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INNOVATIVE INTEGRATED PERIPHYTON TECHNOLOGY FOR SUSTAINABLE AND INTENSIVE NURSERY CULTURE OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

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Abstract: Tank-based freshwater Integrated Periphyton Technology (IPT) and Recirculating Aquaculture System (RAS) were compared in triplicate for 44 days for nursery culture of two separate trials of different Post-Larvae (PL) populations (T1 and T2) from different broodstock and stocking densities of mixed sex giant freshwater prawn *Macrobrachium rosenbergii*. Supplementary feed, alkalinity adjustment, and Carbon to Nitrogen (C:N) ratio were managed with water quality parameters that remained within the suitable range for the duration. Performance characteristics of Survival Rate (SR), Body Weight (BW), and final harvest Food Conversion Ratio (FCR) results were compared. Accordingly, IPT was equivalent to or better than RAS for BWG and productivity in T1 and T2. Meanwhile, in T2, IPT and RAS were similar in terms of SR and BW and achieved an FCR of approximately 2.0. IPT is a zero-discharge system that presents advantages to RAS in terms of no external effluent treatment and the potential for improved FCR through the production of edible biomass within the production volume. With further study and design, IPT could reduce water and land requirements, improve farm economics, decrease environmental impact, and improve the sustainability of intensive aquaculture of *M. rosenbergii*.

Keywords: Integrated Periphyton Technology (IPT), Recirculating Aquaculture System (RAS), sustainability, zero-discharge, environmental impact.

Introduction

Aquaculture in 2018 accounted for 46% of total fish production and 52% of fish for human consumption (FAO, 2020). Notably, the aquaculture production systems need to address a 32% increase in aquaculture production over 2018 levels by 2030, as estimated by the Food and Agriculture Organisation (FAO) in 2020, equivalent to 30% of production from the earth's oceans. However, the projected increase in aquaculture production will not be able to rely solely on past successes in sustainable production (Martinez-Porchas & Martinez-Cordova, 2012).

Such a production increase requires innovative approaches such as intensive aquaculture systems that exert sufficient control over all aspects of the aquaculture production environment to permit high-intensity to ultra-high-intensity production. Accordingly, the sustainability of more intensive aquaculture is assessed by minimising resource intensity by requiring less land and water supply than extensive systems (Tryggvason, 2016). In addition, intensive aquaculture also has the unique capability to be situated closer to urban markets and labour supply to generate less effluent and waste per production unit. It can also utilise urban and peri-urban land designated as commercial, industrial, or agricultural. Building on this, intensive freshwater aquaculture has the added benefit of compatibility with hydroponics in aquaponics and terrestrial farming, hence, the drive to intensify the aquaculture of freshwater species.

The giant freshwater prawn, *Macrobrachium rosenbergii* is a high-value freshwater species that has not been successfully farmed in intensive systems. Although *M. rosenbergii* is readily marketable in sizes above 35 g per prawn, they suffer from Heterogenous Independent Growth (HIG) in males and a separate growth pattern in females. For instance, one group of males, referred to as Blue Claw (BC) males, develop large claws and maintain a position in the culture's social hierarchy that inhibits the growth of the bulk of the culture population.

Moreover, there is a tendency for BC and the lower-ranking Orange Claw (OC) males to exhibit cannibalism (Karplus, 2005). Thus, heterogeneity is the root cause of low productivity and makes uniform products for markets hard to attain. In line with this, investigation of the production of the species in Integrated Periphyton Technology (IPT) and Recirculating Aquaculture System (RAS) could lead to improved productivity and economics. Consequently, this permits the exploitation of *M. rosenbergii* as a high-value freshwater-intensive aquaculture species. Additionally, heterogeneity makes scientific analysis of populations complex due to variance arising from gender sets and the set of male morphotypes, as well as in the case of *M. rosenbergii*.

IPT aquaculture can be compared to RAS and Biofloc Technology (BFT). This includes all water-minimising technological approaches, BFT and IPT achieving very low water exchange rates below 6%, which has been categorised as Super Intensive RAS by FAO, Eurofish, and AKVA Group ASA (Bregnballe, 2015; Vasava *et al*., 2020; Griffiths & Funge-Smith, 2022). Correspondingly, less supplementary feed needs to be produced, transported, and delivered to intensive aquaculture farms since these systems have increased production per unit volume and a lower Food Conversion Ratio (FCR). Note that intensive aquaculture typically requires a more intensive power supply than extensive pond aquaculture. However, an on-site renewable energy supply can be considered. As a result, intensive aquaculture can be optimised as sustainable aquaculture technology. In essence, the experimental technique of comparison of well-established RAS with an innovative IPT culture is a means to obtain results that are distanced from the heterogeneity of the culture species.

IPT is the aquaculture equivalent to Integrated Fixed Film Activated Sludge (IFAS) in wastewater treatment, as BFT is an aquaculture equivalent to suspended growth or activated sludge wastewater treatment. In RAS, a bio-filter equivalent to an IFAS biofilter is located outside the production volume. At the same time, IPT integrates the periphyton biofilter into the production volume and BFT aquaculture integrates suspended biomass within the production volume. Meanwhile, microbial consortia in these systems conduct exchange of nutrients and manage water quality by consuming nutrients from water with the natural objective of consuming all available food from water. This includes carbonaceous organics and nitrogen (Pedros *et al*., 2005; Crab *et al*., 2007). Furthermore, nitrification rates for biofilters exposed to air such as trickling filters or rotating biological contactors (specific surface areas of 100 m^2 m^{-3} to 400 m^2 m^{-3}), used in aquaculture range from about 1.0 g to 4.0 g Total Available Nitrogen (TAN) m^{-2} day^{-1} (Greiner & Timmons, 1998). In addition, fluidised bed filters and plastic bead filters have higher specific surface areas and lower void spaces and generally have lower aerial nitrification rates (0.10 g to 0.5 g TAN m^{-2} day^{-1}) (Greiner & Timmons, 1998).

Integrated biomass such as periphyton or biofloc, in aquaculture production volumes utilises waste feed and excretion from culture species. Remarkably, the availability of biomass to the production species as a nutrition source exerts a positive influence on health and production performance parameters, resulting in increased economic value (Paerl & Pinckney, 1996; Azim & Little, 2006; Crab *et al*., 2010; Nhan *et al*., 2010; Crab *et al*., 2012; Pande *et* *al*., 2013; Defoirdt, 2014; Martínez-Córdova *et al*., 2015; Turan *et al*., 2017; Nevejan *et al*., 2018; Fatimah *et al*., 2019; Biswas *et al*., 2022).

A RAS biofilter is external to the aquaculture production volume and requires flow to be pumped through the external treatment system containing the biofilter (Bell *et al*., 2023; Taufik *et al*., 2023). It typically comprises a solids removal clarifier, which requires mechanical energy and additional area and increases complexity in operation. Bell *et al*. (2023) referred to the RAS biofilter as a periphyton filter. Notably, BFT and IPT are less complex and costly than RAS (Schryver *et al*., 2008) and do not have the space requirements of external treatment or recirculating pumps of RAS. Since IPT and BFT retain all biomass within the production volume, they utilise carbon addition, maintaining a Carbon to Nitrogen (C:N) ratio to facilitate heterotrophic bacteria populations.

Heterotrophic biomass is a beneficial component of periphyton and biofloc (Paerl & Pinckney, 1996; Azim & Little, 2006; Crab *et al*., 2012; Martínez-Córdova *et al*., 2015; Nevejan *et al*., 2018; Biswas *et al*., 2022). In particular, IPT biomass is fixed upon integrated media. It removes the need in BFT for selecting specific aeration equipment or applying additional mechanical energy expenditure to maintain biofloc in suspension such as through excessive aeration (El-Sayed, 2020). Conversely, BFT has faced challenges over 20 years of development that persist today, principally high energy costs and management of residual biofloc solids (Emerciano *et al*., 2021).

IPT aquaculture utilises high specific surface area media with high void space such as that used in IFAS. It supports the co-existence of heterotrophs at the media surface, with consortia of autotrophs and chemotrophs residing in lower oxygen and flow conditions within the media, and non-bacterial single and multi-cellular organisms forming part of an ecosystem on and within the media. Concurrently, the periphyton and media ecosystem optimally utilises waste feed and excretions for biomass available as a live feed to omnivorous species (Martínez-Córdova *et al*., 2015). At the same time, heterotrophic organisms increase their capacity for the management of water quality, health improvement, and waste recycling (Asaduzzaman *et al*., 2010; Hasan *et al*., 2012; Martínez-Córdova *et al*., 2015).

The positive impact of IPT biomass includes improved Survival Rate (SR) and product quality, pathogen deterrence through quorum sensing and probiotic action, and availability as a source of live feed, enzymes, and other beneficial compounds (Crab *et al*., 2007; Khanjani *et al*., 2022). In addition, IPT media within the production volume increases the two-dimensional domain area available to prawns for grazing and safer ecdysis (Coyle *et al*., 2010; Tuly *et al*., 2014). Meanwhile, *M. rosenbergii*, an omnivorous and detritivorous species, graze readily on periphyton organisms (Marlowe, 2006). The use of IPT media simultaneously achieves water quality management, domain improvement, and nutritional and health benefits.

Thoransen *et al*. (2015) reviewed 24 aquaculture growth studies reported in the journal Aquaculture in 2013 and 2014 and discovered that most (83%) applied treatment in triplicate, with an average of 26 fish in each tank (range: 4 to 100). In aquaculture practice at commercial levels, conditions of operation may impact upon consistency of results. Such conditions include seasonality and the origin and number of broodstock employed in aquaculture production. In *M. rosenbergii* culture, comparative data sets are influenced by sex ratio, HIG, and the mix of microbiological consortia. Note that all these factors might have distinct impacts on nursery trials of *M. rosenbergii*. The evaluation of whether IPT is equivalent or better than RAS in the nursery for *M. rosenbergii* is an indicator of the acceptability of IPT for the nursery culture of giant freshwater prawns. Simultaneously, the isolation of the period of experimentation was justified as performance characteristics of nursery culture to 44 days were not expected to be unduly affected either by sexual development or by HIG in males.

Materials and Methods

Experimental Design

IPT was evaluated in comparison with RAS in terms of the respective performance of the two systems with identical inputs. In two trials comparing the performance of IPT with RAS, T1 and T2, the hatchery source of broodstock was different, as was the stocking density (Figure 1). These independent trials provide independent evidence in the comparison of the two treatments. However, the difference in operation between the two treatments in both trials was limited to the location of the biofilm mass supported upon high specific surface area media. The media was present in the production volume for IPT and the RAS biofilter tank for RAS treatments. Moreover, stocked Post-Larvae (PL), supplementary feed, aeration, lighting, and production tank volumes were identical for IPT and RAS in both T1 and T2. As such, comparative performance data supported the evaluation of the relative performance of the two treatments.

The study tanks and biofilters were aerated. Accordingly, Dissolved Oxygen (DO) was kept high and a nitrification rate of 0.45 g TAN m^{-2} day^{-1} was selected for entry to a biofilter design after Losordo and Hobbs (2000). Following this, loading to the biofilter model was determined from 30 kg m^{-3} of prawns, a daily feed ratio of 1.25%, daily feed at peak culture density of 0.38 kg m^{-3}, and supplementary feed crude protein of 32%. Subsequently, the conversion to TAN from crude protein was assumed to be 6.5%. The nitrification rate led to the calculation of the required biofilter area of 20.4 m^2. In line with this, Matala™ type media has a high specific surface area of more than 400 m^2 m^{-3}. Notably, 300 m^2 m^{-3} media was employed such that a sheet of 1 m^2 with a thickness of only 4 cm carried a surface area of 16 m^2 on the surface of fibres while maintaining a free pore space of up to 94% of the mat volume. Meanwhile, the external surface area of such a sheet was approximately 2.16 m^2, including edges.

Such media is designed to be employed as the biological filter component of the submerged biofilter and as a flow-through clarifier with a potential capacity of over 200 kg of wet solids retention per cubic metre. In particular, the current study of the RAS system consisted of sequential plates of media across a flow-through tank, through which water was forced to flow, which performed as an effective clarifier and biofilter. Note that there was no measurable head loss for flow passing through the media due to its 94% void space and low velocity (16 m h^{-1} for the RAS biofilter). Although the specific surface area approached the upper limit for rotating biological contactors, the sequential filter media also had characteristics of plastic bead filters. Additionally, the IPT-integrated periphyton biofilter was identical in terms of the quantity of 4 cm thick media employed in the RAS biofilter.

Experimental Set-up

Nursery PL for experiment T1 originated from Sungai Nerus hatchery, as PL and for experiment T2 from AKUATROP hatchery utilising broodstock obtained from Sungai Pahang, Pahang, Malaysia. Nursery culture was provided for 44 days post-hatchery for

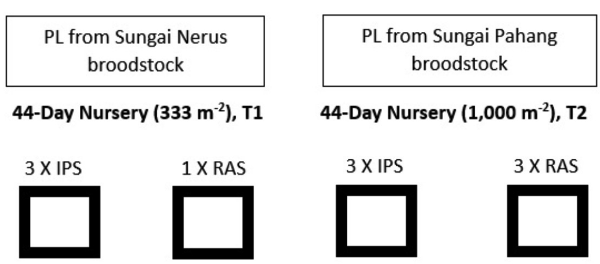

Figure 1: Experimental overview of trials T1 and T2

T1 and T2 at densities of 333 m^{-2} and 1,000 m^{-2}, respectively. Specifically, T1 employed triplicate IPT tanks and a single RAS control and T2 employed triplicate IPT and triplicate RAS culture systems. All treatments of T1 (IPT1-T1, IPT2-T1, IPT3-T1, and RAS-T1) and of T2 (IPT1-T2, IPT2-T2, IPT3-T2, and RAS1-T2, RAS2-T2, and RAS3-T2) were operated with zero water exchange. Tanks of 1.3 m^3 effective volume and 1.5 m^2 bottom area were employed (diameter of 1.4 m and effective depth of 0.85 m) for IPT and RAS production volumes. A diagrammatic arrangement of the IPT and RAS systems is depicted in Figure 2. As such, the RAS tank biofilter element labelled (b) was established in a fibreglass tank of 1.2 m x 1.2 m x 0.8 m with 0.6 m effective water depth.

Note that all tanks were aerated with ceramic diffusers. In addition, "Japan mat" media of high specific surface area (300 m^2 m^{-3}) and high void ratio (94%) was suspended in layers at 200% of tank bottom area (single side) within IPT production volumes. In contrast, RAS system external biofilters contained media quantities and surface area identical to those of IPT to maintain water quality in RAS. Moreover, all RAS tanks utilised open 1 cm mesh screen layers at 200% of the tank bottom area (single side). Each RAS tank was provided with a submersible pump of 6 m^3 hr^{-1} capacity in a biofilter overflow pump tank, producing circulation back to the RAS production volume. Air diffusers in IPT and RAS tanks and RAS biofilter were supplied from a common compressor at a maximum of 60 L min^{-1} per tank.

From the perspective of the grazing area within IPT, the additional external surface area of 3 m^2 of the media provided represented an increase of 200% over the bottom grazing area alone. The periphyton area, based on the specific surface area of the media provided was 27 m^2 per treatment, as summarised in Table 1.

Experimental Method

Fertilisation of tanks was performed in advance of the stocking with a dosage of 100 g fertiliser (Serbajadi brand with N 12%, P 12%, K 17%, and Mg 2%, and trace Mn, Fe, Zn, S, B, Cu, and Mo) to each tank. This is in addition to 50 g liquid organic fertiliser (Mr Garrick) and the systems were kept under aeration for eight days. Meanwhile, the feed schedule was developed as an average for all tanks. It utilises average initial PL mass, providing 3% of estimated surviving prawn mass as feed per day, assuming an 80% SR at 44 days and BW of 2 g in surviving prawns by experiment Day of Culture (DoC) 44. Feeding was twice per day, at 9:00 and 17:00, using PL crumbles (Gold Coin Specialities) with minimum crude protein of 40%, maximum crude fibre of 3%, minimum crude fat of 7%, and free of added antibiotics. Molasses were added as a carbohydrate source to maintain a C:N ratio of 20:1 (Browdy *et al*., 2012).

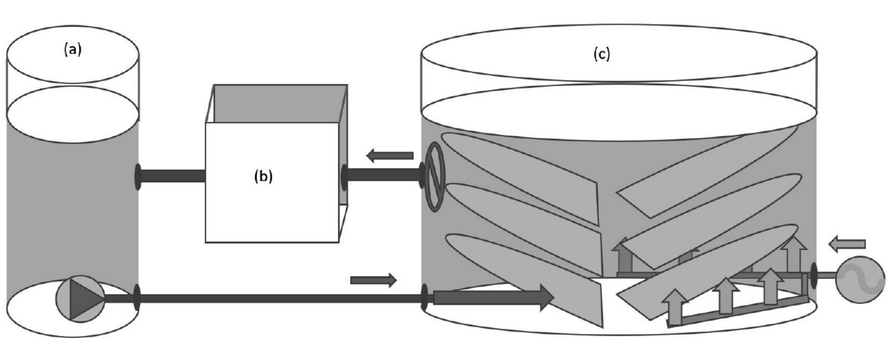

Figure 2: Diagrammatic layout for treatment and control: (a) Pump tank to RAS tanks only, (b) biofilter for RAS tanks only, and (c) production volumes containing high specific surface area fibre mats for IPT and 1 cm HDPE open mesh for RAS, each totalling 3 m^2 single side area

Table 1: Summary of key equipment characteristics for 44-day nursery in IPT and RAS for trials T1 and T2

Parameter	Unit	Quantity for T1	Quantity for T2
Tank effective volume	m^{-3}	1.3	1.3
Tank plan area	m^2	1.5	1.5
Number of IPT tanks	No.	3	3
Number of RAS systems	No.	1	3
Duration of operation	Days	44	44
Media specific area	$m^2\ m^{-3}$	300	300
Media thickness	mm	30	30
Media outer surface area (single side) provided per IPT production tank	m^2	3.0	3.0
Internal biofilm area provided per IPT tank and per RAS biofilter	m^2	27.0	27.0

Moreover, sodium bicarbonate (alkalinity) was added to 13% of feed (Furtado *et al.*, 2015), dissolved, and delivered with dissolved molasses once per day. In addition, water quality parameters of temperature, DO, Total Dissolved Solids (TDS), and pH were measured with a Yellow Springs Instrument portable meter (YSI Professional Plus). Laboratory measurements of water quality samples were made for T1, including Total Suspended Solids (TSS) by filtration at 0.l mm, oven dried, and alkalinity by HCL titration with methyl orange, both approximately weekly, and NH_4-N, NO_2, and PO_4 by Shimadzu UV-VIS Spectrophotometer UV-1800. Water quality measured for triplicate IPT and RAS tanks included temperature (°C), DO (mg L^{-1}), TDS (mg L^{-1}), and pH. These values were measured daily with a YSI handheld meter. For T1, three IPT tanks and one unit of RAS control tank each received 500 PL from the hatchery, which amounted to about 333 m^{-2} bottom area. For T2, three IPT and three RAS tanks received 1,500 PL from the hatchery, which amounted to about 1,000 m^{-2} bottom area.

Statistical Analysis

Statistical analysis of both the Body Weight Gain (BWG) of prawns harvested from each tank and BWG multiplied by the SR experienced by individual tanks (BWG x SR) was performed by Welch Analysis of Variance (ANOVA) and Games-Howell post-hoc analysis using Statistical Package for Social Science (SPSS) version 29.0. BWG alone described the mean mass of prawns produced and BWG x SR yielded a number relevant to the statistical evaluation of the productivity of each tank. Meanwhile, significance was assigned at the 0.05% level. Results were examined for comparisons of averages and the significance of the difference in BWG and BWG x SR for individual tanks from each of the two groups, IPT and RAS. It aims to evaluate the outcome of the hypothesis that IPT yields at least equivalent performance to RAS in the application of *M. rosenbergii* intensive culture.

Results

Water Quality

Water quality measured for IPT and RAS tanks included temperature (°C), DO (mg L^{-1}), TDS (mg L^{-1}), and pH. These values were taken daily with a YSI handheld meter. Water quality for IPT and RAS for trial T1 days and trial T2 was consistent with acceptable aquaculture parameters, with results summarised in Tables 2 and 3. Accordingly, laboratory measurements of water quality samples were made for trial T1, including TSS (filtration at 0.l mm, oven-dried), alkalinity (by HCL titration with methyl orange), and NH_4-N, NO_2, and PO_4 (Shimadzu UV-VIS Spectrophotometer UV-1800).

Trial T1, unionised ammonia, mg NH_3-N L^{-1} concentration, the toxic species was determined from total ammonia, mg NH_4^+-N L^{-1} (Table 3), as approximately 2.3% of NH_4^+-N L^{-1} at 29°C and pH of 7.5. In essence, total average ammonia of 0.09 mg NH_4^+-N L^{-1} for IPT1, IPT2, and IPT3 converted to only 0.001 mg NH_3-N L^{-1} while RAS experienced an average of only 0.005 mg NH_3-N L^{-1}.

Performance Indicators

The feed model used for each of trials T1 and T2 was based on the assumptions expressed in Table 4 below and determined the total feed supplied as delivered to each run, which was used to calculate FCR. The calculated SR, BW, harvest densities (kg m^{-3} and no. m^{-2}), BWG, Average Daily Gain (ADG), and FCR for each of trials T1 and T2, including Standard Deviations (SD) are reported as averages for all the tanks in each treatment set, in Table 4. Input PL and harvested prawns were counted, from which SR was generated while the BW data reported for trial T2 were determined from a subsample.

Table 2: Water quality in treatment tanks IPT1, IPT2, and IPT3 and RAS tanks in trial T1

Trial and Tank	Value	T (°C)	DO (mg L^{-1})	TDS (mg L^{-1})	pH	NH_4-N (mg L^{-1})	TSS (mg L^{-1})	Alk. (mg L^{-1}, $CaCO_3$)	NO_2-N (mg L^{-1})
T1-IPT1	Average	29.5	6.7	266.7	7.6	0.04	2.9	70.4	0.04
	STDEV*	0.6	0.6	55.1	0.3	0.03	2.3	25.4	0.02
T1-IPT2	Average	29.4	6.7	250.3	7.6	0.07	1.9	67.2	0.02
	STDEV*	0.6	0.7	63.6	0.3	0.06	3.3	23.1	0.02
T1-IPT3	Average	29.3	6.7	272.3	7.6	0.03	2.7	87.4	0.30
	STDEV*	0.6	0.7	58.7	0.3	0.02	1.5	43.3	0.46
T1-RAS	Average	28.6	7.1	248.1	7.4	0.21	2.7	50.4	0.56
	STDEV*	0.5	0.7	48.1	0.3	0.24	1.6	22.6	0.47

*STDEV: standard deviation.

Table 3: Water quality in treatment tanks IPT1, IPT2, and IPT3 and RAS tanks in trial T2

Trial and Tank	Value	T (°C)	DO (mg L^{-1})	TDS (mg L^{-1})	pH
T2-IPT1	Average	28.8	7.0	251	7.2
	STDEV*	0.95	1.0	78	0.2
T2-IPT2	Average	28.7	7.1	234	7.2
	STDEV*	0.96	1.0	72	0.2
T2-IPT3	Average	28.5	7.1	226	7.2
	STDEV*	0.87	0.8	78	0.1
T2-RAS1	Average	28.5	8.1	202	7.2
	STDEV*	0.69	0.5	62	0.1
T2-RAS2	Average	28.1	8.3	232	7.3
	STDEV*	0.61	0.5	81	0.2
T2-RAS3	Average	28.7	8.0	226	7.2
	STDEV*	0.60	0.5	68	0.2

*STDEV: standard deviation.

Table 4: Performance indicators for trials T1 and T2

Metric	Feed Model (T1)	IPT (ave.)	IPT (SD)	RAS (single)	Feed Model (T2)	IPT (ave.)	IPT (SD)	RAS (ave.)	RAS (SD)
	Trial T1				Trial T2				
Input PL (No.)	500	500	n/a	500	1,500	1,500	n/a	1,500	n/a
Input mass (g)	0.05	0.05	n/a	0.05	0.05	0.05	n/a	0.05	n/a
Tank volume (m^{-3})	1.3	1.3	n/a	1.3	1.3	1.3	n/a	1.3	n/a
Tank plan area (m^{-2})	1.5	1.5	n/a	1.5	1.5	1.5	n/a	1.5	n/a
Stocking density (no. m^{-2})	333	333	n/a	333	1,000	1,000	n/a	1,000	n/a
Duration (days)	43	43	n/a	43	44	44	n/a	44	n/a
Harvest, (No.)	500	252	78	327	1,500	1,041	n/a	1,116	n/a
Survival Rate, (%)	100%	50%	16%	65%	100%	69%	9%	74%	4%
Average BW, (g)	1.61	1.31	1.00	0.52 ± 0.43	1.29	0.92	± 0.68	0.88	± 0.73
BW x SR	1.61	0.65	0.59	0.34 ± 0.28	1.29	0.63	± 0.45	0.65	± 0.53
ADG, (g d^{-1})	0.040	0.029	n/a	0.011	0.028	0.020	n/a	0.019	n/a
Total feed per tank (g)	1,119	1,119	n/a	1,119	1,935	1,935	n/a	1,935	n/a
FCR	1.40	3.39	n/a	6.58	1.04	2.02	n/a	1.97	n/a

Statistical Analysis for Trials T1 and T2

Input PL for Trials T1 and T2

Data are mean ± SD unless otherwise stated. Samples of 437 and 450 PL were measured for trials T1 and T2, respectively. In two runs of independent-sample t-test (SPSS version 29), a violation of normality was reported in each sample. Furthermore, both data sets had approximately 8% outliers, as assessed by boxplot inspection. With outliers removed, trial T1 sample mean BW was not statistically different from a normal mean, where mean BW differed by -0.00049 (95% -0.0022, 0.0013) from a normal BW mean of 0.0405, t (400) = -0.546, p = 0.585, Hedges' g = 0.027 (95%, -0.125, 0.07). In addition, with outliers removed, trial T2 sample mean BW was not statistically different from a normal mean, where mean BW differed by -0.00049 (95% -0.0022, 0.0013) from a normal BW mean of 0.0287, t (413) = -2.512, p = 0.012, Hedges' g = 0.123 (95%, -0.22, -0.027).

Final Harvest of Trials T1 and T2

Data are mean ± SD unless otherwise stated. A one-way Welch ANOVA was conducted to determine if BW and BW multiplied by SR (BW x SR) were significantly different for the IPT (treatment) and RAS (control) groups for each of trials T1 and T2, using SPSS version 29. In each case, there were outliers (< 5% for trial T1 and < 10.5% for trial T2), as assessed by boxplot, and the assumption of normal distribution was violated for each treatment group, as assessed by the Shapiro-Wilk test ($p < .001$).

Homogeneity of variances was violated, as assessed by Levene's Test of Homogeneity of Variance ($p < 0.001$), and a Welch ANOVA was used as a result, which was considered robust with the large sample "n" reported in Tables 5 and 6. Note that "n" for trial T1 was the full population at harvest. At the same time, it was a representative random sample of the full population at harvest for trial T2. Hence, a one-way Welch ANOVA was conducted to determine if BW and BW x SR at harvest significantly differed for the IPT and RAS groups for each of trials T1 and T2, $p < 0.05$. Tables 5 and 6 summarise means with SDs and rank for BW and BW multiplied by SR (BW x SR) for trials T1 and T2, respectively.

In trial T1, the mean BW at harvest increased from the single RAS control (n = 327, 0.52 ± 0.43), to IPT1 (n = 341, 1.29 ± 1.06), to IPT2 (n = 221, 1.32 ± 1.0) to IPT3 (n = 194, 1.33 ± 0.89) tank harvest BW (Table 5). The BW at harvest was statistically significantly different for individual treatments and control, Welch's $F (3, 471.677) = 113.588$, $p < .001$. At the same time, the mean differences between RAS and each of the IPT treatment tanks (IPT1, IPT2, and IPT3) were statistically significant ($p < 0.001$). In contrast, the difference between the IPT treatment tanks was insignificant. The group means were statistically significantly different ($p < .001$). Therefore, we can reject the null hypothesis and accept the alternative hypothesis. Additionally, it could be observed that the IPT tanks as a distinct group were significantly different and displayed significantly increased BW results compared to the RAS tank as a

Table 5: Summary of means for trial T1 tanks for BW and BW x SR

Tank	N	BW, Mean	BW, STDEV	BW, Rank, Low to High	BW x SR, Mean	BW x SR, STDEV	BW x SR, Rank, Low to High
RAS control	327	0.5163	0.42527	1	0.3360	0.27631	1
IPT T1	341	1.2888	1.05829	2	0.8765	0.71953	4
IPT T2	221	1.3160	0.99500	3	0.5788	0.43804	3
IPT T3	194	1.3325	0.88979	4	0.5198	0.34703	2

Table 6: Summary of means for trial T2 tanks for BW and BW x SR

Tank	N	BW, Mean	BW, STDEV	BW, Rank, Low to High	BW x SR, Mean	BW x SR, STDEV	BW x SR, Rank, Low to High
RAS1	107	0.9274	0.74479	4	0.6581	0.52789	4
RAS2	118	0.6965	0.48101	1	0.5497	0.38011	1
RAS3	68	1.1338	0.95002	5	0.8322	0.69816	6
IPT1	93	1.2394	0.77299	6	0.7662	0.47816	5
IPT2	119	0.7087	0.50243	2	0.5608	0.39743	2
IPT3	101	0.8716	0.66915	3	0.5864	0.45050	3

group. In this trial T1 study, IPT was established to be equivalent to improved performance in comparison with RAS.

In the second part of trial T1, BW was multiplied by SR (BW x SR), as each tank yielded a different average BW and a distinct SR. Specifically, the mean BW x SR at harvest increased from the RAS control (n = 327, 0.34 ± 0.28), to IPT3 (n = 194, 0.52 ± 0.35), to IPT2 (n = 221, 0.58 ± 0.44), to IPT1 (n = 341, 0.88 ± 0.72) (Table 5). The BW x SR at harvest was statistically significantly different for IPT and RAS, Welch's F (3, 521.169) = 67.180, $p < .001$. The mean difference between RAS and each of the IPT treatment tanks (IPT1, IPT2, and IPT3) were statistically significant ($p < 0.001$) and therefore, we can reject the null hypothesis and accept the alternative hypothesis. However, the differences between the IPT treatment tanks IPT1 and IPT2 and IPT1 and IPT3 were also significant. Thus, it could be observed that the IPT tanks as a distinct group were significantly different and displayed significantly increased BW x SR results over the RAS tank as a group. In this trial T1 study of BW x SR, IPT established improved performance compared to RAS.

In trial T2, the mean BW at harvest increased from the RAS2 (n = 118, 0.70 ± 0.48), to IPT2 (n = 119, 0.71 ± 0.50), to IPT3 (n = 101, 0.87 ± 0.67) to RAS1 (n = 107, 0.93 ± 0.74), to RAS3 (n = 68, 1.13 ± 0.95), to IPT1 (n = 93, 1.24 ± 0.77) (Table 6). The BW at harvest was statistically significantly different for IPT and RAS, Welch's F (5, 256.118) = 10.137, $p < .001$. Therefore, we can reject the null hypothesis and accept the alternative hypothesis. Within groups, the highest-performing tank pairs of IPT and RAS, lowest-performing IPT and RAS tanks, and the mid-range performance IPT and RAS tanks were not significantly different as pairs. In this trial T2 study, IPT was considered able to establish an equivalent performance to RAS.

In trial T2, BW was multiplied by SR (BW x SR), as each tank yielded a different average BW and a distinct SR. The mean BW x SR at harvest increased from RAS2 (n = 118, 0.55 ± 0.38), to IPT2 (n = 119, 0.56 ± 0.40), to IPT3 (n = 101, 0.59 ± 0.45) to RAS1 (n = 107, 0.66 ± 0.53), to IPT1 (n = 93, 0.77 ± 0.48), to RAS3 (n = 68, 0.83 ± 0.70) (Table 6). The BW at harvest was statistically significantly different for IPT and RAS, Welch's F (5, 258.733) = 4.495, $p < .001$. Unlike the analysis of BW without the SR factor, the differences between the IPT treatment tanks IPT1 and IPT2 were also significant. In addition, RAS2 differed significantly from RAS3 while RAS2 and RAS3 differed significantly from IPT1 and IPT2, respectively. The group means were statistically significantly different ($p < .05$) as complete groups of IPT and RAS. Therefore, we can accept the null hypothesis and reject the alternative hypothesis. However, it could be observed that the IPT tanks performed equivalently as RAS, pairwise, with IPT2 result close to RAS2, IPT3 close to RAS1, and IPT1 close to RAS3, although RAS3 BW x SR was significantly greater than for IPT1. In this trial T2 study, IPT was considered able to establish an equivalent performance to RAS.

Comparison of means of BW and BW x SR of harvested prawns from trials T1 and T2 are illustrated in charts presented in Figure 3 (top) and (bottom) and Figure 4 (top) and (bottom).

Discussion

Nursery culture is receiving increased attention due to its ability to control the initial growth trial within highly controlled conditions at higher densities than those employed for grow-out. However, an efficient nursery culture has implications for the efficiency of the full farm production cycle, as the operation of a nursery period at higher density reduces the net volume required for the full culture cycle. Coyle *et al*. (2010) assembled a convincing case that implementing a nursery stage increases resistance to predation, cannibalism, and environmental conditions and improves SR. It generally results in higher individual weight, production, and harvest value.

Hence, optimum employment of nursery culture will improve the sustainability and economics of intensive culture, which in turn promotes a culture of the species. Nonetheless,

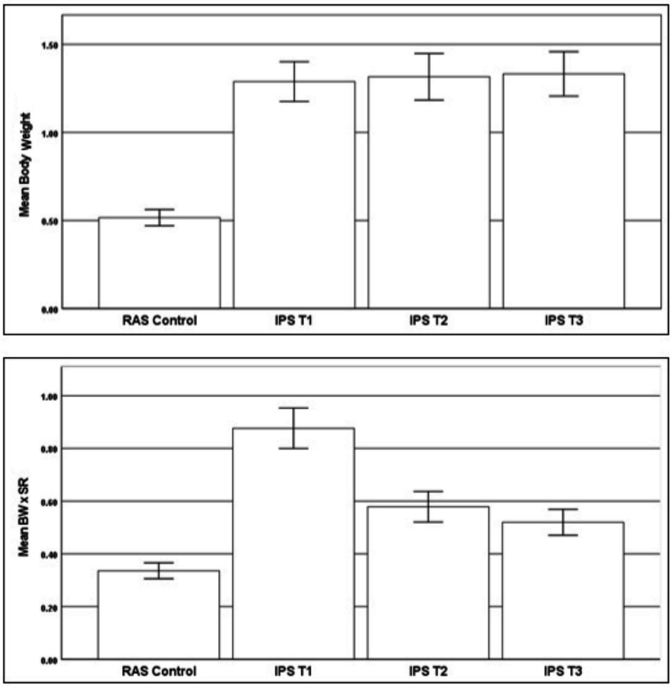

Figure 3: Comparison of means of BW (top) and BW x SR (bottom) for trial T1.
The three IPT tanks were significantly different with higher means than RAS

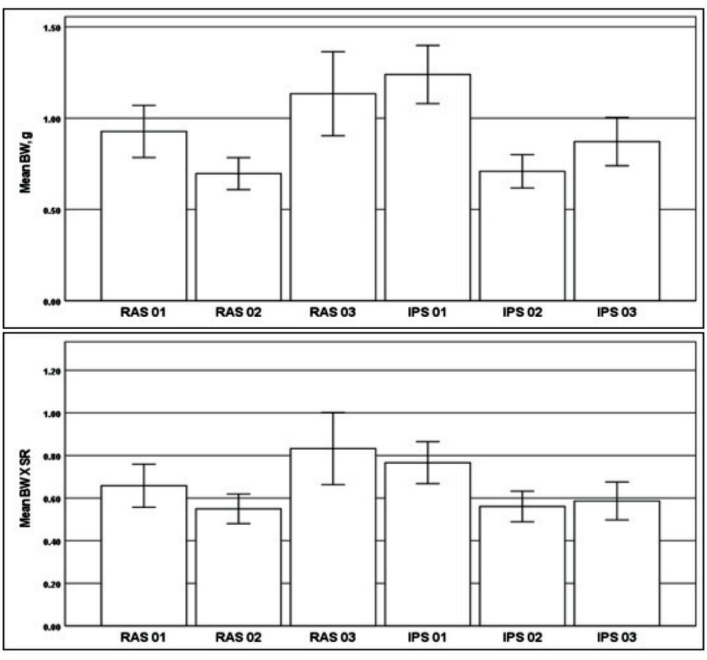

Figure 4: Comparison of means of BW (top) and BW x SR (bottom) for trial T2.
Games-Howell analysis showed no significant difference between pairs of IPT and RAS, demonstrating the equivalence of the two systems

HIG amongst male *M. rosenbergii* and females is also differentiated in their growth curves, which impacts the commercial production of uniform products. These characteristics remain challenging to detect at 44 days of culture. BC male partial harvesting typically begins after 60 days (Rahman *et al*., 2010), which is also when higher-growth individuals referred to as “jumpers” express higher relative growth rates (Karplus, 2005). Before 45 days, HIG did not appear in males, the impact of BC males without the presence of claws was of low significance on the survival and growth of other morphotypes. Hence, it is not considered significant in nursery culture. Nevertheless, further experimentation is required to evaluate the performance of IPT in growing out from 45 days to harvest and evaluation will need to take HIG into account in the experimental design.

IPT provides water quality control and exposes cultured animals to periphyton as a nutrition and health aid. It does so without the external cycling and treatment processes required for RAS. In the current study, we investigated the comparative performance of IPT and RAS to define whether IPT displayed equivalent or better performance characteristics than RAS in the nursery culture of *M. rosenbergii*. In particular, ADG was calculated to help surviving prawns during harvest. ADG for nursery trials T1 and T2 were 0.032 and 0.027 g d^{-1} for IPT tanks and 0.012 and 0.026 g d^{-1} for RAS tanks, respectively. Moreover, nursery ADG in IPT exceeded RAS at both densities. Meanwhile, SR and the combined effect of BW and SR, measured as BW x SR are indicative of nursery productivity. However, if SR is equivalent or better for higher density culture, then, even if BW is reduced in high nursery density, compensatory growth should yield a production advantage in grow out (Marques *et al*., 2010; Marques & Lombardi, 2011; Marques *et al*., 2012).

Averaged 60-day SRs for trials T1 and T2 were 50% and 69% for IPT tanks and 65% and 74% for RAS tanks, respectively. The average BW for trials T1 and T2 were 1.31 g and 0.92 g achieved for IPT tanks and 0.092 g and 0.88 g achieved for RAS tanks, respectively. BW x SR for nursery trials T1 and T2 were 0.065 g d^{-1} and 0.063 g d^{-1} for IPT tanks and 0.034 g d^{-1} and 0.065 g d^{-1} for RAS tanks, respectively. Accordingly, final BW and BW x SR in trial T1 were significantly higher for IPT than RAS. The final BW displayed pairwise equivalence between the triplicate IPT and RAS cultures during the trial T2 nursery stage. In essence, total prawn biomasses harvested from trial T2 were not significantly different between IPT and RAS.

The feeding model strategy as FCR applied for both treatments was 1.4 and 1.04 in trials T1 and T2, respectively, which were considered low applied feeding rates. The FCR achieved by IPT in trials T1 and T2 was 3.39 and 2.02, respectively while for RAS in the two trials, the FCR was 6.58 and 1.97, respectively. The feeding strategies in both trials overestimated productivity. In trial T1, with a feeding strategy at FCR of 1.4, IPT produced higher BW but lower SRs, which was reversed for RAS. Conversely, in trial T2, both IPT and RAS responded well to the feeding strategy and both systems achieved an FCR of about 2.0 for the feed strategy of 1.04. The results suggest an even lower applied feed strategy below FCR of 1.0 might be effective. At an FCR feeding of 1.04, IPT and RAS were similar in terms of SR, BW, and achieved FCR. This could indicate that IPT-integrated media biomass does not improve productivity on these criteria.

A comparative trial of the feeding strategy below an FCR of 1.0 might reveal performance differences between IPT and RAS for *M. rosenbergii* nursery culture. Biswas *et al*. (2022) demonstrated a quantitative reduction in supplementary feeding with higher commercial returns and improved sustainability in brackish water polyculture utilising added substrate to increase available periphyton. At the same time, Azim and Little (2006) reported an increase in periphyton amended production of 30% for carp monoculture, 115% to 210% for carp polyculture, and production of 9 t ha^{-1} $year^{-1}$ in carp polyculture without supplementary feed. Additionally, Haque *et al*. (2014) concluded that periphyton-supported farming of *M. rosenbergii* could yield over 36% improvement in net yield using additives such as corn flour to maintain a high C:N ratio. Overall, the current study established that IPT yields comparative production to RAS with reduced infrastructure requirements, maintaining a lower water exchange rate and waste discharge.

Conclusions

The use of periphyton in IPT systems effectively maintained sufficient biomass at zero discharge, ensuring water quality throughout the culture period without the need for energy-intensive mixing, as required for biofloc suspension in RAS. That is, IPT successfully replaced the external biofilters and mechanical pumps necessary for water quality management in RAS. This study demonstrated that IPT performs comparably to RAS for the nursery culture of *M. rosenbergii* over 44 days. However, the access of prawns to periphyton biomass in IPT did not conclusively result in significant differences in BWG or BWG × SR between IPT and RAS treatments.

Despite this, further research is required to optimise the use of IPT in post-nursery culture, including determining the ideal quantity of media substrate required to maintain water quality, strategies to minimise FCR throughout the full culture cycle, and the potential effects of IPT on Heterogenous Independent Growth (HIG). Additionally, evaluating the nutritional quality of *M. rosenbergii* produced in IPT systems is essential. As such, the development and refinement of IPT for both nursery and full-cycle culture can significantly improve the sustainability of *M. rosenbergii* aquaculture and potentially that of other species. Suppose IPT is successfully assessed in the post-nursery or grow-out stage. In that case, IPT can be applied to existing extensive pond systems to convert them to intensive operation without additional infrastructure while achieving reduced water exchange and waste discharge.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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