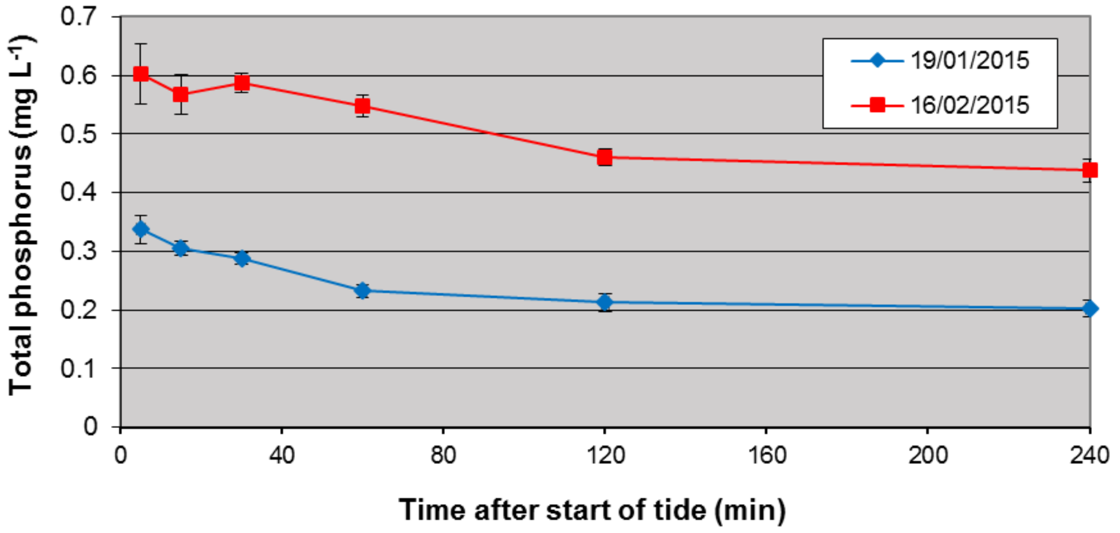

Figure 44 Total phosphorus levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Total ammonia

Total ammonia levels in PASF discharge also declined significantly (P=0.008) during both tidal experiments (Figure 45). The overall mean declines in TAN (both dates inclusive) were not significant (P>0.05) from 5 to 60 min after the start of the tide, and TAN levels at the last two time points (120 and 240 min) were also not significantly different (P>0.05). Higher (P<0.001) mean TAN levels occurred later in Season 2 (4.68 mg L^{-1} on 16/02/15) than earlier in the season (2.39 mg L^{-1} on 19/01/15).

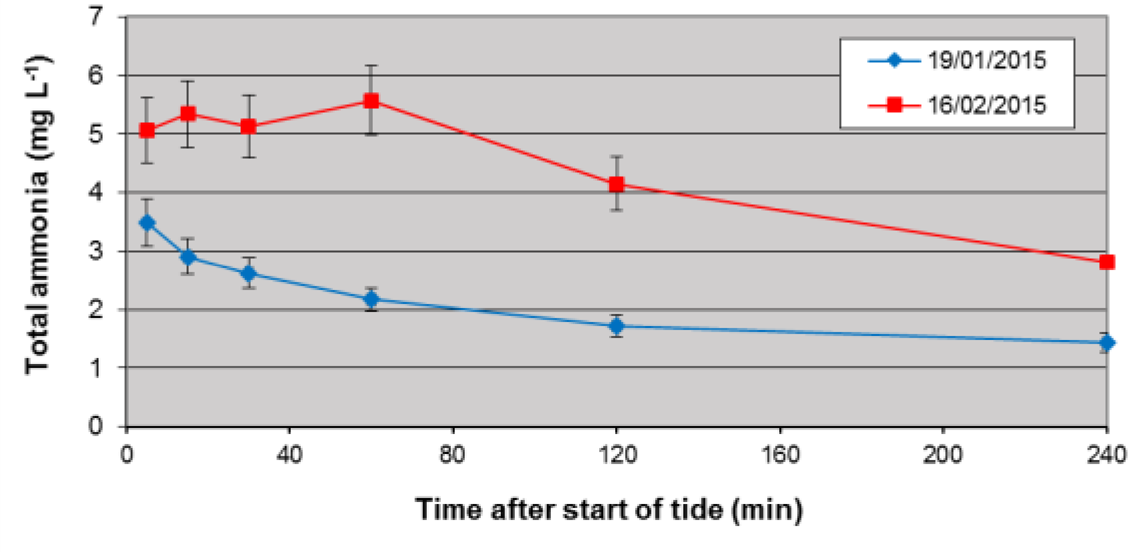

Figure 45 Total ammonia levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Nitrite

Nitrite levels in PASF discharge were not significantly affected by stages of the artificial tide (Figure 46), and there were no differences (P>0.05) between the two sample days in Season 2.

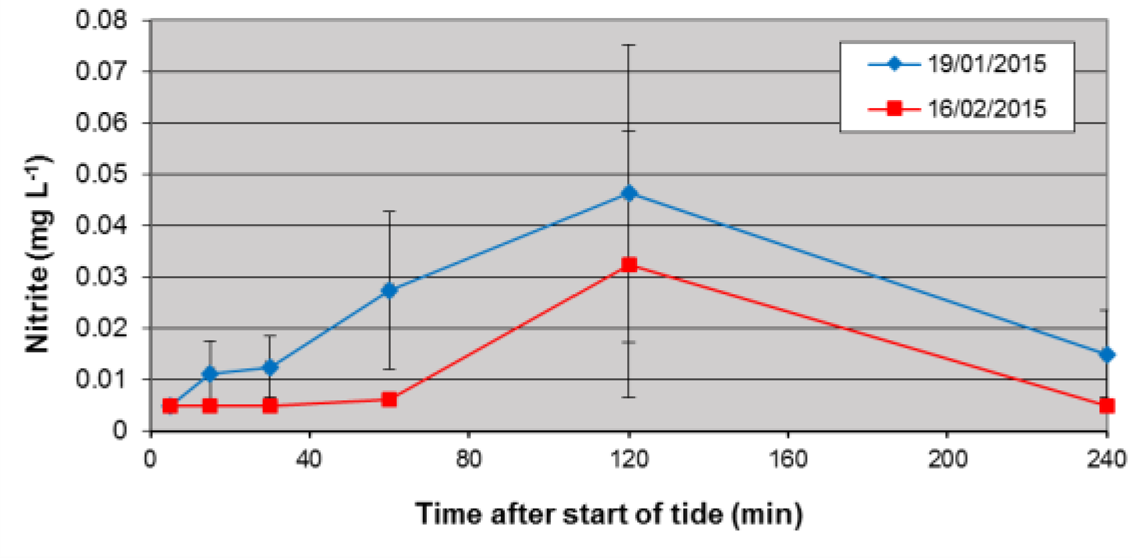

Figure 46 Nitrite levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Nitrate

Nitrate levels in PASF discharge were not significantly affected by different stages of the artificial tide (Figure 47), and there were no differences (P>0.05) between the two sample days in Season 2. As with nitrite, there were large errors associated with means at the 60 and 120 min time periods.

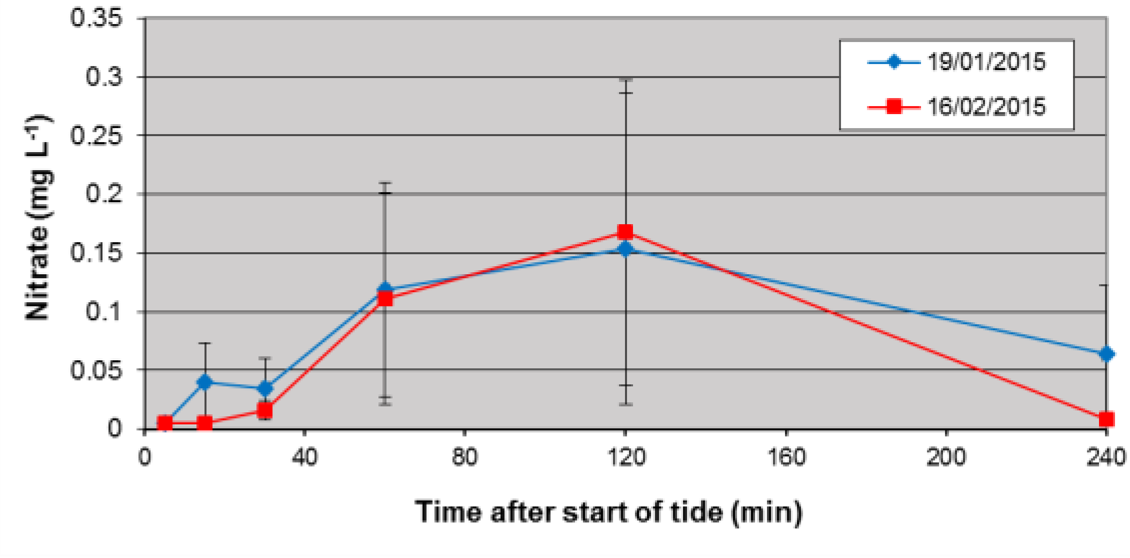

Figure 47 Nitrate levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Dissolved organic nitrogen

Levels of DON in PASF discharge were not affected (P>0.05) by the different stages of the artificial tide (Figure 48). Due to the high errors associated with the means, PASF DON levels also did not differ significantly (P=0.12) between the sample dates, even though on average levels in samples taken earlier in Season 2 (0.64 mg L^{-1} on 19/01/15) were marginally lower than those taken later in the season (1.16 mg L^{-1} on 16/02/15).

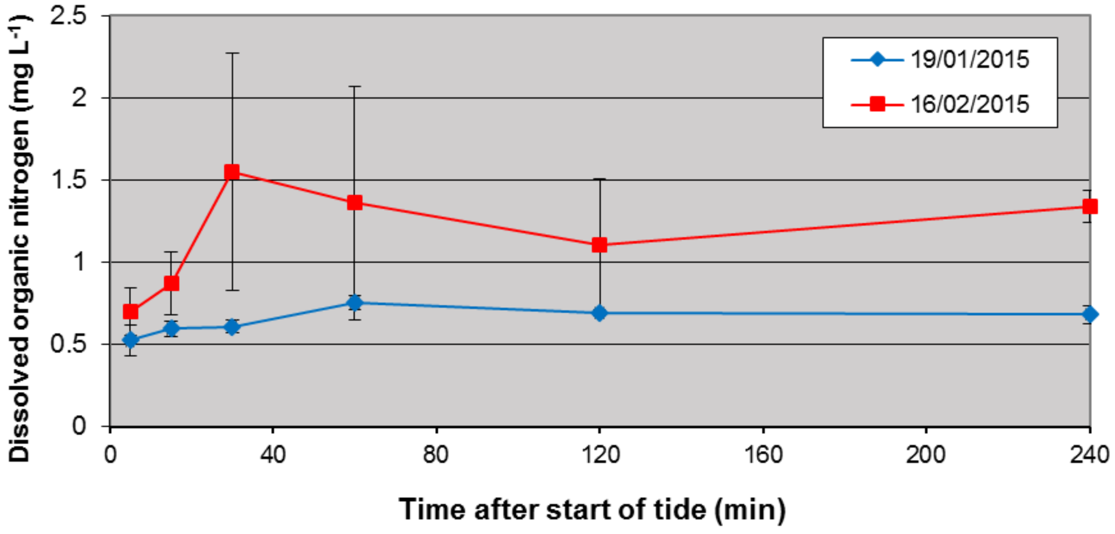

Figure 48 Dissolved organic nitrogen levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Dissolved organic phosphorus

Dissolved organic phosphorus levels in PASF discharge were not significantly affected by different stages of the artificial tide (Figure 49), and there were no differences ($P>0.05$) in DOP levels between the two sample days in Season 2.

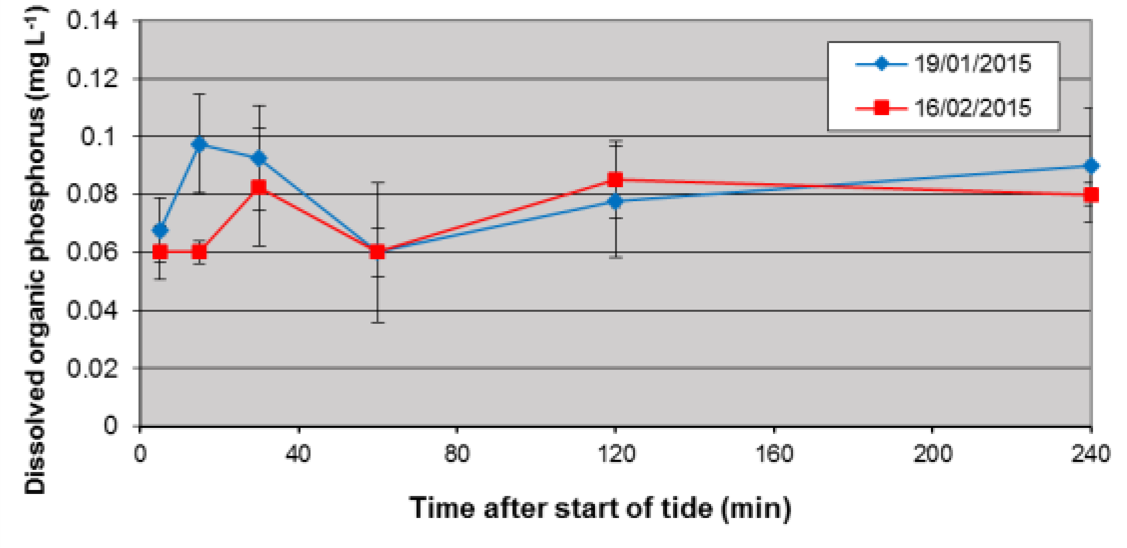

Figure 49 Dissolved organic phosphorus levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Phosphate

Levels of phosphate in PASF discharge were significantly affected ($P<0.001$) by the different stages of the artificial tide (Figure 50); there were steady declines during both experiments. The overall mean declines (both dates inclusive) were significant ($P<0.05$) from 5 to 15 min after the start of the tide, were not significant ($P>0.05$) from 15 to 60 min and from 120 to 240 min, but were significant ($P<0.05$) from 60 to 120 min. Much higher ($P<0.001$) mean phosphate levels occurred later in Season 2 (0.35 mg L^{-1} on 16/02/15) than earlier in the season (0.13 mg L^{-1} on 19/01/15).

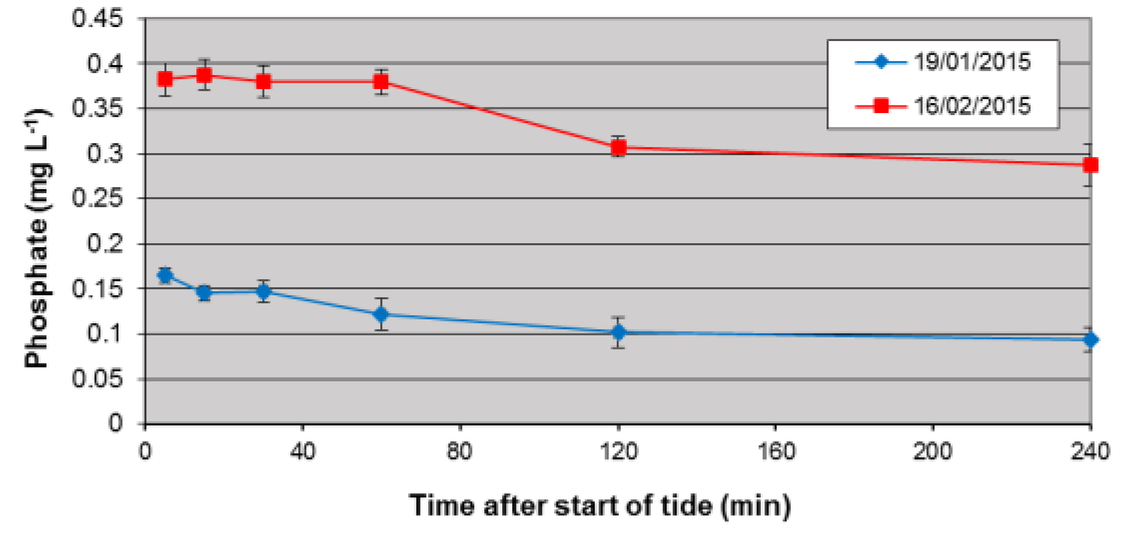

Figure 50 Phosphate levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

Total sulphide

Total sulphide levels in PASF discharge also showed steady declines during both experiments (Figure 51) and there was a significant (P<0.05) interaction between the sample date and time after start of tide (Table 8).

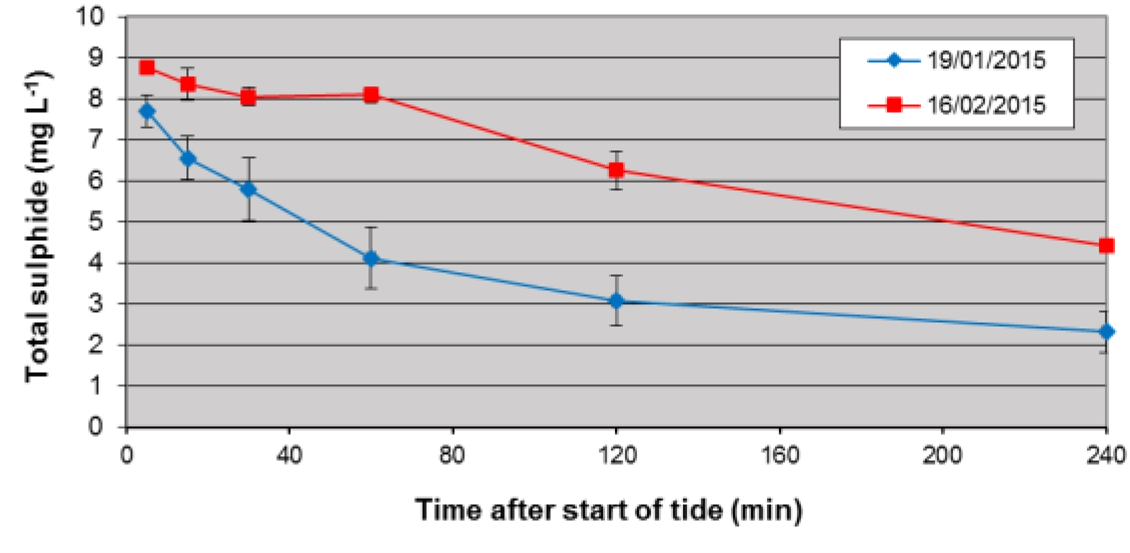

Figure 51 Total sulphide levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

On the earlier sample date (19/01/15) the highest level was produced in the first sample taken (at 5 min post tidal start) but this was similar (P>0.05) to the level measured 10 min later (15 min after the start of the tide). High levels were sustained for longer in the tide on the later sample date (16/02/15) where there were no differences (P>0.05) between levels produced after 5 to 60 min. Except for the first sample taken during the tidal cycle (5 min), samples taken early in the season were lower (P<0.05) at their respective tidal stages than samples taken later in the season.

Table 8 Total sulphide levels (mg L^{-1}) in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2. Different superscripts indicate significant (P<0.05) differences.

Time after start of tide (min)	19/01/2015	16/02/2015
5	7.7^{abc}	8.78^{a}
15	6.55^{bcd}	8.35^{a}
30	5.8^{de}	8.05^{ab}
60	4.13^{f}	8.1^{a}
120	3.08^{fg}	6.25^{cd}
240	2.33^{g}	4.43^{ef}

Total alkalinity

Total alkalinity levels in PASF discharge also showed steady declines during both experiments (Figure 52) except for an apparent increase early in the tidal cycle on the later sample date (16/02/15). There was also a significant (P<0.05) interaction between the sample date and time after start of tide (Table 9) for alkalinity.

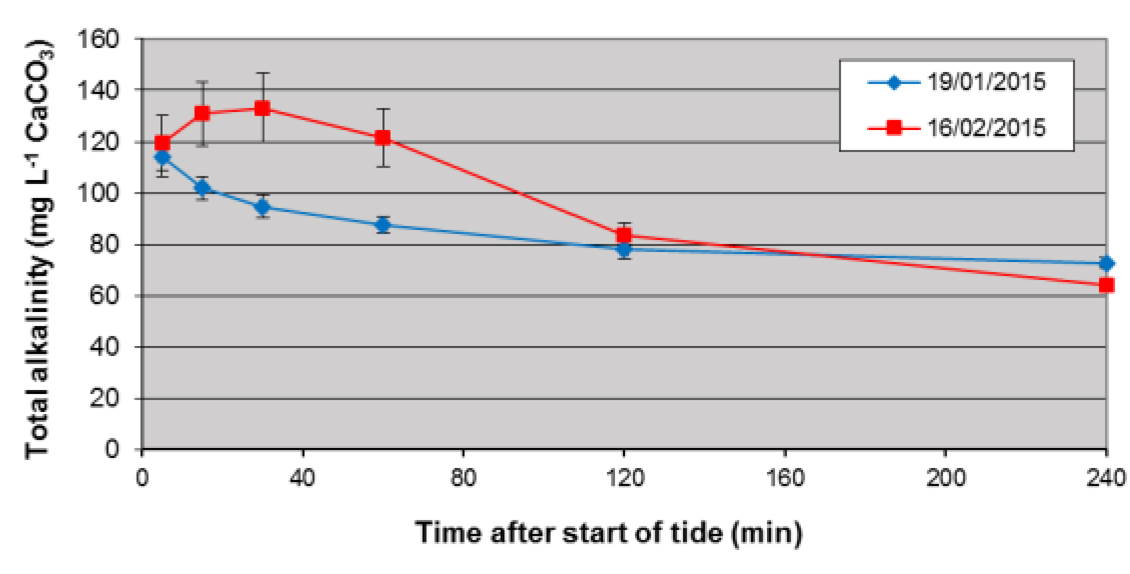

Figure 52 Total alkalinity levels in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2.

On the earlier sample date (19/01/15) the highest alkalinity was again produced in the first sample taken (at 5 min post tidal start) but this was statistically similar ($P>0.05$) to the levels measured up to 55 min later (60 min after the start of the tide). On the later sample date (16/02/15) the highest alkalinity was measured 30 min after the start of the tide, but this was again similar ($P>0.05$) to alkalinities measured between 5 and 60 min after the start of the artificial tide.

Table 9 Total alkalinity levels (mg L^{-1} $CaCO_3$) in discharge from four PASF beds (2 shallow and 2 deep) at different times after the start of an artificial tide on two sample days in Season 2. Different superscripts indicate significant ($P<0.05$) differences.

Time after start of tide (min)	19/01/2015	16/02/2015
5	113.9^{abc}	119.5^{ab}
15	101.9^{bc}	130.8^{a}
30	94.7^{bcde}	133^{a}
60	87.4^{cdef}	121.5^{ab}
120	77.9^{def}	83.8^{def}
240	72.7^{ef}	63.9^{f}

Discussion

It should come as no surprise that species with complementary relationships in nature can be farmed together with mutual benefits in intensive agriculture. Decapods and polychaetes coexist in nature and are important components of many marine benthic communities, so in simplistic terms it can naturally follow that they may be compatible in well-designed farming systems. For example, it may be pertinent that both species in the current study, *Penaeus monodon* and *Perinereis helleri,* are both known to occur in mangrove swamps and estuaries throughout Indo-Pacific regions. Although many commentators consider that polyculture can be less predictable and overly complicated, due to the need to synchronise timelines and manage behavioural conflicts or facility bottlenecks, it can justifiably provide considerable value to operations if the intrinsic values and functional virtues of each species can be meshed and optimised. The partitioning of the PASF system into two sections (i.e., prawn ponds and worm beds) that can either be linked or operated on their own for extended periods provides the flexibility to overcome the imprecision of timelines and procedures associated with managing the production of two very different species from different Phyla (i.e., Arthropoda and Annelida).

The functionality of PASF in a recirculated prawn farm combines ecological and nutritional services where both species benefit in more than one way. The worm beds provide the prawn ponds with a more secure (perhaps beneficial) water supply and a high-quality feed source for its broodstock; and the prawn ponds similarly provide a guaranteed reservoir for controlled water supplies, and a nutritious feed source (algae) for the worm beds. That this algal feed would otherwise be wasted is an added bonus in the PASF arrangement. The particularly efficient ways that waste products from each species and component of this partitioned polyculture system are incorporated and utilised means that 100% recirculation can be practised to capture significant economic, biological and environmental benefits. Compared with other contemporary recirculating aquaculture systems, our PASF design uses a much simpler approach that incorporates less expensive materials, and since it is applicable at small or large scales and flexible for any pond-based mariculture (prawns/shrimp or fish) it may provide the basis for development of biosecure culture systems recently flagged as an important goal for tropical countries in the future (Thitamadee et al., 2016).

Prawn farming is widely viewed as one of the most efficient methods of food production currently known. On a unit per area basis aquaculture can produce more high-value protein than any other agricultural system. Despite some examples of environmental degradation in other countries where developments have been uncontrolled and at much greater scales, prawn farming in Australia is considered sustainable and operating with world's best practice. Currently, settlement ponds are considered best practice for nutrient mitigation in Australia, and they also serve other important on-farm environmental services such as flow mitigation and preventing the escape of culture stock. Since PASF appears to treat prawn pond effluent with equal or better efficacies to those of settlement ponds (see discussion below), there is potential in farm designs to replace some or all of the non-productive settlement ponds with productive PASF beds. There is also potential to reduce the portion of farm area devoted to water treatment and therefore use more of the farm area for prawn production. This latter aspect of PASF adoption is evaluated in our economic modelling (Appendix 1) and alone could provide significant incentive for the future industrialisation of PASF.

Some of the other less-obvious advantages of PASF adoption are vested in the potential for improved nutrition of prawn broodstock and associated flow-on effects (e.g., higher egg production and more vigorous larvae). On-farm production of marine worms will likely lead to higher inclusion levels in prawn broodstock diets, and this may well prove to be one key to the higher reproductive performance of species such as *P. monodon* (see Appendices 2 and 3), which are more difficult to breed in captivity than other commercial penaeids. This may in turn assist in domestication and the reliability of closed-system prawn breeding programs, thereby more fully unlocking the genetic and economic gains that have been recently demonstrated in successful programs (e.g., FRDC, 2014).

This study also provides scientifically validated data regarding the nutrient assimilation capacity of natural systems. There is currently great interest in this due to the concept of maximum sustainable nutrient loads of catchments and tolerable levels of nutrient discharge from anthropogenic nutrient sources, which include aquaculture and other more traditional forms of agriculture (Hansard, 2015). Different vegetation and habitat types provide different levels of assimilation and have different levels of resilience to perturbations, and whilst there have certainly been some robust assessments of the impact of prawn farm effluent on natural environments (Jones et al., 2001; McKinnon et al., 2002a, b), many ecosystem types have not been specifically studied in this regard due to the inherent difficulties in achieving experimentally replicated systems that simulate and provide for relevant loads and species. The present study adds further data of this nature for intertidal sand flats that support populations of polychaetes, and possibly other burrowing animals (e.g., *Callianassa australiensis*) which are common in many Australian estuaries, and shows that these may have particularly high levels of tolerance and capabilities to process and remove significant amounts of nutrients from adjacent waters.

Four of the most important water qualities that exist in most prawn farms' discharge licences in Australia, namely TSS, TN, TP, and (in some cases) chlorophyll *a* were all shown to be significantly reduced by PASF in the present study. Their levels were reduced by up to 92%, 50%, 66%, and 99% respectively, which supports our previous work with PASF. For example, Palmer (2011) reported that PASF removed almost all of the TSS, and provided maximum removal efficacies of 49%, 68% and 93% for TN, TP, and chlorophyll *a*, respectively. To our knowledge these are the greatest reported removal efficacies for any broad-scale method so far developed for land-based mariculture. The shortcomings of this study though are that PASF did not bring TN and chlorophyll *a* levels down below BIRC's discharge maximum, even after several weeks treating the water without prawns or feeding. Background levels of 0.1-0.2 mg L^{-1} of nitrogen and 0.02-0.03 mg L^{-1} of phosphorus are common in BIRC's intake waters, so therefore we also did not achieve zero-net-nutrient discharge from the system.

By comparison, settlement ponds have been shown to provide lesser reductions of up to 60% for suspended solids, up to 20% for total nitrogen and up to 30% for total phosphorus (Preston et al., 2000). So with application at large scale, these present data for PASF appear to offer industrial solutions for the maximum levels of TSS and TP permitted in prawn farm discharge, but importantly, they do not presently offer farmers a way to dispose entirely of the excess nitrogen. Even at the less stringent maximum nitrogen levels applied to many farms from 2005 (3 mg L^{-1}: EPA, 2004/05), final discharge in the present study would have been marginally non-compliant. Again, if this process can be applied at a large enough scale, PASF offers a way to largely eliminate discharge during the season through 100% recirculation and treatment of wastewater, but in the present study it did not prevent the build-up of nutrients in the pond waters during each growing season.

Other important water qualities were also impacted by the PASF beds in the study. The pH of PASF-treated water appeared most affected by the pH of the pond water being treated, although at times there was a tendency for PASF to significantly lower the pH. Deeper beds also appeared to accentuate this pH reduction. At certain times during the prawn culture cycle this will be advantageous, such as when pond pH levels are high due to high levels of photosynthesis, which can cause the toxicity of metabolites such as ammonia to also be higher. Whilst the lowest mean pH level emitted late in Season 2 by PASF (6.68 on 31/03/15) was marginally lower than the preferred range for black tiger prawns (7-9: Australian Prawn Farming Manual, 2006), the large volumes in the ponds could be expected to buffer this for little overall effect in such a large recirculated system. For a flow-through approach where PASF-treated pond water is discharged to waste, pH levels emitted by PASF remained well within the discharge limits of BIRC. However, towards the end of the prawn cropping cycle, farmers battle with low pH caused by accumulated organic matter in the system (e.g., pond sludge in the middle of grow-out ponds). Whilst this is of concern, prawn farmers are well aware of the tendency for low pH towards the end of the crop, and since close monitoring and remedial methods such as liming are commonplace at that time, PASF recirculation should not present any additional challenges in this regard.

Dissolved oxygen levels are significantly reduced by PASF, but there was no evidence that recirculating this low-DO water adversely affected the prawn culture ponds. Low DO in PASF discharge is evidence that the sand bed treatment process is in fact functional. It appears that if levels of DO in PASF discharge are below 1 mg L^{-1}, the water is likely not bypassing the sand filter so that the physical sand filtration processes are at their optima. This is also evidence that the biological aspects of PASF filtration are also being optimised through the decomposition of organic matter, mineralisation of nutrients and harnessing of anaerobic processes that drive off nitrogen in gaseous forms. Further research is necessary to determine if even lower DO could help these biological processes enhance water treatment, particularly to further enhance broad-scale nitrogen removal. Lower DO penetration into sediments could potentially be facilitated by slower percolation rates or different flow patterns, although it is uncertain how this could also affect resident polychaete populations. However, the results of this study suggest that deeper beds emit lower levels of DO, and hence bed depth may be an easily adjusted factor to optimise PASF design in this way in the future.

Dissolved nutrients were also significantly affected by PASF in the study. The increasing ammonia levels towards the end of the trial are evidence of the breakdown of organic matter. Stable neutral pH in the ponds helps to reduce the toxicity of this, and algae in the pond preferentially utilise ammonia before other forms of dissolved nitrogen, so it is quickly scavenged by the healthy algal blooms that prevail in ponds. Concentrations of this and other dissolved nutrients coming from decomposing organic material in the beds (e.g., phosphate) will invariably increase as beds become more blocked, because lower percolation rates directly cause lower dilution rates for this mineralising material at source. Of particular interest are the PASF-reductions of DON, which suggest that it may be processed by benthic microbes more efficiently than those which exist in the pelagic pond-water environment (Burford et al., 2001).

The artificial tidal flow patterns that are intrinsic to the operations of PASF had a pronounced effect on total nitrogen, total phosphorus and dissolved nutrients in the PASF discharge. For most parameters that were affected, there was a slow decline in concentrations during the course of the artificial tide. Highest levels tended to occur in the first hour after the start of the pond water inflow, during the flood tide before the entire bed had been covered. And this flushing seems to have almost abated after two hours when the water body sitting over the bed was at its deepest. Of course, higher head pressure would have caused higher percolation and dilution rates at this time, but as the tide receded, compensatory increases in nutrients did not occur, suggesting that the plug of nutrients had by this time been flushed.

Unfortunately, it is difficult to reconcile precisely how these tidal flow changes affect overall mass balance calculations for nutrient removal by PASF. To fully explore this integrated effect would require a different set of experimental conditions, such as the retainment of all of the flow from particular tides, and at different stages in the season, in a single pool with sampling after it was homogenised. This was beyond the scope and physical capabilities of the present study. However, since this study applied the seasonal sampling analyses from PASF in the middle of tidal flows, it is considered that despite the higher concentrations of nutrients that would have routinely occurred before sampling, the lower flow rates that are intrinsic to PASF operation early in the tidal flow[19], coupled with the lower levels that are emitted at relative times after samples were taken, would have largely countered this effect so that the data generated were indicative of true reduction levels.

Concentrations of dissolved forms of nutrients such as ammonia, phosphate, and sulphide were most heavily impacted by the tidal flushing. This suggests that the decomposition of organic matter and mineralisation of nitrogen and phosphorus continues even during the regular low tide events when the liberated dissolved forms are not continually flushed from the sand bed. Interestingly, nitrite and nitrate levels were not significantly affected by the stage of the artificial tide, but this is not surprising because nitrification is an aerobic process. Percolating water carries oxygen down into the body of the sand bed, but when this ceases and biological demand exceeds supply anaerobic conditions prevail, potentially leading to denitrification and the production of hydrogen sulphide gases. The reason that DON and DOP also did not change significantly during the tidal cycle is unclear, particularly since there was evidence for the release and flushing of other organic compounds (e.g., $CaCO_3$ measured with alkalinity) that would have similarly been released from decomposing organic matter.

More precise, however, are the calculations that can be made from this work for overall nutrient release, since recirculation efficacies are internalised within the closed system during growout, and therefore only volumes and concentrations in ponds at the end of the season are the relevant multipliers. On this basis, our ponds' collective volume (4.8 megalitres), if discharged according to the methods demonstrated in Season 2 and without further (outgoing) treatment by PASF (3.89 mg L^{-1} TN; 0.4 mg L^{-1} TP) would release a total of 58.4 kg ha^{-1} of N and 6 kg ha^{-1} of P. This is well below the past load-based licencing arrangements for many prawn farms in Australia (EPA 2004/05) that permit up to 1 kg ha^{-1} d^{-1} of N and 0.15 kg ha^{-1} d^{-1} of P averaged over the growing season (normally about 120 days). Based on the level of prawn production in Season 2 (3.16 tonne), this equated to 5.91 kg of discharged N, and 0.61 kg of discharged P per tonne of prawns produced, which compares favourably with other coastal land use in sensitive environments. Further improvements can also be expected as PASF optimisations progress (e.g., if deep beds were used throughout the design). The only nutrient losses from the system that are not included in these calculations were those contained within the slurry that is produced as part of the polychaete harvest process. Nutrient

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[19] In previous work described by Palmer (2011), flow rates through PASF did not reach maxima until 60-90 min after the start of artificial tides.

concentrations of this slurry were not assessed in the study, but since this only amounts to several hundred litres with each harvest it can easily be disposed of separately in evaporation pans and landfills so as not to add to farm nutrient releases. Indeed, savings made in the lower landfill-disposal from lower in-pond sludge production (as demonstrated in this study) could compensate for this additional landfill slurry created by the worm harvests.

In accord with these capacities for PASF to remove nutrients from prawn pond waters are its abilities to progressively and reliably treat the inherent very large volumes of prawn pond wastewater, and this totalled more than 78 megalitres during the study period. Shallow beds were less effective in this regard, because they showed a higher degree of clogging and had a greater tendency for tracking (e.g., in Bed 2 on 13/01/15). This difference between shallow and deep beds was shown through the experimental comparisons of maximum discharge rates possible after a significant operational period, and in a practical sense through the necessary use of different discharge-hole sizes for shallow and deep beds to achieve effective and comparative operations. Greater clogging in shallow beds was also demonstrated by the greater need to revert to sand bed short circuits to allow daily draining and drying of the beds according to our preferred water management plan.

It is also apparent that accumulation of organic matter within PASF is largely self-limiting. Organic clogging from overload situations, such as that when treating waters with particularly intense algal blooms, invariably leads to reduced percolation rates and greater degrees of anaerobiosis. Percolation and sand filtration almost cease when the bed is clogged with organic matter. At that point the beds' capacity for water filtration is limited, yet they can still yield a significant amount of worms, as evidenced in Season 1 by those clogged shallow beds harvested late in the season.

Importantly, even when clogged beds were short circuited to allow unfiltered pond water to bypass the sand mass, significant removal of suspended solids and nutrients was still evident. This suggests that the method of short-circuiting beds used in the study still allowed the percolation of some pond water through the sand matrix for continued water treatment. Alternatively, processes that occur above the sand mass (e.g., settlement, nutrient absorption by benthic algae) still provide useful wastewater treatments, though not with efficacies demonstrated by fully-functional PASF beds.

The wet-weather operational mode, as is necessary when heavy rain threatens uncovered PASF beds to protect the polychaetes from very low salinities, was investigated fortuitously on one occasion each season. Since each of these modes of operation (i.e., dry- and wet-weather) are necessary parts of the management regime for an outdoor PASF that can operate successfully in all weather conditions, this study included all conditions that were presented during the study period, even though it was not specifically designed to compare these different modes. Nevertheless, there is evidence in the results that the wet operational mode may lead to somewhat reduced efficacy for TSS removal, improved efficacy for reduction of turbidity, and increased levels of sulphide in PASF discharge. The effectiveness of this wet weather operational strategy in protecting the vulnerable worm beds from low salinities was well tested when cyclone Marcia passed by the study site in Season 2; between 20th and 22nd February 2015, 323 mm of rain fell on Bribie Island (BOM, 2015), causing a reduction of about 7 ppt in salinity in the ponds, but with close management no appreciable impact on the worms.

The dissolved nutrients which constantly feed back into the pond from PASF could have several positive effects on ecosystem stability and health. The combination of phytoplankton removal by PASF and nutrient supply may help keep algal blooms in a state of physiological youth. The dissolved nitrogen and phosphorus that PASF supplies would ensure that pond phytoplankton are constantly supplied with primary nutrients so that bloom failure is reduced. The conversion of sulphide to sulphate would ensure this important nutrient also does not become limiting. A broad mix of other essential nutrients and trace elements are also in constant supply from PASF, as is demonstrated in the analyses of biofilm that typically flows with the treated water (see Appendix 4). This mix includes aluminium, calcium, iron, magnesium, phosphorus, potassium, arsenic, boron, bromine, copper, iodine, molybdenum, samarium, silicon, strontium, titanium, vanadium and zinc, many of which are routinely supplied in balanced commercial fertilisers. The higher alkalinities that PASF supplies may also help maintain this stabilising quality in ponds to assist in pH management, and possibly offer savings in the use of lime.

This study also investigated several important aspects associated with the polychaete productivity of PASF. One developmental option investigated was the potential to stock PASF beds with young, ready-to-settle larvae (nectochaetes), as has been reported from Thailand for *Perinereis nuntia* in more-closely-managed culture systems that treat the incoming water with ozone (Poltana et al., 2007), and as could be more suitable for large-scale industrial PASF expansions. In PASF, however, the culture beds are fed with prawn pond waters with less control over the mix of organisms and other water qualities, such that prevailing benthic conditions in the sand beds are also largely uncontrolled. Since PASF is also mainly framed as an outdoor activity, prevailing severe weather conditions could add further to potentially harsh environments that the fragile worm larvae would need to endure to survive with direct stocking activities. Although this approach to stocking PASF was discounted as an option in earlier work because it gave very low survival (<1%: Palmer, 2010), it was revisited in the present study under varying conditions including different underlying bloom types that may have improved larval survival (i.e., chlorophytes + copepods versus diatoms + rotifers). But despite these differing conditions, the survival of larvae was still much lower than that of one-month-old juveniles.

Polychaete biomass production levels of around 1 kg m^{-2} were achieved in Season 1 from the high-density stocking of one-month-old juveniles (i.e., 6,000 m^{-2}) and the operational methods described in this report. In Season 2, lower densities of one-month-old juveniles were applied (2000 m^{-2}), and as expected this resulted in much larger worms that are well suited to fishing bait markets (and prawn broodstock feeds) although there was lower overall biomass production (0.7 kg m^{-2}). The different sand bed depths that were studied (130 and 230 mm) did not appear to significantly affect polychaete production levels, and sand bed clogging also did not adversely affect polychaete production, as long as a simple set of rules that maintained worm bed ecosystem health and included daily sun drying of beds was applied. Although not covered in detail in this report, the harvesting and processing of worms from PASF were practical know-how-based processes that were eventually handled by supervised casual workers in the program, as long as a standardised set of methods were applied.

The use of live wild-captured polychaetes in prawn broodstock diets is very topical at present because this has been identified with potential for vertical transmission of disease (Vijayan et al., 2005) and is considered to have promoted the spread of new diseases in cultivated penaeids in Asia and other parts of the world (Thitamadee et al., 2016). The quandary here lies in the need for large quantities of polychaete biomass for commercial penaeid operations, and the preference of hatcheries for this to be live because it may give better results than frozen or pasteurized forms. The development of PASF not only addresses these concerns by providing a more-controlled and sustainable way to produce large quantities of polychaete biomass, but it can also provide this at levels of biosecurity that are equal to the cultivation system with which it is linked (e.g., systems for specific pathogen free stock). Whilst we have not identified any amplification of disease in any segment of our recirculating system, further concerns regarding the use of PASF-worms that may be contaminated by wastewater from insecure culture systems are the subject of future planned research. Purging to remove contaminants in gut contents, and further disinfection treatments which ensure that live polychaetes hold no pathogen threats but retain their unique nutritional values, seem to be the best directions for research designed to make this useful product more widely available in locations where serious diseases are endemic.

Finally, there may be minor safety issues associated with the use of PASF that should be openly discussed. The distinctive odour of sulphide gas can be quite prevalent near the drainage outlets of PASF, and it is important for operators to be aware of the associated hazards of this gaseous by-product. Hydrogen sulphide gas has broad-spectrum toxicity to humans at very low levels (MSDS#1010) so its inhalation at any level should be avoided. In solution at high concentrations this can also form sulphuric acid which is corrosive and with contact can cause severe skin or eye damage. Caution should therefore be exercised in restricted spaces where PASF discharge is flowing, and when handling the most concentrated solutions such as those which are emitted from PASF in the first few minutes after the start of artificial tides. In this regard it is worth noting that similar anaerobic, sulphide-rich conditions also develop in the settled sludge-pile that develops in the middle of most prawn ponds. Whilst the levels of ventilation in these areas generally reduce the potential for poisoning via inhalation, this can also cause skin irritation with prolonged contact.

Conclusion and future needs

This study has demonstrated the application of PASF in a fully recirculated prawn production system, over two successive production seasons, and under conditions that have tested its resilience to environmental (e.g., adverse weather events) and commercial (e.g., economic) imperatives. It has begun the process of optimising the nutrient removal functionality of PASF, documented its current capabilities in terms of water treatment and polychaete production, and engaged industry in meaningful ways to help drive industrial uptake. This novel approach to prawn production was successful at producing commercial quantities and qualities of black tiger prawns at densities that are directly relevant to the present Australian industry. Given that Australia is considered one of the leading innovators in the sustainable production of this species of prawn, this study also has world-wide application.

This study has also demonstrated a 100% recirculation strategy that has many potential advantages over flow-through models, including better within-season biosecurity and surety of unpolluted water supplies, high feed conversion and growth, low sludge production, and zero nutrient emissions during the production phase. Importantly though, this study does not demonstrate an ability to practically achieve zero-net-nutrient discharge from broad-scale prawn production systems. To go some way towards achieving this would likely require a tertiary water treatment phase downstream of PASF, which assimilates dissolved nutrients in a form that can be regularly removed from the system (e.g., seaweed: Palmer, 2008, 2010; Lawton et al., 2013; Mata et al., 2015). Instead in the present work, we have channelled those dissolved nutrients into an alternative plant-based product (i.e., pond phytoplankton) that is already part of the prawn production process. However, the disposal of grow-out pond waters at the end of each season is an essential part of biosecurity and pond health protocols for prawn farmers, and excess nitrogen in the system, even following several weeks of PASF operations without any feeds added, tended to marginally exceed broadly applied licenced discharge levels.

As well as the significant advantages mentioned above, the PASF system offers production of a high-value by-product (polychaete biomass) which is particularly useful to the prawn farming industry as an important component of broodstock prawn maturation diets. In fact, during the course of the study, this by-product of the PASF system has become a business option that may stand alone from the water treatment mechanisms it facilitates. This does not suggest that these two functions (i.e., water treatment and polychaete production) should be divorced in future developments, because they are synergistic in several ways. The pond algae that feed the worms in PASF may improve their specific nutritional contents and provide the ability to conveniently supply food in liquid form to the worm beds, which saves on labour and alternative feeds for better worm production economics. Additionally, the continual removal of senescing algae and minimisation of silt loads in prawn culture ponds appears to provide physical and chemical improvement, which may contribute to better prawn pond ecosystem health. The annual harvest of worms from the PASF beds clears the filter beds of accumulated inorganic silt, provides economic returns that can easily pay for the PASF operation, and facilitates the reuse of the same sand in the filters each year.

In particular, the excellent growth rates of prawns in the recirculated ponds in both successive seasons suggests that this most important aspect of prawn production did not suffer from the complete recirculation of PASF-treated wastewater during the prawn production cycle. This study has investigated and provides data for many of the most important aspects of PASF wastewater treatment for consideration by industry, including farm area needs, water volumes able to be treated, nutrient removal efficacies and relevance to current nutrient discharge licences, useful functionalities and practicalities involved in its application, and economics of installations and long term use. Whilst this style of recirculating aquaculture system has greater complexity when compared with traditional flow-through prawn farming models, this may not be avoided in future more advanced operations, and we expect this change in practice could be afforded by the significant advantages it appears to offer.

Application at large enough scales to be applied in industrial settings seems to be the next major hurdle in the development of PASF. Whilst the PASF process appears scalable with sufficient materials and business commitment to do so, there are related services that simultaneously must be built to higher levels for this to occur and be economically viable. One of these necessary services for upscaling is the substantial worm seedstock supply that would be needed to facilitate industrial expansions. Since polychaete breeding and associated seedstock production technologies are not readily available in the public domain, and are largely restricted to a small number of companies worldwide, this will involve technology transfers and/or research and developments in the wider prawn farming industry. Two possible commercialisation approaches are presently envisaged; 1) where centralised seedstock producers or PASF operators service the needs of industry, and/or 2) where prawn farming companies develop expertise in this area to service their own needs. These options are currently being explored with industry and other prospective partners.

Another uncertainty regarding the potential industrialisation of PASF is the dramatic increase in polychaete biomass that it will create with widespread adoption, and how this will affect existing polychaete markets and structures upon which economic models are based (see Appendix 1). Whilst moderate portions of the produce from an industrial-scale PASF could be absorbed within the farming system, and particularly within the associated prawn hatcheries for great potential benefit in prawn seedstock production, new markets and uses for polychaete biomass that are supplementary to bait and prawn broodstock feeds will be essential for market stability and ongoing profitability of this practice. These uncertain aspects of PASF industrialisation are yet to be realised, but should be anticipated in the forward planning of pathways for expansion of PASF in the future.

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References

Australian Prawn Farming Manual. 2006. The State of Queensland, Department of Primary Industries and Fisheries. ISSN 0727-6273.

BOM. 2015. Bureau of Meteorology, Daily Rainfall Data, Bribie Island. http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=136&p_display_type=dailyDataFile&p_startYear=&p_c=&p_stn_num=040978

Boyd, C.E. 1979. Water quality in warm water fish ponds. Agricultural experiment station, Auburn University, Alabama.

Burford, M.A., Peterson, E.L., Baiano, J.C.F., Preston, N.P. 1998. Bacteria in shrimp pond sediments: their role in mineralizing nutrients and some suggested sampling strategies. *Aquaculture Research* 29(11), 843-849.

Burford, M.A., Jackson, C.J., Preston, N.P. 2001. Reducing nitrogen waste from shrimp farming: an integrated approach. The New Wave, Proceedings of the Special Season on Sustainable Shrimp Culture, Aquaculture 2001. *Eds.* C.L. Browdy and D.E. Jory, 35-43.

Chen, J.-C., Liu, P.-C., Lei, S.-C. 1990. Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. *Aquaculture* 89(2), 127-137.

CSIRO, 2015. http://www.csiro.au/en/Research/AF/Areas/Aquaculture/Premium-breeds/Black-tiger-prawn

EIFAC. 1986. Report of the working group on terminology, format and units of measurement as related to flow-through and recirculation system. European Inland Fisheries Advisory commission. Tech. Pap., 49. 100 pp.

EPA. 2004/05. Environmental Protection Agency, Operational Policy: Marine Prawn Aquaculture. Licensing wastewater releases from existing marine prawn farms in Queensland Reviewed and Approved 2004/05. https://www.ehp.qld.gov.au/licences-permits/business-industry/pdf/wastewater-prawn-farm-em1862.pdf

FRDC. 2014. http://frdc.com.au/stories/Pages/how_to_build_a_better_prawn.aspx

Funge-Smith, S.J., Briggs, M.R.P. 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. *Aquaculture* 164(1-4), 117-133.

GenStat. 2015. GenStat for Windows, Release 16.1. VSN International Ltd., Oxford.

Hansard. 2015. Joint Select Committee on Northern Australia: Opportunities for expanding the aquaculture industry in Northern Australia. Tuesday, 15 September, 2015. Commonwealth of Australia, Canberra.

Harrison, K.E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *J. Shellfish Res*., 9, 1–28.

Jones, A.B., O'Donohue, M.J., Udy, J., Dennison, W.C. 2001. Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses. *Estuarine, Coastal and Shelf Science* 52(1), 91-109.

Kawahigashi, D.K. 1998. Overview of commercial maturation technology in the Western hemisphere. World Aquaculture Society Conference Proceedings, Brazil, 98, 381–392.

Kuhn, D.D., Smith, S.A., Flick, G.J. 2011. High nitrate levels toxic to shrimp. *Global Aquaculture Advocate* November/December 2011, 36-37.

Lawton, R.J., Mata, L., de Nys, R., Paul, N.A. 2013. Algal bioremediation of waste waters from land-based aquaculture using Ulva: selecting target species and strains. PLOS One, 8(10), e77344.

Mata, L., Magnusson, M., Paul, N.A., & de Nys, R. 2015. The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi*: biomass and bioproducts. *Journal of Applied Phycology*, 1-11.

MBD Energy 2015 http://www.abc.net.au/news/2015-01-01/qch-algae-ready-for-expansion-in-2015/5995836

McKinnon, A.D., Trott, L.A., Alongi, D.M., Davidson, A. 2002a. Water column production and nutrient characteristics in mangrove creeks receiving shrimp farm effluent. *Aquaculture Research* 33(1), 55-73.

McKinnon, A.D., Trott, L.A., Cappo, M., Miller, D.K., Duggan, S., Speare, P., Danidsom, A. 2002b. The trophic fate of shrimp farm effluent in mangrove creeks of North Queensland, Australia. *Estuarine, Coastal and Shelf Science* 55(4), 655-671.

MSDS#1010. Materials Safety Data Sheet – Hydrogen sulfide. http://www.google.com.au/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved=0CBwQFjAAahUKEwiq35yghJfIAhWkPKYKHVq7DRQ&url=http%3A%2F%2Favogadro.chem.iastate.edu%2FMSDS%2Fhydrogen_sulfide.pdf&usg=AFQjCNFF70eCfvW16qeW9R7jvU-gphesYg&bvm=bv.103388427,d.dGY

Palmer, P.J. 2008. Polychaete-assisted sand filters – prawn farm wastewater remediation trial. National Landcare Programme Innovation Grant No. 60945 Technical Report. 61 p.

Palmer, P.J. 2010. Polychaete-assisted sand filters. *Aquaculture* 306, 369-377.

Palmer, P.J., Wang, S., Borchert, T., Dutney, L., Nicholson, S. 2010. Nursery pond management for cobia fingerling and juvenile production. In: Cobia aquaculture under Queensland conditions: A project within the Queensland Aquaculture Development Initiative 2007-09. P. J. Palmer (Ed). Unpublished Report. The State of Queensland, Department of Employment, Economic Development and Innovation. pp. 55-66.

Palmer, P.J. 2011. Commercial application of polychaete sand filters for wastewater treatment and broodstock feeds. Landcare Sustainable Practices Grant No. SEQC1418 Technical Report. 34 p.

Palmer, P.J., Wang, S., Houlihan, A., Brock, I. 2014. Nutritional status of a nereidid polychaete cultured in sand filters of mariculture wastewater. *Aquaculture Nutrition* 20, 675-691.

Poltana, P., Lerkitkul, T., Pongtippatee-Taweepreda, P., Asuvapongpattana, S., Wongprasert, K., Sriurairatana, S., Chavadej, J., Sobhon, P., Olive, P.J.W., Withyachumnarnkul, B. 2007. Culture and development of the polychaete *Perinereis* cf. *nuntia*. *Invertebrate Reproduction and Development* 50(1), 13-20.

Preston, N.P., Jackson, C.J., Thompson, P., Austin, M., Burford, M.A., Rothlisberg, P. 2000. Prawn farm effluent: composition, origin and treatment. Fisheries Research & Development Corporation, Technical Report No. 96/162, 71 p.

Robertson, C.H., Burford, M.A., Johnston, A. 2003. Recirculation prawn farming project. Natural Heritage Trust, Coast and Clean Seas, Project Number 717511. ISSN 0727-6281.Final Report QO03014, 41 p.

Rowell, J.G. and Walters, R.E. 1976. Analysing data with repeated observations on each experimental unit. *Journal of Agricultural Science* 87, 423-432.

Shigueno, K. 1975. Shrimp culture in Japan. Association for international technical promotion Japan. 150 p.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W., Itsathitphaisarn, O. 2016. Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture* 452, 69-87.

Thomas, H., Schiettecatte, L.-S., Suykens, K., Koné, Y.J.M., Shadwick, E.H., Prowe, A.E.F., Bozec, Y., de Baar, H.J.W., and Borges, A.V. 2009. Enhanced ocean carbon storage from anaerobic alkalinity generation in coastal sediments. *Biogeosciences* 6, 267-274.

Vijayan, K.K., Raj, V.S., Balasubramanian, C.P., Alavandi, S.V., Sekhar, V.T., Santiago, T.C. 2005. Polychaete worms - a vector for white spot syndrome virus (WSSV). *Diseases of Aquatic Organisms* 63, 107-111.

Appendix 1 – Economic projections for application of PASF

Introduction

To provide prospective users of PASF with enough information to evaluate the costs and cash flows associated with variable-sized farm-based installations, an economic decision tool was developed with the assistance of an economist with considerable experience with aquaculture models. The project team worked closely with Mr Bill Johnston, Senior Principal Agricultural Economist with the Industry Analysis Unit of the Queensland Department of Agriculture and Fisheries to develop a model with realistic cost/benefit estimates that are applicable to prawn farms in Australia.

Economic model background

The model input uses the range of actual construction and operational costs (GST not included) associated with the PASF prototype that was installed at BIRC in 2013, and operated under commercially-relevant experimental conditions during two prawn production seasons in 2014 and 2015. The model also uses realistic worm production data and market prices generated during recent and previous PASF developmental research.

Although there are several practical and operational reasons for application of the specified prototype (as constructed at BIRC) at much larger scales, it is still unclear if this is entirely feasible. For example, an installation involving two hectares of PASF beds may require several design modifications, such as larger beds with more level sand surfaces, and different water supply and filtered-water-release mechanisms. However, such modifications could still be expected to use similar basic design and filtration rate principles, and most of the same materials, so that the costs and production figures would be largely unchanged. Of course, economies at very large scale, such as much larger beds than in the current prototype, could be expected to reduce costs further, and this model attempts to predict some of these factors.

In 2014 and 2015, experimental work at BIRC applied this water treatment system at a rate of 15% of total farm area. Total farm area at BIRC (3765 m^2) included 2 x 1600 m^2 prawn culture ponds (total of 3200 m^2) plus a total of 565 m^2 set aside for water treatment and recirculation. The water treatment/ recirculation element of this system included 10 x 54 m^2 PASF beds (540 m^2) plus an associated small area (25 m^2) used for PASF water reticulation (e.g. recirculation sump, water distribution manifold and pipes – see Figure 2).

Decision tool design

The model incorporates several linked Microsoft Excel worksheets which tally the costs and benefits, and projects the revenues over a 20-year period. Adjustable variables within worksheets (yellow boxes) can be manually modified to investigate the effect of changes to the design and operation on economics. Fixed costs and calculated values (red and brown boxes) are protected and cannot be modified. The following worksheets are incorporated:

Bed parameters

Input variables include the size of beds, the depth, weight and cost of sand, and the design and costs of pond liners, side supports, bed drainage and sumps.

Polychaete production

Input variables include the juvenile polychaete stocking density, the grow-out period and the bed dry-out period (nominally 10 and 2 months, respectively, in a 12-monthly cycle). The model assumes that economies of scale in seed production would reduce the price of juvenile worms. In scaling predicted worm production according to different juvenile stocking densities, a simplified relationship is applied which assumes a linear relationship from 0 to 5,000 juveniles m^{-2}, with a maximum production of 1.5 kg from stocking densities of 5,000 m^{-2} and over (see Figure 53 below). These estimates take into consideration that some beds will likely be harvested before they reach their full production potential at 10 months of age, but it should be recognised that harvesting beds too early (before age of 4-5 months) will invariably reduce harvest rates.

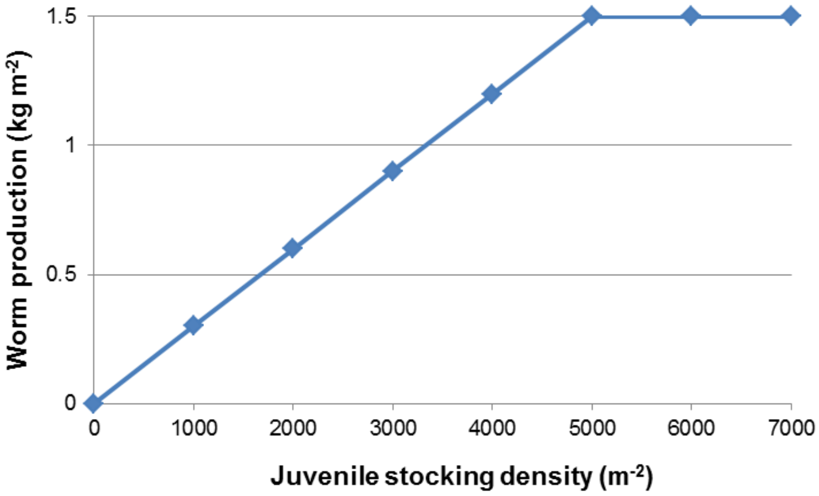


Figure 53 Estimated worm production from different juvenile stocking densities in PASF

Post production

The anticipated costs of processing, packaging, freight and marketing can be captured in this worksheet. Additionally, variable percentages of large and small worms are assumed to attract different premiums – here it is assumed that small worms (<0.6 g) are mainly sold as frozen product, and large worms (>0.6 g) are mainly sold into live markets. The sale prices for these forms of product (live and frozen) are also provided as variable inputs, to account for the potential depression of market prices that could be caused by dumping large amounts of new product on existing markets. In this regard, we expect that businesses would internalise the use of some of their own farm-reared worms, thereby replacing all of those that would otherwise have been purchased from other sources by the business, and then sell surpluses in ways that best maintain existing price structures (e.g. find new markets as production and availability increase).

Labour

The costs of farm labour are captured in this worksheet. Different input options are provided for casual or permanent employees with allowance for various on-costs.

Operating

Farm operating expenses are captured in this worksheet. These may include costs associated with various fuel supplies, repairs and maintenance, electricity, administration, phones, vehicle costs, site fees, etc. Costs associated with electricity and fuel usage are not automatically scaled to the number of beds, and need to be adjusted manually when this multiplier changes (for a guide please see associated comments in the Operating worksheet). Unleaded petrol is currently used in the worm harvest process, and as a guide, allowance should be made to use about 100 L to harvest 540 m^2 of PASF bed. Electricity is generally used for pumping pond water to the beds (e.g. about 5,000 kWh per 540 m^2 of PASF), but if PASF-treated water is to be fully recirculated and gravity flow back to the ponds is not possible, electricity and pumping costs would double.

Capital

This worksheet captures the range of capital costs not already captured in previous worksheets, and provides a list of all capital costs and a total capital outlay. It allows the user to build in variable costs, useful life, and salvage values for each item. It assumes that the project will run for 20 years.

Summary

Summary statistics are provided in this worksheet. This entails an output summary, a cost structure summary, and economic indicators such as annual returns, internal rate of return (IRR), benefit/cost ratios and payback periods. A breakdown of costs per kilogram of worms is provided for various major groupings, along with a 20-year graph of discounted cumulative cash-flows.

Risk analysis

A risk analysis worksheet equipped with a stochastic simulator is also included whereby the user can adjust the likelihood of different levels of yearly production and different sales prices for the worm biomass produced. As a general rule, risks will vary for different businesses in different operating environments, and also for how well different managers manage those risks. Since these rates of change may vary for different businesses (for example, better managers will have varied skews of the risk curve), these rates are not fixed and need to be manually adjusted in the spreadsheet when maximum production is changed. The stochastic simulator accounts for uncontrolled factors that can affect production, such as prevailing favourable or unfavourable weather during production.

Prawn farm benefits

There are several potential additional economic benefits for prawn farming businesses that are not captured by the financial summary and risk projection outputs of the model. These benefits are significant and are itemised and described below, but only the first benefit is costed in this stand-alone spreadsheet because the others are too complex to cost with different farms having different multipliers.

1. Conversion of existing settlement pond area into prawn production ponds

The model captures this element of benefit in the worksheet entitled “Prawn Farm Benefits”, but not in the IRR or payback periods of the model. For farms currently using 30% of farm area for water treatment, the system can potentially free up a further 15% of farm area for prawn production at current intensities. This reasonably assumes that the PASF beds can treat wastewater with at least equivalent efficacies to those of settlement ponds.

2. Improved water treatment facilitates farm expansions or further intensifications

Improved nutrient removal by better wastewater treatment systems will allow farms to increase their prawn productivity without increasing their nutrient discharges. This assumes that PASF beds can treat wastewaters with better efficacies than existing settlement ponds (as suggested by current and previous results). As identified above, the feasibility of applying the PASF technology at large prawn farms will depend on modified designs that can be applied at greatly increased scales.

3. Improved hatchery productivity from using more worms in prawn broodstock diets

Producing polychaete worms on farms will improve their availability on farms for greater surety of supply and quality. Quite aside from the reduction of broodstock feed costs, survival, health and vigour of prawn broodstock will likely be improved by feeding them higher levels of nutritious polychaete worms (see Appendix 2). It is also widely accepted that feeding live worms to prawn broodstock also improves their nauplii output. Furthermore, it is also considered likely that this will also improve the vigour of prawn larvae for less rearing problems (e.g. better moulting at critical stages), and lower overall hatchery costs.

4. Improved biosecurity in hatcheries and farms

Using feeds sourced from outside of the farming system carries biosecurity risks that could affect long-term economic viability. This risk will be greatly amplified if businesses move towards Specific-Pathogen-Free or -Resistant strains through specialised breeding practices. Whilst it is difficult to place a price on this benefit, most operators would agree that any disease outbreak in the farming system could have catastrophic effect on business viability.

5. Facilitation of prawn domestication

Growing prawn broodstock will be facilitated by the ability to provide them with better feeds that will likely include polychaete worms (see Appendix 3). Better survival, growth and reproductive output would mean that less stock and facilities need to be allocated to breeding programs. Given the current situation where domesticated broodstock generally have low reproductive output, this aspect of their management may prove vital for successful prawn domestication and selection programs, particularly when viewed over longer terms when consistent reproductive results are vital.

Model output – worked example

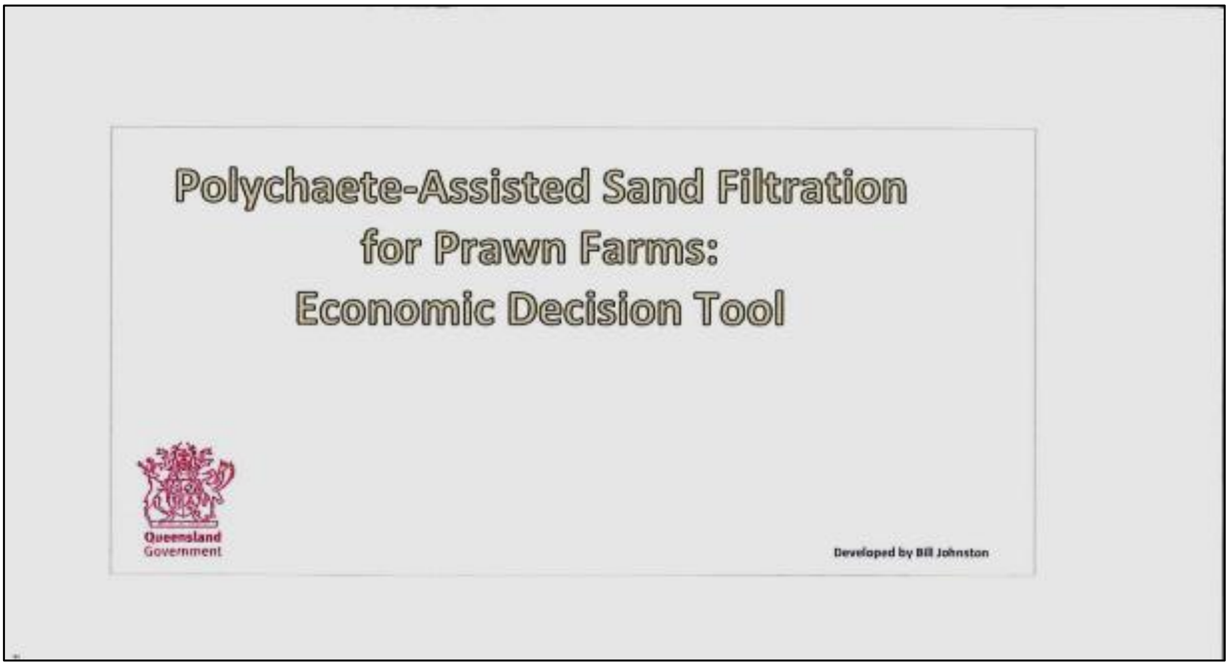
To provide an example of the Decision tool's output, the prototype operation at BIRC is used as a case study for a well-established farm that has low capital investment needs to accommodate the new PASF operation. For example, it is assumed that such a farm would: a) use existing on-site vehicles to manage the new worm culture operation; b) use existing storage sheds and offices; c) need to construct a live worm holding facility costing $10,000; d) need to modify their existing workshop costing $1,000; e) need to build a worm harvester costing about $3,000; and f) have gravity flow from the PASF beds back to the ponds and/or to waste.

The worked example (see Figures 54 – 62) assumes a worm stocking density of 3000 m^{-2} and assumes that the worms are sold at current industry bulk prices. Electricity costs used assume there is gravity flow from the beds back to the prawn ponds in a fully recirculated design, even though the current prototype at BIRC uses a 3-phase submersible pump (purchase cost of around \$5,000) to return the treated water to ponds. Two transfer pumps (Onga 142) are alternatively used to supply the 10 PASF beds with water at the BIRC demonstration facility.

Worm production is averaged across 20 years and because the first harvest doesn't occur until year 2 the average is diminished slightly, giving slightly lower than expected annual production (448 kg) and revenue (\$80,705) in the Summary Statistics worksheet. Farm labour (\$63.88 kg^{-1}) and juveniles (\$46.92 kg^{-1}) represent the highest costs for worm production in this small scale scenario, but it is possible that these costs would be substantially reduced with the expected economies of larger scale. For example, when the number of prototype PASF beds is increased to 278 (= 1.5 ha), which is equal to the worked example for a 10 ha prawn farm as shown in Figure 63 (see below), labour costs are dramatically reduced to levels comparable with other operational costs (e.g. \$2.30 kg^{-1}) and juvenile costs are reduced by about 77% to \$10.83 kg^{-1}. It is also important to note that for this larger-scale installation, there would be a substantial increase in the number of juveniles required to stock the beds at the same density (e.g. 45×10^6 for 3,000 m^{-2}).

The key economic indicators for this small-scale worked example suggest a payback period of about 5 years with an IRR of 26.5% (Figure 61). By comparison, the increased-scale example discussed above suggests a payback period of less than 2 years and IRR of 169%.

The worked example for the stand-alone "Prawn Farm Benefits" spreadsheet (Figure 63) uses a 10-ha farm (total ponded area) producing an average of 10 tonnes of prawns $ha^{-1} y^{-1}$ with a profit margin of \$3.00 kg^{-1}. It assumes that the farm operated with 30% of farm ponded area as settlement ponds before the development, and that 50% of this was converted to PASF. The outcome here is for an additional \$45,000 profit per year, derived entirely from increased prawn production.



Polychaete-Assisted Sand Filtration
for Prawn Farms:
Economic Decision Tool

Queensland
Government

Developed by Bill Johnston

Figure 54 PASF economic decision tool – Title page.

Bed Dimensions and Requirements

Bed Dimensions

Average length of beds	9.30	metres
Average width of beds	5.80	metres
Bed surface area	53.94	square metres
Number of beds for the farm	10	
Total surface area of beds on the farm	539	square metres

Sand Required

Average depth of sand within beds	0.20	metres
Volume of sand per bed	10.79	cubic metres
Total volume of sand required for the beds	108	cubic metres
Weight per cubic metre of sand	1.40	tonnes
Total tonnes of sand required	151	tonnes
Cost of sand	$40.30	per tonne
Total cost of sand	$6,086.59	

Pond Liner

Width of poly liner (2mm thickness) rolls purchased for beds	7.00	metres
Length of poly liner required per bed)	10.50	metres
Cost per metre of poly bed liner (delivered)	$42.50	per metre
Cost of pond liner per bed	$446.25	
Total cost of liner	$4,462.50	

Bed Supports

Number of timber stakes required per bed	64	
Cost per timber stake	$2.25	
Cost of timber stakes per bed	$144.00	
Total cost of timber stakes	$1,440.00	

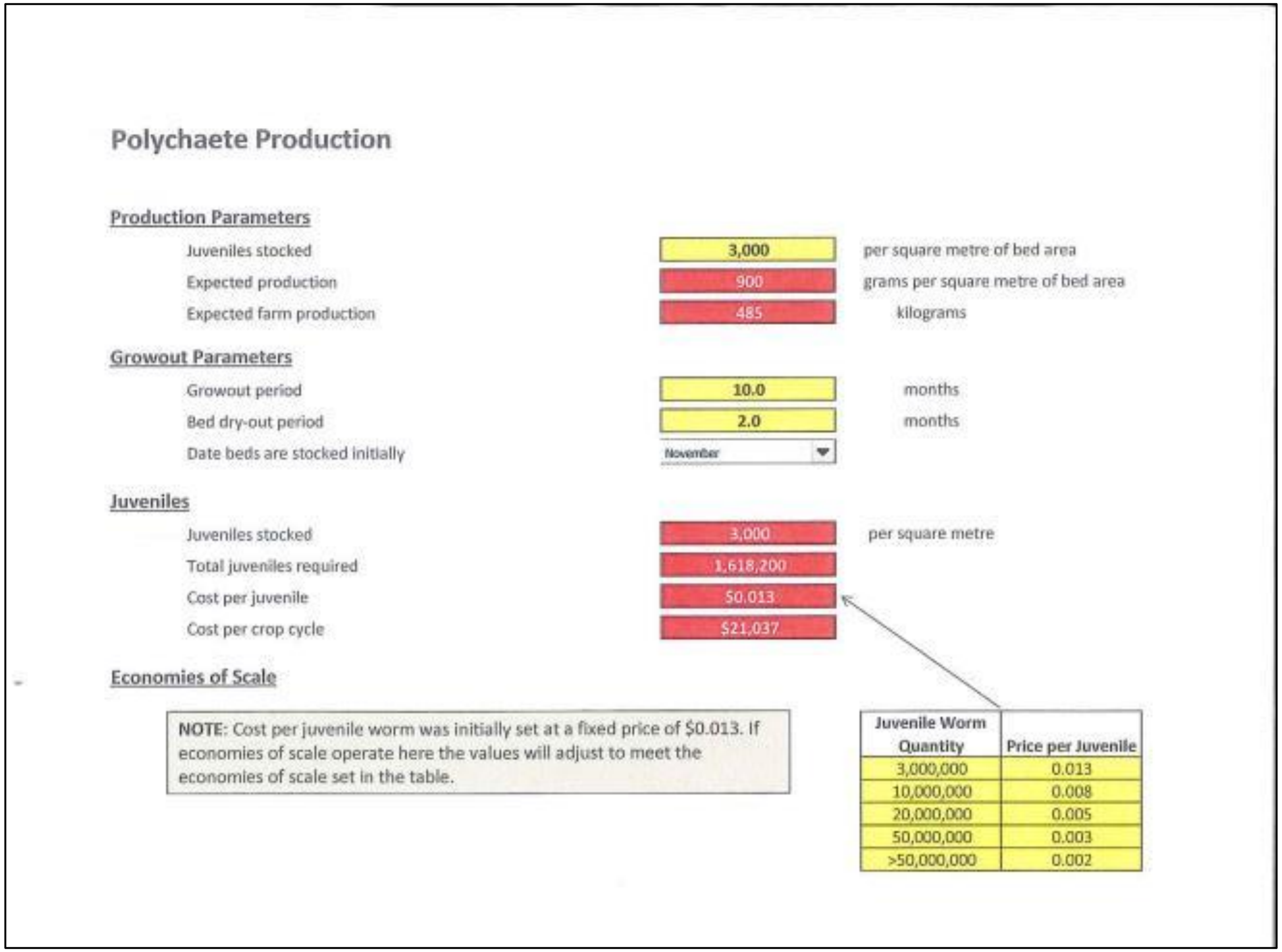
Bed Drainage

Number of ag pipe (50mm) drainage lines per bed	11	
Length of ag pipe drainage lines	5.6	metres
Total length required (all beds)	616	metres
Cost per metre of ag pipe	$1.30	per metre
Total cost of ag pipe	$800.80	
Cost per metre of ag pipe sock	$0.22	per metre
Total cost of ag pipe sock	$135.52	
Additional cost of joiners, grommets and other	$700.00	
Total cost of bed drainage	$1,636.32	

Bed Sumps

Length of PVC pipe per bed (vertical 250mm)	1.5	metres
Total length of pipe required	15	metres
Cost of pipe	$50.00	per metre
Total cost of pipes	$750.00	
Concrete bases required	10	
Concrete cost per base	$10.00	
Total cost of bases	$100.00	

Figure 55 PASF economic decision tool – Bed Parameters worksheet (worked example).



Polychaete Production

Production Parameters

Juveniles stocked	3,000	per square metre of bed area
Expected production	900	grams per square metre of bed area
Expected farm production	485	kilograms

Growout Parameters

Growout period	10.0	months
Bed dry-out period	2.0	months
Date beds are stocked initially	November	

Juveniles

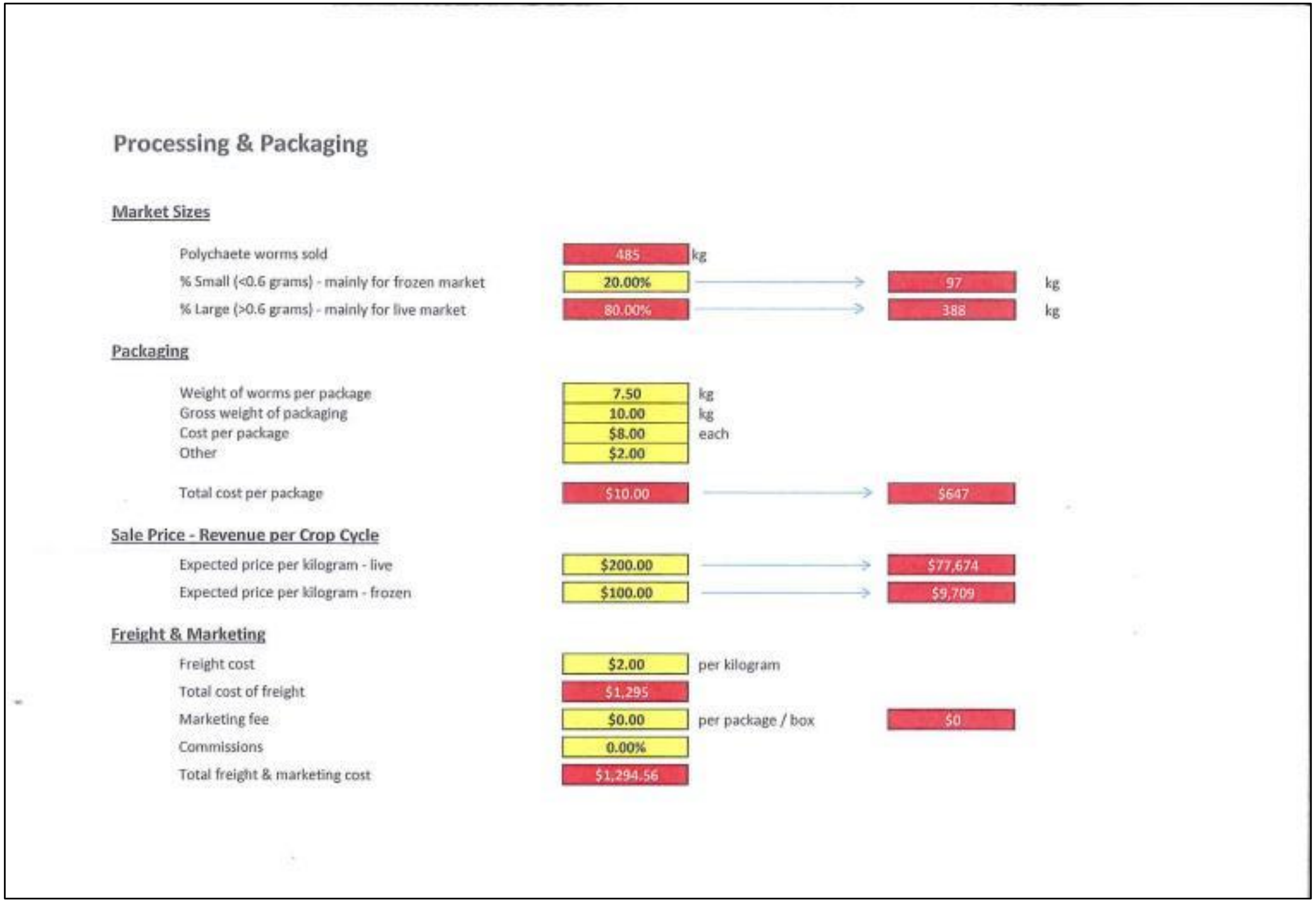
Juveniles stocked	3,000	per square metre
Total juveniles required	1,618,200	
Cost per juvenile	$0.013	
Cost per crop cycle	$21,037	

Economies of Scale

NOTE: Cost per juvenile worm was initially set at a fixed price of $0.013. If economies of scale operate here the values will adjust to meet the economies of scale set in the table.

Juvenile Worm Quantity	Price per Juvenile
3,000,000	0.013
10,000,000	0.008
20,000,000	0.005
50,000,000	0.003
>50,000,000	0.002

Figure 56 PASF economic decision tool – Polychaete Production worksheet (worked example).



Processing & Packaging

Market Sizes

Polychaete worms sold	485	kg		
% Small (<0.6 grams) - mainly for frozen market	20.00%		97	kg
% Large (>0.6 grams) - mainly for live market	80.00%		388	kg

Packaging

Weight of worms per package	7.50	kg	
Gross weight of packaging	10.00	kg	
Cost per package	$8.00	each	
Other	$2.00		
Total cost per package	$10.00		$647

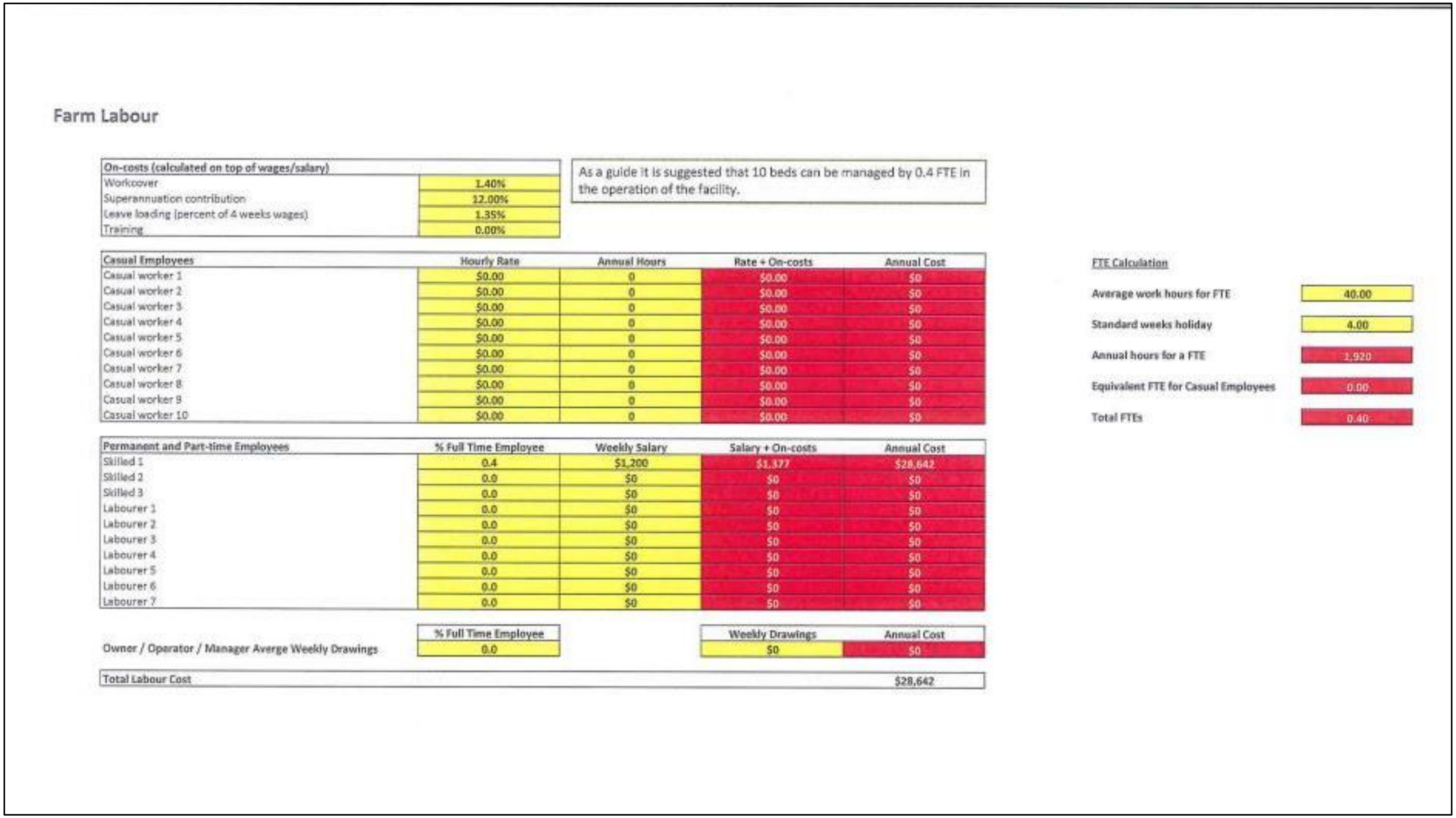
Sale Price - Revenue per Crop Cycle

Expected price per kilogram - live	$200.00	$77,674
Expected price per kilogram - frozen	$100.00	$9,709

Freight & Marketing

Freight cost	$2.00	per kilogram	
Total cost of freight	$1,295		
Marketing fee	$0.00	per package / box	$0
Commissions	0.00%		
Total freight & marketing cost	$1,294.56		

Figure 57 PASF economic decision tool – Post Production worksheet (worked example).



Farm Labour

On-costs (calculated on top of wages/salary)	
Workcover	1.40%
Superannuation contribution	12.00%
Leave loading (percent of 4 weeks wages)	1.35%
Training	0.00%

As a guide it is suggested that 10 beds can be managed by 0.4 FTE in the operation of the facility.

Casual Employees	Hourly Rate	Annual Hours	Rate + On-costs	Annual Cost
Casual worker 1	$0.00	0	$0.00	$0
Casual worker 2	$0.00	0	$0.00	$0
Casual worker 3	$0.00	0	$0.00	$0
Casual worker 4	$0.00	0	$0.00	$0
Casual worker 5	$0.00	0	$0.00	$0
Casual worker 6	$0.00	0	$0.00	$0
Casual worker 7	$0.00	0	$0.00	$0
Casual worker 8	$0.00	0	$0.00	$0
Casual worker 9	$0.00	0	$0.00	$0
Casual worker 10	$0.00	0	$0.00	$0

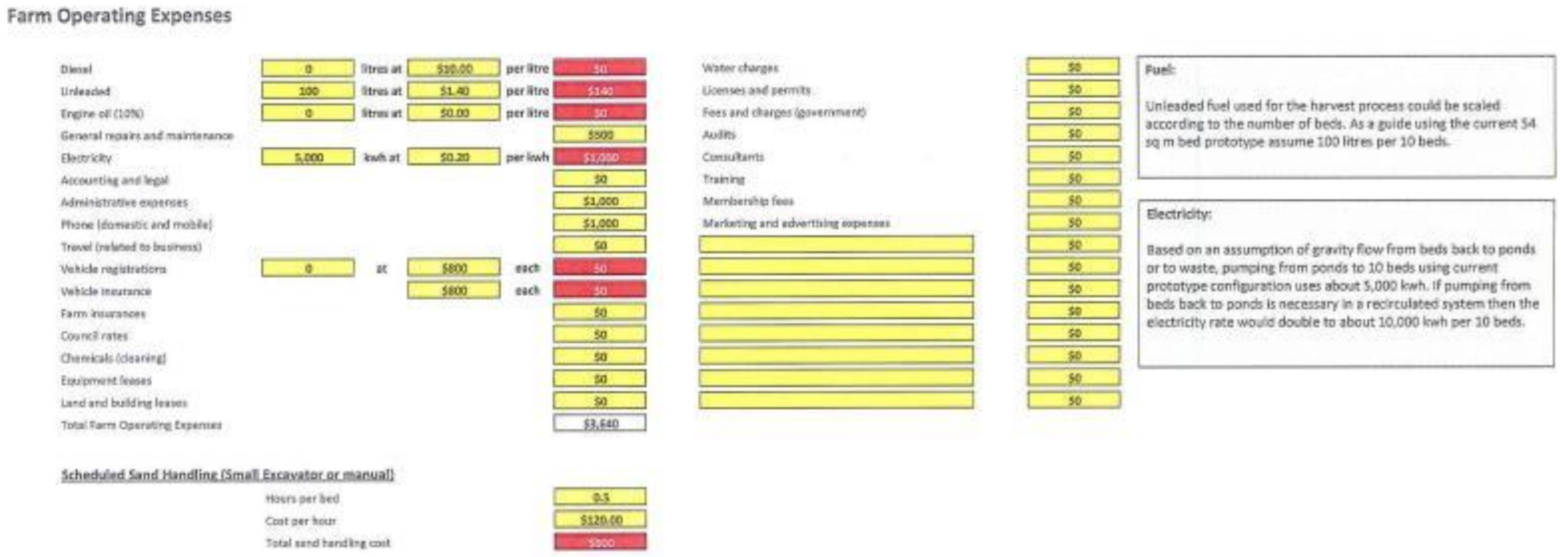
Permanent and Part-time Employees	% Full Time Employee	Weekly Salary	Salary + On-costs	Annual Cost
Skilled 1	0.4	$1,200	$1,377	$28,642
Skilled 2	0.0	$0	$0	$0
Skilled 3	0.0	$0	$0	$0
Labourer 1	0.0	$0	$0	$0
Labourer 2	0.0	$0	$0	$0
Labourer 3	0.0	$0	$0	$0
Labourer 4	0.0	$0	$0	$0
Labourer 5	0.0	$0	$0	$0
Labourer 6	0.0	$0	$0	$0
Labourer 7	0.0	$0	$0	$0

	% Full Time Employee	Weekly Drawings	Annual Cost
Owner / Operator / Manager Averge Weekly Drawings	0.0	$0	$0

Total Labour Cost	$28,642

FTE Calculation	
Average work hours for FTE	40.00
Standard weeks holiday	4.00
Annual hours for a FTE	1,920
Equivalent FTE for Casual Employees	0.00
Total FTEs	0.40

Figure 58 PASF economic decision tool – Labour worksheet (worked example).



Farm Operating Expenses

Diesel	0	litres at	$10.00	per litre	$0
Unleaded	100	litres at	$1.40	per litre	$140
Engine oil (10%)	0	litres at	$0.00	per litre	$0
General repairs and maintenance					$500
Electricity	5,000	kwh at	$0.20	per kwh	$1,000
Accounting and legal					$0
Administrative expenses					$1,000
Phone (domestic and mobile)					$1,000
Travel (related to business)					$0
Vehicle registrations	0	at	$800	each	$0
Vehicle insurance			$800	each	$0
Farm insurances					$0
Council rates					$0
Chemicals (cleaning)					$0
Equipment leases					$0
Land and building leases					$0
Total Farm Operating Expenses					$3,640

Water charges	$0
Licenses and permits	$0
Fees and charges (government)	$0
Audits	$0
Consultants	$0
Training	$0
Membership fees	$0
Marketing and advertising expenses	$0
	$0
	$0
	$0
	$0
	$0
	$0
	$0
	$0

Fuel:

Unleaded fuel used for the harvest process could be scaled according to the number of beds. As a guide using the current 54 sq m bed prototype assume 100 litres per 10 beds.

Electricity:

Based on an assumption of gravity flow from beds back to ponds or to waste, pumping from ponds to 10 beds using current prototype configuration uses about 5,000 kwh. If pumping from beds back to ponds is necessary in a recirculated system then the electricity rate would double to about 10,000 kwh per 10 beds.

Scheduled Sand Handling (Small Excavator or manual)

Hours per bed	0.5
Cost per hour	$120.00
Total sand handling cost	$600

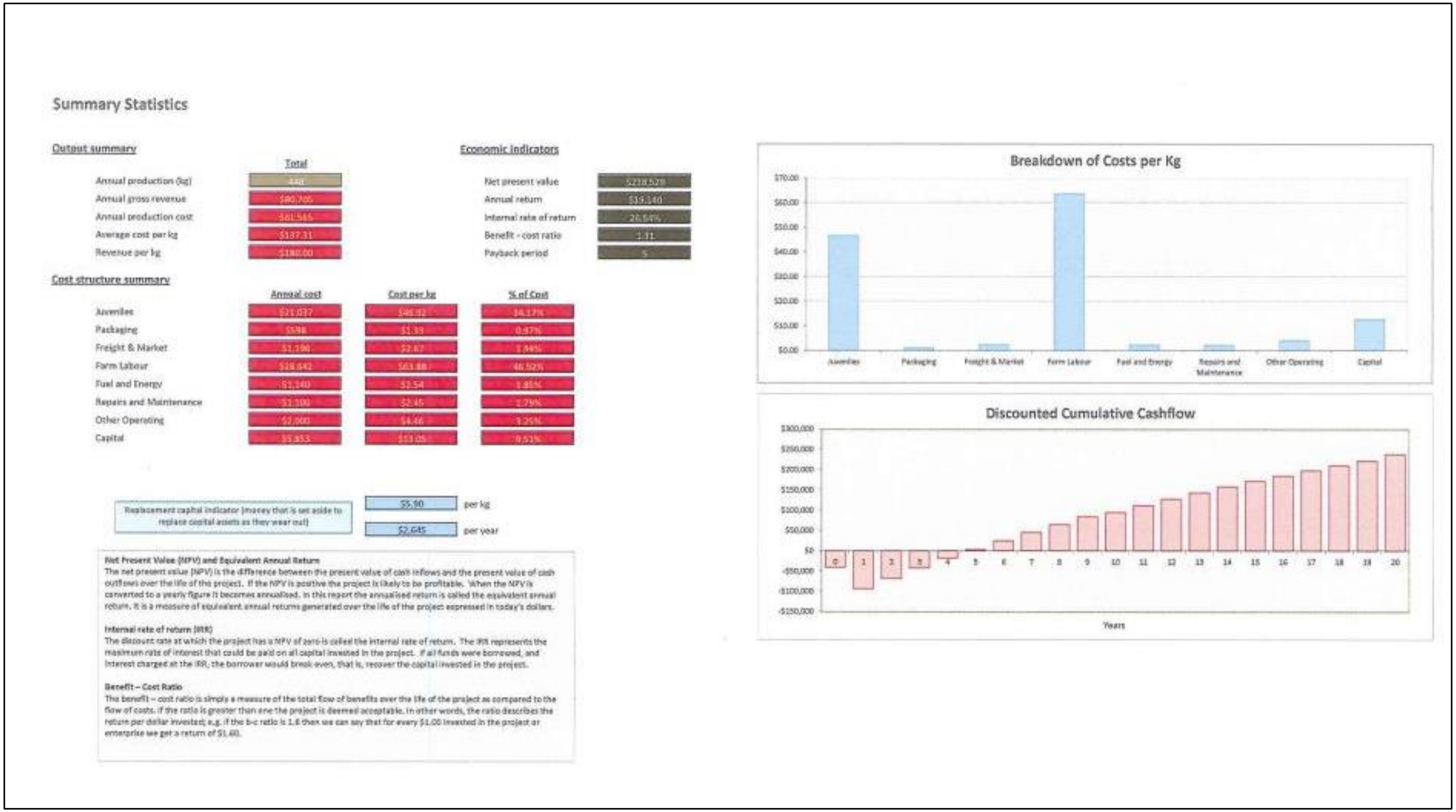
Figure 59 PASF economic decision tool – Operating worksheet (worked example).

Capital Cost

Project Length (Years) 20

Capital Item	No. of items	Cost of items	Total cost	Purchase year	Life (years)	Salvage value
Land and Buildings						
Land	-	$0	$0	0	0	0%
Sheds and office	0	$0	$0	0	20	40%
Staff accomodation	0	$0	$0	0	0	0%
Workshop	0.2	$5,000	$1,000	0	20	40%
Live worm holding facility	1	$10,000	$10,000	0	20	40%
Other	-	$0	$0	0	0	0%
Electricity connection	-	$0	$0	0	0	0%
Vehicles and Machinery						
Utilities	0	$40,000	$0	0	10	40%
Motorbikes / 4 Wheelers	0	$15,000	$0	0	10	40%
Tractor (Second Hand)	0	$0	$0	0	0	0%
Mower/Slasher	0	$0	$0	0	0	0%
Harvester	1	$3,000	$3,000	0	5	50%
PASF Beds (including Internal Drainage)						
Bed poly liner	10	$446	$4,463	0	10	0%
Sand	151	$40	$6,087	0	20	40%
Timber stakes	640	$2.25	$1,440	0	1	90%
Ag pipe rolls (50mm diameter x 100m)	616	$1.30	$801	0	10	10%
Ag pipe sock (65mm) - metres required	616	$0.22	$136	0	2	0%
Other (end caps, grommets, joiners)	1	$700	$700	0	10	50%
Bed sumps (150mm PVC pressure pipe)	15	$50	$750	0	10	0%
Concrete sump bases	10	$10	$100	0	15	0%
Main Underground Drainage						
Pipe (150mm UPVC)	12	$40	$480	0	20	0%
Fittings	2	$40	$80	0	20	0%
Individual bed underground drainage (50mm HDPE)	3	$235	$705	0	20	0%
Pond Water Transfer						
Pumps	2	$800	$1,600	0	1	0%
Safety switches	2	$100	$200	0	1	0%
Timers	2	$28	$28	0	1	0%
Pond transfer pipes (50mm HDPE)	5	$464	$2,320	0	20	20%
Sump, Transfer and Water Supply						
Sump pump (for filtered water return to ponds)	0	$5,000	$0	0	5	10%
Sump float switch	0	$200	$0	0	3	0%
Manifold fittings	1	$300	$300	0	10	50%
Filtered water return pipes (50mm HDPE)	2	$464	$928	0	10	20%
Sump tank (2000 litre tank)	1	$600	$600	0	20	100%
Suction pipe fittings	-	$298	$298	0	1	0%
Main water supply fittings	-	$2,110	$2,110	0	10	20%
Bottom Outflow						
Main sub-surface connector pipe (50mm HDPE)	1	$235	$235	0	20	0%
PVC standpipes for drainage control	30	$21	$630	0	10	0%
Fittings	-	$705	$705	0	10	0%
Overflow						
Short internal adjustable standpipes (50mm PVC)	5	$21	$105	0	20	0%
Fittings	-	$178	$178	0	10	0%
Other Infrastructure and Equipment						
Generator	0	$0	$0	0	0	0%
Water monitoring equipment	0	$0	$0	0	0	0%
Harvesting equipment	-	$0	$0	0	0	0%
Processing equipment	-	$0	$0	0	0	0%
Bed cleaning equipment	-	$0	$0	0	0	0%
Workshop tools and equipment	-	$0	$0	0	0	0%
Other capital items	-	$0	$0	0	0	0%
Other capital items	0	$0	$0	0	0	0%
Other capital items	0	$0	$0	0	0	0%
Total capital outlay			$39,977			

Figure 60 PASF economic decision tool – Capital worksheet (worked example).



Summary Statistics

Output summary

	Total
Annual production (kg)	448
Annual gross revenue	$80,706
Annual production cost	$61,565
Average cost per kg	$137.31
Revenue per kg	$180.00

Economic indicators

Net present value	$218,529
Annual return	$19,140
Internal rate of return	26.54%
Benefit - cost ratio	1.31
Payback period	5

Cost structure summary

	Annual cost	Cost per kg	% of Cost
Juveniles	$21,037	$46.92	34.17%
Packaging	$598	$1.33	0.97%
Freight & Market	$1,196	$2.67	1.94%
Farm Labour	$28,642	$63.88	46.52%
Fuel and Energy	$1,140	$2.54	1.85%
Repairs and Maintenance	$1,100	$2.45	1.79%
Other Operating	$2,000	$4.46	3.25%
Capital	$5,853	$13.05	9.51%

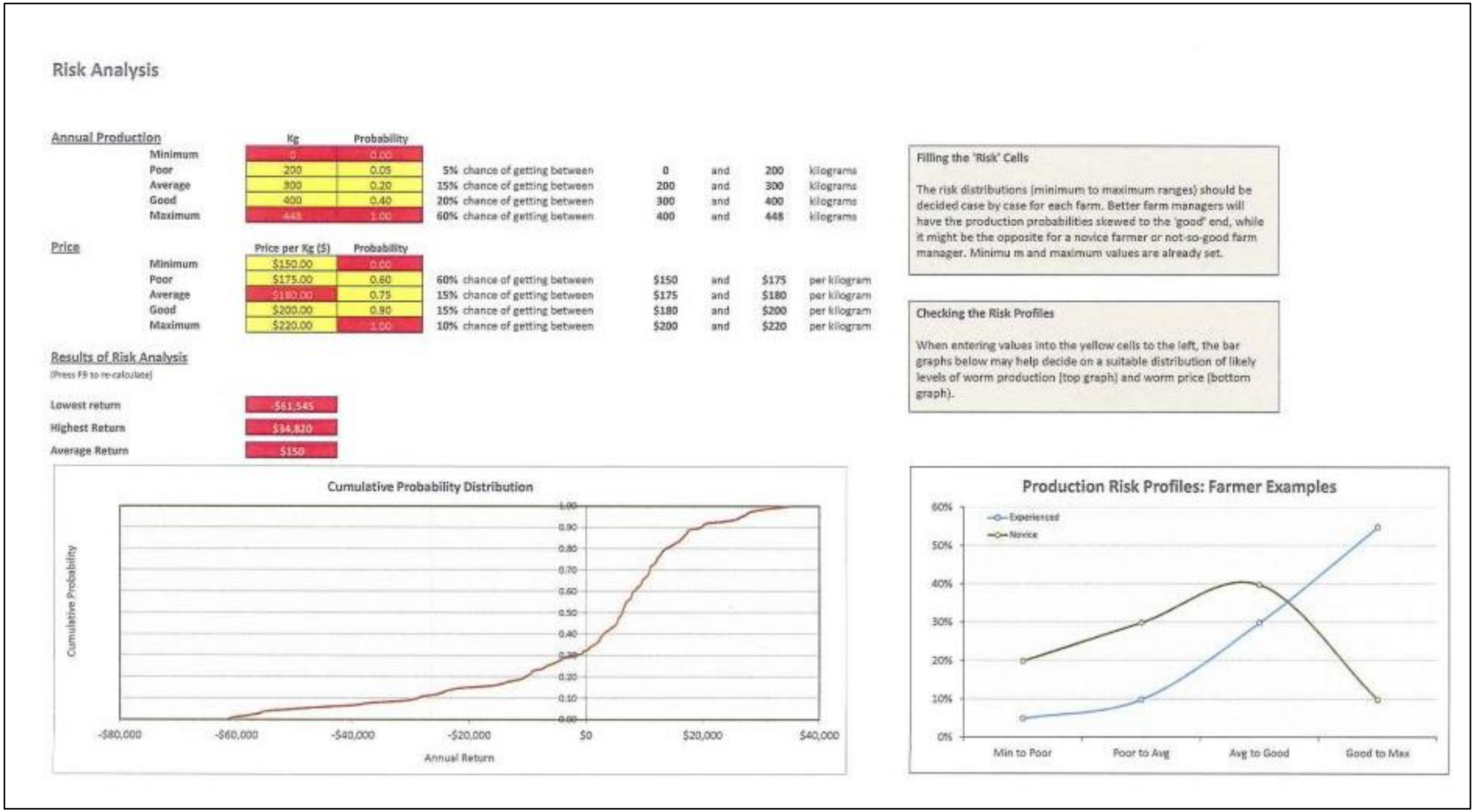
Replacement capital indicator (money that is set aside to replace capital assets as they wear out)	$5.90	per kg
	$2,645	per year

Net Present Value (NPV) and Equivalent Annual Return
The net present value (NPV) is the difference between the present value of cash inflows and the present value of cash outflows over the life of the project. If the NPV is positive the project is likely to be profitable. When the NPV is converted to a yearly figure it becomes annualised. In this report the annualised return is called the equivalent annual return. It is a measure of equivalent annual returns generated over the life of the project expressed in today's dollars.

Internal rate of return (IRR)
The discount rate at which the project has a NPV of zero is called the internal rate of return. The IRR represents the maximum rate of interest that could be paid on all capital invested in the project. If all funds were borrowed, and interest charged at the IRR, the borrower would break even, that is, recover the capital invested in the project.

Benefit – Cost Ratio
The benefit – cost ratio is simply a measure of the total flow of benefits over the life of the project as compared to the flow of costs. If the ratio is greater than one the project is deemed acceptable. In other words, the ratio describes the return per dollar invested; e.g. if the b-c ratio is 1.6 then we can say that for every $1.00 invested in the project or enterprise we get a return of $1.60.

Figure 61 PASF economic decision tool – Summary worksheet (worked example).



Risk Analysis

Annual Production

	Kg	Probability					
Minimum	0	0.00					
Poor	200	0.05	5% chance of getting between	0	and	200	kilograms
Average	300	0.20	15% chance of getting between	200	and	300	kilograms
Good	400	0.40	20% chance of getting between	300	and	400	kilograms
Maximum	448	1.00	60% chance of getting between	400	and	448	kilograms

Price

	Price per Kg ($)	Probability					
Minimum	$150.00	0.00					
Poor	$175.00	0.60	60% chance of getting between	$150	and	$175	per kilogram
Average	$180.00	0.75	15% chance of getting between	$175	and	$180	per kilogram
Good	$200.00	0.90	15% chance of getting between	$180	and	$200	per kilogram
Maximum	$220.00	1.00	10% chance of getting between	$200	and	$220	per kilogram

Results of Risk Analysis
(Press F9 to re-calculate)

Lowest return	-$61,545
Highest Return	$34,820
Average Return	$150

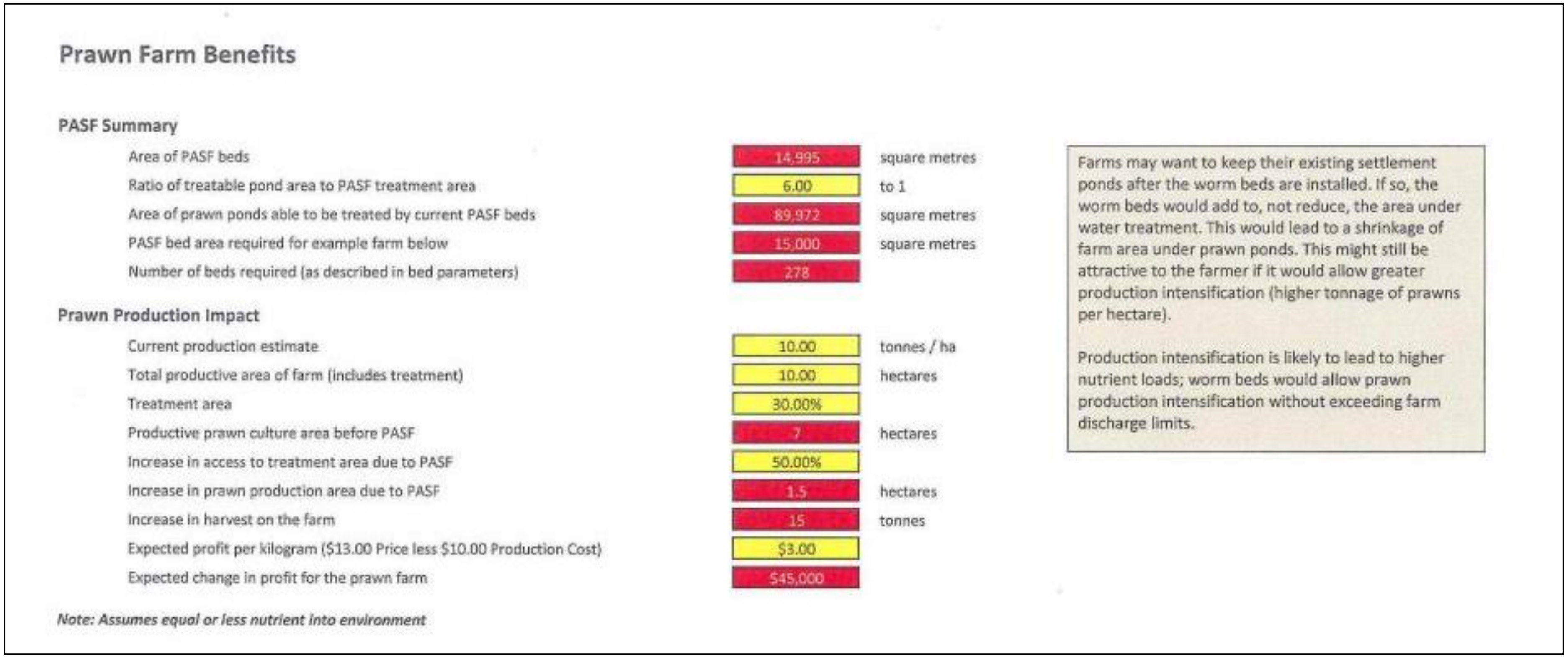
Filling the 'Risk' Cells

The risk distributions (minimum to maximum ranges) should be decided case by case for each farm. Better farm managers will have the production probabilities skewed to the 'good' end, while it might be the opposite for a novice farmer or not-so-good farm manager. Minimu m and maximum values are already set.

Checking the Risk Profiles

When entering values into the yellow cells to the left, the bar graphs below may help decide on a suitable distribution of likely levels of worm production (top graph) and worm price (bottom graph).

Figure 62 PASF economic decision tool – Risk Analysis worksheet (worked example).



Prawn Farm Benefits

PASF Summary		
Area of PASF beds	14,995	square metres
Ratio of treatable pond area to PASF treatment area	6.00	to 1
Area of prawn ponds able to be treated by current PASF beds	89,972	square metres
PASF bed area required for example farm below	15,000	square metres
Number of beds required (as described in bed parameters)	278	
Prawn Production Impact		
Current production estimate	10.00	tonnes / ha
Total productive area of farm (includes treatment)	10.00	hectares
Treatment area	30.00%	
Productive prawn culture area before PASF	7	hectares
Increase in access to treatment area due to PASF	50.00%	
Increase in prawn production area due to PASF	1.5	hectares
Increase in harvest on the farm	15	tonnes
Expected profit per kilogram ($13.00 Price less $10.00 Production Cost)	$3.00	
Expected change in profit for the prawn farm	$45,000	

Farms may want to keep their existing settlement ponds after the worm beds are installed. If so, the worm beds would add to, not reduce, the area under water treatment. This would lead to a shrinkage of farm area under prawn ponds. This might still be attractive to the farmer if it would allow greater production intensification (higher tonnage of prawns per hectare).

Production intensification is likely to lead to higher nutrient loads; worm beds would allow prawn production intensification without exceeding farm discharge limits.

Note: Assumes equal or less nutrient into environment

Figure 63 PASF economic decision tool – Prawn Farm Benefits worksheet (worked example).

Further information

For further information on the “PASF for Prawn Farms: Economic Decision Tool”, Mr Johnston can be contacted at Maroochy Research Station, 47 Mayers Road (PO Box 5083), Nambour, Queensland 4560 or at Bill.Johnston@daf.qld.gov.au.

Appendix 2 – Polychaete maturation diet report: qualitative summary

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Explanatory note

The following presents a general summary of results from a *Penaeus monodon* broodstock trial carried out by CSIRO staff at the Bribie Island Research Centre (BIRC) in 2014. These data derive from a collaborative research project between CSIRO, an industry partner, and the Queensland Department of Agriculture and Fisheries (DAF). Qualitative/descriptive results on female maturation, and egg and nauplii numbers per spawning are presented.

Overview

The trial was conducted by CSIRO at BIRC to evaluate the effect of different maturation diets on the reproductive output of a domesticated Giant Tiger Prawn (*Penaeus monodon*) broodstock. The diets varied in levels of polychaetes included (two levels, referred to here as lower and higher) and in whether polychaetes were provided as live or frozen feeds. The polychaete worms used were *Perinereis helleri* produced in polychaete-assisted sand filters at BIRC by DAF.

It is worth noting that broodstock used in the trial were not at optimal age or condition at the time the experiment was conducted. As a consequence the overall survivorship and reproductive outcomes were lower than would typically be expected for these domesticated broodstock. Moreover, it should be noted that the experiment was modest in terms of animal numbers, which reduces the certainty of conclusions that can be drawn from the trial. However, notwithstanding these caveats, the results did suggest that the polychaete species cultured by DAF staff at BIRC have great promise as a key dietary ingredient to be used in prawn broodstock diets to improve maturation and reproductive outcomes.

Methods

Several dozen domesticated *P. monodon* broodstock were transported to BIRC in September 2014 and introduced into four 10,000 L maturation tanks. Each tank had a thin layer of sand spread on the bottom. Equal sex ratios in each tank facilitated natural mating (no artificial insemination was performed). Over the following five weeks, different maturation diets which varied in levels of polychaetes (approximately 20% or 50% of total dry weight of ingredients) as live (L) or frozen (F) ingredient were applied to the different tanks. Other portions of the experimental diets consisted of a range of fresh-frozen invertebrates and a semi-moist pellet. Each ingredient was fed individually as a ‘portion’, with each portion (for all dietary components) consisting of the same approximate dry weight of ingredient. Prawns in each tank were fed three times per day.

Over the course of the trial, the maturation tanks were maintained at water temperatures of 29°C ± 0.5, and flowthrough of seawater at 5 L min^{-1}. Each tank of prawn broodstock was provided with its respective dietary treatment ration from day 1, with females ablated for commencement of reproductive evaluation on day 18, and the evaluation ended on day 37. Ovarian development of the females was closely monitored on a daily basis from the ablation day onwards, with ripe females transferred to individual spawning tanks for spawning evaluation (egg and nauplii numbers per spawning).

Female maturation assessments were based on the percentages of females that attained different progressive stages of ovarian development. They were scored through visual examination of the dorsal exoskeleton, using a standard 0-4 scale, where stage 0 indicated no apparent development and stage 4 indicated that prawns were ripe and ready to spawn.

Results

Survival

Overall, the survival of the stocks was moderately low. During the conditioning phase (pre-ablation) and the evaluation phase (post-ablation) female mortalities were particularly high. No major trends of survivorship relating to the main diet-treatments were evident.

Female maturation

On average, the degree of ovarian development was highest in the L50 (live worm 50%) treatment. This was particularly evident in the higher percentage of females attaining stage 4 maturation prior to ablation, and in the higher number of females attaining stage 4 maturation over the course of the evaluation period.

On average, the percentage of females maturing was next-best in the F50 (frozen worm 50%) treatment - however, it is notable that the percentages of females reaching stage 4 prior to ablation was higher in the L20 (live worm 20%) than the frozen treatments.

Collectively, the 20% treatments (L20, F20) had lower percentages of females maturing than the 50% treatments. In general, similar results were provided by the two 20% treatments, with the exception of better maturation to stage 4 in the L20 compared with F20 prior to ablation.

Overall, the main findings were for higher levels of ovarian maturation to stage 4 in the L50 treatment. Stage 4 ovaries were only found prior to ablation in the L treatments, and the typically higher levels of maturation (to stages 3/4 and 4) occurred in the 50% treatments following ablation.

Egg and nauplii production

Egg numbers per spawning were higher, on average, in the L than the F treatments. The nauplius hatch rate was also typically higher in the L than F diet-treatments. Percentages of nauplii hatching per spawning were typically quite low (averages for all treatments were less than 25%). This was particularly low in the F20 treatment, and higher, on average, in the L than F diet-treatments. The resulting nauplii numbers per spawning were quite low across all treatments, but higher, on average, in the L than F diet-treatments.

Overall, the results suggested that the L diet-treatments outperformed the F treatments.

Final note

Given the limitations of the current experiment, it is recommended that this experiment be repeated to potentially validate these encouraging findings. Due to the potential for high variability in results, and to assist in providing more robust comparisons between these treatments in the future, it may be necessary to use a minimum of 30 females per diet-treatment at the commencement of the evaluation period. It is also recommended that the conditioning period entails a minimum of 30 days, during which time mortalities should be low. The nature of this type of research means that poor stock condition can overly compromise treatment comparisons, so we further recommend that minimum reproductive performances of test animals be applied as a proviso for this important research.

Appendix 3 – Prawn broodstock cultured at BIRC using PASF and tested under commercial hatchery conditions

Introduction

Many of the largest prawn farming companies in Australia are considering their options to invest in captive breeding programs for their main production species, *Penaeus monodon*. This is being driven by expectations of improved performance from selectively bred stocks, and the escalating costs associated with the collection of wild broodstock which can be of variable quality and uncertain biosecurity status. This is a challenging pursuit because *P. monodon* is widely considered the most difficult commercially-farmed penaeid to domesticate (Coman et al., 2006; 2007). High stock health and rapid growth rates are both imperatives for cultured prawn broodstock, particularly if they are to reliably meet industrial needs within a 12-monthly production cycle. Where stock health directly influences survival, growth needs to be fast enough to provide large mature animals for effective use in large-scale production systems. Many captive breeding programs for this species have faltered due to poor reproductive performance, suggesting that this is a key area for research and investment in the future.

Recent literature on this subject suggests that feeding polychaetes can aid in the growth, survival and reproductive performance of *P. monodon* (Meunpol et al., 2005; Leelatanawit et al., 2014; Chimsung, 2014). Hence, methods that can produce suitable polychaetes for use as broodstock feeds will be very useful in these endeavours. Additionally, whilst selection may play a part in the longer-term suitability of stock for captive breeding, focus on culture environments and husbandry are primary needs that appear to have played a very important role in advances made to date (Coman et al., 2005). Along with supplies of polychaetes for critical broodstock nutrition, the development of PASF offers the potential to improve broodstock culture environments. Water treatment and recirculation offer better control of water temperatures, and limits the inflow of seawater thereby reducing the potential for influx of potential pathogens and disease. Given the likely scale of broodstock production to economically service future commercial needs, pond rearing methods (for which PASF is well tailored – see main report) may be unavoidable. This could be particularly so if the selection environment is to appropriately simulate commercial grow-out environments. Therefore, despite its somewhat poor track record in Australia (see Discussion below), pond rearing should be taken into consideration for future domestication designs.

Given this situation it was considered prudent in the current study to investigate the suitability of prawns grown in PASF-recirculated ponds for breeding. Towards the end of Season one, a small number of prawns grown in each of the PASF-recirculated ponds were transferred to either 1) a smaller covered pond to on-grow to larger sizes, or 2) a recently harvested production pond after refilling. These prawns were then overwintered in the covered pond at low stocking density (<1 m^{-2} - to minimise competition for feeds), using a standardised feeding plan which included live polychaetes from the progressive harvest of PASF at BIRC. Limited water exchange was applied to maintain highest-possible water temperatures without supplemental heating – only the natural heat retention and solar collection aspects of the covered pond were applied so that production costs were minimised. Then, at a time typically suitable for industrial seed production in Queensland, the health status and reproductive performance of these on-grown prawns were assessed. It was anticipated that if adequate survival, growth, health and reproductive performance could be demonstrated in this work, it may help renew the confidence of industry to utilise pond-rearing techniques for broodstock production in the future, and in turn help validate PASF as a useful tool for production of *P. monodon* broodstock.

Materials and methods

One covered 200 m^2 broodstock culture pond (N1 – see Figure 64) at the Bribie Island Research Centre (BIRC) was filled with screened (300 µm) seawater on 18/3/14, and the next day 30 of the larger males and females from the drain harvest of pond G2 were stocked. This initially provided a stocking density of 0.3 prawns m^{-2} and total prawn biomass of 3.1 kg. These prawns were only fed artificial pellet feed (Ridley Grower 1 – 1.5% of the initial stocking body weight) once per day for the first 1.5 months after stocking. One Force 7 aspirator was used to provide continuous aeration and water circulation. To minimise the addition of cool water through the winter grow-out period no water exchange (other than periodic top-up to account for seepage and evaporation) was applied. Aqua blue dye (one satchel on fill) was applied to limit the growth of filamentous macro-algae, and a green Chlorophyte bloom developed and persisted for the majority of the culture period.

Figure 64 Pictures of outside (left) and inside (right) the covered broodstock pond (N1) at BIRC.

To also investigate the potential for recently harvested production ponds to provide a temporary on-growing environment for broodstock, 30 of the larger males (47.9 ± 1.1 g) and 30 of the larger females (55.8 ± 1 g) from the drain harvest of grow-out pond G1 were also stocked (on 21/03/14) into a production pond (G2) two days after it had been fully harvested and subsequently refilled with screened (300 µm) seawater. This provided a very low stocking density (0.0375 prawns m^{-2}) and total prawn biomass of 3.1 kg in the 1600 m^2 pond. One Force 7 aspirator provided continuous water aeration and slow circulation and a light green mixed phytoplankton bloom developed and persisted during the experiment. The prawns in this pond were also fed once daily with Ridley Grower 1 at a rate of 1.5% of body weight d^{-1}. This amount of artificial feed was low given the size of the larger pond, and given the amount of natural feeds and detritus that were present in the pond (after harvest and refilling) which likely provided a large portion of their nutrition. These prawns were re-harvested after 46 days (on 6/05/14), sexed, re-weighed and also introduced into the covered broodstock culture pond.

Table 10 provides a summary of all of the stock sources, stocking dates and average body weights for prawns when introduced into the covered broodstock culture pond (N1). The final stocking density after both groups of prawns were stocked was 0.58 m^{-2}. From early in May (9/5/14) and thereafter, artificial pellet feeding was gradually increased to compensate for predicted prawn biomass growth, still feeding at about 1.5% of prawn biomass per day. Also early in May this artificial feed was replaced with coarsely chopped pilchards or squid (freshly thawed) every two or three days (2% per day using their wet weights), and from mid-May broken marine worms (PASF-produced *Perinereis helleri*) along with the mucus-laden silt from the weekly PASF-bed harvests at BIRC were also added to the pond. In June and July this feeding regime was modified to include 1% pellet in the morning and 1% wet diet (either pilchards, squid or broken worms) in the afternoon. In August the broken worms became more dominant in this feeding plan, and in September it became the only wet feed added on a daily basis.

Table 10 Stocking data for covered broodstock culture pond N1 in Season 1.

Stocking date	Source ponds	Sex	Number stocked	Average body weight (g)	Standard error body weight (g)
19/03/14	G2	Males	30	48.9	0.8
19/03/14	G2	Females	30	55.3	1.3
6/05/14	G2 from G1	Males	24	66.4	1.3
6/05/14	G2 from G1	Females	32	81	1.4

The prawns in the broodstock culture pond were disturbed as little as possible, and water temperatures fell to about 24°C in mid-winter. On 6/8/14 a random sample of three males and three females were removed with a cast net for inspection of condition and weight assessments. These were later returned to the pond in good condition. On 22/8/14, another three males and three females were similarly removed for inspection and weighing, and these were preserved with Davidson's solution and later submitted for health screening to the Biosecurity Queensland Veterinary Laboratories.

On 29/9/14 the majority of broodstock from the culture pond (N1) were harvested and transferred (via a 1,000 L road-transport tank) to the commercial hatchery of Truloff Prawn Farms at Alberton in south-east Queensland. On arrival the prawns were inspected, and males and females were stocked equally into two standard broodstock maturation tanks. These prawns were fed to satiation four times per day, with frozen/thawed squid, mussel, ox liver, and frozen *P. helleri* that had been grown in the PASF system at BIRC. Seven days later (on 6/10/14) these prawns were treated with the same spawning procedures that this successful commercial hatchery routinely applies to wild *P. monodon* broodstock (e.g. unilateral eyestalk ablation of females and natural mating). Ablated prawns were monitored for the next three weeks taking notes on their moulting frequency, ovarian developments, and later recording their spawning results.

Results

There was high survival and considerable growth of prawns when they were initially stocked at low density into the refilled production pond. Over the 46 days in this environment, males had 87% survival and females had 100% survival. Males and females during this time also increased in size by 38.7% and 46.8 %, respectively – about 1% body weight increase per day (Figure 65). They were in excellent condition (no visible necrosis, long antennae) when transferred to the covered pond.

Excellent condition was also observed for prawns sampled from the covered pond on 6/8/14 (Figure 66). High condition and survival (close to 100% for males and females) was again apparent when the prawns from the broodstock culture pond were harvested prior to transfer to the commercial hatchery (Figure 67). Although only a few sample points were possible, growth appeared to be continuous despite the moderate temperatures they experienced through the winter period.

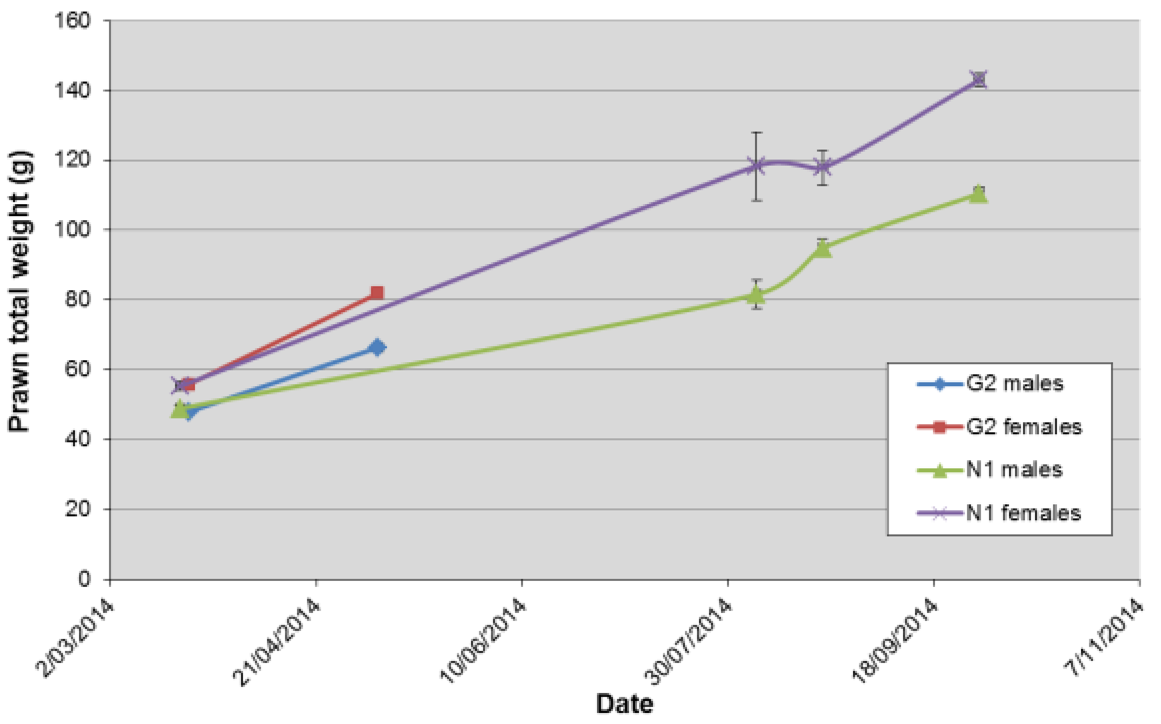

Figure 65 Mean (± se) weights of prawn broodstock grown in refilled production pond (G2) and covered pond (N1).

Health assessments made by the Biosecurity Queensland Veterinary Laboratories suggested that the animals were generally in good health, but had lymphoid organ spheroidosis which was suggestive of a moderate level of virus exposure. Four of the six prawns tested positive to Gill-Associated Virus (GAV) by polymerase chain reaction (PCR). All the prawns in the sample were PCR-negative for Mourilyan Virus (MoV), Taura Syndrome Virus, White Spot Disease and Infectious Hypodermal and Haematopoietic Necrosis Virus (one was inconclusive for IHHNV). Two of the three females inspected had early-stage maturing oocytes and the third had immature oocytes. All (three) of the male prawns had a percentage of sperm abnormalities consisting of pale enlarged and hollow sperm (see Chong et al., 2014). Sections of the testes had 20-25% of this syndrome, whilst sections of the vas deferens had 5-20% pale enlarged sperm that were loose from the bulk of normal sperm in the forming spermatophore.

Figure 66 Pictures showing four different prawns sampled on 6 August 2014.

On arrival at the hatchery 20-25 of the females were already impregnated, with healthy looking spermatophores visible in their thelyca. The frequency of moults after ablation (on 6/10/14) is provided in Figure 68. Several females moulted within three days of ablation, and most males (45) and females (43) moulted within two weeks. Two females and one male died two days after ablation.

Figure 67 Harvested prawn broodstock being transferred to road transport tank.

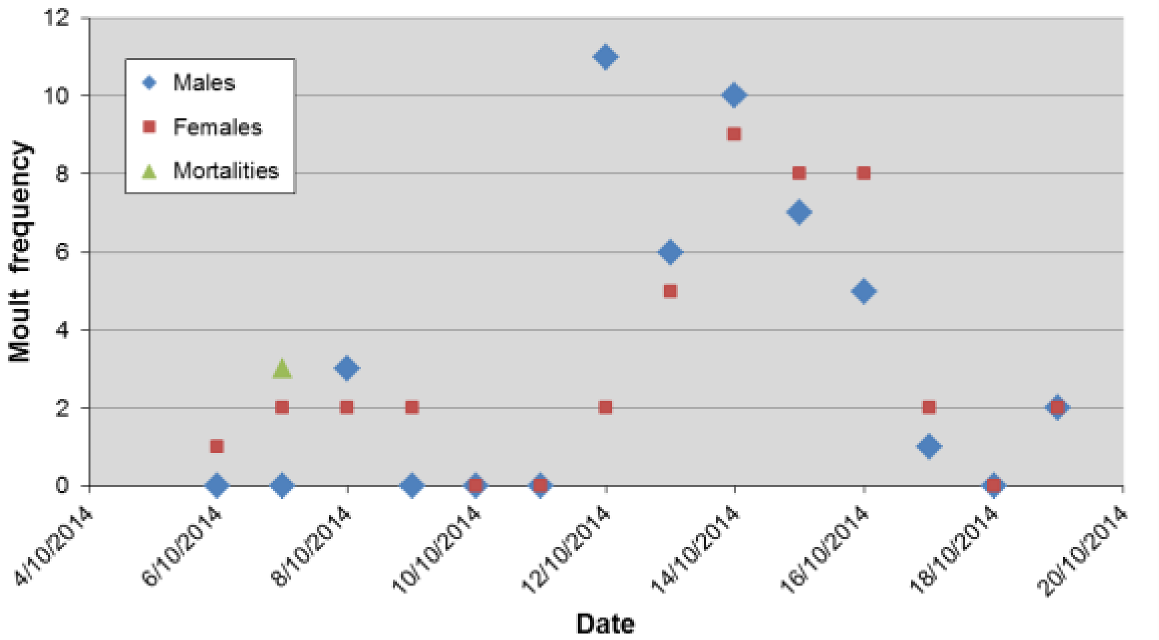

Figure 68 Frequency of moulting and mortalities of broodstock following unilateral eyestalk ablation.

A summary of the spawning results is provided in Table 11. In total 27 females spawned, and only three of these released eggs that were not fertilised. Their oocyte development was initially slow and somewhat discontinuous compared with the better synchrony and shorter timeframe which high-quality wild broodstock generally demonstrate after ablation. As a group, significant egg development became apparent on 19/10/14 when two females displayed stage 1 eggs[20], five females displayed stage 2, and one had achieved stage 3. The following day (20/10/14) the majority of females reached stage 2-3. A total of 1.14 x 10^6 nauplii were produced over the next several days. Those spawns which hatched provided an average of 44,600 nauplii per female. Some of these nauplii were cultured according to standardised practices to produce approximately 300,000 postlarvae, which appeared to be of acceptable commercial quality.

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[20] Stage 4 is ripe and ready to spawn.

Table 11 Spawning results for cultured broodstock transferred from BIRC to commercial hatchery in Season 1.

Date	Number of females	Total nauplii produced	Nauplii per female
22/10/2014	1	0	0
23/10/2014	4	350,000	87,500
24/10/2014	2	10,000	5,000
25/10/2014	4	350,000	87,500
26/10/2014	2	0	0
27/10/2014	0	0	0
28/10/2014	5	100,000	20,000
29/10/2014	5	300,000	60,000
30/10/2014	4	30,000	7,500

Discussion

P. monodon is well known to be difficult to domesticate. Since it is the largest known species of prawn (recorded with body weights up to 440 g: Deshmukh and Sawant, 1990) their relatively large reproductive sizes (see below) appear to place considerable demands on their nutritive intake, and great onus on achieving optimal feeding and husbandry practices. Commonly, eggs spawned from domestic stock have significantly lower hatch rates than wild stocks (Coman et al. 2005). This and other variable factors make it difficult to assess the causative nature of reproductive failures or address the suitability of culture stock with genetic selection. As a result, most commercial prawn farming operations in Australia currently rely on wild-caught broodstock.

However, there are also several difficult-to-manage issues associated with the complete reliance on wild broodstock. Primarily, their collection relies on the abundance of wild stocks and favourable fishing weather at suitable times for hatchery production. The availability and willingness of experienced trawler operators to tailor their operations to live capture and meticulous handling, so that the animals can be transported to hatcheries in very good condition, also changes from year to year. The escalating costs of mounting wild collection activities, and the unpredictable quality and performance of collected stock compound these difficulties. Furthermore, the nature of using wild broodstock does not facilitate high levels of biosecurity; the screening of collected animals for pathogens cannot address this given the regular discovery of new diseases. And of course, the use of wild broodstock does not allow for potential advances from selective breeding.

In the simplest of terms domestication should involve the selection of potential breeders from each successive generation and their placement into suitable culture systems, so they can become reproductively active at times that are suitable for the creation of the next generation. In northern Australia, farms often aim to stock ponds by August each year, so that much of their stock can be harvested for Christmas seafood markets in late December. Servicing this means that hatcheries need to be spawning broodstock in July each season. In southern Queensland, where there is a later onset of summer growing temperatures, the focus in on stocking ponds in October and November each year. In this later situation, broodstock need to be ready for spawning in September or early October.

This yearly seasonal production cycle creates challenging growth imperatives for the production of cultured broodstock, and this is accentuated in Australia by a significant period of relatively low slow-growth ambient temperatures during winter. But importantly, research conducted in Australia over the last decade has seen improvements in growth and reproductive performance of *P. monodon* broodstock held in captivity, and some reported successful programs (e.g. FRDC, 2014) demonstrate that the potential genetic gains are worth pursuing. For example, work by CSIRO (see Coman et al., 2005; 2013) has reported significant improvements in the growth and reproductive performance of cultured broodstock, such as the 200% weight gains for broodstock grown in tanks in 2003 compared with those grown in 1997. Importantly, whilst genetic selection partially contributed to this result, this was largely attributed to husbandry improvements (Coman et.al., 2005). That work compared raceways and tanks and concluded that those approaches offered better, more controlled and biosecure environments than larger open ponds for rearing prawn broodstock, and that future efforts should integrate husbandry improvements with genetic selection. The difficulties faced by a subsequent institution-based breeding program for *P. monodon* in 2005 (Burke et al., 2009) compounded these considerations and further supported the dismissal of rearing protocols that involved outdoor ponds in Australia. In that case significant percentages (13-53%) of *P. monodon* broodstock reared in outdoor ponds at BIRC exhibited gonopore necrosis and poor fertility, although the cause of these problems was not specifically identified.

Fresh natural feeds are thought to significantly contribute to broodstock quality for many commercial penaeid species. Prawns have long been known to consume a vast array of organic materials including phytoplankton and other plant matter, crustaceans and decaying animal material (Dall, 1968). In aquaculture ponds, *P. monodon's* dietary intake has also been shown to include plants, crustaceans and detritus, in addition to commercial feeds (Focken et al. 1998). The burrowing habits of *P. monodon* (Moller and Jones, 1975) would also suggest that benthic organisms like polychaetes would be important in their diet. Since they tend to inhabit a very broad range of habitats (variable salinities of 1-45 ppt., depths of 0-110 m, sandy to muddy substrates: FAO, 2016), in the wild they are likely exposed to a wide variety of benthic feeds, which fulfils their nutritional requirements and leads to the high performance seen in wild-caught broodstock.

However, researchers do not know exactly how to replicate this in captivity using commercially available feeds, and therefore must rely on assumptions made from the range of species which have been studied. For example, most hatcheries use squid as the base diet because it is relatively inexpensive and readily available. Memon et al. (2012) found that the spermatophore quality of banana prawns (*Penaeus merguiensis*) improved when they were fed squid, and they attributed this to its high lipid content. On the other hand, others (e.g. Meunpol et al., 2005) have successfully used polychaetes as a more specific guide to the fatty acid content of diets fed to pond-reared male *P. monodon*. The unusually broad range of fatty acids found in some polychaete species like *Perinereis helleri* (e.g. 30 detected by Palmer et al., 2014) may help to address any deficiencies in these basal diets. Indeed Leelatanawit et al. (2014) also recently showed that spermatophore weights and sperm counts improved in *P. monodon* when they were fed sand polychaetes (*Perinereis nuntia*) rather than commercial pellets. Yet artificial pellets very conveniently supply concentrated forms of protein and reliable levels of vitamins making them a useful inclusion when strong growth is necessary.

Problems experienced by domestication programs for *P. monodon* have in the past been attributed to smaller than optimal breeding stock, which can be a function of several factors including their age, their culture densities, and the water temperatures and feeds offered. These factors also need to be balanced with industrial imperatives such as the cost-effectiveness of culture operations and facilities; therefore, low-cost options, such as the use of otherwise empty production ponds and avoidance of powered heating systems for overwintering, are worthy of consideration. This study has demonstrated that stocking selected broodstock into recently harvested and re-filled production ponds is a simple, low-cost method to substantially increase the body size and broaden the nutritional base (with natural feeds) of potential broodstock. This approach easily affords the use of low stocking densities which reduce competition to encourage fast growth. It also provides a simple method to achieve the temporary physical separation of families for later introduction into more controlled breeding facilities. As farm managers will attest, harvesting many production ponds at the end of a season can be logistically difficult, and in some situations when farm ponds are being progressively harvested this may be a convenient way to better manage different broodstock families. Furthermore, prawns re-harvested a month or two later from these temporary holding ponds would be much larger and therefore more able to tolerate PIT[21] or eye-tagging to allow future identification of their particular families or specific individuals for farm-based breeding designs. As long as water temperatures are sufficiently high to maintain growth, ponds could still be given enough time to dry before re-filling for the next crop; and if the stock previously harvested from the re-filled pond were healthy and free from disease, this could provide a very useful way to broaden the domestication design without additional costs other than those associated with pond re-filling.

Mature female *P. monodon* tend to be larger than mature males. Females can be found carrying spermatophores in their thelyca from body weights of 60 g, but > 82 g is considered necessary for reproductive females; on the other hand males over 33 g have been found with spermatophores (FAO, 2016). In commercial practice, considerably larger sizes for males (88 g) and females (117 g) can be recommended (e.g. Coman et al. 2005), and given these indicative sizes, the 12-mo-old animals produced in the present study (males of 111 g; and females of 143 g) were easily large enough for commercial breeding. The particularly long period to spawn after ablation seen in the present study (16-24 days) appears to be typical of domesticated lines of this species (e.g. 15-19 days reported by Coman et al., 2006). Given the pronounced effect that appropriate feeding can have during the last month of final maturation for this species (Coman et al., 2007), it remains to be seen if this period can be shortened by feeding better diets during this time; for example, much higher inclusions of polychaetes, which is facilitated by PASF.

The necessary *P. monodon* broodstock production timelines that are applicable in Australia (highlighted above) also influence the demand for essential prawn broodstock maturation feeds for both domesticated and wild-caught broodstock. Whilst frozen broodstock feeds (e.g., squid, mussel) can be stockpiled and stored for use when necessary, live feeds such as cultured polychaetes must be produced with these timeframes in mind. Assuming that these feeds are most needed one to two months before such broodstock are spawned, in general they need to be readily available from May through to October each year. This timeframe fits nicely into the PASF polychaete production

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[21] A passive integrated transponder (PIT) tag is an electronic microchip encased in biocompatible glass casing which is implanted into the animal to allow later identification.

schedules that have been demonstrated in the present study (see Table 6) to support prawn production in southern Queensland, whereby PASF beds can be stocked in November and be ready for harvest from May through to October each year.

Although these prawn broodstock were grown for only about 50% of their culture period in a system that used PASF recirculation, this experiment represents the first step towards validating this style of culture system for the culture of *P. monodon* broodstock. The results certainly suggest that this recirculation through PASF did not compromise the prawn's quality as broodstock. In fact, given the range of beneficial factors that this recirculation approach may provide (e.g. supply of bacterial film rich in micronutrients to broaden dietary intake; alkalinity control for water quality stability – see Appendix 4 and nutrient results in main report) there may be real advantages in doing so for the entire production period. This could be a useful avenue for future husbandry research with PASF.

Reviewing the methods used herein, we suggest that future work of this nature use purged, unbroken worms rather than broken worms for more repeatable results. Although the nutritional profile of broken worms was probably very similar to whole worms, this has not been tested. And although they were fed soon after harvest and before there had been any putrefaction or deterioration from bacterial infection of the damaged worm biomass, the broken worms were not purged, and therefore represented a potential biosecurity concern, as did the silt-laden mucus from the worm harvest which was also added to the broodstock pond with intention to further broaden the prawns' nutritional base. Whilst the purged worms produced in Season 1 tested PCR-negative for all endemic viruses, broken worms and the silt-laden mucus were not tested, introducing uncontrolled factors into the experiment. Given the precedence of viral contamination in unpurged worms (e.g. MoV in *P. helleri*; Palmer et al., 2014) this is an important consideration for future research.

Interestingly, given the detection of pale, enlarged and hollow sperm in the histopathological examinations of a small sample of the prawn broodstock, the successful spawning results suggest that the occurrence of this deformity does not entirely compromise male fertility. The spawning results demonstrated that many of these cultured first-generation broodstock were viable, and the hatchery operators advised that the reproductive results from these were commercially scalable.

Conclusion

This study demonstrates the potential for PASF to enhance the production of domesticated prawn broodstock. When applied as a water treatment agent, PASF could be used to fully recirculate water to provide available nutrients and trace elements that maintain phytoplankton blooms and healthy ecosystems, and minimise water exchange in the culture systems for better biosecurity and temperature controls. Polychaetes cultured in such a system should have similar biosecurity status to the prawn broodstock and therefore should not represent a disease threat, even when used live and at high inclusion levels in their diet. Following the transfer of cultured prawn broodstock to final maturation facilities the polychaetes could be conveniently harvested and confidently fed to contribute to their optimal reproductive performance.

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References

Burke, M. J., Coman, G., Kenway, M., Macbeth, M., Cowley, J., Knibb, W. (2009). Practical, feasible and low cost genetic selection of *Penaeus monodon* for increased profitability. Fisheries Research and Development Corporation, Project Report No. 2005/205.

Chimsung, N. 2014. Maturation diets for black tiger shrimp (*Penaeus monodon*) broodstock: a review. *Songklanakarin Journal of Science and Technology* 36(3): 265-273.

Chong, R.S-M., Cowley, J.A., Paterson, B.D., Coman, G.J., Mann, D.L., Arnold, S.J., Prior, H.C., Wood, A.T., Amigh, M.J. 2014. Hollow sperm syndrome during spermatogenesis in the giant tiger shrimp *Penaeus monodon* (Fabricius 1798) from eastern Australia. *Aquaculture Research* 46(11): 2573-2592.

Coman, G.J., Arnold, S.J., Callaghan, T.R., Preston, N.P. 2007. Effect of two maturation diet combinations on reproductive performance of domesticated *Penaeus monodon*. *Aquaculture* 263 (1–4): 75-83.

Coman, G.J., Arnold, S.J. Peixoto, S., Crocos, P.J., Coman, F.E., Preston, N.P. 2006. Reproductive performance of reciprocally crossed wild-caught and tank-reared *Penaeus monodon* broodstock. *Aquaculture* 252(2–4): 372-384.

Coman, G.J., Arnold, S.J., Wood, A.T., Preston, N.P. 2013. Evaluation of egg and nauplii production parameters of a single stock of domesticated *Penaeus monodon* (Giant Tiger Shrimp) across generations. *Aquaculture* 400-401: 125-128.

Coman, G.J., Crocos, P.J., Arnold, S.J., Keys, S.J., Preston, N.P. 2005. Growth, survival and reproductive performance of domesticated Australian stocks of the giant tiger prawn, *Penaeus monodon*, reared in tanks and raceways. *Journal of the World Aquaculture Society* 36(4): 464-479.

Dall, W. 1968. Food and feeding of some Australian penaeid shrimp. FAO Fish Rep., 3(57): 643–656.

Deshmukh, V.D. and Sawant, A.D. 1990. The record size for the giant tiger prawn *Penaeus monodon* Fabricius. *Marine Fisheries Information Service, Technical and Extension Series*. 105. pp. 14-15.

FAO, 2016. Aquaculture feed and fertilizer resources information system.
http://www.fao.org/fishery/affris/species-profiles/giant-tiger-prawn/nutritional-requirements/en/

Focken, U., Groth, A., Coloso, R.M. and Becker, K. 1998. Contribution of natural food and supplemental feed to the gut content of *Penaeus monodon* Fabricius in semi–intensive pond system in the Philippines. *Aquaculture* 164: 105–116.

FRDC. 2014. http://frdc.com.au/stories/Pages/how_to_build_a_better_prawn.aspx

Leelatanawit, R., Uawisetwathana, U., Khudet, J., Klanchui, A., Phomklad, S., Wongtripop, S., Angthoung, P., Jiravanichpaisal, P., Karoonuthaisiri, N. 2014. Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*). *Aquaculture* 433: 266-275.

Memon, A.J., Ikhwanuddin , M., Talpur , A.D., Khan , M.I., Fariddudin , M.O., Safiah J., Abol-Munafi , A.B. 2012. To determine the efficiency of different fresh diets in improving the spermatophore quality of banana shrimp *Penaeus merguiensis* (De Man, 1888). *Journal of Animal and Veterinary Advances* 11: 1478-1485.

Meunpol, O., Meejing, P., Piyatiratitivorakul, S. 2005. Maturation diet based on fatty acid content for male *Penaeus monodon* (Fabricius) broodstock. *Aquaculture Research* 36: 1216-1225.

Moller T.H., Jones D.A. 1975. Locomotory rhythms and burrowing habits of *Penaeus semisulcatus* (de Haan) and *P. monodon* (Fabricius) (Crustacea: Penaeidae). *Journal of Experimental Marine Biology and Ecology* 18(1): 61-77.

Palmer, P.J., Wang, S., Houlihan, A., Brock, I. 2014. Nutritional status of a nereidid polychaete cultured in sand filters of mariculture wastewater. *Aquaculture Nutrition* 20, 675-691.

Appendix 4 – Growth and condition trials for *Penaeus monodon* grown with different levels of PASF recirculation and biofilm

Introduction

Some penaeid shrimp like *P. monodon* are carnivorous but most are also highly omnivorous. Many are known to consume a range of organic matters including plants, animals, bacteria and detritus, as well as manufactured feeds. Their dextrous ability to sort through detritus with their maxillipeds enables this very broad diet. They generally feed by grinding collected materials into a paste with their feeding mandibles before ingestion, and this makes their ingested feeds readily digestible and further adds to their feeding efficiencies. These attributes make them particularly good feed converters in outdoor pond environments. Many studies have focussed on their use of natural feeds in culture ponds, but their use of bacterial biomass has found recent favour, and particularly bioflocs that are generated in culture ponds with various management approaches. Bacterial biomass is also being explored for shrimp diets by several agencies around the world because it appears to provide additional proteins (Burford et al., 2004) and bioactives that enhance prawn growth (CSIRO, 2015).

Of particular interest in this study is the considerable biofilm material that develops on the inside of drainage pipes and around the PASF bed outlets where sulphur is apparently being oxidised (Palmer, 2010). Strands of bacterial slime can at times also be relatively well represented in the PASF-bed discharge. It develops quickly on the insides of pipes and regularly sloughs off in the flow of water; most noticeably following flow changes or any physical disturbances. The burrowing activities of the worms in the beds act to enhance oxygen penetration into and across the entire area of the bed, and therefore are likely to further stimulate this sulphur oxidation and biofilm development within the sand beds. This process is likely also used by the worms either directly as food or indirectly through the food chain it supports (bacteria, protozoans, copepods, etc.), and it is hypothesised that prawns might also benefit directly from this sulphur-bacteria-based food source.

To investigate the potential for PASF-treated water carrying particles of this biofilm to provide additional nutrition for growth of prawns in a recirculated system, three controlled trials were performed during the second experimental season (2014-15). These trials were designed to compare the growth of prawns in pond-water with different levels of PASF recirculation and PASF biofilm supply. Nutritional analyses were also performed for samples of the PASF biofilm to assess its potential food value.

Materials and methods

A 12-tank experimental system was arranged in an outdoor locality next to the recirculation sump of the PASF system at BIRC (Figure 69). Each tank had a water volume of 1700 L (1550 mm diameter x 900 mm deep), aeration (one air-stone with moderate air flow), and pond-water and/or PASF-filtered water supplies. Tanks were fully covered with one layer of 70% shade cloth to prevent bird predation and reduce light levels. On two occasions in Season 2, average-sized prawns were removed from the ponds in the PASF recirculation system, individually weighed and stocked into each tank for growth comparisons under replicated experimental conditions. On a third occasion towards the end of Season 2, juvenile prawns were sourced from CSIRO for similar comparisons.

Figure 69 The experimental system showing tanks (left) and water supply from recirculation sump (right).

The first trial began on 3 December 2014 using prawns collected from feed trays in the ponds. Fifteen prawns with average (± se) weights of 1.8 ± 0.08 g were individually weighed and collectively stocked into each tank; this was two weeks after PASF operations had begun and just as anaerobic sediments began to develop in the PASF beds. Water supplies to the tanks were managed so that three levels of PASF recirculation were applied. A total of approximately 87% exchange per day was applied to all tanks. In tanks with low recirculation this involved only the supply of water from the prawn ponds in the larger integrated system: this treatment provided operational controls for experiments and a baseline of 4.5% PASF recirculation (being used at that time in the larger integrated pond-culture system). Medium or high recirculation rates were applied by replacing approximately 25 or 50%, respectively, of the pond-water exchange with PASF-treated water from the recirculation sump. To achieve this it was necessary to take into consideration the longer (almost double) timeframe for pumping from the sump compared with pumping from the ponds, which was part of the normal daily operations of the larger integrated system as shown in Table 12 below.

Table 12 Daily exchange and flow rate calculations* used for different rates of PASF-treated water recirculation in the first growth trial.

Water source	Low Recirc	Medium Recirc	High Recirc
Pond-water	100% = 4.1 L min^{-1}	75% = 3.1 L min^{-1}	50% = 2.1 L min^{-1}
PASF-treated water	0% = 0 L min^{-1}	25% = 0.5 L min^{-1}	50% = 1.0 L min^{-1}

*Calculations based on 1,700-L tanks with 6 hr pond-water pumping per day, and 12 hr PASF-treated-water pumping per day.

The second trial began on 9 January 2015 using 12 prawns per tank. Their average (± se) starting size was 12 ± 0.28 g and they were collected with a cast net from the culture ponds in the integrated system. This trial was undertaken when white sulphurous deposits were becoming increasingly prevalent in the recirculation sump. Water from the ponds was supplied to each tank to provide 100% exchange per day (7.5 hr x 3.8 L min^{-1}). Unlike in the first trial, where biofilm application was via different rates of recirculated-water supply from the sump, biofilm was collected and added directly to tanks at different rates. Every one or two days during the trial, a small plunger (19-mm diameter poly pipe) was used to dislodge biofilm from the inside of the drainage pipes that were discharging into the sump. This allowed the biofilm to be quickly collected at high concentrations for adding directly to the experimental tanks. This biofilm concentrate was pooled in a bucket with PASF-filtered water, gently mixed to provide a homogeneous suspension, and volumetrically split between the tanks according to three treatments that provided low, medium and high biofilm supplies. The amounts of biofilm added to tanks each day were assessed via settlement (2 hr) of the concentrate in 1-L Imhoff cones as shown in Table 13 below.

Table 13 Daily settled volumes (mL) of biofilm supplied to tanks in the second growth trial.

Date	Low biofilm supply	Medium biofilm supply	High biofilm supply
10/01/2015	0	1	2
11/01/2015	0	17	34
12/01/2015	0	15	30
13/01/2015	0	9	18
14/01/2015	0	15	30
15/01/2015	0	15	30
16/01/2015	0	25	50
17/01/2015	0	22	44
18/01/2015	0	15	30
19/01/2015	0	6	12
20/01/2015	0	20	40
21/01/2015	0	12	24
22/01/2015	0	27	54
23/01/2015	0	13	26
24/01/2015	0	16	32
27/01/2015	0	10	20
28/01/2015	0	7	14
29/01/2015	0	12	24
30/01/2015	0	15	30
31/01/2015	0	8	16
1/02/2015	0	7	14
2/02/2015	0	8	16
3/02/2015	0	12	24
4/02/2015	0	0	0
Total settled volumes added	**0**	**297**	**594**

The third trial began on 14 April 2015 using 12 prawns (1.3 ± 0.03 g average) per tank. Instead of shade cloth, clear plastic was used to cover each tank to provide higher, more stable water temperatures. A flow rate of 4 L min^{-1} was applied to each tank with 9 hr pumping per day (2160 L = 127 % exchange per day). Biofilm was collected and assessed each day and added directly to the experimental tanks (Table 14) in similar fashions to those methods used in the second trial.

In all three trials each treatment was replicated four times and treatments were randomised within the experimental set-up (Low = Tanks 3, 7, 9, 12; Medium =Tanks 1, 6, 8, 10; High = 2, 4, 5, 11). No supplemental feeds were added to the experimental tanks during the first two trials, but in the third trial artificial feed (Ridley Starter 1) was added to each tank once daily at a rate of 2% of the averaged total biomass of prawns stocked into the tanks. In all trials disturbances of the prawns after stocking and during the trial were minimised. Water temperatures in the tanks were monitored with submersible max/min thermometers (Tanks 1, 2 and 3) and water flow rates were recalibrated on a weekly basis. All prawns were harvested and individually weighed after 33 days in the first trial, after 25 days in the second, and after 27 days in the third.

Table 14 Daily settled volumes (mL) of biofilm supplied to tanks in the third growth trial.

Date	Low biofilm supply	Medium biofilm supply	High biofilm supply
15/04/2015	0	17	34
16/04/2015	0	10	20
17/04/2015	0	5	10
18/04/2015	0	0	0
19/04/2015	0	0	0
20/04/2015	0	48	96
21/04/2015	0	13	26
22/04/2015	0	20	40
23/04/2015	0	8	16
24/04/2015	0	17	34
25/04/2015	0	0	0
26/04/2015	0	0	0
27/04/2015	0	16	32
28/04/2015	0	15	30
29/04/2015	0	16	32
30/04/2015	0	10	20
1/05/2015	0	18	36
2/05/2015	0	7	14
3/05/2015	0	0	0
4/05/2015	0	10	20
5/05/2015	0	17	34
6/05/2015	0	10	20
7/05/2015	0	9	18
8/05/2015	0	24	48
9/05/2015	0	0	0
10/05/2015	0	0	0
Total settled volumes added	**0**	**290**	**580**

Samples of suspended biofilm particles in the water column of the recirculation sump were also collected for specific analyses on three separate occasions (26/08/14, 15/01/15 and 27/01/15). In 2014 collection was with a fine-meshed net which concentrated the material into a small volume (100 mL) of PASF-filtered water for analyses. In 2015 drainage pipes were physically disturbed (as described above) to dislodge biofilm material and provide concentrates; for analyses these were settled (2 hr) in several 1 L Imhoff cones and pooled to provide total 1 L volumes each containing approximately 360 mL and 800 mL of settled biofilm on the 15/01/15 and 27/01/15, respectively. These concentrates were frozen in an ultra-freezer (-80°C) prior to testing by Australian Laboratory Services Pty Ltd for elemental and nutritional contents.

Prawn survival and weight change results were analysed by one-way AVOVA using GenStat (2015) and LSD pairwise comparison of means.

Results and Discussion

Water temperatures fluctuated by several degrees diurnally in the outdoor experimental system. In the first trial water temperatures predominantly ranged from 26 to 31°C, in the second trial they ranged from 27 to 33°C and in the third trial they ranged from 22 to 26°C.

Survival rates (Table 15) were similarly ($P>0.05$) high for all treatments in each trial. Trial 2 provided the lowest survival rates and an overall deterioration of prawn condition in all treatments, which was presumably due to the larger size of prawns in that trial and their subsequent need for a high-protein diet for growth. Nevertheless, these survival results demonstrate that greater levels of PASF-filtered water exchange/recirculation (up to approx. 50% per day trialled) and biofilm supply to those routinely applied at large scale in the integrated system would not likely be detrimental to prawn survival.

Weight increases occurred in all treatments in the first and third experiments which utilised small unfed and fed prawns, but weight losses occurred in the second experiment which used larger unfed prawns. In the growth results provided (Figure 70), although there appeared to be the tendency for marginally improved growth or reduced weight loss with the medium supply levels of PASF biofilm (e.g., $P=0.06$ in trial 2), weight changes were not significant ($P>0.05$) in all three trials.

Table 15 Mean (± se) survival rates (%) for prawns supplied with different levels of PASF biofilm in each growth trial. Within rows, numbers with different letters are significantly different ($P<0.05$).

Trial no.	Biofilm supply		
	Low	**Medium**	**High**
1	75 ± 4.19 a	76.7 ± 6.38 a	80 ± 7.2 a
2	60 ± 2.72 a	61.7 ± 1.67 a	60 ± 3.85 a
3	100 ± 0 a	87.5 ± 4.17 a	95.8 ± 4.17 a

The nutritional and elemental contents of biofilm concentrates provided in Tables 16, 17 and 18 show that the biofilm materials flowing from PASF beds are potentially rich sources of several elements including aluminium, calcium, iron, magnesium, phosphorus and potassium. Several other elements represented in low but measureable amounts included arsenic, boron, bromine, copper, iodine, molybdenum, samarium, silicon, strontium, titanium, vanadium and zinc. Many of these elements are essential and/or trace nutrients utilised by phytoplankton in the ponds. However, the degree of their incorporation in biofilm matrices and availability for plant uptake were not assessed.

The levels of food-related substances were generally low and variable in the three samples analysed. Carbohydrates ranged from 0.2 to 0.9 g 100 g^{-1}, and protein (up to 1.1 g 100 g^{-1}) was only detected in the first two samples analysed. No lipids were detected in any of the biofilm concentrates. Since these levels were measured in moist concentrates containing 94-96% water, their levels were somewhat higher when considered after conversion into more conventional dry matter (DM) measures: protein up to 19 g 100 g^{-1} DM; carbohydrate up to 24 g 100 g^{-1} DM.

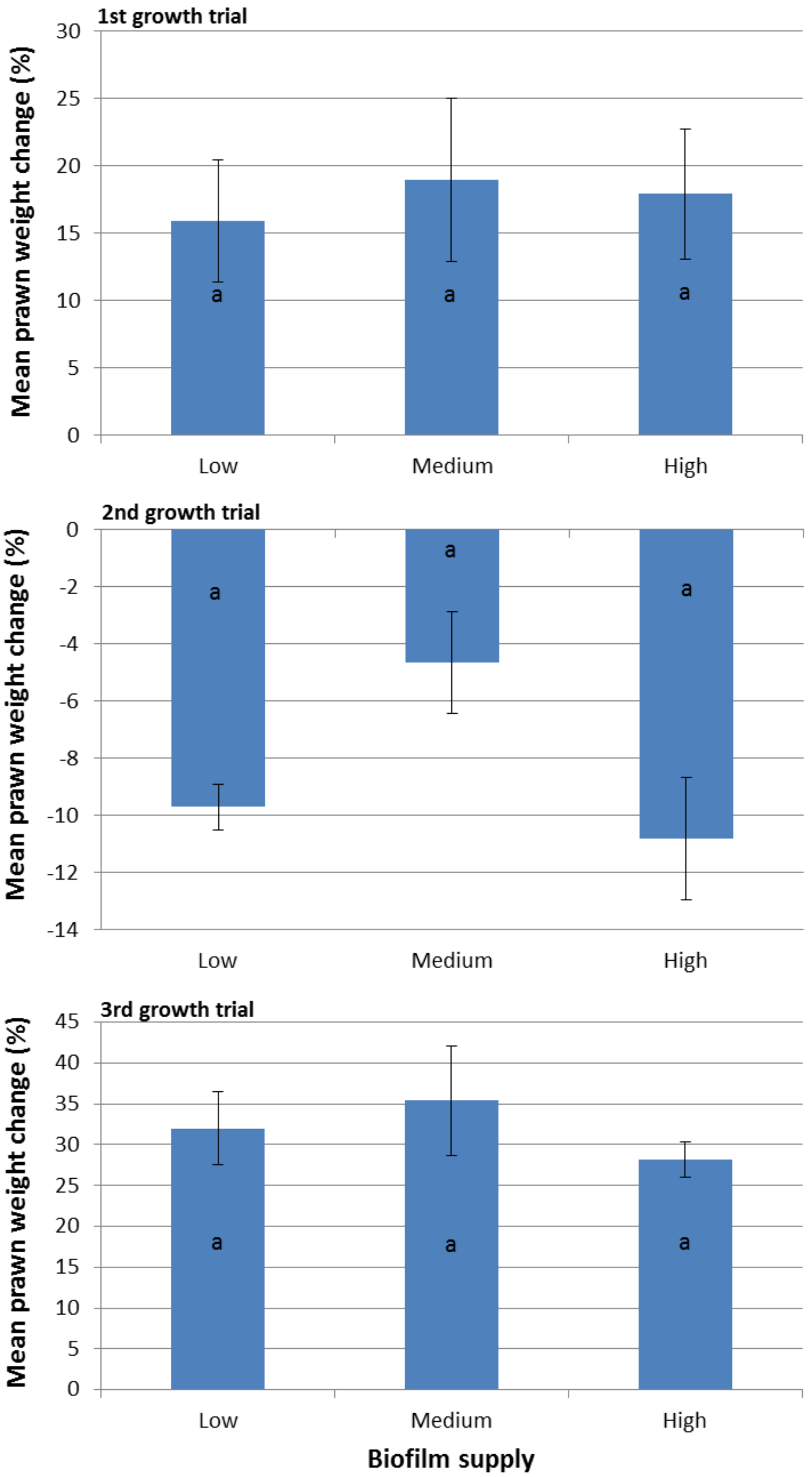

Figure 70 Mean prawn weight change expressed as a percentage of starting weights after supplying different levels of PASF biofilm in the three growth trials. Within trials, columns with different letters are significantly different ($P<0.05$).

Table 16 Nutritional and elemental composition of biofilm concentrate (100 mL) collected from the sump of the PASF recirculation system on 26/08/14. Data are reported on a wet matter basis.

Nutritional Substance	**Amount**	**Unit**	**Elemental Substance**	**Amount**	**Unit**
Total Dietary Fibre	0.6	g/100g	Lanthanum	<1	mg/kg
Ash	4.2	g/100 g	Lead	<1	mg/kg
Cholesterol	<1.0	mg/100g	Lithium	<1	mg/kg
Energy	27	kJ/100g	Lutetium	<1	mg/kg
Moisture	94.3	g/100g	Magnesium	880	mg/kg
Protein	1.1	g/100g	Manganese	<1	mg/kg
Fat - Total	<0.1	g/100g	Mercury	<1	mg/kg
Fat - Saturated	<0.1	g/100g	Molybdenum	1.3	mg/kg
Fat - Monounsaturated	<0.1	g/100g	Neodymium	<1	mg/kg
Fat - Polyunsaturated	<0.1	g/100g	Nickel	<1	mg/kg
Fat - Trans	<0.1	g/100g	Niobium	<1	mg/kg
Carbohydrates - Total	0.2	g/100g	Osmium	<1	mg/kg
Sugar - Total	<0.1	g/100g	Palladium	<1	mg/kg
Sodium	710	mg/100g	Phosphorus	290	mg/kg
Elemental Substance			Platinum	<1	mg/kg
Aluminium	70	mg/kg	Potassium	110	mg/kg
Antimony	<1	mg/kg	Praseodymium	<1	mg/kg
Arsenic	1.9	mg/kg	Rhenium	<1	mg/kg
Barium	<1	mg/kg	Rhodium	<1	mg/kg
Beryllium	<1	mg/kg	Rubidium	<1	mg/kg
Bismuth	<1	mg/kg	Ruthenium	<1	mg/kg
Boron	4.1	mg/kg	Samarium	17	mg/kg
Bromine	15	mg/kg	Scandium	<1	mg/kg
Cadmium	<1	mg/kg	Selenium	<1	mg/kg
Calcium	35	mg/kg	Silicon	<1	mg/kg
Cerium	<1	mg/kg	Silver	<1	mg/kg
Cesium	<1	mg/kg	Strontium	6.7	mg/kg
Chromium	<1	mg/kg	Tantalum	<1	mg/kg
Cobalt	<1	mg/kg	Tellurium	<1	mg/kg
Copper	1.5	mg/kg	Terbium	<1	mg/kg
Dysprosium	<1	mg/kg	Thallium	<1	mg/kg
Erbium	<1	mg/kg	Thorium	<1	mg/kg
Europium	<1	mg/kg	Thulium	<1	mg/kg
Gadolinium	<1	mg/kg	Tin	<1	mg/kg
Gallium	<1	mg/kg	Titanium	3.1	mg/kg
Germanium	<1	mg/kg	Tungsten	<1	mg/kg
Gold	<1	mg/kg	Uranium	<1	mg/kg
Hafnium	<1	mg/kg	Vanadium	2.9	mg/kg
Holmium	<1	mg/kg	Ytterbium	<1	mg/kg
Indium	<1	mg/kg	Yttrium	<1	mg/kg
Iodine	2.7	mg/kg	Zinc	11	mg/kg
Iridium	<1	mg/kg	Zirconium	<1	mg/kg
Iron	3000	mg/kg			

Table 17 Nutritional and elemental composition of biofilm concentrate (1 L*) collected from the sump of the PASF recirculation system on 15/01/15. Data are reported on a wet matter basis.

Nutritional Substance	**Amount**	**Unit**	**Elemental Substance**	**Amount**	**Unit**
Total Dietary Fibre	<1	g/100g	Lanthanum	<1	mg/kg
Ash	3.7	g/100 g	Lead	<1	mg/kg
Cholesterol	<1.0	mg/100g	Lithium	<1	mg/kg
Energy	12	kJ/100g	Lutetium	<1	mg/kg
Moisture	95.6	g/100g	Magnesium	1900	mg/kg
Protein	0.4	g/100g	Manganese	<1	mg/kg
Fat - Total	<0.1	g/100g	Mercury	<1	mg/kg
Fat - Saturated	<0.1	g/100g	Molybdenum	<1	mg/kg
Fat - Monounsaturated	<0.1	g/100g	Neodymium	<1	mg/kg
Fat - Polyunsaturated	<0.1	g/100g	Nickel	<1	mg/kg
Fat - Trans	<0.1	g/100g	Niobium	<1	mg/kg
Carbohydrates - Total	0.3	g/100g	Osmium	<1	mg/kg
Sugar - Total	<0.1	g/100g	Palladium	<1	mg/kg
Sodium	1100	mg/100g	Phosphorus	98	mg/kg
Elemental Substance			Platinum	<1	mg/kg
Aluminium	31	mg/kg	Potassium	390	mg/kg
Antimony	<1	mg/kg	Praseodymium	<1	mg/kg
Arsenic	<1	mg/kg	Rhenium	<1	mg/kg
Barium	<1	mg/kg	Rhodium	<1	mg/kg
Beryllium	<1	mg/kg	Rubidium	<1	mg/kg
Bismuth	<1	mg/kg	Ruthenium	<1	mg/kg
Boron	10	mg/kg	Samarium	<1	mg/kg
Bromine	41	mg/kg	Scandium	<1	mg/kg
Cadmium	<1	mg/kg	Selenium	<1	mg/kg
Calcium	280	mg/kg	Silicon	130	mg/kg
Cerium	<1	mg/kg	Silver	<1	mg/kg
Cesium	<1	mg/kg	Strontium	5.8	mg/kg
Chromium	<1	mg/kg	Tantalum	<1	mg/kg
Cobalt	<1	mg/kg	Tellurium	<1	mg/kg
Copper	<1	mg/kg	Terbium	<1	mg/kg
Dysprosium	<1	mg/kg	Thallium	<1	mg/kg
Erbium	<1	mg/kg	Thorium	<1	mg/kg
Europium	<1	mg/kg	Thulium	<1	mg/kg
Gadolinium	<1	mg/kg	Tin	<1	mg/kg
Gallium	<1	mg/kg	Titanium	<1	mg/kg
Germanium	<1	mg/kg	Tungsten	<1	mg/kg
Gold	<1	mg/kg	Uranium	<1	mg/kg
Hafnium	<1	mg/kg	Vanadium	<1	mg/kg
Holmium	<1	mg/kg	Ytterbium	<1	mg/kg
Indium	<1	mg/kg	Yttrium	<1	mg/kg
Iodine	<1	mg/kg	Zinc	<1	mg/kg
Iridium	<1	mg/kg	Zirconium	<1	mg/kg
Iron	280	mg/kg			

*A settled volume of approximately 360 mL was contained in the concentrate.

Table 18 Nutritional and elemental composition of biofilm concentrate (1 L*) collected from the sump of the PASF recirculation system on 27/01/15. Data are reported on a wet matter basis.

Nutritional Substance	Amount	Unit	Elemental Substance	Amount	Unit
Total Dietary Fibre	<1	g/100g	Lanthanum	<1	mg/kg
Ash	2.9	g/100 g	Lead	<1	mg/kg
Cholesterol	<1.0	mg/100g	Lithium	<1	mg/kg
Energy	15	kJ/100g	Lutetium	<1	mg/kg
Moisture	96.2	g/100g	Magnesium	1500	mg/kg
Protein	<0.1	g/100g	Manganese	<1	mg/kg
Fat - Total	<0.1	g/100g	Mercury	<1	mg/kg
Fat - Saturated	<0.1	g/100g	Molybdenum	<1	mg/kg
Fat - Monounsaturated	<0.1	g/100g	Neodymium	<1	mg/kg
Fat - Polyunsaturated	<0.1	g/100g	Nickel	<1	mg/kg
Fat - Trans	<0.1	g/100g	Niobium	<1	mg/kg
Carbohydrates - Total	0.9	g/100g	Osmium	<1	mg/kg
Sugar - Total	<0.1	g/100g	Palladium	<1	mg/kg
Sodium	880	mg/100g	Phosphorus	56	mg/kg
Elemental Substance			Platinum	<1	mg/kg
Aluminium	8.5	mg/kg	Potassium	300	mg/kg
Antimony	<1	mg/kg	Praseodymium	<1	mg/kg
Arsenic	<1	mg/kg	Rhenium	<1	mg/kg
Barium	<1	mg/kg	Rhodium	<1	mg/kg
Beryllium	<1	mg/kg	Rubidium	<1	mg/kg
Bismuth	<1	mg/kg	Ruthenium	<1	mg/kg
Boron	8.3	mg/kg	Samarium	<1	mg/kg
Bromine	32	mg/kg	Scandium	<1	mg/kg
Cadmium	<1	mg/kg	Selenium	<1	mg/kg
Calcium	200	mg/kg	Silicon	39	mg/kg
Cerium	<1	mg/kg	Silver	<1	mg/kg
Cesium	<1	mg/kg	Strontium	4.3	mg/kg
Chromium	<1	mg/kg	Tantalum	<1	mg/kg
Cobalt	<1	mg/kg	Tellurium	<1	mg/kg
Copper	<1	mg/kg	Terbium	<1	mg/kg
Dysprosium	<1	mg/kg	Thallium	<1	mg/kg
Erbium	<1	mg/kg	Thorium	<1	mg/kg
Europium	<1	mg/kg	Thulium	<1	mg/kg
Gadolinium	<1	mg/kg	Tin	<1	mg/kg
Gallium	<1	mg/kg	Titanium	<1	mg/kg
Germanium	<1	mg/kg	Tungsten	<1	mg/kg
Gold	<1	mg/kg	Uranium	<1	mg/kg
Hafnium	<1	mg/kg	Vanadium	<1	mg/kg
Holmium	<1	mg/kg	Ytterbium	<1	mg/kg
Indium	<1	mg/kg	Yttrium	<1	mg/kg
Iodine	<1	mg/kg	Zinc	<1	mg/kg
Iridium	<1	mg/kg	Zirconium	<1	mg/kg
Iron	46	mg/kg			

*A settled volume of approximately 400 mL was contained in the concentrate.

Assuming that 1 mL of settled biofilm weighed 1 g, conversions using the amounts of settled material in each analysed sample suggest that up to 1.1 g 100 mL^{-1} of protein and 0.8 – 2.3 g 100 mL^{-1} of carbohydrate can exist in moist settled PASF biofilm. Given that biofilm settled volumes totalling approximately 300 mL for the medium supply and 600 mL for the high supply were applied during the second and third trials, this meant that a total of about 3.3 g and 6.7 g of protein, and 2.5–6.8 g and 5–13.5 g of carbohydrate were applied in the medium and high level biofilm supply treatments, respectively, during the entirety of each of the last two trials.

Considering the total biomasses of prawns in tanks at the end of each trial (means of 99.8 g in Trial 2 and 19.5 g in Trial 3), these total protein and carbohydrate levels supplied to the tanks over about one month were, in fact, more akin to daily feeding rates recommended for prawns of these size classes (i.e., 4-7% of body weight daily for 2-12 g prawns: Australian Prawn Farming Manual, 2006). Hence, only marginal effects on growth could have been expected when the prawns were relying on these nutrients exclusively (Trials 1 and 2). However, the lack of consistent trends for improved growth from increasing biofilm availability suggests that this prawn species may only have a limited ability to utilise these forms of protein and carbohydrate. The low level of co-feeding with a balanced artificial feed in the third trial (2% of body weight per day) addressed the potential for the biofilm to substantially enhance the assimilation of artificial feed; but again, this did not provide significant effects on growth. It is therefore apparent that the contributions to prawn growth of protein and carbohydrates provided by PASF biofilms may only be of practical use when prawns are very small and when their overall feed demands are low.

Summary

The three growth trials undertaken during Season 2 demonstrate that only minor growth advantages may be provided to prawns from the supply of biofilms generated in a PASF system. This was apparent in weight change results under fed- and unfed conditions. The study did not demonstrate significant weight gains in prawns of various sizes that were exposed to relatively high levels of PASF biofilm, although weight losses appeared to be minimised at medium biofilm levels when other suitable feeds for the prawns were lacking. Survival did not appear to be affected by different levels of PASF recirculation or biofilm availability, suggesting that high levels of recirculation (up to approx. 50% exchange per day), should they be applied specifically for water treatment measures, would not adversely affect survival rates in recirculated ponds.

The nutritional breakdown of the biofilm materials generated by the PASF beds supported these growth findings whereby the levels of protein and carbohydrates measured were low, and no discernible lipids were detected. This suggests that the biofilm had limited food value in its own right for prawns in the experimental system. However, this does not preclude potential benefits of PASF recirculation for plankton and ecosystem health which are of vital importance in prawn culture ponds. For example, a wide range of nutrients and trace elements were apparent in the biofilm concentrates, and it is possible that these could provide several advantages in the maintenance of algal blooms and other microbial processes in the pond environment.

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References

Australian Prawn Farming Manual, 2006. Australian prawn farming manual: Health management for profit. The State of Queensland, Department of Primary Industries and Fisheries. ISSN 0727-6273.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture* 232, 525-537.

CSIRO. 2015. http://www.csiro.au/en/Research/AF/Areas/Aquaculture/Better-feeds/Novacq-prawn-feed

GenStat. 2015. GenStat for Windows, Release 16.1. VSN International Ltd., Oxford.

Palmer, P.J. 2010. Polychaete-assisted sand filters. *Aquaculture* 306, 369-377.