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DEVELOPMENT AND ASSESSMENT OF INTEGRATED PERIPHYTON SYSTEM FOR REARING OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

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DOCTOR OF PHILOSOPHY

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DAVID MARIONI

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Institute of Tropical Aquaculture and Fisheries Universiti Malaysia Terengganu

2025

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DEDICATION

To my late father

Dr. Peter Marioni *who passed away during my Ph.D. study*

and

To my late beloved mother

Mrs. Kathleen Mary Marioni *who passed away during my Ph.D. study*

To my siblings

George, Mary, and Christopher Marioni

To

My beloved wife, Sasikalah

I would like to express my sincere gratitude to my principal supervisor, Professor, Mhd Ikhwanuddin, Ph.D., and my co-Supervisor, Associate Professor Ts. Dr. Nor Azman Kasan, Ph.D., for their continuous support during my Ph.D.

Thanks a lot

May God bless

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Doctor of Philosophy

DEVELOPMENT AND ASSESSMENT OF INTEGRATED PERIPHYTON SYSTEM FOR REARING OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

DAVID MARIONI

MAY 2024

Main Supervisor	**:**	**Professor Muhammad Ikhwanuddin Abdullah, Ph.D**
Co-Supervisor	**:**	**Associate Professor ChM. Ts. Dr. Nor Azman Kasan, Ph.D**
Co-Supervisor	**:**	**Professor Abol Munafi Ambok Bolong, Ph.D**
Faculty/Institute	**:**	**Institute of Tropical Aquaculture and Fisheries**

Giant freshwater prawn, *Macrobrachium rosenbergii* is a freshwater prawn species indigenous to Malaysia, proven unsuited for aquaculture at densities effective for large-scale commercial projects, while Malaysia seeks to increase national food production. Integrated Periphyton System (IPS) was compared to Recirculating Aquaculture Systems (RAS) as intensive aquaculture systems which could increase aquaculture densities of *M. rosenbergii*, by using more sophisticated infrastructure with higher capital cost to improve farm economics while reducing land area, water consumption, and environmental impact per unit production. RAS effluent treatment systems incorporate biofilters external to production volumes, while IPS integrates an equivalent biofilter substrate into the production volume. *M. rosenbergii* is readily marketable in sizes above 35 grams per prawn but suffers from Heterogenous Individual Growth (HIG) in males, and a separate growth pattern in females, making uniform products hard to attain. The general objective of this study was to prepare, operate, and characterize a representative

IPS for the sustainable culture of *M. rosenbergii*, including three specific objectives. The production efficiency of IPS was compared with RAS in nursery and post-nursery to 104 days of culture (DoC) with mixed sex *M. rosenbergii*, showing IPS equivalent or better than RAS tank systems based on performance in mixed sex trials up to post-nursery (grow out), on a range of parameters. Performance of IPS for mixed sex and all-male *M. rosenbergii* up to post hatchery of 90 to 104 DoC, and of all-male *M. rosenbergii* in IPS commercial production from hatchery to 195 DoC, were evaluated. Results indicated a pond production capacity for IPS culture of all-male *M. rosenbergii* of over 8 mt hectare^{-1} year^{-1}, with other production characteristics equivalent or better than most researched applications globally. IPS design, operational parameters, and feed model, were developed for the target species in IPS tanks and ponds, providing useful tools and insights for further application of IPS in research and commercialization. As IPS relies on periphyton within the system, it is concluded that the periphyton species maintained on IPS biofilter media need to be studied to uncover how these microbial species contribute to improving water quality and enhance prawn growth.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN DAN PENILAIAN SISTEM PERIPHYTON BERSEPADU UNTUK TERNAKAN UDANG GALAH, *Macrobrachium rosenbergii*

DAVID MARIONI

MEI 2024

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Fakulti/Institut : Institut Akuakultur Tropika dan Perikanan

Udang galah, *Macrobrachium rosenbergii* adalah spesies udang air tawar asli Malaysia, telah terbukti tidak sesuai untuk akuakultur pada kepadatan yang optimum untuk projek komersial berskala besar, manakala Malaysia berusaha untuk meningkatkan pengeluaran makanan negara. Sistem Periphyton Bersepadu (IPS) dibandingkan dengan Sistem Akuakultur Kitar-semula (RAS) sebagai sistem akuakultur intensif yang boleh meningkatkan kepadatan akuakultur *M. rosenbergii*, dengan menggunakan infrastruktur yang lebih canggih dengan kos modal yang lebih tinggi untuk meningkatkan ekonomi ternakan sambil mengurangkan keluasan tanah, penggunaan air, dan kesan alam sekitar bagi setiap unit hasil pengeluaran. Sistem rawatan efluen RAS menggabungkan biofilter di luar isipadu pengeluaran, manakala IPS mengintegrasikan substrat biofilter yang setara ke dalam ispadu pengeluaran. *M. rosenbergii* mudah dipasarkan dalam saiz melebihi 35 gram per udang tetapi mengalami Pertumbuhan Individu Heterogen (HIG) pada jantan, dan corak pertumbuhan berasingan pada betina, menjadikan hasil pengeluaran yang seragam sukar dicapai. Objektif umum kajian ini adalah untuk menyediakan,

mengendalikan, dan mencirikan IPS yang mewakili untuk ternakan mampan *M. rosenbergii*, termasuk tiga objektif khusus. Kecekapan pengeluaran IPS dibandingkan dengan RAS di tapak semaian dan selepas semaian kepada 104 hari ternakan (DoC) dengan jantina campuran *M. rosenbergii*, menunjukkan IPS setara atau lebih baik daripada sistem tangki RAS berdasarkan prestasi dalam percubaan jantina campuran sehingga pasca-semaian (membesar), pada julat parameter. Prestasi IPS untuk jantina campuran dan *M. rosenbergii* semua jantan sehingga pasca-penetasan 90 hingga 104 DoC, dan *M. rosenbergii* semua jantan dalam pengeluaran komersial IPS daripada penetasan hingga 195 DoC, telah dinilai. Keputusan menunjukkan kapasiti pengeluaran kolam untuk ternakan IPS bagi semua jantan *M. rosenbergii* lebih 8 mt hektar^{-1} tahun^{-1}, dengan ciri pengeluaran lain adalah setara atau lebih baik daripada kebanyakan aplikasi yang dikaji di seluruh dunia. Reka bentuk IPS, parameter operasi dan model permakanan, telah dibangunkan untuk spesies sasaran dalam tangki dan kolam IPS, menyediakan alat dan pandangan berguna untuk aplikasi IPS selanjutnya dalam penyelidikan dan pengkomersialan. Memandangkan IPS bergantung pada periphyton dalam sistem, adalah disimpulkan bahawa spesies periphyton yang dikekalkan pada media biofilter IPS perlu dikaji untuk melihat bagaimana spesies mikrob ini menyumbang kepada peningkatan kualiti air dan meningkatkan pertumbuhan udang.

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The Universiti Malayasia Terengganu (UMT) is an aquatic science and conservation focused campus, and it was my great fortune to work under the Institute of Tropical Aquaculture and Fisheries, AKUATROP. The facilities at UMT are outstanding matched only by the dedicated staff and productive energy on campus. AKUATROP's 4,000 m^2 marine and freshwater experimental facility provided an ideal venue for my experimental trials and is complemented by the extensive laboratories at Penyelidikan INOS dan AKUATROP, as well as by laboratories, equipment and facilities made available at other Faculties within UMT.

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I dedicate my PhD thesis to my beloved parents, for their enormous support, sacrifices, unconditional love and always keeping me in their prayers. I'm assured that they were proud of my pursuit even though they are not able to share its conclusion with me, God rest their souls.

Sasikalah, my wife/best friend/business partner, has made the difficulties of the past five years easier to bear and made the good times sweeter. Your constant support and encouragement during necessary separation and obsessive dedication of time, including throughout COVID events, allowed me to prepare this thesis successfully.

APPROVALS

I certify that an Examination Committee has met on 5TH May 2024 to conduct the final examination of David Marioni, on his Doctor of Philosophy thesis entitled **"Development and Assessment of Integrated Periphyton System for Rearing of Giant Freshwater Prawn, *Macrobrachium rosenbergii*"** in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

Prof. Dr. Mohammed Yusuf Chisti, Ph.D
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ASSOC. PROF. ChM. Ts. DR. NOR AZMAN KASAN, Ph.D
Director
Institute of Tropical Aquaculture and Fisheries
Universiti Malaysia Terengganu

Date

This thesis has been accepted by the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Doctor of Philosophy.

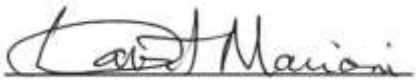
_____

ASSOC. PROF. ChM. Ts. DR. NOR AZMAN KASAN, Ph.D
Director
Institute of Tropical Aquaculture and Fisheries
Universiti Malaysia Terengganu

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UMT or other institutions.



DAVID MARIONI

Date: 30 September 2025

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LIST OF ABBREVIATIONS

ADG	Average Daily Gain, g d^{-1}
ANOVA	One-way Analysis of Variance
BC	Blue Claw male
BFT	Biofloc Technology
BW	Body Weight, g
BWG	Body Weight Gain, g
CH	Carbohydrate
CP	Crude Protein
C/N	Carbon to Nitrogen ratio
d^{-1}	Per day
DO	Dissolved Oxygen
DoC	Days of Culture
FAO	Food and Agriculture Organization, of the United Nations
FCR	Feed Conversion Ratio
g	gram
HIG	Heterogenous Individual Growth
IPS	Integrated Periphyton System
kg, kg^{-1}	Kilogram, per kilogram
L	Liter
m^2, m^3	square meter, cubic meter
m^{-2}, m^{-3}	Per square meter, per cubic meter
mg, mL	Milligram, milliliter
mg L^{-1}	milligrams per liter

mt	metric tonne
mt ha^{-1} $year^{-1}$	metric tonne per hectare per year
OC	Orange Claw male
° C	degrees Celsius
PHB	Poly-Beta-Hydroxybutyrate
PL	Post Larvae (upright swimming prawn)
RAS	Recirculating Aquaculture System
SGR	Specific Growth Rate, %
SM	Small Male
SOTE	Specific Oxygen Transfer Efficiency
spp.	species
SPSS	Statistical Package for the Social Sciences
SR	Survival Rate
SSA	Specific Surface Area
STDEV	Standard Deviation
T	Temperature
TAN	Total Available Nitrogen
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
USD	American dollar
W	Watt
°	degree
<	less than
>	more than

%	percentage
±	plus minus
2-D	two dimensional
3-D	three dimensional

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CHAPTER 1

INTRODUCTION

1.1 Research background

Significant increases in aquaculture production are required to meet food security demands, which also form a significant proportion of the demands placed on the resource constrained capacity of the earth, where such demands vary depending on the method of aquaculture production. Design basis for aquaculture production systems needs to address a 32 % increase in aquaculture production over 2018 levels by 2030, as estimated by FAO (FAO, 2020). Aquaculture in 2018 accounted for 46 % of total fish production and 52 % of fish for human consumption (FAO, 2020). With aquaculture production now approximately equivalent to the production from earth's oceans, increase of aquaculture production of 32 %, is roughly equivalent to 30% of production from earth's oceans. In further perspective, this effectively requires duplication of about 30 % of all existing aquaculture production inclusive of supporting resource allocations such as land area. The projected increase in aquaculture production will unlikely be able to rely solely on past successes in sustainable production (Martinez-Porchas & Martinez-Cordova, 2012). Meeting such a production increase requires innovative approaches to satisfy the critical need for input resources to production.

Aquatic products constitute a major global trading sector, that is subject to scrutiny of quality control as part of the food chain. Certification programs, increasingly strict permitting requirements, and the market demand for responsibly sourced aquatic products, place aquaculture production under pressure to scientifically assess and document the full supply chain, and to detail production protocols.

Standards are driven by the market which increasingly demands employment of progressively more sustainable technology and operational practices. Permitting of necessary new facilities requires attention to impacts on resources (e.g., land and water supply), on the environment (including biodiversity), on sustainability (e.g., climate change, transportation, and energy consumption), and attention to social and economic impacts (e.g., employment and technical capacity). Commercial aquaculture production needs to incorporate measures to achieve certification and permitting, while managing impacts effectively, to meet market demands (FAO, 2020). Globally, the status quo of aquaculture production systems still rests with extensive pond aquaculture, which is much less effective in utilization of resources than intensive, or high productivity systems. This reinforces the need for further research, development, and implementation of intensive aquaculture systems.

The Integrated Periphyton System (IPS) defined and developed under the current study utilizes a biofilter as in Recirculating Aquaculture Systems (RAS), but with biofilter substrate located within the production volume. IPS requires a carbon source supplement as is employed in Biofloc Technology (BFT). IPS, RAS, and BFT are leading aquaculture systems designed to produce more sustainably and intensively. While the IPS maintains biomass within the production volume similarly to BFT, it is an entirely different process. IPS represents a fixed film approach where biomass is contained on and within high specific surface area media with little suspension in the water column. In BFT, biofloc biomass is mainly suspended in the water column. IPS biofilm biomass on and within IPS media is referred to as periphyton. IPS periphyton is integrated to the aquaculture production volume by the provision of substrates that support periphyton attachment. There are abundant references to applications of RAS and BFT, but IPS lacks identification as a distinct type of aquaculture system.

RAS utilizes biomass as do IPS and BFT. However, RAS differs in that the biomass, retained on media in a 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). Production species do not have access to the biomass growing on RAS substrate, except to the extent it is released from the biofilter into the recirculating water. Implementation of commercial

RAS had been impeded by uncertainty of assured revenue, resulting from lack of precedents, lack of prototyping of specific systems and culture species, and slow rate of change within rural based aquaculture industry. The economics of RAS have been compared unfavorably with extensive aquaculture production, due to perceived higher capital investment, higher technical level of operation, and higher operation and maintenance cost. The high energy requirement of RAS was discussed by Badiola *et al.* (2018). The overriding fact is that pond culture has remained as the status quo in global terrestrial aquaculture production (FAO, 2020), which has resisted intensification. Modern aquaculture investment in intensive aquaculture requires higher returns and technological control of operations, which can be delivered far more effectively by properly designed and managed intensive systems (Badiola *et al.*, 2012). Hence, there is a need for more study and assurance of the performance of intensive systems.

Intensive aquaculture systems have the potential to meet critical aquatic production needs. These systems exert sufficient control over all aspects of the aquaculture production environment to permit highly intense to ultra-high intensity production, minimizing resource intensity by requiring less land and water supply than extensive systems (Tryggvason, 2016). They have the unique capability to be situated closer to urban markets, generate less effluent per unit production, and can utilize both urban and peri-urban land designated commercial, industrial, or agricultural. The aquaculture production sector is responding with intensive, commercial RAS farms such as employed by the biggest farmer in Asia, Charoen Pokphand Foods (CPF), which is planning full conversion to indoor operations (Gibson, 2018), and represented by the global increase in new intensive, large-scale, land-based RAS salmon farms of capacity amounting to 700,000 mt by 2030 (Fletcher, 2019). New RAS facilities frequently reference continuous improvement of resource efficiency during planning announcements, meet and exceed environmental standards, and minimize land, water, and energy consumption. RAS engineering achieves payback in terms of facilitation of permitting, inclusive of setting the stage for future expansion of facilities. Investments in RAS are not without risk, with failures of large investments having been documented globally. However, the decisions currently being taken to invest in large scale RAS reflect the ability of RAS to deliver sustainable aquaculture. RAS at

small to very large scale, and for multiple species, is absorbing the attention of investors in aquaculture. Beyond permitting advantages, other commercial advantages will accrue as major projects establish successful production, including consistent operation, and improved food conversion ratio (FCR), growth rate, disease resistance, and survival rate (Tryggvason, 2016). In the context of global needs for expansion, there is considerable capacity to be served by alternative systems (FAO, 2020). There is a growing number of BFT aquaculture farms but relatively few commercial IPS farms. Additional, documented experience in BFT and IPS operation is necessary if further investment and commercial permitting is to be achieved.

BFT is an aquaculture production process that maintains a principally heterotrophic suspended biomass within the production volume, to maintain water quality, while the biomass consumes waste feed and excreta within the production volume. BFT utilizes a suspended growth approach as a cost-effective means of achieving intensive culture in tanks and ponds, while being able to remove most or all the external treatment system requirements used in RAS. Like RAS, BFT technology permits very low water exchange rates (Vasava *et al.*, 2020; Griffiths & Funge-Smith, 2022), and it is becoming commercially important in providing new aquaculture production capacity. BFT is less complex and costly than RAS (Schryver *et al.*, 2008). BFT does not have the space requirements of external treatment or recirculating pump of RAS but requires carbon addition to maintain a C/N ratio mainly to facilitate heterotrophic bacteria populations. IPS aquaculture can be compared to both RAS and BFT. RAS, BFT, and IPS are all water minimizing technological approaches, and BFT and IPS achieve very low water exchange rates below 6 %, which has been categorized as Super Intensive RAS by FAO, Eurofish, and AKVA (Bregnballe, 2015). This low water exchange rate equates to evaporative replacement only, with no operational water discharge, except for cleaning transition between cycles.

IPS utilizes periphyton biomass growing on surfaces within the production volume (Biswas *et al.*, 2022). Periphyton is distinct from biofilm, which is a term generally reserved for incidental bacterial films in piping and process elements. The general term used for fixed film biomass in aquaculture is periphyton, and it is grown upon various materials termed “substrates” within production volumes. Application of

high Specific Surface Area (SSA) media mat substrates is considered new to aquaculture. Rather than investigate the specific surface area of substrates, the total dimensional area of a netting has been considered by many researchers to define the addition of periphyton (Biswas *et al.*, 2022). Integrated Periphyton Systems (IPS) is a term for aquaculture employing media surface area where periphyton resides, within the production volume. High SSA media is considered as an optimum means for intensification of periphyton-based biomass and has specific impacts on aquaculture production. The current study examines advantages and disadvantages of an advanced form of periphyton treatment, wherein porous, synthetic media mat, with high specific surface area, and high internal void space, is used as substrate.

In marine aquaculture studies, the media Aquamat™ has been used, and is like Matala™ (Matala, 2024) media used in the current study. These medias are more expensive than many other types of low specific surface area substrates that have been employed in prior studies and applications, such as bamboo or palm fronds. The current study defines and analyses how the use of Matala™ media, as a central technology component of IPS, can influence the production performance of freshwater culture species through reduction in water exchange requirements, maintenance of water quality, and reduction or removal of waste management requirements, like applications of Aquamat™ in brackish or saline water. The current study proposes that integrated periphyton applications need to be clearly identified as a third zero discharge aquaculture production system, distinct from RAS and BFT. In particular, the application of IPS demonstrates capacity for high intensity culture of Giant freshwater prawn, *Macrobrachium rosenbergii*.

While there is a lack of recognition of periphyton aquaculture with media as a distinct class of intensive aquaculture, there are also inconsistencies within the field of study of this class of aquaculture. For example, in definition of periphyton area. The important distinction between media external area accessible to culture species, and media interior specific surface area available to microbes is not considered in much of the literature. There is a wide range of approaches taken, rather than a consistent analysis of the characteristics of periphyton and their impact.

Researchers have also struggled to define the biofiltration effect of periphyton within the production zone because positive effect is complicated by periphyton substrate as domain improvement. In study by Suantika *et al.* (2012), a RAS was provided with vertical textile for *M. rosenbergii* domain expansion without consideration of periphyton activity, with biological activity by biofilm relegated to a separate trickling biofilter compartment. This is a case where periphyton substrate was considered only as domain improvement, Specific surface area of nylon netting was not defined in a periphyton study by Biswas *et al.* (2022).

A hard surface media such as bamboo or palm frond has biomass carrying capacity of 1 m^2 of biofilm per 1 m^2 of media surface. Specific surface area is measured by the surface area provided per volume of the substrate. Matala™ type media has high specific surface area up to more than 400 m^2 m^{-3} such that a sheet of 1 m^2 with a thickness of only 4 cm carries surface area of 16 m^2on the surface of fibers, while maintaining a free pore space of up to 94 % of the mat volume. The external surface area of such a sheet is 2.16 m^2, inclusive of edges. A large volume of periphyton research involves biofilm on hard, two-dimensional surfaces, that is integrated into extensive and BFT ponds. Azim and Little (2006) discussed the addition of hard substrates for periphyton formation with media area measurement related to the pond surface area rather than a quantified specific surface area. A similar basic approach was taken by other researchers to periphyton studies with *M. rosenbergii* (Thapa *et al.*, 2020; Chavez *et al.*, 2015; Tuly *et al.*, 2014; Mamun *et al.*, 2012; Asaduzzaman *et al.*, 2008 and 2010). Documented experience in IPS operation using three-dimensional web matrix, i.e., using Aquamat™ media, is mainly available for saltwater or brackish water aquaculture. For purposes of this study which employs Matala™ media equivalent to Aquamat™ media, the substrate will be referred to as IPS media. Additional research into three-dimensional periphyton media applications is necessary if further investment and commercial permitting of IPS aquaculture is to be achieved.

1.2 Problem statements and research justification

Periphyton aquaculture system studies for freshwater prawns are few but they predominantly analyse extensive pond culture using hard substrate addition (Thapa *et al.*, 2020; Chavez *et al.*, 2015; Tuly *et al.*, 2014; Mamun *et al.*, 2012; Asaduzzaman *et al.*, 2008 and 2010; Azim and Little, 2006). Biofilter media is different from hard substrate in that there is a greater diversification of microbiological population from the surface to the centre of the media. Biofilter media used in RAS is also able to be used as IPS media. RAS biofilter applications as examples of intensive aquaculture exist in greater numbers than for periphyton aquaculture with IPS media. Direct comparison of IPS with RAS is therefore a means of obtaining an evaluation of IPS as an intensive aquaculture system in comparison with a more established such system.

Mixed sex *M. rosenbergii* culture populations exhibit both gender ratios and ratios of male morphotypic differentiation of Heterogenous Individual Growth (HIG). HIG morphotypic differentiation in males includes small males (SM), orange claw (OC) males, blue claw (BC) males. Market size is maximum for BC males, and these are the desirable market product, hence gender and HIG influence productivity of *M. rosenbergii* aquaculture. Gender and HIG are seldom reported together with research outputs for mixed sex rearing of *M. rosenbergii* in BFT or RAS, or within periphyton aquaculture system studies. There is a gap in the literature with respect to understanding sexual transition to male or female in the species, such as to which factors influence transition and how growth is impacted. There is a need for more research examining gender and HIG in periphyton aquaculture, inclusive of stocking with all male *M. rosenbergii*. This study investigates techniques to address achievement of uniform market product and includes investigation of culture of all-male *M. rosenbergii*. With the advent of all-male hatchery stock in Malaysia experimentation was able to provide results for IPS culture of both mixed sex and all-male *M. rosenbergii*, addressing a further gap of literature defining the differences between all-male and mixed sex culture.

The bulk of studies consider a nursery phase, while another smaller group of studies extend to a post-nursery phase of up to 4 months in duration. There is a lack of studies of grow out culture from 4 months up to 6 months with achievement of market

size product of above 35 g. Study of mixed sex culture is confounded by the difficulties of gender determination up to 3 months or more, and further complexities of hierarchical mixed sex culture of the species. In *M. rosenbergii* culture, the evolution and maintenance of the sex ratio between male and female, and Heterogenous Independent Growth (HIG) of males, affect growth performance and marketability.

Periphyton culture system studies, which are a form of IPS, tend to be limited to evaluation of extensive, low population density, pond systems, and to using organic media of low specific surface area, e.g., bamboo or branches, without any internal web matrix accessible to microbial consortia at low dissolved oxygen levels. There are low oxygen regimes within both periphyton biofilm and BFT biofloc that permit diversity of microbial consortia, ranging from aerobic to anaerobic. There are different functional aspects to three-dimensional periphyton media; one is the outer surface area on which biofilm resides, with aerobic conditions and interacting directly with the moving aqueous environment, and the other is the interior of three-dimensional fibrous matrix with depth that allows for relatively permanent occupation of facultative and anaerobic bacteria. Three-dimensional, web media like Matala™ permits aerobic, to facultative, to anaerobic microbial populations to establish permanent zones, providing a more robust ecosystem interaction amongst the microbial communities (Paerl & Pinckney, 1996). This study focuses on presenting an integrated periphyton based aquaculture system (IPS) with a level of technological input that matches or exceeds what is provided in BFT and RAS systems. Therefore, the current study is addressing a class of periphyton in aquaculture, wherein the substrate includes an internal web. The substrate used in IPS (Matala™ media) is like Aquamat™ media used in prior research discussed in more detail in Chapter 2.

The current study addresses the limitations on the intensity and productivity of *M. rosenbergii* culture, that exist despite decades of prior studies and commercial practice. Marine shrimp culture is not comparable due to the radical differences between fresh and saltwater aquaculture. Intensities routinely achieved for marine species such as for Whiteleg shrimp, *Litopenaeus vannamei*, as high as 60 mt ha^{-1} per cycle, are not expected for *M. rosenbergii*. However, *M. rosenbergii* culture typically

produces at about 6 mt ha^{-1} per year (for two cycles). This study recognizes some root causes of the differences in achieved intensity, i.e., HIG, and establishes evidence for aquaculture solutions to increase productivity.

As discussed in the above section, use of inferential statistics requires considerable effort, but provides invaluable insight. Aquaculture studies routinely deviate from pure statistical experimental design. The primary reason is that aquaculture experimentation often trends towards applied science, and scales of experimentation are larger. The resources and cost required to establish such experiments typically limit replication in experimental design. Literature review dealing with this, and other issues is presented in Chapter 2.

1.3 Research questions

1.3.1 The system

The major focus of the current study is to elaborate an Integrated Periphyton System (IPS) approach to aquaculture such that it can be considered as an alternative to BFT or RAS technology in specific applications. IPS is a distinct class of zero discharge aquaculture system that utilizes the externalized biofilter of RAS, but within the production volume (internalized). IPS fixes the biomass, which is similar to BFT biofloc, onto biofilter media within the production volume, without dependence on suspended biofloc. The features and potential advantages of IPS need to be elaborated in aquaculture of a suitable species. For the selected freshwater species, *M. rosenbergii*, these advantages are outlined as follows:

IPS media increases available domain territorial area suited to prawn grazing upon, resting, and use during ecdysis, in the form of two sides of the cross-sectional area of the added substrate, that is additional to bottom area. Periphyton on these external substrate surfaces, and on pond bottom are accessible to the production species. The available periphyton area is most important for species like *M.*

rosenbergii which do not significantly occupy the water column, preferring bottom or substrate surfaces. McCallum *et al.* (2018) pointed out that *M. rosenbergii* utilizes substrate for dual purposes, positioning for ecdysis, or moulting, and for foraging. The main foraging ground is still the bottom of the tank where supplementary feed resides. However, substrate external surfaces form habitat that is separated from the main foraging ground. Prawns using this substrate area are subject to reduced incidence of foraging related cannibalism, and energy expenditure to avoid dominant individuals, while still able to graze on periphyton (Karplus, 2005). Cannibalism in *M. rosenbergii* results from larger males competing for food or mates or finding prawns in ecdysis which can easily be consumed. As such, substrate may reduce stress on individuals, by providing safer domain, and may even reduce disparities in growth from Heterogenous Individual Growth (HIG) as a result. Higher survival and more uniform growth improve productivity with potential for more intensive aquaculture of *M. rosenbergii*.

Integrated periphyton biomass is fixed on and within the periphyton media, in sufficient quantities to achieve water quality management. Paerl and Pinckney (1996) detailed the different microbial regimes of an ecosystem within fixed media from core to surface and their role in processing carbon, nitrogen, and sulfur. Heterotrophs in the surface layer generate their own wastes, feeding other bacterial species of nutrient recycling consortia in the ecosystem, e.g., anaerobic, chemotrophic, and autotrophic bacteria, which are protected within the periphyton biofilm and within the substrate in conditions of lower flow and dissolved oxygen (DO) (Crab *et al.*, 2007). Periphyton biofilter can produce over 10 g dry weight of fresh biomass m^{-2} d^{-1} and flexibly uptake both Total Available Nitrogen (TAN) and NO_3-N (Guttman, *et al.*, 2018). The periphyton biomass removes the need for a stand-alone biofilter in a separate tank (as in RAS), or for suspension of biomass (as in BFT).

Culture species not preferential to feeding on biomass in the water column include grazers and detritovores, which can preferentially utilize periphyton biomass. *M. rosenbergii* have access to the heterotrophic outer layer of biomass on the media, as a feed source, where they will graze for food. Heterotrophic biomass production is enhanced through the addition of low-cost carbon. Various multicellular organisms

may feed on heterotrophs, and in turn become a higher trophic level live feed source for the culture species (Nie *et al.*, 2017). Advantages of both health and nutrition accrue through grazing on live feed sources, together with advantages of displacement of supplementary feed requirement and reduction of cannibalism. Digestive enzymes from internals of consumed bacteria are transferred to the prawn, reducing digestive energy requirements, and facilitating feed utilization (Pandey *et al.*, 2014; Crab *et al.*, 2007).

The periphyton biomass not only consumes waste products excreted by the culture species, but also dissolved waste feed. Conversion of waste to biomass retains more value of input feed, while reducing the need to process waste (Crab *et al.*, 2007). Food Conversion Ratio (FCR) is reduced due to the contribution to nutrition, health, and growth performance from biomass cultured in the IPS (Crab *et al.*, 2007). This is true for zero or low discharge systems where the dissolved matter is retained within the production volume, not discharged to the environment.

Periphyton biomass has less capacity to retain pathogens than does biofloc biomass. It can maintain equivalent or higher microbial density in given production volume than an equivalent BFT system. Quorum sensing effect suppresses pathogen expression due to high heterotrophic population. Anti-pathogenic compound PHB in bacteria of periphyton biomass can also be ingested by culture species and accumulate to increase disease resistance (Crab *et al.*, 2007). Kha *et al.* (2016) showed that PHB enhanced *Artemia* fed to *M. rosenbergii* larvae significantly increased survival rate.

Production species beneficially utilize only about one quarter to one third of supplementary feed for growth, in conventional aquaculture production systems (Avnimelech, 2006). The capability to achieve value from a larger proportion of supplementary feed has potential to unlock both economic benefits, and lower carbon footprint of feed production and transport. Ecosystem management of wastes, and minimized water exchange, have the potential to reduce or remove considerable environmental impacts of organic loaded wastewater discharges (Iber & Kasan, 2021).

Browdy *et al.* (2012) estimated that a conventional pond rearing system might be considered to produce about 2,000 kg of fish per hectare, and to lose about 45,000

m^3 of water to evaporation, seepage, and drainage per year, which results in a water demand of 45 m^3 kg^{-1} fish and a production rate of 0.2 kg fish m^{-2}. On the other hand, water conserving, intensive aquaculture production, was estimated to consume 1 m^3 water kg^{-1} fish at a production rate of 10 to 100 kg fish m^{-2}.

An IPS technological component was designed (refer to Appendix 1) that was implemented into rearing tanks for mixed sex, and all-male *M. rosenbergii* culture, and into ponds for commercial all-male *M. rosenbergii* culture. Combining biofilter surfaces into production volumes added ecological components, processes, and intermediaries specific to IPS to the basic aquaculture production tank as set out in simple tabulation in Table 1.1. The table describes ecosystem components of the IPS that are developed between the physical system (production volume, periphyton substrate framework, aeration equipment), the environment (water, sunlight, natural oxygen, receiving atmosphere), and the operational inputs (artificial aeration, supplementary feed and carbon source, adjustment of alkalinity/Ca/Mg, and the culture species). The stoichiometry of exchanges between components is presented in Figure 1.1.

Table 1.1: Ecological components of integrated periphyton culture

INPUTS → FACILITY → ↓ RESOURCE ↓	**PRODUCTION VOLUME WATER & WATER INPUT**	**AERATION ENERGY & EQUIPMENT**	**FEEDING EQUIPMENT**	**PERIPHYTON MEDIA ON SUBSTRATE FRAMEWORK**	**↓ OUTPUTS ↓**
CULTURE SPECIES JUVENILES	DOMAIN / TERRITORY, GRAZING AREA	CULTURE SPECIES RESPIRATION	SPECIES UPTAKE & DIGESTION (FEED, EXOVIUM & PERIPHYTON)	PERIPHYTON ORGANISMS: HETEROTROPHS AUTOTROPHS CHEMOTROPHS MULTI-CELLULAR ORGANISMS	**HARVEST OF CULTURE SPECIES**
AERATION (DRIVEN AIR) & ATMOSPHERIC DISSOLVED OXYGEN (DO)	CULTURE SPECIES DO REQUIREMENT	MICROBIAL AND ALGAL DO UPTAKE	OXIDATION	PERIPHYTON ZONES: AEROBIC FACULTATIVE ANAEROBIC	**CO2**
PREPARED FEED	SOLUBILIZATION	AMMONIFICATION	ORGANIC CARBON	LIVE FEED	**INERTS**
PREPARED CARBON, Mg, Ca & ALKALINITY SOURCES	SOLUBILIZATION, C:N RATIO	MICROBIAL C & N UPTAKE	MICROBIAL DIGESTION	NITRIFICATION / DENITRIFICATION	**RESIDUAL BIOMASS**
NATURAL MICROBES	SOIL / PERIPHYTON MEDIA / WATER	MICROBIAL AND ALGAL RESPIRATION	INGESTION OF SOLUBLE WASTES	MICROBIAL ECOSYSTEM	**WATER EXCHANGE (MINIMUM)**
SUNLIGHT	PHOTOSYNTHESIS	ALGAE, PHYTOPLANKTON	CULTURE SPECIES EXCRETIA INPUT	ALGAE	**EVAPORATION**

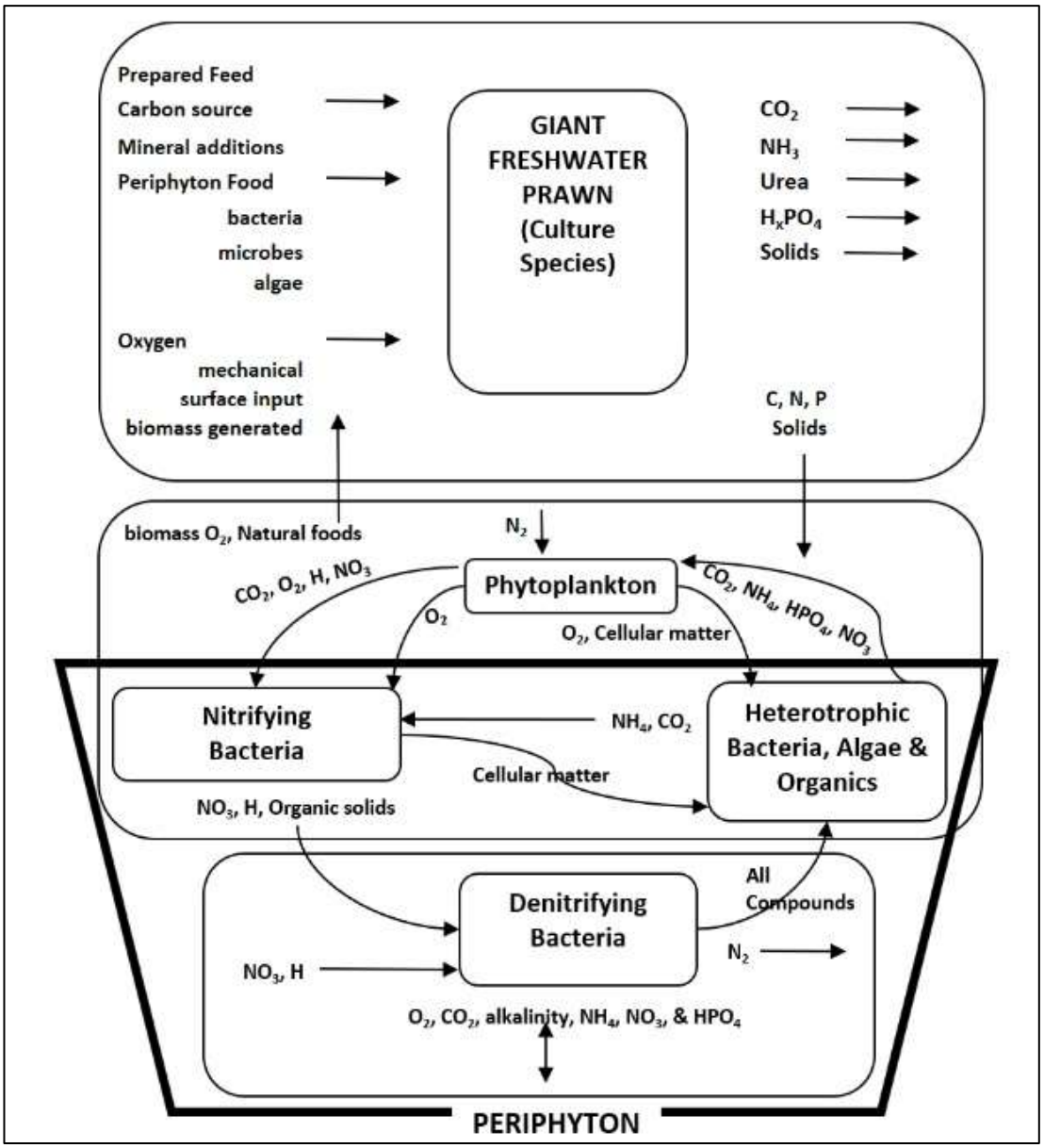

Figure 1.1: Stoichiometric exchanges within an IPS system

For the above reasons, IPS culture of *M. rosenbergii* has the potential to enable higher density production than is typically recorded either in the literature or reported in commercial practice for alternative aquaculture practices.

1.3.2 The species

M. rosenbergii, was selected as a target species with which to demonstrate IPS for various reasons. Production of *M. rosenbergii* in Malaysia has lagged from an early

lead several decades ago, to the current day when numerous nations (ASEAN, China, Bangladesh, etc.) produce the species at orders of magnitude greater annual volumes than in Malaysia. Malaysia can capitalize on the species that has a place in a wide range of local cuisine, especially if local production is demonstrated as economically viable for expansion.

There are some additional opportunities for the species in Malaysia. Malaysia is a rice growing country with some 600,000 hectares under rice paddy. Rice-prawn farming, utilizing freshwater prawn is extensively practiced in other rice growing countries, but not yet in Malaysia. The species is a freshwater species suitable to co-culture in freshwater rice paddy. The species also has potential in aquaponics culture, including urban aquaculture production.

M. rosenbergii is an omnivorous species (inclusive of carnivorous, herbivorous, detritivorous, and coprophagic characteristics) adapted to grazing as opposed to feeding from the water column, hence is a good candidate for utilizing periphyton domain area for grazing (Brown *et al.*, 2010).

M. rosenbergii exhibits large growth pattern distinctions between Heterotrophic Individual Gain (HIG) in male morphotypes and the non-hierarchical female, where these growth patterns impact on culture economics due to resultant lack of product uniformity at harvest. Means to reduce competitive, hierarchical, and cannibalistic stress are explored, and exploited in the IPS system design, and measures are identified to potentially improve harvest uniformity.

All-male *M. rosenbergii* hatchery stock is now available from several hatcheries in Malaysia, and the opportunity exists to compare the performance of mixed sex with all-male culture in an IPS, to further the objective of achieving sustainable, high intensity production and optimum economics. Changing sex ratio in culture may improve definition of the impacts of gender related features of the aquaculture of *M. rosenbergii*. Comparison of all-male with mixed sex culture may provide insight into influences on HIG amongst males. Through comparative survival rate analysis, the influence of presence of females on HIG or cannibalism may also be

clarified. Culture of all-males also removes complex variables related to the female sex. One main objective is more uniform *M. rosenbergii* product at harvest.

Sexual differentiation including presence of claws is not a factor in the nursery stage. Stocking populations in higher density nursery phases may be followed by lower density grow out phases. Thereby, overall farm culture volume per animal is reduced over the full life cycle. Nursery stocking of smaller animals can protect them from predators, and results in their having less distance to travel to supplementary feed (Coyle *et al.*, 2010). This was emphasized as having large positive impact on survival rate of the first two months of culture of *M. rosenbergii* in commercial applications (Mr. Kamaruzzaman Hosain, Department of Fisheries, Bangladesh – personal correspondence, 2022). Movement from high to low density environment can stimulate growth, referred to as compensatory growth (Coyle *et al.*, 2010; Karplus & Sagi, 2010; Karplus, 2005).

Partial harvest of larger animals, i.e., after 4 months, yields product for sale, while also achieving reduced density in remaining culture animals, and unlocking compensatory growth. Although HIG in male *M. rosenbergii* restricts growth of most males (Ranjeet & Kurup, 2002), removal of larger Blue Claw (BC) males through partial harvesting at a target body weight (BW) stimulates smaller males to rapid growth after each partial harvest. Less energy is also expended by smaller males, after partial harvests, in evading the larger ones (Suneetha *et al.*, 2018). These techniques manage relationships between density, and HIG, resulting in overall higher productivity per unit area under aquaculture, and uniformity of product in harvest.

Separation of post-nursery stock is also investigated. Post-nursery stock at 30 Days of Culture (DoC) is still not practical to separate by sex. However, post-nursery *M. rosenbergii* stock does exhibit size differentiation, and different size groups are expected to have different growth potentials. The larger sizes are also expected to contain a higher proportion of males. Separation of stock would allow customized feeding and density of different groups. There may be potential to reduce HIG by managing mass-separated populations of post-nursery stock, because of the way that morphological differentiation follows mass.

The purpose of the study is to elaborate some characteristics of IPS systems in different phases of high-density *M. rosenbergii* culture. The study aims to establish precedent for further research into the complex set of contributing factors that make higher density culture feasible. A series of experiments is carried out with RAS as control. IPS prototype scale experimentation is utilized to evaluate and demonstrate innovations inherent in IPS, specific to production of the target species.

Malaysian *M. rosenbergii* aquaculture has effectively stagnated for decades from a peak production level in 2000 of only 1,380 mt year^{-1} (New *et al.*, 2010), with 2019 output estimated at 400 mt year^{-1} (Habib, 2017; Banu & Christianus, 2016). Globally, the introduced species is a high value culture species generating enormous economic value in aquaculture, having risen to an estimated 2018 production of 237,124 mt, valued at USD 1.93 billion (Pillai *et al.*, 2021). This rise was partially attributed by Pillai *et al.* (2021) to recent developments in genetic improvement (Pillai *et al.*, 2021). A reliable, modular, intensive culture system to rear *M. rosenbergii* is needed to permit organic expansion of commercial aquaculture, for specific application in Malaysia. The general conclusions of the study may be applied to hatchery, nursery, and grow-out stages of culture, and to other aquaculture production species with particular emphasis on omnivorous species.

The current study analyses the characteristics of *M. rosenbergii* to optimize culture of the species, where the major, relevant characteristics can be discussed as follows.

M. rosenbergii is a freshwater species, and freshwater culture is readily adaptable to many terrestrial locations including freshwater ponds, rice paddy fields, urban tank aquaculture farms, or as a culture species within aquaponics systems. Understanding of the interaction of the species with periphyton, and IPS technological components will be useful. *M. rosenbergii* is an omnivore species which will graze readily on attached, and other biomass, i.e., biomass supported by IPS.

M. rosenbergii is identified with Malaysia and is present with brood stock in many Malaysian rivers, while the Government of Malaysia and Malaysian private

sector have identified the opportunity for increased production and marketing in Malaysia (Banu & Christianus, 2016).

M. rosenbergii maintains a high market price, globally, and locally where a typical RM65.00 per kg paid ex-farm for 35 g product mass and above assumes that morphology of Blue Claw (BC) male has been achieved. BC males are the most attractive and saleable *M. rosenbergii* product. The potential of *M. rosenbergii* as a high value aquaculture species for commercial production increases if higher intensity production can be supported, with improved aquaculture system economics.

HIG in males, and distinct growth of females result in non-uniform *M. rosenbergii* product at harvest, decreasing market value (Ranjeet & Kurup, 2002). Means to decrease impact of HIG include the use of IPS, use of mixed sex post-nursery size separation, sequential partial harvests, and culture of all-male stock. Size separation using mesh sizes was conducted by Daniels and D'Abramo (1994) and Daniels *et al.* (1995). All-male hatchery technology is fully developed and accessible for *M. rosenbergii* stock in Malaysia, which may yield additional production optimization.

1.4 Objectives of the study

The objectives of the current study were distilled to a realistic program which could provide relevant evidence in support of the further research and implementation of IPS as an intensive aquaculture system contributing to sustainable aquaculture. The resultant general objective of this study was to prepare, operate, and characterize a representative IPS as a sustainable means of culture of *M. rosenbergii*. The specific objectives of this study were the following:

1. to compare the production efficiency of IPS with RAS in nursery and post-nursery to 104 DoC with mixed sex *M. rosenbergii*.

2. to evaluate performance of IPS for mixed sex and all-male *M. rosenbergii* up to post hatchery above 90 DoC, and all-male in IPS experimental and commercial production from hatchery to 195 DoC.
3. to develop useful culture system design and operational parameters, and feed model for culture of the target species, *M. rosenbergii* in IPS tanks and ponds.

1.5 Hypothesis

In the experimental comparisons of IPS and RAS, treatments were carefully expressed with identical system unit sizing. The treatments were distinguished only with respect to the presence of periphyton media within the IPS production tanks, whereas the external RAS biofilter utilized equivalent media to that used in IPS. Various environmental factors including temperature, amount of food, living space available per prawn, pH and temperature of the water, and prawn age and phase of growth were maintained as equivalent between IPS and RAS. IPS and RAS utilized post larvae from the same hatchery batch and were operated within identical time frames. The RAS tanks provided equivalent domain areas to the media contained in IPS tanks through the addition of equivalent surface of open mesh of low specific area, which was done to remove the domain area factor from complicating evaluation of periphyton media presence. The evaluation compared the effect of additional periphyton surface area, as specific surface area of three-dimensional media within IPS production tanks, with no such periphyton area within RAS production tanks.

As a scientific hypothesis, this setup was considered testable; that is, there existed the possibility of disproving it. Many hypotheses may be proposed to explain a particular phenomenon.

For objective (1), to compare the production efficiency of IPS with RAS in nursery and post-nursery to 104 DoC with mixed sex *M. rosenbergii*, and one way to state the explanation, or hypothesis, that had been chosen to test is:

Hypothesis 1: Treatment group, IPS or RAS, defined by presence or absence of periphyton biofilter substrate within the production volume, affects prawn growth.

Prediction 1a: Prawns grown with periphyton present in the production volume will have improved survival rate in comparison with RAS.

Prediction 1b: Prawns grown with periphyton present in the production volume will have improved harvest body weight in comparison with RAS.

Prediction 1c: Prawns grown with periphyton present in the production volume will have improved combined productivity of Body Weight (BW) x Survival Rate (SR), BW x SR, in comparison with RAS.

Because RAS is capable of high intensity culture, comparison with IPS is tested under high intensity culture conditions.

It is far more difficult to establish a provable hypothesis that relates Heterogenous Individual Growth (HIG) in males, and separate female growth pattern, to performance of IPS or RAS. However, observation of statistical experiments can yield useful observation.

For objective (2), to evaluate performance of IPS for mixed sex and all-male *M. rosenbergii* up to post hatchery above 90 DoC, and all-male in IPS experimental and commercial production from hatchery to 195 DoC, one way to state the explanation, or hypothesis, that had been chosen to test is:

Hypothesis 2: HIG in males and separate female growth pattern prevent uniform product of stock at harvest of *M. rosenbergii*. The technique of separating

prawns into low and high average individual mass groups at post-nursery (above 30 days post-hatchery) stage is feasible in terms of speed and ease of operation, whereas sexual differentiation is too difficult at this age. The two groups are expected to have differentiated populations, where the larger average mass prawns are expected to be made up of more faster growing males. Sexual differentiation at above 90 DoC will be practicable through morphological identification, which will also permit identification of HIG groupings of males, i.e., Small Male (SM), Orange Claw (OC) male, and Blue Claw (BC) male. Separation has the potential to produce more uniform harvest product. All-male culture has the potential to improve performance and delivery of uniform BC males more effectively by removal of females. There will be positive improvement in production with all-male because of removal of male-male competition for mating with female individuals.

Prediction 2a: Prawns separated by mass post-nursery (above 30 days post-hatchery) will develop as populations with separate characteristics, with respect to SR, BW, and BW x SR during culture. Sex determination by morphology at above 90 DoC will permit association of performance with gender and HIG of two weight groups, hence evaluation of the mass differentiation technique will be quantifiable.

Prediction 2b: Prawns separated into different average mass groups at post-nursery (above 30 days post-hatchery) age will develop as group populations with different sex ratios, HIG, and performance characteristics, which will be determinable at above 90 DoC post-hatchery. Gender and HIG will be related to SR, BW, and BW x SR, to quantify the potential efficiency of a separation technique.

Prediction 2c: Performance of all-male may be improved over mixed sex *M. rosenbergii* with removal of one differentiated group, the female gender, leading to improved efficiency in production of uniform product.

For objective (3), to develop useful culture system design and operational parameters, and feed model for culture of the target species, *M. rosenbergii* in IPS tanks and ponds, one way to state the explanation, or hypothesis, that had been chosen to test is:

Hypothesis 3: Biomass is used in RAS, BFT, and IPS to manage water quality, operate at zero discharge, and provide advantages of nutrition and health to culture species, which together support sustainable aquaculture. Research typically elaborates on addition of supplementary feed and carbon, or alkalinity control. However, without displaying the growth model of the culture species, there is lack of correlation of supplements to performance. The resultant changing mass in ponds arises from phases of growth, density change arising from mortality, different growth patterns for sex and changing morphology, and partial harvesting. Food Conversion Ratio (FCR) is an important economic consideration in aquaculture and to optimize FCR the feed model needs to address the potentials for impact on culture species mass throughout the culture period. Sustainability is most successful if it brings improved economics and the optimization of FCR is of major significance in lowering aquaculture costs.

Prediction 3: Adjusting the FCR accurately towards or below 1.0 will achieve better economic performance through reduced waste feed, while ensuring consistent growth performance measured with BW, SR, Average Daily Growth (ADG), Specific Growth Ratio (SGR), and areal yield (i.e., mt ha^{-1} $year^{-1}$) of the culture species, and economic utility of the feed model will be quantifiable by minimum FCR for maximum performance.

CHAPTER 2

LITERATURE REVIEW

2.1 Sustainability of intensive aquaculture

The significant increases anticipated in aquaculture production place large resource demands on earth's capacity, which vary in type and scale depending on the means of aquaculture production employed. Design basis for aquaculture production systems needs to address the next 33 % increase in aquaculture production, as estimated by FAO to be in place by 2030 (FAO, 2020), which in perspective, effectively requires duplication of over a third of every piece of existing aquaculture production from entirely new resource allocations. The world's resource capacity has been exceeded by annual consumption since the 1970's and has increased such that the date of exceedance occurs on 2 August 2023, for 2023 (Miller *et al.*, 2023). With aquaculture production capacity currently equivalent to that of the entirety of earth's oceans, another third of equivalent planetary ocean production capacity is envisioned, by 2030! Freshwater fish carries 75% of global aquaculture production (i.e., over 80 Mt in 2017), yet is underrepresented in the literature (Naylor *et al.*, 2021), The projected increase in aquaculture production will unlikely be able to rely solely on past successes in sustainable production (Martinez-Porchas & Martinez-Cordova, 2012; Martínez-Córdova *et al.*, 2015). Meeting such a production increase requires innovative approaches to satisfy the critical need for input resources to production, while conforming to the criteria for sustainable aquaculture (Ghamkhar *et al.*, 2021).

Aquaculture is receiving global attention that recognizes the cross-border nature of its impacts, with the global demand side market (customers) requiring quality control, i.e., certification programs (Boyd *et al.*, 2020). Design basis to achieve sustainable aquaculture expansion requires indexing of aquaculture practices that includes more scope than economic performance alone (Volpe *et al.*, 2013; Valenti *et al.*, 2011; Bridson *et al.*, 2020). Responsible sourcing of aquatic products extends to

sustainable aquaculture, where permitting of new facilities requires assessment of the impacts resulting from resource use, as well as environmental impacts.

2.2 Integrated biomass aquaculture

Martinez-Porchas and Vargas-Albores (2017) reviewed the status of microbial metagenomics in aquaculture, describing studies of microbial diversity, consortia, antibiotic resistance, and probiotics, amongst others and pointed out the criticality of more study into high intensity aquaculture production systems, including both BFT and periphyton systems. In the case of BFT, a production of 100 to 600 kg ha^{-1} day^{-1} of protein is possible from waste and carbon addition, but the means to optimize the metabolism of the complex microbial consortia responsible, relative to cost of inputs and value of outputs, requires further investigation (Martinez-Porchas & Vargas-Albores, 2017).

An example of analysis of the aquatic microbial diversity in RAS, BFT, and periphyton, using tilapia was investigated by da Silva *et al.* (2016) with the conclusions that higher final weight resulted from both BFT and periphyton applications, but that periphyton demonstrated bacterial consortia carrying lower pathogenic potential. Farook *et al.* (2019) described genetic detection of the range of diseases affecting *M. rosenbergii*.

Periphyton in aquaculture, as maintained in IPS, may be represented by study of autotrophic nitrifying biofilm as carried out by Kindaichi *et al.* (2004) who showed that periphyton biofilm was diverse and stable, that carbon metabolism coexisted with nitrifiers and denitrifiers, and that periphyton represented an efficient food web preventing accumulation of metabolites or wastes. Kumar *et al.* (2019) used a substrate media mat known as Aquamat™, and reared *L. vannamei* in saltwater, as did Audelo-Naranjo *et al.* (2010; 2011; 2012) in a series of experiments. Aquamat™ has similar characteristics to Matala™ media mat used in the current study.

RAS is a well-established high intensity, low water consuming, aquaculture system (Fudge *et al.*, 2023; Bregnballe, 2015). Implementation of commercial RAS has been historically impeded by uncertainty of assured revenue. The economics of RAS have been compared unfavourably with less intensive methods of aquaculture production. Perceived higher capital investment, higher technical level of operation, and increased operation and maintenance cost are claims made about RAS in comparison with extensive pond culture. The overriding fact is that extensive pond culture is the status quo in global terrestrial production.

The commercial aquaculture decisions currently being taken to invest in large scale, technologically intense RAS reflect on the ability of RAS to control operational and environmental factors, which assists in overcoming permitting hurdles that influence land and resource allocation (Fudge *et al.*, 2023). The ability to permit bio secure facilities near urban centres, also allows the reduction of distance to market, important in reducing carbon footprint due to product transport. RAS facilities close to urban centre markets for aquaculture product also move aquaculture employment into urban centres with corresponding reductions in carbon footprint possible, along with potential social advantages. The same advantages belong to both BFT and IPS.

Beyond permitting advantages, which have immediate commercial implications, improved Food Conversion Ratio (FCR), growth rate, and survival rate are potential commercial advantages enabled by RAS (Fudge *et al.*, 2023; Bregnballe, 2015). Browdy *et al.* (2012) estimated that a conventional, extensive pond rearing system might be considered to produce around 2,000 kg of fish ha^{-1} and to lose about 45,000 m^3 of water to evaporation, seepage, and drainage per year. This results in water demand of 45 m^3 kg^{-1} fish at a production rate of 0.2 kg fish m^{-2}. Water conserving RAS production, on the other hand was estimated to consume 1 m^3 water kg^{-1} fish at a production rate of 10 to 100 kg fish m^{-2}.

While RAS maintains water quality, and drastically reduces water consumption, in high intensity culture, it is not designed to recycle wastes to positive probiotic and nutrition inputs. Only about one third of supplementary feed is beneficially utilized by production species in conventional aquaculture production

systems, i.e., extensive pond aquaculture, (Avnimelech, 2006). A capability to achieve production from a larger proportion of input feed reduces supplementary feed cost, and results in a lower carbon footprint for feed transport for aquaculture production (Ghamkhar *et al.*, 2021). This capability belongs to both BFT and IPS.

Improved sustainability of aquaculture production needs to be considered against the backdrop of the huge projected increase in aquaculture production. While the status quo of aquaculture production is largely based on extensive systems, increase in production should be implemented using the most sustainable production systems, which requires certainty of economics to attract appropriate capital investment (FAO, 2020). Intensive aquaculture systems have unique potential to meet critical resource needs. They have the unique capability to be situated close to market, require less land and water supply than extensive systems, generate less effluent per unit production, and may utilize peri-urban land designated commercial, industrial, or agricultural. RAS, for example, exerts control over all aspects of the aquaculture production environment sufficiently to permit high to ultra-high intensity culture, carrying less and potentially minimum resource intensity (Fudge *et al.*, 2023; Bregnballe, 2015). The aquaculture production sector is responding with commercial RAS farms including Asia's biggest farmer, Charoen Pokphand Foods (CPF) planning full conversion to indoor operations (Gibson, 2018) and, with the advent of large-scale land-based salmon farm RAS (Bregnballe, 2015). However, there are constraints involved with intensive requirements for capital, labour, energy, water, and management to conduct aquaculture in intensive RAS (Engle, 2023). Competing systems with RAS are Biofloc Technology (BFT), well established in Thailand (Griffiths 2022), and other intensive aquaculture systems including the Integrated Periphyton System (IPS) identified and examined in this study, which reduce footprint, complexity, or cost.

Bell *et al.* (2023) described a RAS which included a series of periphyton media biofilters and a clarifier in addition to the production species vessel. The media biofilters were described as periphyton biofilters, which is relevant to the current study. Periphyton biofilters are used within the production species vessel in IPS. IPS would incorporate sludge removal from the production vessel if required, either during or

following a production cycle. The RAS periphyton media biofilters used in the current study were Matala™ type (Matala, 2024) in a horizontal cross flow configuration. As such, suspended solids are reliably trapped by sequential mats, eliminating the need for a standalone clarifier. Matala™ media is tested to provide over 200 kg of wet solids retention per cubic meter.

The important feature of intensive aquaculture systems is that they do not rely on water exchange to manage water quality. These systems retain added feed and additives in the production volume without dilution and discharge in water exchange. Intensive BFT and IPS systems rely on production volume ecosystems to cycle nutrients and additives, through exchange between microbial biota including algae, bacteria, and multi-cellular organisms. Some of the ecosystem biomass is consumed as feed by the production species. Hence, intensive BFT and IPS systems can reduce the supplementary supply of feed and additives into production volumes.

Both fixed film and suspended growth microorganism cultures can positively impact economics of intensive aquaculture production of commercial species, through conservation of feed value, further reduction of footprint, and water conservation. Crab *et al.* (2007) confirmed the advantages of nitrogen removal and conversion to nutrition in both biofloc and periphyton aquaculture systems. Ruby *et al.* (2018) provided a review of the potential of periphyton based aquaculture. Water consumption, feed utilization, land requirement, geographic and social location, and effluent discharge are key areas influencing sustainability. Their management is increasingly pivotal in successful permitting of production facilities and of capacity expansion. Investors are led to invest in intensive systems for these reasons.

2.3 The role of microbes

Design of biological wastewater treatment facilities involves use of microorganisms to consume nutrients (pollutants), after which the microorganisms are separated through economical means. This technology began development in the 1800’s (Lofrano & Brown, 2010). Two main types of biological treatment are

suspended growth (the biofloc equivalent) and fixed film (the periphyton equivalent). Microbial consortia co-exist in these systems, process wide ranges of compounds, and conduct exchange of nutrients. They manage water quality with the natural objective of consuming all available food from water, including carbonaceous organics and nitrogen (Pedros *et al.*, 2005).

BFT is a well-developed field with prolific examination in both research and commercial settings, developed over the past several decades (Crab, *et al.*, 2012; Avnimelech *et al.*, 2009). It is a respected means to integrate water treatment and waste management in-situ of aquaculture production. BFT is a suspended growth system where the microbial biomass is distributed within an aerated tank, with mixing providing the energy for suspension of biofloc. BFT nutrients are continuously recycled and reused, and sustainable growth of microorganisms within the production volume is benefited by minimum or zero water exchange that avoids loss of nutrients. BFT microorganisms (biofloc) have two major roles: (i) maintenance of water quality; and (ii) nutrition (Emerenciano *et al.*, 2013; Khanjani *et al.*, 2022). BFT dates to about 1990 onwards with much acclaim owing to the work of Avnimelech (1999), progressing to "Biofloc Technology – A Practical Guide Book" in 2009 (Avnimelech *et al.*, 2009). More recent research defines the health benefits of biofloc that include quorum sensing, and reuse of bacterial components through consumption (Browdy *et al.*, 2012).

Periphyton biomass develops similarly to that developed in external biofilters in RAS. RAS biofilters tend to be more restrictive, low-light environments highly dominated by bacteria (Avnimelech, 2006). BFT and IPS in ponds include algae production, which becomes incorporated to biofloc and periphyton, respectively. Use of periphyton in production volumes is steadily increasing (Martinez-Cordova *et al.*, 2015). The original definition of periphyton only included algae, fila and cladocerans, but has evolved as an assemblage coating submerged surfaces that includes algae, bacteria, protozoans, and free-swimming microorganisms such as rotifers. The presence of algae in periphyton is a significant difference from RAS biofilters, and a similarity with BFT biofloc content. In aquaculture, periphyton includes all aquatic biota attached to substratum together with associated detritus and microorganisms, and

surface area of any type can be used for periphyton production (Ruby *et al.* (2018); Azim, 2001). The potential of periphyton-based aquaculture production systems was discussed by Azim in his Ph.D. Thesis, Wageningen University (Azim, 2001). Azim and Little (2006) specified periphyton growing on hard surface substrates in later research. IPS includes periphyton on hard substrates. In the IPS of the current study, media web matrix provides a longer-term residence of non-aerobic microbial biota. Azim and Little (2006) considered periphyton that resided within a BFT environment, which is not representative of a pure IPS application. The review of periphyton in aquaculture presented by Ruby *et al.* (2018) limited discussion of the concept of specific surface area, other than outer surface of rigid media. The use of Aquamat™, which is like the Matala™ media adopted in the current study, was mentioned without reference or specifics. Audelo-Naranjo *et al.* (2010) evaluated Aquamat™ versus no substrate in a marine *L. vannamei* aquaculture system over 32 days finding 13 % improved shrimp biomass yield, and significantly higher protein content in shrimp, and conducted further experiments exploring the benefits of Aquamat™ for marine culture of *L. vannamei* (Audelo-Naranjo *et al.*, 2011; 2012). Kumar *et al.* (2017; 2019) evaluated Aquamat™ versus no substrate in a marine *L. vannamei* aquaculture system over 120 days and found that it increased profitability, with inclusion of the higher capital cost for Aquamat™. A commercial marine aquaculture operation for *L. vannamei* was studied by Monroy and Peterson (2001) with Aquamat™ employed over 20 % of the 45-ha farm area. Aquamat™ was evaluated as having achieved payback within a single cycle.

Inside production tanks, waste products and un-consumed feed supplynutrition, and form the basis of an even more expansive ecosystem, with the commercial species under production at the highest trophic level. Biomass acts as an in-situ biofilter to improve or manage water quality. The biomass is exposed to the commercial species and utilized as feed by it, providing both nutrition and health benefits (Nevejan *et al.*, 2018; Crab *et al.*, 2010; Turan *et al.*, 2017; Defoirdt, 2014; Nhan *et al.*, 2010; Pande *et al.*, 2013; Fatimah *et al.*, 2019).

Significant research has evidenced that the presence of biomass in production volumes supports quorum sensing based resistance to disease outbreak for many fresh

water and sea water species, but specifically for *M. rosenbergii* (Nhan *et al.*, 2010; Pande *et al.*, 2013). Quorum sensing in integrated biomass systems is enhanced because these systems use an added carbon source to increase the C/N ratio, in support of large heterotrophic bacterial populations. The presence of these large heterotrophic populations is one mechanism of deterrence within the category of positive impact on disease from quorum sensing. Girard (2019) elaborated the multiple mechanisms of pathogen signaling which can be disrupted to the detriment of pathogen proliferation.

In addition, biomass may prove to contain, or to be manipulated to contain, compounds like those provided by added probiotics (Nevejan *et al.*, 2018; Martinez-Cordova *et al.*, 2015). Biomass which is consumed protects production species from infection by pathogenic bacteria through accumulation of poly-beta-hydroxybutyrate (PHB), sourced from the biomass (Crab *et al.*, 2012). Kha *et al.* (2016) established that PHB enriched *Artemia* fed to *M. rosenbergii* larvae significantly improved survival rates especially when associated with higher lipid content of feed.

Further, bacteria in consumed biomass themselves utilize digestive enzymes, and these may be directly employed, after consumption, by the production species' digestive system (Pandey *et al.*, 2014; Crab *et al.*, 2007; Martínez-Córdova *et al.*, 2015). Bacterial biomass may also encourage the development and presence of zooplankton, providing trophic upgrading of waste feed and by-products, producing another trophic level of organisms as feed for the production species (Nie *et al.*, 2017). Both BFT and IPS maintain biomass with the above advantages. It will be important to both maintain and highlight the distinctions between biofloc and periphyton in future investigations to permit optimization of the different opportunities presented by their different elements and operational mechanisms.

2.4 BFT as integrated biomass, suspended growth aquaculture

Biofloc technology (BFT) is a suspended complex of microbes, algae and detritus. The extracellular polymeric substances produced by bacteria are the glue that binds these materials together as biofloc (Schryver *et al.*, 2008). Bioflocs are effective

at capturing both suspended solids and dissolved contaminants, as is the case for Activated Sludge in treatment of wastewater. The presence of periphyton upon media has the same capacity. BFT is the utilization of suspended growth biomass integrated into aquaculture production. The integrated suspended growth approach provides biodegradation of water borne nutrients. Genomic research carried out by Vargas-Albores *et al.* (2019) suggested that both nitrogenous and carbonaceous compounds could be processed by bacteria within a unit of BFT system.

Study of BFT initially focused on performance in management of water quality (Crab *et al.*, 2007). Reviews of BFT applications included periphyton systems in evaluation against the criteria of performance, and demonstrated the contribution of integrated microbial systems to benefits in health, nutrition, economics and, sustainability in aquaculture production (Azim & Little, 2006; Nevejan *et al.*, 2018; Martínez-Córdova *et al.*, 2015). One key factor in the adoption of BFT is the ability and preference of the production species to consume feed suspended within the water column (Kent *et al.*, 2011) whereby BFT biofloc can become feed. Biofloc can be manipulated to produce a larger floc, however planktivorous species remain the most natural match for BFT. The ability of *L. vannamei* to consume and digest suspended microbes and microalgae was established by Kent *et al.*, (2011).

Extensive reviews of the development of BFT technology have described a range of applications within the past decade. Significant success has been noted at a commercial level with respect to the probiotic and nutritional benefits of BFT, but the technology is far from having become the status quo, or business-as-usual in aquaculture production (Crab *et al.*, 2012; Suneetha *et al.*, 2018; Emerenciano *et al.*, 2013).

BFT has demonstrated that integrated biomass systems are effective from an economic perspective, and more applications and study will further define whether BFT is suitable to meet the criteria of super intensification matching RAS performance, and for which production species it will be commercially advantageous to employ at nursery and / or grow out stages. Both BFT and periphyton aquaculture production systems employ microbial matrices within the production volume.

Microbial matrices are resilient with both types being able to support both nitrification and carbon conversion simultaneously. The advantages of including periphyton in a BFT system for *L. vannamei* intensive production (300 shrimp m^{-2}) were revealed where periphyton acted as an additional food source in study conducted by Ferreira *et al.* (2016). Schveitzer *et al.* (2013) performed a similar experiment finding that, even with low biomass development on periphyton media, that weight gain, final mass, and survival rate increased with periphyton addition, and concluded that periphyton media most likely reduced stress in the culture species.

Reduced light in the pond environment resulting from periphyton substrate addition may positively influence feeding behavior, and levels of hierarchical stress. Inserted material barriers supporting periphyton may complicate suspension and aeration requirements of BFT, which is somewhat more energy intensive than periphyton due to the requirement of biofloc suspension.

2.5 IPS as integrated periphyton biomass in aquaculture

Fixed film microorganisms, or periphyton, consist of bacteria, algae and plankton in a matrix bonded with extracellular polymeric substance (EPS) produced by the (bacteria and algae) and attached to a surface (Pandey *et al.*, 2014). Within an aquaculture production volume, surface area is needed on which the biofilm may form and reside, and the added material provided to increase surface area is typically referred to as substrate. Substrate can be provided by addition of media, either living and structural (i.e., vegetative roots) or purely structural (i.e., mesh). Fixed film microbiology will vary depending on the complexity of the media, which has also been studied in detail in wastewater treatment engineering applications (Eding *et al.*, 2006; Choi *et al.*, 2013). Media used in biological treatment of wastewater, has been shown capable of processing aquaculture waste. Aquaculture researchers and commercial operations have adopted the technology within RAS, BFT and periphyton systems.

On a two-dimensional (2-D) fixed film surface the microbiology of inner, intermediate, and outer layers typically house aerobic, facultative, and anaerobic

bacteria, respectively, within thin layers, like what occurs in trickling filter and recirculating biological contactors. These layers work cooperatively utilizing microbiological consortia interaction and molecular diffusion to process and cycle nutrients in the water (Ebeling *et al.*, 2006; Paerl & Pinckney, 1996). Typically, the anaerobic layer at the inner level will generate gases which can force biofilm to "slough off", or to fall off from the surface. Where biomass is exposed to the production species it is subject to grazing by the culture species. In the case of living 2-D media, e.g., seaweed surface, or vegetative root surface, there are beneficial exchanges between the living media and the attached growth microorganisms, and water quality is influenced by the living media as well as by the attached growth (Morgan *et al.*, 2008).

The media may alternatively be provided with three-dimensional (3-D) depth in the form of crevasses in the material surface, cavities, layers of porous media, or thick fibrous media. The thick fibrous media used in the current study (Matala™ media) is designed for biological wastewater treatment with high specific surface area, and high pore space volume (greater than 90 %). 3-D effects include creation of zones inside the media that have lower agitation and oxygen supply, supporting facultative and anaerobic bacteria. In the current study, IPS is operated with a quantity of the media set up as layers within the production tank such that the surface of the media layers is accessible to *M. rosenbergii*. The IPS can be seen as an aerated submerged biofilter. Aeration turbulence keeps biomass in the water of the tank in contact with the media which brings about adherence of biomass to the media. The adhered biomass becomes periphyton, which absorbs nutrients, and to which suspended solids adhere, and provides clarification of the water volume in operation as a result. RAS systems of the current study, on the other hand, are provided with separate periphyton biofilter tanks. RAS biofilters are flow through systems, whereby the media is present across the direction of flow, and flow must pass through the high void space media (94 % void space). Velocity from inlet to outlet of RAS biofilter is very low (about 16 m h^{-1} or 0.0044 m s^{-1}). Low velocity ensures that flow is distributed across each compartment media face, and that biomass attaches to the substrate such as occurs in lamella filters. Matala™ media has a capacity of over 200 kg of wet solids m^{-3}. RAS biofilter tanks are aerated.

The RAS biofilters operate as fixed bed filters. Bregenballe (2015) defined the operation of these filters wherein fine organic particles are removed as these substances adhere to the bacterial film. The fixed bed filter operates as a fine mechanical filtration unit removing microscopic organic material and leaving the water very clear. A moving bed filter would not have the same effect as the constant turbulence of water would make any adhesion impossible. RAS biofilter chambers are aerated. The media provides filtration equivalent to the action of a clarifier and secures biomass from passing through the biofilter tank. Bell *et al.* (2023) demonstrated an equivalent RAS design but with a standalone clarifier. In the current study, multiple media sections contribute to cumulative total clarification. Bell *et al.* (2023) referred to their RAS biofilter as a periphyton filter.

The IPS of the current study design moves the periphyton filter into the production vessel and dispenses with external filters and clarifier. Design of the periphyton biofilter employed in RAS units is duplicated in terms of media type and quantity in the IPS units. The sizing of the biofilters for RAS and IPS is identical and derived from Losordo and Hobbs (2000), as further described in Chapter 3. Nitrification rates for biofilters exposed to air, such as trickling filters or rotating biological contactors (specific surface areas of 100 to 400 m^2 m^{-3}), used in aquaculture range from about 1.0 to 4.0 g TAN m^{-2} day^{-1} (Greiner & Timmons, 1998). Fluidized bed filters and plastic bead filters have higher specific surface areas, lower void spaces and generally have lower aerial nitrification rates (0.10-0.5 g TAN m^{-2} day^{-1}) (Greiner and Timmons, 1998). The current study utilized a submerged fixed film of fibrous Matala™ media, through which water was forced to flow by directional control. There was no head loss for flow passing through the media because of its 94 % void space and low velocity (16 m h^{-1} for the RAS biofilter). Although specific surface area approached the upper limit for rotating biological contactors the sequential filter media also has characteristics of plastic bead filters. The study filters were aerated and dissolved oxygen kept high, and a nitrification rate of 0.45 g TAN m^{-2} day^{-1} was selected.

3-D effects near the surface, where oxygen is not limited, provide refuge for development of additional trophic levels, such as zooplankton, which feed on

periphyton. In a C/N modified system, heterotrophic bacteria are supported which congregate at the surface and near surface, acting as food for culture species and zooplankton. Prior study has claimed to have not found correlation of substrate with nutrition (Ahsan *et al.*, 2014), however substrate such as bamboo does not constitute 3-D media. More complex media is typically able to support a more complex ecosystem. Research into the microbiology of 3-D media exists as studies of biofiltration (or biological treatment) media in wastewater treatment systems, and in biofilters external to aquaculture production tank volumes of commercial species. There were no studies found which evaluate the advantages of 3-D media in comparison with 2-D media, supplied within production volumes.

The integrated periphyton, IPS approach in aquaculture production can be considered as a biofilter element that is integrated to the production tank of a commercial species. Different types of RAS biofilter have been ranked as multistage water treatment units, which emit high levels of bacterial biomass to be recirculated into the production tank (Malone & Pfeiffer, 2006). The microbiological population of external biofilters is employed to improve water quality and is not typically designed to convert waste feed to useful biomass. Hence, biomass does not become subsequently available as a probiotic or nutritional source to the commercial species, as noted by Avnimelech (2006). However, some evidence of biofilters improving FCR and nutrition over non-biological treatment types constituted early recognition that integrated production of microorganisms would have a positive impact on aquaculture production. Such study has compared periphyton and BFT, based on their performance in management of water quality (Crab *et al.*, 2007).

Studies and reviews of 2-D periphyton media applications have described the use of both synthetic (e.g., meshes or plastic surfaces) and natural (e.g., bamboo or tree branches) fixed film media. Periphyton aquaculture system studies for freshwater prawns have most frequently analysed extensive pond culture using hard, natural substrate addition such as branches or twigs (Chavez *et al.*, 2015; Martínez-Córdova *et al.*, 2015; Tuly *et al.*, 2014; Mamun *et al.*, 2012; Asaduzzaman *et al.*, 2008 and 2010; Azim & Little, 2006). Thapa *et al.* (2020) utilized synthetic mesh without

providing insight into specific surface area. Studies of 3-D media have generally used the Aquamat™ product and have been mainly limited to saltwater aquaculture study.

Mounting research interest exists to determine the extent to which biofloc can be manipulated to improve the characteristics of biofloc, and to positively influence production performance and quality. Martinez-Cordova *et al.* (2015) reviewed progress in the field and produced an updated table outlining manipulation of control parameters (C/N ratio, energy, DO, temperature, pH and ionic levels and, light exposure) to produce influence in biofloc composition, size, structure and stability.

Despite being considered extensive rather than intensive, Azim *et al.* (2001) found that systems rearing fish in ponds with periphyton could yield up to about 5 mt ha^{-1}, without supplementary feed. *M. rosenbergii* were reared at 20,000 juveniles ha^{-1}, still an extensive system, by Haque *et al.* (2014) who concluded that periphyton-supported farming, could yield over 36 % improvement in net yield through using additives such as corn flour to maintain a high C/N ratio. Periphyton applications have been shown to maintain higher metabolism, versatility, functional redundancy, and more consistent carbon-source utilization, than biofloc (Lyons & Dobbs, 2012). Hence, further exploration of the potential for manipulation of periphyton with use of carbon supplementation is supported.

2.6 Giant freshwater prawn, *M. rosenbergii*, biology, culture intensity and performance

Dinh and Nguyen (2014) described the evidence for heritable component of male morphotypes and conducted statistical analysis of sex and adult morphotypes. The definitive volume describing the target species, *M. rosenbergii* is “Freshwater Prawns Biology and Farming”, edited by Michael Bernard New (Ed: New, Wiley-Blackwell, 2010), and this volume covers the species comprehensively, up to the date of the latest edition. Brown, *et al.* (2010) produced the chapter “Biology”, and Karplus and Sagi (2010) the chapter “The Biology and Management of Size Variation” within this volume. For the purposes of the current study, characterization of male morphology resultant from HIG is important. Kuris *et al.* (1987) took prior studies into

account and clarified the morphological differentiation of males, showing clear observational distinction of small males (SM), orange claw (OC), and blue claw (BC). Raanan *et al.* (1991) reviewed growth, size, rank, and maturation. Tidwell *et al.* (2015) investigated both all-male and mixed sex *M. rosenbergii*, and the separation of OC and BC for both.

Giant Prawn 2017 was an exhibition held in Bangkok, Thailand, March 2017, organized by Michael New, and Dr. K.R. Salin, Asian Institute of Technology. In this definitive conference of the status of *M. rosenbergii* aquaculture, total production of all *Macrobrachium* species in 2014 was reportedly 217,000 mt, valued at USD 1.2 billion, while in 2016, only 1,500 hectares were reportedly dedicated to *M. rosenbergii* farming in Malaysia with total estimated annual prawn production between 300 and 500 mt, with yields per hectare of 1 to 2 tonnes, and survival rates of 20 to 30 % (Merican, 2017). Pillai *et al.* (2021) have reported yield for *M. rosenbergii* alone in 2018 of 237,124 mt valued at USD 1.946 billion, attributed to recent success in genetic improvement. Factors contributing to reported low return on investment included hatchery supply quality and quantity, low yield per hectare (reported as 2 mt ha^{-1} in Thailand), and long culture period (reported as 8 to 12 months). The yields are extremely low when compared to yields typically obtained by farming marine Whiteleg shrimp, or tilapia (Merican, 2017).

In Malaysia, the supply of mixed sex and all-male *M. rosenbergii* PL post hatchery is adequate and improving, while some hatcheries engage in genetic enhancement and/or biosecure hatchery operations. After the 2017 conference, Pillai *et al.* (2021) reported that *M. rosenbergii* culture to 35 to 40 grams has been achieved in 90 days in Thailand, with concurrent 80 to 85 % survival rate and intensity of about 15 m^{-2} improvement, which is indicative of harvesting of BC males. There is good reason to believe that Malaysian hatcheries can work towards supporting similar achievement by engaging in genetic enhancement. The main factors which will stimulate *M. rosenbergii* aquaculture in Malaysia are low FCR, and improved performance (yield per hectare). The sale price of RM65.00 kg^{-1} ex-farm for 35 g prawns (28 per kilogram) allows farmers to harvest starting from partial harvest at about 4 months through to 8 months for final harvest, until genetic programs improve

performance as described above. High ratios of BC males are typical at 35 g and above. Because the market does not presently demand larger prawns, i.e., significantly bigger than 35 g, justification of additional months in the cycle is typically not considered of value by farmers.

2.6.1 BFT and IPS for culture of *M. rosenbergii*

The central purpose of the current study is evaluation of intensive, integrated biomass aquaculture systems in rearing the target species, *M. rosenbergii*, with emphasis on the innovative development and application of IPS based culture. There are abundant examples of periphyton- and BFT-based aquaculture of a variety of species of fish and shrimp, and strong evidence for commercial potential has been assessed in the many available reviews. Some fish are planktivores that consume biofloc readily, whereas herbivores, detritivores and omnivores prefer periphyton (Martínez-Córdova *et al.*, 2015). Freshwater prawns are assumed to favor periphyton grazing, as it is more efficient for their feeding approach. Prawns are also considered typically coprophagous (D'Abramo & New, 2010).

Periphyton supported culture has been examined at full-scale most often with extensive pond systems. Pandey *et al.* (2014) have summarized research on the effects of a variety of substrates. Organic materials (e.g., bamboo, bagasse, straw, etc.) have been generally favored by extensive pond farmers, in line with their low-cost operation philosophy. Significant positive indications for commercial production have been demonstrated for rohu (*Labeo rohita*), common carp (*Cyprinus carpio*), penaeid shrimp (*Fenneropenaeus paulensis*, *Penaeusesculentus*, *L. vannamei*, *P. monodon*, *M. rosenbergii*, and *Farfantepenaeus merguiensis*), tropical rock lobster (*Panulirus ornatus*), and bottom feeders in general. Azim and Little (2007) have reported increase in periphyton amended production of 30 % for carp monoculture, and 115 % to 210 % for carp polyculture, and production of 9 t ha^{-1} $year^{-1}$ in carp polyculture without any supplementary feed (Azim & Little, 2007).

BFT is considered viable for *M. rosenbergii*, *L. vannamei*, and tilapia such as *Oreochromis niloticus x Oreochromis aureus* (Crab *et al.*, 2012), therefore inclusive of both marine and freshwater species. Emerenciano *et al.* (2013) summarized the history of BFT from development in France in the 1970's with different penaeid species, through the 1990's in Israel and USA with tilapia and Whiteleg shrimp, *L. vannamei* (Emerenciano *et al.*, 2013). In 1988, a Belize Aquaculture farm produced about 11 to 26 mt ha^{-1} $cycle^{-1}$ of Whiteleg shrimp in ponds. Emerenciano *et al.* (2013) further reported BFT is employed in large-scale shrimp farming in Asia, Latin and Central America, and smaller operations in USA, South Korea, Brazil, Italy, China, and other countries. The distinct differences between marine and freshwater shrimp include their occupation of the water column, feeding and communal behaviors, and the impact of saltwater on mineral content, biota intensity, etc. There is little value in comparison between BFT of Whiteleg shrimp and *M. rosenbergii*, mainly because of these differences including those imposed by salinity. Tierney *et al.*, 2020 described analysis of *L. vannamei* for impacts of substrate inclusion to production volume in 50-day nursery RAS, at densities of 1,500 and 3,000 m^{-2}, and recommended high densities combined with substrate due to higher biomass generation. While the result is not incompatible with general conclusions reached in similar trials with *M. rosenbergii*, the marine food chain is dependent on saltwater. Marine biotas react differently to substrate, and their different characteristics and composition affect how the marine culture species utilizes them, and these variables are assumed to overwhelm the utility of direct comparison to freshwater applications.

It is considered reasonable to include BFT culture results in assessment of IPS potential, i.e., for freshwater applications, and there are no reports of a technologically complete IPS approach to culture of *M. rosenbergii*. Densities of *M. rosenbergii* culture are somewhat inconsistently reported. A further negative impact of HIG is the difficulty it creates to sample *M. rosenbergii* from ponds and obtain a representative sample. Feed trays may be dominated and populated by a few BC males removing their usefulness in population measurement. The following review of references to densities of culture of *M. rosenbergii*, provides limited detail on reported performance available from experimental or commercial scale culture.

Global stocking densities were reviewed in New *et al.* (2010), and selected figures are reported in Table 2.2 (New & Kutty, 2010). Sahu *et al.* (2013) reported on field trials at smallholdings in India. Average PL mass of 0.02 g was stocked at 3.75 to 5 m^{-2}. FCR of 1.65 to 1.78 and survival rates of 66 to 91% were reported over cycle times ranging from 188 to 219 days (about 6 to 7 months). Average prawn mass produced was reported as 60 to 90 g. Average production was reported as 1.7 to 3.3 mt ha^{-1} assumed to be on a per year basis. Banu and Christianus (2016) reported that Malaysian production ranged from 0.9 to 3.15 mt ha^{-1} $cycle^{-1}$, which points out a lack of consistent commercial methodology, as well as low production efficiency.

Only general conclusions can be reached with review of the reported global commercial densities and production figures as found in Table 2.1. PL average stocking weight for nursery is typically 0.01 to 0.02 g, while global stocking densities have shown high variability. The detected trend for nursery densities appears to indicate preference for around 200 m^{-2}. Higher survival is important in this initial nursery stage from about 30 up to 90 days. Subsequent juvenile stocking density as second stage nursery, of 40 m^{-2}, appears anyway like grow out densities. From the global review, grow out production of about 3 mt ha^{-1} $cycle^{-1}$ appears typical from the commercial review of conventional, extensive culture.

Table 2.1: Global commercial characteristics reported in Chapter 17 of "Freshwater Prawns Biology and Farming"

Country	PL	Juvenile Nursery	Growout
China	300 to 4,000 m^{-3} (30-day)	1,500 to 3,000 m^{-3} (30-day)	5 to 23 m^{-2} (2 to 3 mt ha^{-1} $year^{-1}$) 45 to 65 m^{-2} (6-7 mt ha^{-1} $year^{-1}$)
India	3 to 5 m^{-2}	2 to 3 m^{-2}	
Indonesia	50 to 100 m^{-2} (60-day)	10 to 20 m^{-2}	
Malaysia	120 to 150 m^{-2}	450 to 1,000 m^{-2}	10 to 15 m^{-2}
Taiwan	100,000 to 500,000 m^{-3} (45-day)		

Country	PL	Juvenile Nursery	Growout
Thailand	19 to 208 m^{-2}	20 to 40 m^{-2} (dominant < 20 m^{-2})	
	60 to 80 m^{-2}	> 40 m^{-2}	
Brazil	400 to 600 m^{-2} (60-day)		5 to 20 m^{-2}
USA	400 to 800 m^{-2}		2 to 7.5 (with substrate) m^{-2} 25 m^{-2} (Hawaii)

Further review of academic research driven culture provides further insight into potentials, starting with research of *Macrobrachium* other than *M. rosenbergii*. Marques *et al.* (2000), working with *Macrobrachium amazonicum*, compared nursery growth for 20 days at 2, 4, 6, 8, and 10 m^{-2}, with nursery growth for 60 days at 100, 200, 300, 400, 600, and 800 m^{-2}. In the 20-day experiment, prawn biomass produced was highest for lowest densities. In the 60-day runs, produced biomass was highest for 400, 600 and 800 m^{-2} densities, while highest mean weight occurred at 100 and 200 m^{-2}, and survival was equivalent for all six densities during the 60-day run. Marques *et al.* (2010) studied PL stocking at 4, 6 and 8 m^{-2} for 28 days, followed by juvenile stocking at 200, 400 and 800 m^{-2} for a further 62 days. Juveniles achieved consistent survival of about 95 % at all densities but highest prawn biomass production was achieved at 800 m^{-2}. Marques *et al.* (2011) then cultured PL at 50, 400, 1,200 and m^{-2} for 60 days, moving each group to grow out at 12 m^{-2}, and found best growth and lowest FCR in the 400 and 800 m^{-2} stock in the latter stage. It was concluded that higher density in earlier stages promoted a growth spurt in the subsequent stage, where density was reduced, and this growth was termed “compensatory growth”. Marques *et al.* (2012) continued with nursery of 0.16 g prawns at 400, 800, and 1,200 m^{-2} for 71 days, with average size achieved of 0.94, 0.61 and 0.48 grams, respectively, all achieving approximately 95 % survival. Prawns were transferred to 20 m^{-2} cages for the next 30 days and the stock from highest density outperformed other densities in terms of average weight, and FCR, again supporting a compensatory growth effect. Compensatory growth has been further considered by researchers of *M. rosenbergii* (Coyle *et al.*, 2010; Karplus & Sagi, 2010; Karplus, 2005). Performance of *M. amazonicum* is generally lower than for *M. rosenbergii*, and the outcomes of research support equivalent or higher nursery densities for *M. rosenbergii*.

Academic-driven culture of mixed sex *M. rosenbergii* with conventional, non-BFT and non-IPS technology, is peripherally relevant to the current study and provides some insights. Aflalo *et al.* (2014) showed that selective harvesting technique, removal of larger prawns in the latter weeks of a cycle, produced the same net production as a single final harvest. However, while single final harvesting gave a size range of 30 to 150 g, selective harvesting produced a more uniform output of 55 to 60 g with better cash flow management. Banu and Christianus (2016) conducted first phase grow out culture on 5.8 g prawns at 20, 30 and 40 m^{-2} densities, and found similar average masses after 120 days.

As discussed above, results of *M. rosenbergii* culture with BFT are relevant to IPS culture. Perez-Fuentes *et al.* (2013) cultured average weight 0.025 *g M. rosenbergii* over 180 days at density 37 m^{-2}, achieving 85 % survival. Average masses for two BFT treatments were 11.5 and 15.2 g. However, these results are well below achievable results from conventional methods reported above. Perez-Rostro *et al.*, (2014) worked with *M. rosenbergii* over 180 days at density 34 m^{-2}, achieving 85 % survival and FCR of 2.27. The BFT system only supplied water to replace losses from evaporation, meaning no wastewater was produced in operation, and represented a 96 % water saving over conventional culture. Hence, while BFT culture of *M. rosenbergii* may not produce exceptional results, it is workable in water constrained situations and does remove both environmental impact of wastewater discharge, and the need for additional wastewater treatment equipment. Negrini *et al.* (2017) worked with *M. rosenbergii* in a BFT system, over 60 days at densities 50, 100, 150, 200, and 250 m^{-2}, from 0.32 g average mass of input prawns. Tank sizes were only 50 L and depth shallow. Total biomass produced was highest for the highest density, but 50 m^{-2} density produced highest survival rate and lowest FCR. This study recommended 50 m^{-2} density for commercial grow out operations. Global review has indicated that a density of around 200 m^{-2} is more appropriate in the first 60 days for optimum output.

Coyle *et al.* (2003) used vertical substrate netting at about 50 % of bottom area in grow out of 0.6 g average weight prawns, for 106 days at density of 6 m^{-2}, for average weight of 46 g, FCR of 2.3, and survival rate of 94 %. The calculated yield was 2.6 mt ha^{-1} $cycle^{-1}$. Yield in this experiment defined opportunity for commercial

production at lower density. Compared with 40 m^{-2} density however, a commercial operation would require over 500 % more area for a similar production.

Marlowe (2006) conducted nursery culture at 4,600 and 4,750 m^{-2} for 60 and 40 days, respectively, using 3 to 4 layers of horizontal mesh substrate, obtaining 70 and 63 % survival, respectively. Grow out at density of 2.74 m^{-2} was conducted with vertical net substrate in ponds, estimated above 100 % of pond area, for 30, 60 and 126 days, with survival ranging from 82 to 88 %. The substrate application in nursery phase indicated performance potential to support high densities. Applied to grow out, the low density applied did not demonstrate increase over typical commercial culture results.

Asaduzzaman *et al.* (2008) conducted grow out for 120 days of average 5 g prawns using substrate area equivalent to 100 % of bottom area, at 2 m^{-2}, and achieved 72 % survival and 46 to 56 g prawns, yielding 480 kg ha^{-1}, which they translated to 1.5 mt ha^{-1} $year^{-1}$. Azaduzzaman *et al.* (2010) applied substrate area equivalent to 100 % of bottom area, at 10, 15 and 20 m^{-2}, for 120 days, starting from a 5 g average mass. Rather than reporting performance, they indicated that periphyton had high value in terms of improving results, and recommended use of substrate periphyton to support higher density culture.

However, Mamun *et al.* (2012) conducted nursery culture of average 0.06 g PL with substrate at low density of 4 m^{-2}, comparing substrate types of branches, netting and bamboo, for 100 days, resulting in 66 % survival for all substrates. The highest average weight of 7 g prawns was achieved for branches and netting. The low yield in this instance demonstrates that more evidence is required even in low density culture to establish an advantage in commercial practice. Tuly *et al.* (2014) conducted grow out culture of average 0.4 g prawns with bamboo substrate at density of 20 m^{-2}, over 150 days, resulting in 63 % survival and average 56 g prawns. FCR was high at 3.15 for production of 1.4 mt ha^{-1} $cycle^{-1}$. The result was in line with good global commercial performance.

Haque *et al.* (2014) utilized BFT and periphyton combined grow out at density 2 m^{-2} for 90 days. BFT was operated with C/N ratio of 20:1 using maize starch, but

bamboo substrate was presumed to be 50 to 100 % of bottom area. 5.4 g average weight prawns were reared to average 34 g with 84 % above 33 g, at 76.4 % survival rate, and extremely low FCR of 0.36. While distinction between periphyton and BFT biomass in contribution to the outcomes could not be determined, 428 kg ha^{-1} for the 90-day cycle translated to up to about 1.7 mt ha^{-1} $year^{-1}$. The results of this prior study revealed commercial potential in achieving reasonable yield with extremely low FCR, albeit at very low density. A most effective grow out operation with periphyton was conducted by Chavez *et al.* (2015) with grow out culture of average 2 g prawns, utilizing substrate of bundled twigs, at density of 20 m^{-2}, over 160 days, resulting in approximately 85 % survival for 46 to 52 g prawns. Hence, periphyton has the potential to permit highest productivity at higher density in grow out.

The availability of all-male PL in Malaysia, either produced in local hatchery or imported from Thailand, stimulated examination of their potential in the current study. Saddiqi *et al.* (1997), cultured average 1.8 g all-male in concrete tanks at 5, 10, 15 and 20 m^{-2} densities, over 168 days. The survival rate, average mass, and FCR, ranged from 93 % to 70 %, 29.6 g to 17.4 g, and 3.7 to 5.6, respectively, which were not exceptional as commercial results. Sagi *et al.* (1985) found that all-male could generate 4.7 mt ha^{-1} in 150 days, or 9.4 mt ha^{-1} $year^{-1}$. The commercial follow-through of this potential has not been truly demonstrated to date. Nair *et al.*, (2006) found all-male over 60 % more profitable than both mixed sex and all-female *M. rosenbergii*, albeit with only about 3.6 mt ha^{-1} $year^{-1}$. Phansawat *et al.* (2022) were able to use visual detection of the male gonopore complex at 43 days (0.78 g) to separate sexes. All-males grew to 93 g in 183 days, compared with mixed sex reaching 69 g in 225 days. All-male culture could generate 2.08 mt $harvest^{-1}$, compared with mixed sex generation of only 1.27 mt $harvest^{-1}$. Thus, there is evidence that all-male represent a strong commercial potential in *M. rosenbergii* aquaculture. However, Banu *et al.* (2016) reared all-male *M. rosenbergii* over 120 DoC, identifying three male morphotypes (SM, OC, and BC) before additional grow out of 80 days. At the total of 200 DoC, a mean of only 20 to 26 g Body Weight (BW) in nursery for densities from 20 to 40 m^{-2} was achieved.

2.6.2 Nitrogen compounds in the rearing of *M. rosenbergii*

M. rosenbergii is a robust species in terms of its ability to manage higher levels of Total Available Nitrogen (TAN). Braga *et al.* (2022) tested the species in nursery conditions for 58 days at high intensity of 375 m^{-2} for both BFT and water exchange system (WES) treatments. Survival rates for both treatments were both 65 % at maximum tested TAN level of 40 mg L^{-1}, while water exchange in BFT was only one tenth of that in the water exchange system. Azaduzzaman (2008) experimented with varying C/N ratios from 10 to 20 in periphyton and non-periphyton systems, finding that periphyton system operation resulted in lower NO_2, TAN, and NO_3 levels, while total nitrogen levels were similar for the two treatments. Values for NH_4-N and NO_2-N were well below the 0.5 mg L^{-1} level, which is the level where no deleterious effects were noted by Valenti *et al.* (2010). Negrini (2017) also found NH_4-N and NO_2-N were well below these levels in investigation of *M. rosenbergii* in BFT for densities ranging from 50 to 250 m^{-2}. The recommendation was not based on impacts from higher levels of NH_4-N and NO_2-N, but on performance factors.

2.7 Statistics of aquaculture research

The current study has used larger volume tankage rather than aquariums, in the belief that volume and depth are features which impact the commercial aquaculture environment. As a result, aquarium scale experiments may not yield results reflecting community effects operating at commercial scale. The IPS used in the current study provides 3D mat substrate with additional grazing area to support growth and survival at higher densities, essentially increasing effective volume. The RAS used in the current study provides grazing area alone to support growth and survival at higher densities. Higher densities benefit from more grazing area.

Statistical power increases with increased effect size, the number of replicate tanks and the number of aquatic species within each replicate tank, while statistical power is reduced with increased variance and number of treatments tested (Ling & Cotter, 2003; Ling, 2007; Smart *et al.*, 1998). Smart *et al.* (1998; 1997) suggested the use of experimental cross-over design by replicating the treatment application over

time in a single experimental unit. Treatments performed successively on the same experimental unit, means that the treatment effect would be compared within tanks. Phases B1, B2, and B3 of the current study were not strictly cross-over studies but the same tanks were used in each case.

Thorarensen *et al.* (2015) have evaluated fish growth studies in review of the literature. They point out that experimental designs are hindered by resource constraints from providing optimal number of rearing units, or of optimal population in each tank (n). The survey of publications by Thorarensen *et al.* (2015) suggested that over 80 % of 24 growth studies published in the Journal Aquaculture over a full year applied treatments in triplicates with populations per tank ranging from 4 to 100. Araujo (2008) suggested that more replicates (at least three) are better. However, the use of two tanks instead of three could be acceptable with considerations such as allocating the control to the lesser number of tanks (Araujo, 2008). Professor V.T. Balinas, University of Philippines Visayas suggested a range of 4 to 8 replicates for agricultural research, and that most aquaculture research uses 3 replicates based on little expected variation. At this point in time, it has become clear that factors such as sex ratio, the microbiome of culture species and of the environment are relevant to experimental results. Aquaculture as an applied science frequently does not report on idealized statistical experiments but does scientifically observe statistical events and use inferential statistics to evaluate performance. Experimental design must balance resources and reliability of statistical analysis (Thorarensen *et al.*, 2015).

After evaluation of the variance of data in such growth studies, Thorarensen *et al.* (2015) suggested that variance is similar for different aquaculture species, and that the same levels of replication and of n are suitable for studies on different species of fish. The current study is aware that HIG has a large impact on *M. rosenbergii* culture, and that study could isolate periods of low HIG growth, from periods of high HIG growth. In the current experiment on *M. rosenbergii*, it was considered most effective to separate the nursery stage to 43 DoC as an experiment (in Phases B1 and B2). Separation of the first 60 DoC of grow out (DoC 44 to 104) was also considered a period of growth where less variance would result (in Phases B3 and B4). The results of these separated periods decreased variance by limiting changes over their durations

for the two treatments. Growth in subsequent DoC, i.e., post 100 days, sees the full development of BC males, such that partial harvest can be considered. Also, the consecutive experiments supported reflection on the dynamic of change between the first and second periods of growth.

The current study populations were always greater than 100, hence the minimum difference in mean BW body-mass of different treatment groups that could be detected with triplicate tanks would be 19 % of the grand mean or slightly less (Thorarensen *et al.*, 2015). Analysis with a mixed model ANOVA approaches population responses more closely than with non-linear models (Thorarensen *et al.*, 2015). Oksanen (2001) makes the point that a decision to refrain from using inferential statistics in the context of less than perfectly replicated experiments is as irrational as a hiker declining to hike a trail when the known trail ends a bit before the actual destination. The experimental designs employed in the current study used inferential statistics to observe relationships in a progressive series. While experiments were less than perfectly replicated, it has been applied as common practice in aquaculture research, and the nature and progression of the current study sought to extract the most useful data possible under typical constraints.

2.8 Summary and implications

The current study finds support for its hypotheses as follows:

Periphyton biomass is produced in-situ on and within an appropriate media, which will uptake both culture species waste excretions and waste feed in an intensive tank culture, and the biomass generated is beneficial as high value feed, converting waste to replacement of supplementary feed (Emerciano *et al.*, 2013; Avnimelech, 2006; Avnimelech *et al.*, 2009; Avnimelech, 1999; Crab *et al.*, 2012; Haque *et al.*, 2014; Crab *et al.*, 2007). Haque *et al.* (2014) described how a combination of BFT and periphyton was able to rear prawns with an FCR of 0.36. Martinez-Cordova *et al.* (2015) elaborated that BFT, and by extension IPS, maintain in-situ microbiota populations by minimizing water exchange.

High specific surface area media with high void space improves the co-existence of surface heterotrophs using oxygen, with other bacterial consortia residing in lower oxygen and flow conditions within the media, while additionally supporting multi-cellular organism culture on the surface and in the media void space. A diverse ecosystem most effectively processes wastes to active biomass and delivers multiple nutrition opportunities to culture species (Pedros *et al.*, 2005; Lofrano & Brown, 2010; Paerl & Pinckney, 1996). Paerl and Pinckney (1996) particularly defined the capabilities of aquatic consortia microenvironments.

Carbon addition in the form of carbohydrates, i.e., molasses, enhances heterotrophic organisms, which ensures that water quality is maintained. Heterotrophs are complementary to the full consortia of organisms in the periphyton media layers, contributing to a maximum of nutrition and health promoting biomass (Crab *et al.*, 2007; Pandy *et al.*, 2014; Martinez-Cordova *et al.*, 2015). Crab *et al.* (2012) defined how inexpensive carbon sources equated to improved water quality with decrease in supplementary feed requirements, contributing to economic realization of sustainable aquaculture.

Omnivore and detritivore species are well adapted to grazing on periphyton biomass and may prefer it to supplementary feed. They may prefer grazing to feeding from the water column (Martinez-Cordova *et al.*, 2015). Brown *et al.* (2010) described how *M. rosenbergii* feed within the water column at night and on the bottom during daylight hours. Kawamura *et al.* (2018) described the mechanism by which *M. rosenbergii* collects and feeds upon pelleted feed, which with sinking feed is a bottom-feeding mechanism. Moller (1978) described *M. rosenbergii* PL ability to access live feed in the water column.

Periphyton, integrated biofilter (IPS) processes both excreted waste and waste feed into primary biomass, while maintaining water quality parameters acceptable to the culture species, without an external biofilter, and while minimizing waste discharge to the environment (Emerciano *et al.*, 2013; Crab *et al.*, 2007; Martinez-Cordova *et al.*, 2015). Emerciano *et al.* (2013) described the function of integrated microorganisms in aquaculture production volumes as having the major roles of

maintenance of water quality, in generation of microbial protein, and as nutrition source to the prawns which reduces FCR.

Periphyton media surface area is used by high density populations of surface-area-dependent species, utilizing tank volume more effectively. Increased domain area reduces competitive, hierarchical, and cannibalistic pressures between cultured individual animals (McCallum, *et al.*, 2018). This is a logical extension of the presence of feed on the substrate in the production volume, and the feeding mechanism of *M. rosenbergii* (Kawamura *et al.*, 2018; Indulkar & Belsare, 2004, McCallum *et al.*, 2018).

Periphyton integrated aquaculture will exert improvement in health (survival rate), and nutrition (FCR and growth rate), over non-periphyton aquaculture for certain aquaculture species. Nonetheless, such improvements hinge on low water exchange that maintains the in-situ microbiota populations needed (Martinez-Cordova *et al.*, 2015).

The benefits of IPS in providing water quality management, supplementary feed, and health benefits, will balance or outweigh the costs of system equipment elements, carbon supplement, and additives, in comparison with extensive ponds, RAS, or BFT, in culture of the target species, *M. rosenbergii*. Kumar *et al.* (2019) provided convincing evidence that utilization of periphyton Aquamat™, like Matala™ media employed in the current study, demonstrated an economic improvement over economics of more extensive methods. Although a marine system stocking *L. vannamei*, the system demonstrated improved economic return of semi-intensive shrimp farming, increasing net income generation by over 35 %, and reducing both the breakeven point and feed cost in a field trial of 120 days at low 20 m^{-2} density.

Table 2.2 sets out the conceptual experimental components, the attributes related to the selected culture species, and the main outcomes. The table is prepared in consideration of specific application to the target species, *M. rosenbergii*.

Table 2.2: Advantages of IPS for Giant freshwater prawn aquaculture

IPS Component and Operational aspect	Attributes	Outcomes
Substrates: A: Substrate with 2 sides (typically angled) B: Substrate Tubular units for ecdysis protection	Domain territorial area and security are both increased (Refer to Appendix 1)	• prawn intensity is calculated based on available surface area, resulting in potential for higher stocking density of tank volume • separated habitat for prawns is more secure during ecdysis. Grazing movement on substrate differs from horizontal bottom grazing
Media consortia: Edible biomass (algae, bacterial consortia, multi-cellular animals) is fixed on and within the periphyton media	Live feed, probiotic microbiota and enzymes are constantly available to culture species in grazing	• all the advantages of Biofloc Technology pertain (feed for nutrition benefits, amplification of useful consortia by low-cost carbon source, consortia optimize waste nutrient conversion to protein, and consortia transfer enzymes and other beneficial molecules that assist digestion to culture species) • economical feed replacement with periphyton biomass • added available grazing decreases cannibalism and stress • no energy requirement to maintain biomass suspension
Media Consortia: Integrated biofilter	Water quality management without external biofiltration	• smaller footprint with no external facilities • water quality control integral to culture mass • water quality control responds to all impacts immediately as consortia

IPS Component and Operational aspect	Attributes	Outcomes
		have very fast evolution to advantage changed conditions
Media Consortia: Waste digestion	Waste management with conversion to feed	• smaller footprint with waste management integral to culture mass • waste value preserved as high value feed replacement
Media: Biomass with minimum pathogen is fixed on periphyton media surfaces	Pathogens are exposed to large heterotrophic populations and deterred from multiplying	• consortia neutralize waste from availability to pathogens, and suppress pathogens by quorum sensing mechanism
Progressive population density management: Volume and density appropriate to animal size. Selective harvesting	Ability to split growth stages and sexes. Removal of larger males to remove barrier to growth in smaller males. Sex identification after about 45 - 90 days	• Selective harvesting maintains growth of small males, removing hierarchical pressure, increasing achievable stocking density at each size range • Morphological and gender separation by visual identification can be possible during population transfer
Single sex (case of all-male): Remove complication of managing dual sexes	Reduced competition due to no female presence. Higher growth potential and marketability of males accessed	• Removing one gender to reduce sexual competition, to reduce stress between stages of male development • Removing less-desirable females to target uniform BC males

Suitably angled periphyton substrate (the first item of Table 3.4), increases available domain territorial, and resting area, while it prevents excessive solids accumulation. Prawn preference for moulting on an incline is noted by McCallum *et al.* (2018). Prawn intensity may be calculated based on available substrate area, or on

plan area of tankage. The latter, which is most common in the literature, results in higher calculated stocking density of tank.

Tubes have long been used in prawn aquaculture for protection during ecdysis, much as they have been used for protection during gestation and birth for finfish, and their value has been established for *M. rosenbergii* as well (Moller, 1978). The design developed (Appendix 1) provides a maximum number of tubes, with flexibility of design dimensions, for a minimum of material used. Substrate with high specific surface area and void space supports an ecosystem of nutrient conversion pathways by microbiota. Periphyton biomass becomes fixed on and within the periphyton structural media, implementing IPS efficiently. If dissolved oxygen is sufficient, no mixing energy is required to maintain suspension of biofloc, as it is fixed in attachment to the media. In ponds, suspended matter typically includes a large proportion of algae, which also becomes part of periphyton through attachment. The production species have access to the periphyton as a feed source. The tube design was deployed in all phases of the program. In the commercial Phase C, the tubes were deployed as a separate string, rather than being attached to the media as is shown in Appendix 1.

More species are capable of grazing on periphyton biomass than typically ingest suspended biofloc. Biofloc needs to maintain a floc size to be accessible to some species, whereas periphyton is grazed by herbivores, detritivores, and omnivores. Advantages of nutrition will accrue through grazing on this live feed source. Periphyton biomass production is enhanced through addition of low-cost carbon source, amplifying production of heterotrophic bacteria, as is carried out in BFT. The periphyton substrate protects a full range of nutrient recycling bacterial consortia, e.g., anaerobes, chemotrophic, and autotrophic bacteria, by providing gradient from oxygenated surface to low flow and low oxygen interior. Matala™ substrate employed in both the experiments and commercial phase had very high surface area to volume. Matala™ brand FSM400 provided 400 m^2 m^{-3}, while retaining 94 % free void volume (http://www.matala.com.tw/biomedia/matala-filter-media.html). This material is like Aquamat™ media, employed by other researchers (Audelo-Naranjo *et al.*, 2010; 2011; 2012; Siddiqui *et al.*, 1997). High free volume in substrate provides a favorable environment for production of multi-cellular animals feeding on produced bacteria,

which ultimately become multi-cellular, live feed for the culture species. The constant availability of live feed, while displacing supplementary feed, may further reduce cannibalism, and may encourage growth of SM and OC males through provision of available grazing area with less competition pressure from BC males. Digestive enzymes used by bacteria are transferred to the prawn upon ingestion, and remain viable in reducing prawn metabolic energy requirements, while facilitating feed utilization. Live microbial and multicellular feed, produced from waste, improves economics by reducing FCR of supplementary feeding.

Integrated biofilter of an IPS or BFT maintains water quality while removing the need for a stand-alone biofilter in a separate tank. Mechanical systems for biofilter recycle, and their energy requirements are removed, reducing operation and maintenance costs, and there is a net reduction in system footprint with removal of the need to separately treat and manage effluent, in comparison with RAS. The water exchange rate for the systems approaches zero, except for evaporation and cleaning losses.

Periphyton biomass (Table 3.4) not only consumes excreted waste products, but also waste feed. As in BFT, more value of supplementary feed is retained for growth, reducing FCR while reducing or removing the need to process waste. Just as BFT biomass generates health advantages, periphyton biomass provides equivalent or better services (Table 3.4). Periphyton has less tendency to retain pathogens than does biofloc. Yet, periphyton can be designed to maintain equivalent or higher microbial density in a unit production volume in comparison with biofloc. Quorum sensing effect suppresses pathogen expression due to present and active non-pathogenic microbial biomass, representing high populations of non-pathogenic bacteria. In addition, anti-pathogenic compound PHB can be ingested from bacteria and accumulate in prawn to increase disease resistance.

Culture populations can be split to greater production volumes as they mature. Changing populations of culture species at various stages of growth maintains optimum densities during different stages of maturation (Table 3.4). Selective harvesting is another means to achieve this benefit in *M. rosenbergii* culture. Over the

last third of a growth cycle, repeated removal of large males removes the hierarchical pressure on smaller males that restrains them from further growth.

Lastly, due to the advancements in hatcheries over the past decade, all-male *M. rosenbergii* PL are readily available. Cost is approximately 50 % more (i.e., RM0.13 vs RM0.08 per PL), but the impact of higher price on overall economics of farming is relatively small, provided there is performance improvement. It has been postulated that removal of females from the population can reduce aggressive tendencies in *M. rosenbergii* males since there is no competition to mate with females. Less aggression can reduce stress and cannibalism. Furthermore, females grow differently than males, typically reaching a final plateau at, or below a marketable 35 g. The presence of females in marketed product can decrease uniformity, and there is also a preference for males in the market with BC male claws being identified with the *M. rosenbergii* product. Results from Phases B5, B6, and B7, and the Phase C commercial farm results, were generated with IPS culture of all-male *M. rosenbergii* stock.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter of the thesis outlines the design and methodology of the research. The methodologies employed during the project are observational, and experimental, with addition of a simulated growth and feeding model refined over the course of experimentation. The experimental design included an initial phase minus culture species to permit start-up and verification of the technological approach, the Integrated Periphyton System (IPS). Subsequent experimental phases developed triplicates of treatment for correlational and comparative analysis to substantiate aspects of the hypotheses in Phases B1 to B4 for mixed sex *M. rosenbergii*, and Phases B5 to B7, and C for all-male *M. rosenbergii*. A patent application was developed (approved) as an elaboration of the core technology under development (Refer to Appendix 1) which elaborates a means of implementing an IPS in full-scale pond aquaculture. Full-scale commercial aquaculture production employing the technology permitted presentation of preliminary results from commercial operation as Phase C implemented by the author, for culture of all-male *M. rosenbergii*. Observational data was collected in each phase, outside of instrumented, laboratory analytical, or other physical measurement method (e.g., temperature, length, or mass). Observed behaviour of culture species included observation of morphological features, and assessment of sex ratio for cultured individual animals. The growth and feeding simulation, as an excel spreadsheet model, was refined for adoption by the commercial operation providing insight into conversion of experimental parameters to full-scale commercial operational parameters.

The timeline of the experimental research is presented in summary in Table 3.1, with summary description of the key contribution of each major activity. Table 3.1 also associates the applicable objectives with each individual phase. Experimental methodology for Phases A, B1 to B7, and C is described in Table 3.2. Figure 3.1 presents the experimental organization of Phases B1 to B3 which compared IPS with RAS. The culture of two different hatchery populations of *M. rosenbergii* for a 30 DoC nursery period was examined in Phases B1 and B2. Subsequent post-nursery culture of a smaller average mass subset of Phase B2 nursery harvest was examined in Phase B3. Phase B4 examined an IPS grow out of larger average mass subset of prawns from nursery Phase B2.

Table 3.1: Timeline and research contribution of phases

From	To	Phase Code	Activity Description	Contribution
4-Oct-18	**23-Nov-18**	A	Periphyton Media Test 1.5 m^3 tank, 1.5 m^2 with 1.3 m^3 liquid volume	Operational Integrated Periphyton System (IPS) module without culture species
19-May-19	**31-Jun-19**	B1	Triplicate IPS Treatment vs single RAS Control - Areas of 1.5 m^2	44-day experimental output at 333 m^{-2} mixed sex PL Study objective 1
2-Mar-20	**13-Apr-20**	B2	Triplicate IPS Treatment vs Triplicate RAS Control - Areas of 1.5 m^2	43-day post hatchery experimental output at 1,000 m^{-2} mixed sex PL Study objectives 1, 2 & 3
14-Apr-20	**15-Jun-20**	B3	Triplicate IPS Treatment vs Triplicate RAS Control - Areas of 1.5 m^2	Phase B2 extended from Day 43 to 104-day post hatchery at reduced densities, for smaller average mass subset Study objectives 1, 2 & 3
14-Apr-20	**15-Jun-20**	B4	Single IPS Treatment in 8 m3 tank	Phase B2 extended from 43-day to 104-day post hatchery at 220 m^{-2}, for larger average mass subset Study objectives 2 & 3
27-Jun-22	**31-Jul-22**	B5	Four 10m x 10m Hapa nets suspended in an IPS managed pond	0 to 35-day post hatchery experimental

From	To	Phase Code	Activity Description	Contribution
				output at 250 m^{-2} all-male PL Study objectives 2 & 3
1-Aug-22	**31-Aug-22**	B6	Triplicate 230 Litre IPS with all-male	36- to 64-day post hatchery experimental output at 200 m^{-2} from Phase B5 harvest Study objectives 2 & 3
1-Sep-22	**1-Oct-2022**	B7	Triplicate 230 Litre IPS with all-male	65- to 95-day post hatchery experimental output at 200 m^{-2} from Phase B6 harvest Study objectives 2 & 3
27-Jan-22	**10-Aug-22**	C	Commercial observation of a single all-male pond culture	Full cycle all-male from hatchery PL up to 95 DoC nursery, and grow out to total 195 DoC Study objectives 2 & 3

Table 3.2: Experimental methodology summary

Phase	Activity Description
A	Periphyton Media were constructed of layered mesh material to achieve a specific surface area per unit volume of media and high pore space such that periphyton biofilm could be assumed to occupy the surface area of the 3-dimensional filamentous material volume.
	Aeration, feed, carbon source (molasses), and alkalinity source (sodium bicarbonate) were provided in proportion to an equivalent prawn stocking density of the tank and supplementary feed, but without culture animals. The media periphyton was being tested for ability to consume the input TAN, while maintaining water quality parameters.
	Two tanks of 1.5 m^2 were operated in parallel, each containing the calculated media quantity, and Water Quality was monitored for 50 days.
B1	Periphyton Media material was exchanged for a commercially manufactured polypropylene fibre mat with 300 m^{-2} m^{-3} specific surface area, 94% void space, in sheets of 30 mm thickness.
	Three tank replicates of IPS treatment (1.5 m^2), and a fourth RAS tank (1.5 m^2) with attached biofilter, were operated for 44 days culture of mixed sex *M. rosenbergii* at 333 m^{-2}.

Phase	Activity Description
B2	Triplicate IPS Treatment versus triplicate RAS Control (all 1.5 m^2) were operated in parallel for 43 days in parallel.
	Media installation was altered to 30 degrees from vertical.
	Resulting harvest was sampled, weighed, and counted. Produced prawns were separated to two sets of small and large prawns.
B3	Triplicate IPS Treatment vs Triplicate RAS Control (all 1.5 m^2)
	Selected small prawn sets from Phase B2 harvest were returned to the 3+3 tanks for further growth at lower densities.
	Both triplicate sets of tanks were operated for a further 60 days before production was weighed and counted.
B4	Single IPS Treatment in 8 m^3 tank
	Large prawns from Phase B2 harvest were transferred to an IPS tank of 8 m^3 for further growth at a density of about 220 m^{-2}.
	8 m^3 tank was operated for a further 60 days before production was weighed and counted.
B5	HAPA nets of 10 m x 10 m x 1 m depth were suspended in IPS equipped larger pond, pond J2, of 1,800 m^2. All male *M. rosenbergii* were stocked at 250 m^{-2} for 35 days of nursery.
B6	Triplicate IPS tanks of 0.33 m^2 were stocked at 200 m^{-2} with all male *M. rosenbergii* from Phase B5 for 30 days of post-nursery.
B7	Triplicate IPS tanks of 0.33 m^2 were stocked at 100 m^{-2} with all male *M. rosenbergii* from Phase B6 for additional 30 days of post-nursery.
C	Commercial high intensity pond aquaculture utilizing IPS for all-male *M. rosenbergii* from hatchery PL to 195 DoC

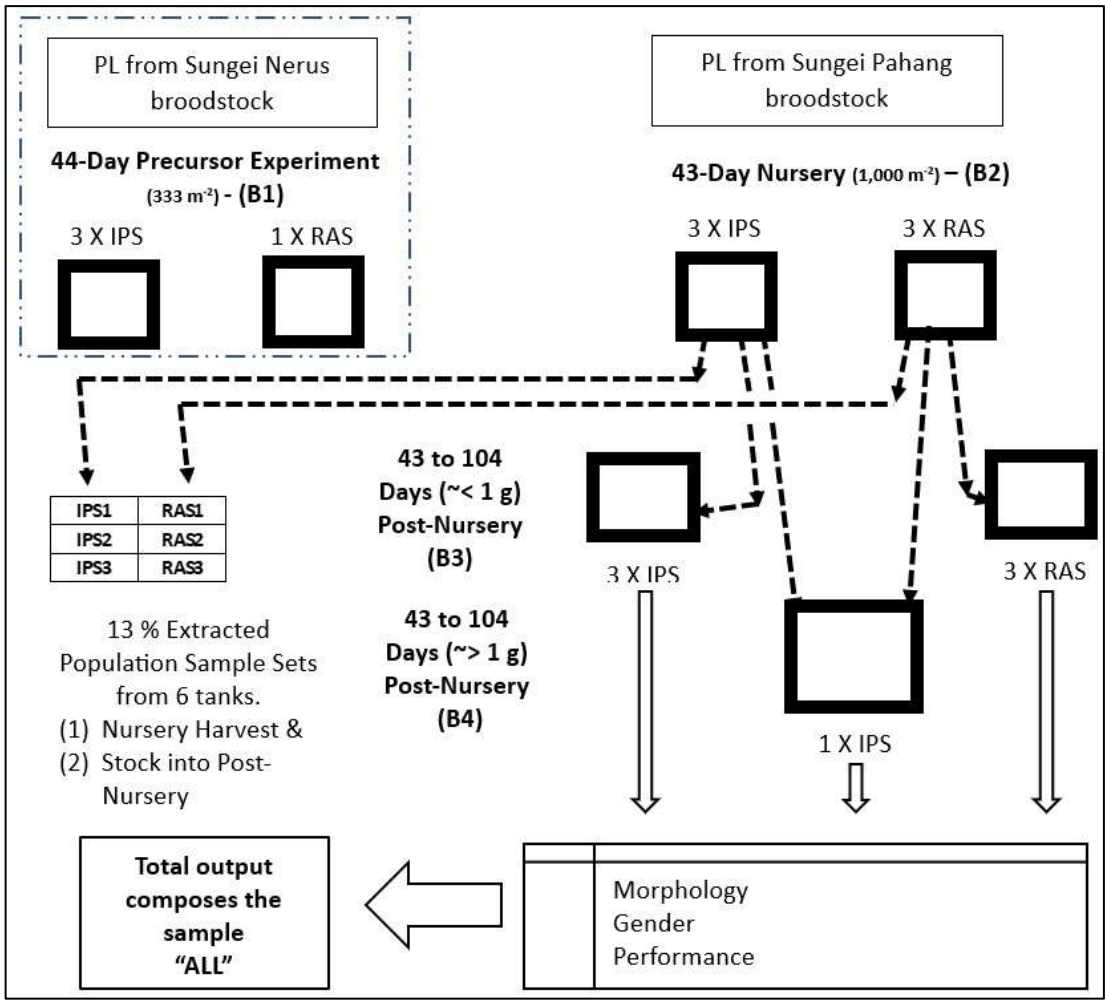

Figure 3.1: Experimental organization of Phases B1, B2, B3, and B4

The use of biofilter media throughout the experimental phases was not applied to minimize media quantities. In all phases, the media specific surface area exceeded the requirement of the biofilter model, originally applied in Phase A. Appendix 2 (b), sets out the applied biofilter area versus design requirement for each phase. Observational methodology for the stages is described in Table 3.3.

Table 3.3: Observational methodology

Phase Code	Common Activity Description	Observational Details
A	A biofilter design model was used to size the media and develop a synthetic feed and additives program (aeration, feed, carbon, and alkalinity sources)	Water quality parameters T, pH, DO, TDS, NH_4-N, TSS, and alkalinity were recorded.
B1	A feed and additives model determined the supplements (feed, carbon, and alkalinity sources) required by a mixed sex culture population. Water quality parameters T, pH, DO, TDS, NH_4-N, TSS, and alkalinity were recorded. Performance characteristics of SR, ADG, FCR, BWG, were recorded. Residual biomass was collected and measured at harvest.	440 separate input PL and input population were sampled by length and mass.
B2		Input was separated by size criteria, and for each of the two harvest sets, small and large prawns, morphological and performance data were collected for comparison.
B3 to B4		Harvest was broken into two groups, small below 1g and remaining large prawns, counted, and weighed. Female, Blue Claw (BC) males, and non-BC males were grouped and counted with respective weights of the groups. Input prawn data was from Phase B2.
B5	A feed and additives model determined the supplements (feed, carbon, and alkalinity sources) required by an all-male culture population. Water quality parameters T, pH, DO, TDS, NH_4-N, TSS, and alkalinity were recorded.	Performance characteristics of SR, ADG, FCR, BWG, were recorded at harvest. Total transfer of 200 prawns to Phase B6 was weighed for average mass. A separate sample of 44 prawns were measured and weighed. Total harvest of Hapa nets was measured and recorded.
B6 and B7	A feed and additives model determined the supplements (feed, carbon, and alkalinity sources) required by an all-male culture population.	Input prawns from Phase B5 and sequential harvest populations from Phases B6 and B7 were sampled by length and mass. Water quality parameters T, pH, DO, TDS, NH_4-N, TSS, and alkalinity were recorded. Performance characteristics of SR, ADG, FCR, BWG, were recorded. Residual biomass was collected and measured at Phase B7 harvest.

Phase Code	Common Activity Description	Observational Details
C	A feed and additives model determined the supplements (feed, carbon, and alkalinity sources) required by an all-male culture population.	Commercial observation of all-male pond culture, in which nursery and grow out to a total of 195 days was evaluated. Commercial sales records permitted evaluation of farm efficiency. Water quality parameters T, pH, DO, TDS, NH_4-N, TSS, and alkalinity were recorded for the subject pond, and additionally for a set of 5 IPS ponds over a 3-month period.

3.2 Design of the IPS

It is necessary to demonstrate that the IPS design will maintain water quality, and that the target species will respond favourably to its special characteristics. While a greater number of treatments with replications may be possible in smaller, aquarium tanks, the prototype scale system is considered essential to effectively evaluate community performance of the target species, relevant to commercial operation. The hierarchical male morphology, HIG, and the separate morphology of females justify working with a larger population to reflect community performance.

Post larvae (PL) received from hatchery have been retained from egg hatching until full conversion of the population is achieved, where PL have rotated and resemble miniature adult prawns. PL after rotation are typically about 14 to 20 days from hatching for mixed sex and for all-male. Release of PL from the hatchery typically occurs as the last PL of a batch achieve conversion. All inputs of PL from hatchery, released to experiments, have been designated as PL of zero (0) days. Subsequent treatment was designated to start from PL-0 up to conclusion of treatment.

The treatment system (IPS), in comparison with a control (RAS) system, was operated over a 43-day nursery phase, Day of Culture (DoC) 0 to DoC 43, with definitive results of treatment versus control. First phase grow out from DoC 43 up to DoC 104 was carried out on selected populations and included testing and generation

of proof of concept for commercial culture. Trial of the IPS operating with all-male was separately established at similar densities to mixed sex trials. Commercial scale results available from operation of a commercial IPS system, stocked with all-male population, are also reported.

3.3 Phase A: establishment of IPS running system

The experimental objective is based upon wastewater treatment principles whereby the periphyton media consists of more than just a 2-dimensional surface for biomass. The bulk of aquaculture literature does not describe the 3-dimensional potential of periphyton, other than to model biofiltration such as is used in RAS. The experimentation utilizes the principles of wastewater treatment by fixed film biomass through the employment of 3-dimensional substrate (Appendix 1), within which an ecosystem of microbes exists and integrates anaerobic, facultative, and aerobic bacteria in conversion of compounds of waste, inclusive of recycling microbial waste. Following the principles developed in BFT, carbon source is added to match the calculated nitrogen supply in a 20:1 ratio, which supports aerobic heterotrophic bacteria. Biomass in turn forms a good live feed, processes wastes quickly, and deters the development of pathogenic organisms.

3-dimensional media was constructed from screen mesh layers, and preliminary evaluation carried out to determine if the aerobic environment, together with the periphyton media would manage the water quality in the production volume. Five layers of HDPE insect mesh were formed into plates of 0.5 m^2 each. Three layers were assembled on frames in each tank with two plates per layer, resulting in a total of 3.0 m^2 of the mesh layer media. Given mesh thread diameter of about 0.3 μm with spacing of 0.75 mm in two directions, an approximate surface area of 16.8 m^2 for the 3 m^2 of mesh layer media was obtained. Using commercial media with 300 m^2 m^{-3} and 30 mm thick provides a similar surface area of 17 m^2 from the same substrate area. This fact supported conversion to commercial substrate used in later experimental phases, through to commercial operation. Commercial substrate was considered more uniform and long-lasting than hand-made substrate, hence more amenable to

commercial operations. Two tanks, each of 1.3 m^3 effective volume, were aerated by diffuser and air supply from compressor was maximum 60 L min^{-1} per tank.

No culture species were included in Phase A; hence no culture tubes were provided. The experiment established qualitative evaluation of whether water quality could be maintained under conditions of excess feed in the production volume, using high specific surface area media. Alternatively, the model tested the result of taking a RAS biofilter media set and inserting it into the production volume. In effect, Phase A was a simple evaluation of periphyton biofilter capacity which would be used in IPS production volumes, and in RAS biofilter modules in later experiments.

A model from NC State University (Lasordo & Hobbs, 2000), that was developed to size external biofilter for RAS aquaculture tanks, was a principal guide in formulation of calculations of feed, total available nitrogen (TAN), nitrification, required biofilter surface area, and to check oxygen requirements, as reflected in Appendix 2 (a) and the following Table 3.4. Appendix 2 (b) describes the media deployment in later phases.

Table 3.4: Biofilter model for Phase A

Parameter	Quantity	Unit	Origin, Calculation, or Description
Tank effective volume	1.3	m^{-3}	Actual tank capacity. 870 mm water depth used
Peak culture density	30	kg m^{-3}	Used to calculate feed addition at peak culture density
Culture mass (prawns)	39.2	kg	
Daily feed ratio	1.25	%	
Daily feed at peak culture density	490	g	Feed per day determines the TAN loading applied.
Crude Protein (CP)	32	%	CP content of feed
Conversion to TAN from CP	6.5	%	TAN from protein
Daily TAN from feed	0.01	kg d^{-1}	TAN from applied feed to be removed by the biofilter component of IPS

Parameter	Quantity	Unit	Origin, Calculation, or Description
Conversion to TAN from bulk feed	2.08	%	TAN from protein in bulk feed
Target residual TAN	1	mg L^{-1}	(Or lower) Calculation purpose
Passive nitrification (PN)	10	%	Non-biofilter nitrification allowance
Daily TAN from feed after PN	0.009	kg d^{-1}	TAN to biofilter
Alkalinity requirement as sodium bicarbonate	73.5	g d^{-1}	TAN kg d^{-1} x 7; 15 % of feed
Biofilter area for TAN removal	0.45	gTAN m^{-2} d^{-1}	
Required biofilter area	20.4	m^2	
Media specific area	200	m^2 m^{-3}	
Oxygen required by feed added	0.147	kg d^{-1}	30 % of daily feed
O_2 for passive nitrification	0.005	kg d^{-1}	TAN removed in passive nitrification x 4.57
O_2 for biofilter nitrification	0.042	kg d-1	TAN removed in biofilter x 4.57
Total O_2 required	0.193	kg d-1	
Specific Oxygen Transfer Efficiency (SOTE)	2.45	%	Estimated from average dirty water using fine pore diffuser SOTE at 0.7 m x 3.5 %/m
Total O_2 required for SOTE	7.9	kg d^{-1} oxygen	
Air required at 21% O_2	37.6	kg d^{-1} air	
Air volumetric requirement	20.4	m^3 d^{-1}	1.84 kg m^{-3}
	0.9	m^3 h^{-1}	15 L min^{-1}
Factor of Safety (FOS)	1.5		Air supply margin
Air at FOS	21.3	L min^{-1}	

Total alkalinity is reported in units of calcium carbonate. Adding 1 mole of sodium bicarbonate is equivalent to adding 0.5 moles of calcium carbonate. Adding

168 g (2 moles) of sodium bicarbonate is equivalent to adding 1 mole (100 g) of calcium carbonate, because calcium carbonate can accept 2 hydrogen ions vs. only 1 for sodium bicarbonate. A desired 50 mg L^{-1} alkalinity is equivalent to 50 mg L^{-1} calcium carbonate. Therefore, the requirement was to add 84 mg L^{-1} Na-HCO_3, which in 1,300 L is 109 g. Alkalinity was adjusted with 109 g of sodium bicarbonate per day to balance supplementary feed of 245 g per day.

In later operations, this was adjusted to 13 % of feed (Furtado *et al.*, 2014). Martins *et al.* (2017) demonstrated that the use of sodium bicarbonate is effective on both alkalinity and pH adjustments at around 15 % of feed consumption in BFT systems. TAN in the biofilter model is generated at 2.08 % of feed, as per Table 3.3-1, and alkalinity is added at 7 times the TAN input or about 15 % of feed, hence there is agreement. Furtado *et al.* (2015) described that for every gram of TAN converted to NO_3, 4.18 g of O_2 and 7.07 g of alkalinity are consumed, which again agrees with the model. 0.17 g of nitrifying bacteria biomass are produced, and this also consumes alkalinity of about 0.64 g per gram of TAN. Heterotrophic bacteria are noted to require only half the alkalinity of nitrifying bacteria (Furtado *et al.*, 2015).

Daily water quality analysis was performed by handheld probe (YSI Professional Plus) for two tanks in parallel containing identical media, and receiving identical feed and alkalinity supply, without carbon addition. Data collected included temperature, dissolved oxygen, total dissolved solids, pH, and NH_4-N. Laboratory analysis was performed to determine total suspended solids (TSS) [filtration at 0.1 mm and oven dried], NH_4-N [Shimadzu UV-VIS Spectrophotometer UV-1800], and alkalinity [titration method], according to Siang (2013).

3.4 Phase B1: first phase of experimentation with mixed sex *M. rosenbergii*

Phases from B1 onward were carried out with the target species. The employed tank systems in B1 (Replicate and Control) utilized commercially available media, “Japan Mat”, of high specific surface area (300 m^2 m^{-3}) and high void ratio (94 %), in replacement of the media developed in Phase A. The “Japan Mat” a 3-dimensional mat

of layered polypropylene line, was of similar construction to Matala™ mat used in later experiments. IPS replicates had placements of biofilter media within the production volume, while media was contained in a separate biofilter vessel in RAS (control) culture units. Daily water input was provided for replacement of evaporated water only. Other equipment elements of the IPS and RAS systems are described below, along with their culture species population, operational parameters, measurement, and observation. Figure 3.2 depicts the general layout for treatment and control of both the IPS and RAS systems in a common diagram. Main tank diameter of 1.4 m gave a footprint area of about 1.5 m^2, and tank depth of 0.85 m gave a volume of about 1,300 L. Media area per tank was the same used in phase A, at 3 m^2. Hence, one side of the media alone, provided 200 % of the tank bottom plan area. Considering the exposure of both sides of the media provides 400 % of the tank bottom plan area. Higher substrate exposed area was applied than virtually all reported studies employing substrate. The RAS tank biofilter element labelled (b) in Figure 3.2 was established in a fiberglass tank of 1.2 m x 1.2 m x 0.8 m with 0.6 m effective water depth, aerated with ceramic diffusers. Water from tank (c) overflowed to tank (b) for the RAS system, then by gravity flowed into tank (a) where a pump returned the flow to tank (c). In the IPS, tank (c) overflowed directly to tank (a) and flow was recycled.

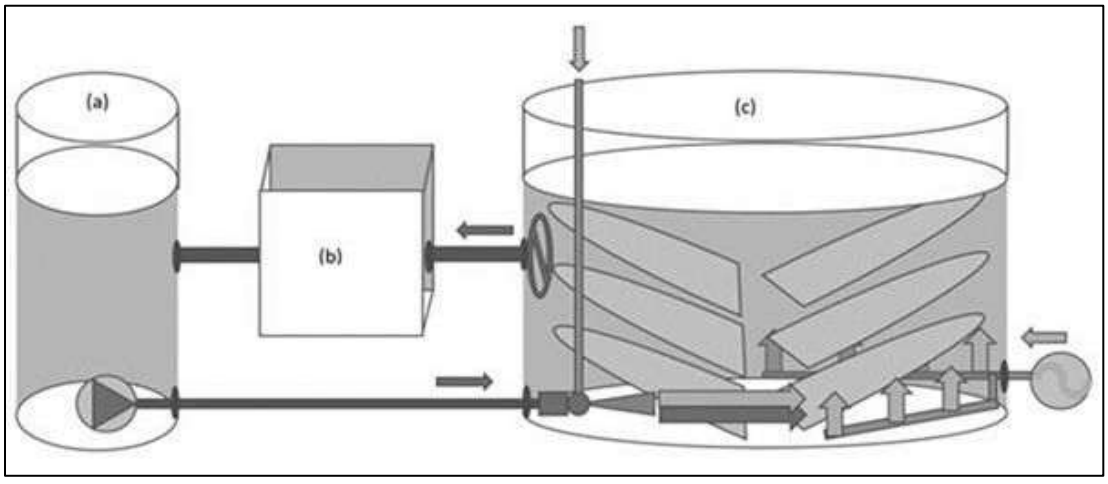

Figure 3.2. General layout for treatment and control in Phase B1: (a) pump tank to venturi aerator common to both IPS and RAS, (b) biofilter for RAS only, (c) periphyton polypropylene fibre mats for IPS, and 1 cm^2 HDPE mesh for RAS, each with a 3 m^2 single side area (1 m^2 per layer).

3.4.1 Phase B1: three (3) replicated IPS treatment tank systems

The three IPS replicates constructed as the main experiment were independent systems (Figure 3.2). Each system contained high specific surface area periphyton substrate biofilter sheets (Matala™ FSM 300 m^2 m^{-3}, 30 mm thick), integrated to the production volume for waste recycling and water quality control, and with surface area useable for prawn resting or grazing (Appendix 2 b).

Ancillary systems to the main tank included:

- Overflow from main tank to a separate filtration device to remove particulate,
- A recirculation pump of 6 m^3 hr^{-1} to maintain filtration and adequate circulation flows,
- Jet valves, utilizing recirculation flow, to add oxygen while delivering recirculation water to the tank and promoting circulation in the tank, and
- Air diffusers supplied from a compressor at the base of tank, beneath the periphyton substrate biofilter. Air was supplied from compressor at a maximum of 60 L min^{-1} per tank.

3.4.2 Phase B1: single control RAS system

A single independent RAS system was constructed as control, where the biofilter was external to the production volume in a separate vessel (Figure 3.2). The separate RAS biofilter vessel held the same quantity of media as was present in the IPS treatment production tanks (Appendix 2 b).

A layered mesh substrate for prawn habitation with low specific surface area, was provided in the RAS production tank, which minimized biofilm area at less than 10 % of periphyton while mimicking the resting area provided by substrate in IPS treatment tanks. This method removed the variable of rest area from the comparison, with active periphyton media in IPS removed from RAS.

Ancillary systems to the main tank included:

- Overflow from main tank through a screen to retain particulate,
- Overflow from filtration to standalone biofilter (with equivalence to periphyton biofilter capacity in the treatment tanks),
- Overflow from biofilter tank through a screen to retain biofilter produced particulate,
- A recirculation pump of 6 m^3 hr^{-1} to maintain filtration and adequate circulation flows,
- Jet valves, utilizing recirculation flow to add oxygen while delivering recirculation water to the tank and promoting circulation in the tank, and
- Air diffuser at the base of tank, beneath the substrate layers (non-periphyton). and in the stand alone biofilter, with air supply from compressor up to 60 L min^{-1} for the RAS tank

3.4.3 Phase B1: species populations, operation, measurement, and observation

The first phase of experimentation (Phase B1) included observation and measurement of the input PL from Sungei Nerus hatchery, and a final harvest sample. The experiment ran for 44 Days of Culture (DoC) from 19 May 2019 to 31 June 2019.

Fertilization of tanks in advance of stocking was accomplished by adding 100 g fertilizer (Serbajadi brand with N 12 %, P 12 %, K 17 %, & Mg 2 %, and trace Mn, Fe, Zn, S, B, Cu, & Mo) to each tank, along with 50 g liquid organic fertilizer (Mr. Garrick brand), and the systems kept under aeration for eight days. Subsequently, *M. rosenbergii* of 20 days post nauplii age, were cultured for 44 days.

A sample of 437 PL was extracted from the PL population received from the hatchery before stocking of Phase B1, with caliper measurements taken at time of weighing (tip of rostrum to tip of telson), and subsequent measurement with computer microscope (Nikon AZ100M Multizoom with NIS Elements imaging software), with pictures retained.

Three IPS tanks and one unit of RAS control tank (Figure 3.4-1) each received 500 PL from the hatchery, which amounted to 385 individuals m^{-3}, and about 333 m^{-2} bottom area (over 1.5 m^2), in the 1.3 m^3 tanks. Top surface of media provided 200 % additional surface area, which could reduce density to 111 m^{-2} (over 4.5 m^2), and to 67 m^{-2} (over 7.5 m^2) if area of both sides of media was included. This phenomenon is a major basis for increased density with addition of substrate to production volumes.

Feeding was twice per day, at 9:00 and 17:00, using PL crumbles (Gold Coin Specialities) with minimum crude protein 40 %, maximum crude fibre 3 %, minimum crude fat 7 %, free of added antibiotics. Feed was added as 5 % of mass for 14 days, and 7 % of mass for the remaining 30 days. Molasses, as carbohydrate source, was added to maintain a C/N ratio of 20:1 (Browdy *v.*, 2012), using the following, derived formula:

$$Molasses_{required} = TAN_{protein} \; x \; CP_{feed} \; x \; N_{release} \; x \; Feed \; (g) \; x \; PN \; x \; CN \; x \; \frac{1}{CH} \quad (1)$$

where,

Total Available Nitrogen (TAN) in protein = 6.5 %

CP_{feed} = 40 % crude protein

$N_{release}$ = 25 % waste feed + 50 % excreted N = 75 %

PN = (1 - % passive nitrification) = 90 %

CN = 20 (desired C/N ratio)

CH = carbohydrate content in molasses by mass = 50 %

Carbohydrate in feed was considered to reduce the requirement for supplementary carbon source by about 30 %, resulting in molasses addition of about 50 % of feed by weight. Sodium bicarbonate (alkalinity) was added as 13 % of feed (Furtado *et al.*, 2014), dissolved and delivered with molasses once per day. IPS and RAS were fertilized a week in advance of stocking. Two days before stocking, the calculated first day feed, molasses, and sodium bicarbonate was pre-dissolved in water and distributed to treatment replicates, but half and half to each of the RAS control and its attached biofilter.

Water quality parameters of temperature, dissolved oxygen (DO), TDS, and pH were measured with a handheld meter (YSI Professional Plus). Laboratory measurements of water quality samples were made, including TSS (filtration at 0.l mm, oven dried), and alkalinity (by HCL titration with methyl orange), approximately weekly, and NH_4-N, NO_2, and PO_4 (Shimadzu UV-VIS Spectrophotometer UV-1800), according to Siang (2013).

Final harvest occurred at 44 DoC. 341, 321, 194, and 327 individuals were harvested from each of treatment tanks IPS1, IPS2, IPS3 and control tank RAS, respectively, for a total of 1,083 samples. Individual samples were weighed and measured by callipers, individually. 50 individuals from each of the tank samples (total of 200 pieces), were photographed and measured by measuring microscope (Nikon AZ100M), with scaled pictures retained. Calliper measurement identified to each weight was used in statistical analysis. Performance was evaluated through statistical

comparison of means for length and mass, as well as through calculation of growth parameters of Survival Rate (SR, %), Body Weight Gain (BWG, g), Average Daily Gain (ADG, g d^{-1}), Specific Growth Rate (SGR, %), and Food Conversion Ratio (FCR), as follows:

$$Survival\ Rate\ (\%) = \frac{Number\ at\ cycle\ end}{Number\ at\ cycle\ beginning}\ x\ 100 \quad (2)$$

$$Body\ Weight\ Gain\ (BWG, g) = Mean\ final\ prawn\ BW - Mean\ initial\ prawn\ BW \quad (3)$$

$$Average\ Daily\ Gain\ (ADG, g\ d^{-1}) = \frac{BWG}{T_2 - T_1} \quad (4)$$

$$Specific\ Growth\ Rate\ (SGR, \%\ d^{-1}) = \frac{In\ (W_2) - In\ (W_1)}{T_2 - T_1}\ x\ 100 \quad (5)$$

where,

W_1 = Initial live prawn BW (g) at time T_1 (days)

W_2 = Final live prawn BW (g) at time T_2 (days)

$T_2 - T_1$ = Duration of the experiment (days) = Days of Culture (DoC)

In(x) = Natural logarithm (of x).

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Feed\ given\ within\ production\ cycle\ (kg)}{Animal\ weigh\ gain\ within\ production\ cycle\ (kg)} \quad (6)$$

3.5 Phase B2: second phase of experimentation with mixed sex *M. rosenbergii*

Phase B2 was run similarly to Phase B1 but with higher density, for 43 days. The tankage for Phase B2 was altered from that used in Phase B1 in the following ways.

1) Three separate (triplicate) RAS control tanks were established, each with external biofilter, for comparison with the three (triplicate) IPS treatment tank sets.
2) Jet aeration was removed except for the control RAS tanks so that circulation was maintained (6 m^3 hr^{-1}) from biofilter tanks to RAS culture tanks, which then overflowed back to biofilter, to continuously cycle culture tank water through the attached, external biofilter tanks.
3) Tanks were provided with in tank lighting (one 15 W unit per tank) for 12 hours per day and shade cloth covers provided to ensure equal light distribution to each individual tank.

The tanks were fertilized without culture species for 8 days using 100 grams of chemical fertilizer (N:12 %, P:12 %, K:17 %, Mg:2 %, with trace Mn, Fe, Zn, S, B, Cu, & Mo), and 50 grams of a pelletized organic fertilizer. Feeding was twice per day, at 9:00 and 17:00, using PL crumbles (Gold Coin Specialities) with minimum crude protein 40 %, maximum crude fibre 3 %, minimum crude fat 7 %, free of added antibiotics. Feed was added as 5 % of mass for 14 days, and 7 % of mass for the remaining 29 days. Molasses, as carbohydrate source, was added to maintain a C/N ratio of 20:1 (Browdy *et al.*, 2012), resulting in molasses addition of about 50 % of feed by weight. Sodium bicarbonate (alkalinity) was added as 13 % of feed (Furtado *et al.*, 2014), dissolved and delivered with molasses once per day.

3.5.1 Phase B2: species populations, operation, measurement, and observation

Phase B2 experimentation was a single nursery stage growth to harvest. The input hatchery PL, and a final prawn harvest sample were observed and measured. A separated, sacrificial sample of 450 pieces of hatchery PL was weighed.

Three IPS tanks and 3 RAS control tanks each received 1,500 pieces of hatchery PL, which amounted to a density of 1,154 individuals m^{-3}, and 1,000 m^{-2}. Water quality measurements throughout operation included daily dissolved oxygen, temperature, TDS, and pH measurements via handheld probe [YSI Professional Plus].

The final harvest occurred at a total culture period of 43 DoC. Individuals were divided to two groups, large and small, where small individuals were estimated around 1 g BW, or below. A group of prawns was weighed and measured to provide a basis for visual size appraisal. Large specimens, approximately above 1 g BW, constituted 397, 405, and 313 individuals from IPS1, IPS2, and IPS3, respectively, and 235, 293, and 304 from RAS1, RAS2, and RAS3, respectively. Small specimens constituted 530, 782, and 696 individuals from IPS1, IPS2, and IPS3, respectively, and 829, 891, and 797 from RAS1, RAS2, and RAS3, respectively.

Temperature, dissolved oxygen, TDS, and pH were recorded daily. The data from these inputs, measurements, and associated observations are recorded in Chapter 4.

3.6 Phases B1 and B2: comparison with RAS of two IPS nursery runs with different densities and hatchery origin of mixed sex *M. rosenbergii*

A first series of experiments was designed to compare operation in tank-based Giant freshwater prawn, *M. rosenbergii* aquaculture of an Integrated Periphyton System (IPS) in tanks as treatment, with a Recirculating Aquaculture System (RAS) system as control. IPS and RAS were evaluated based on growth performance and subsequent survival rate of mixed sex *M. rosenbergii*, and by comparing Feed Conversion Ratio (FCR), Body Weight (BW, g), Survival Rate (SR, %), and Average

Daily Gain (ADG, g d^{-1}). Population was 500 in Phase B1 (IPS500 triplicate, and RAS500) of 40 days and 1,500 in Phase B2 (IPS1500 triplicate, and RAS1500 triplicate) of 32 days. An applied feed schedule managed feed, alkalinity adjustment, and C/N ratio through addition of molasses. IPS culture volumes were clear water systems, where periphyton biofilm provided ecosystem functions. There were three replications for each treatment, excepting Phase B1 where a single RAS system was compared with triplicate of IPS. Initial and final weights and SR were calculated with standard deviation. BW and BW multiplied by SR (BW x SR) were examined statistically by Welch ANOVA with Games-Howell post hoc testing, to evaluate performance and productivity.

3.7 Phase B3: third phase of experimentation with mixed sex *M. rosenbergii*

In the third phase of experimentation (Phase B3), the set of small prawns, mass below about 1 g BW, that originated as the product of Phase B2, were redistributed to the original triplicate sets of IPS and RAS tanks for a further 60 days of culture. In analysis of these tanks, they were coded as IPS_{small} and RAS_{small}. Population was decreased by about 13 % loss to sampling and 7 % mortalities during transfer. After subtraction of losses from sample extraction, and in the processing and transfer, the inputs in terms of populations and densities into Phase B3 are recorded in Table 3.5.

Table 3.5: Input individuals to Phase B3 and initial density

Size	System	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3
Tank population	IPS	400	599	519	N/A		
Density, Individuals m^{-3}		307	461	399			
Tank population	RAS	N/A			613	704	647
Density, Individuals m^{-3}					472	542	498

Equipment characteristics of IPS and RAS tanks employed in Phase B3 are given in Table 3.6.

Table 3.6: Summary of key equipment characteristics for all Phase B3 tanks for 60 DoC

Parameter	Unit	Quantity, IPS and RAS
Tank effective volume	m^{-3}	1.3
Tank depth	m	0.85
Tank plan area	m^2	1.5
Number of IPS tanks	No.	3
Number of RAS systems	No.	3
Duration of operation	Days	60
Media specific area	m^2 m^{-3}	300
Media thickness	mm	30
Media outer surface area (double side) provided per tank	m^2	6.0
Media outer surface area (double side) provided per unit tank volume	m^2 m^{-3}	4.6
Internal biofilm area provided per tank	m^2	27
Internal biofilm area provided per unit tank volume	m^2 m^{-3}	20.8

The transferred individuals were retained in their original tanks, which maintained a population imbalance that had resulted between individual tank outputs. Differences in densities retained from the 43 DoC nursery into Phase B3, resultant from retention of derived populations to their originating tanks, were considered valuable for observation.

Fertilization of tanks in advance of stocking was not possible due to immediate stocking from the identical tanks. Small dosage of 20 g fertilizer (Serbajadi brand with N 12 %, P 12 %, K 17 %, & Mg 2 %, and trace Mn, Fe, Zn, S, B, Cu, & Mo) to each tank, along with 10 g liquid organic fertilizer (brand Mr. Garrick) was observed with immediate commencement of feeding. An average BW of 0.62 g was calculated for the total transferred population, and the average number of prawns per tank, 581, was used as the starting point, for the feed model. The SR assumed by the feed model for the 60 DoC was assumed to be 80 %, and the 20 % mortality was distributed linearly over the 60 days, with an average BWG of 2 g. Feeding at 3 % of modelled, cultured prawn mass per day resulted in lower targeted production and lower feed addition, than was employed in the first stage nursery of either Phase B1 or B2. Feeding was twice per day, at 9:00 and 17:00, using PL crumbles (Gold Coin Specialities) with minimum crude protein 40 %, maximum crude fibre 3 %, minimum crude fat 7 %, free of added antibiotics. Molasses, as carbohydrate source, was added to maintain a C/N ratio of 20:1 (Browdy *et al.*, 2012; Marioni *et al.*, 2020). Water quality parameters of temperature, dissolved oxygen (DO), TDS, and pH were measured with a portable handheld meter (YSI Professional Plus).

Prawns were harvested, and weighed individually, and gender recorded, separately for each tank. Gender assessment was based on identification of males by three main morphotypes, namely Small Male (SM), Orange Clawed (OC) male and Blue Clawed (BC) male, with the remainder identified as female.

3.8 Phase B4: fourth phase of experimentation with mixed sex *M. rosenbergii*

In the fourth phase of experimentation (Phase B4), the set of large prawns, mass approximately above 1 gram BW, that originated as the product of Phase B2, were transferred to a single large 10-tonne IPS_{large} tank of about 3 m diameter, plan area 7.07 m^{-2}, with 8 m^3 water volume (1.1 m depth), for a further 60 DoC. After some losses in the sampling (13 %) and mortality during transfer (7 %), the inputs to IPS_{large} were 1,558 individuals with a total mass of 2,263 g, average BW of 1.45 g, which translated to about 220 individuals m^{-2}, 195 m^{-3}, and 283 g m^{-3}. Equipment characteristics of IPS_{large} employed in Phase B4 are given in Table 3.7.

Table 3.7: Summary of key equipment characteristics for IPS_{large} tank for 60 DoC

Parameter	**Unit**	**Quantity, IPS_{large}**
Tank effective volume	m^{-3}	8
Tank depth	m	1.13
Tank plan area	m^2	7.1
Number of IPS tanks	No.	1
Number of RAS systems	No.	N/A
Duration of operation	Days	60
Media specific area	$m^2\ m^{-3}$	300
Media thickness	mm	30
Media outer surface area (double side) provided per tank	m^2	16
Media outer surface area (double side) provided per unit tank volume	$m^2\ m^{-3}$	2
Internal biofilm area provided per tank	m^2	144
Internal biofilm area provided per unit tank volume	$m^2\ m^{-3}$	18

Fertilization of IPS_{large} was accomplished by adding 100 g fertilizer (Serbajadi brand with N 12 %, P 12 %, K 17 %, & Mg 2 %, & trace Mn, Fe, Zn, S, B, Cu, & Mo), along with 50 g liquid organic fertilizer (brand Mr. Garrick), and the system kept under aeration for eight days ahead of stocking.

The feed schedule developed for IPS_{large} assumed harvest BW of 7 g, utilized average initial PL mass, provided 3 % of estimated surviving prawn mass per day, and assumed 80 % SR by 60 DoC. Feeding was twice per day, at 9:00 hour and 17:00 hour, using PL crumbles (Gold Coin Specialities) with minimum crude protein 40 %, maximum crude fibre 3 %, minimum crude fat 7 %, free of added antibiotics. Molasses carbohydrate source was added to maintain a C/N ratio of 20:1 (Browdy *et al.*, 2012; Marioni 2020). Water quality measured for all tanks included temperature (° C), Dissolved Oxygen (DO, mg L^{-1}), Total Dissolved Solids (TDS, mg L^{-1}), and pH with readings taken daily with a handheld meter (YSI Professional Plus).

Prawns were harvested, and weighed individually, and gender recorded, separately for each tank. Gender assessment was based on identification of males by three main morphotypes, namely Small Male (SM), Orange Clawed (OC) male and Blue Clawed (BC) male, with the remainder identified as female.

3.9 Phase B5: fifth phase of experimentation, first phase with all-male *M. rosenbergii*

Owing to practicality, Phase B5 was conducted as part of the Hapa net stocking process for a commercial IPS pond, stocking all-male, *M. rosenbergii* prawns, obtained from a local Malaysian hatchery (GK Aqua, Port Dickson, Negeri Sembilan, Malaysia). Four sets of Hapa 40 mesh (0.4 mm mesh) nets of 10 m x 10 m x 1 m depth were hung within an 1,800 m^2 pond equipped with IPS media, aerated by surface aerators. Each Hapa was fed according to the developed feed model for the phase, and C/N ratio was modified with molasses to 20:1, with addition of sodium bicarbonate for alkalinity and pH adjustment as 15 % of feed. Water quality was monitored both in the pond and directly in the suspended Hapa nets. Density of all-male, post hatchery PL in the Hapa nets was 250 m^{-2}. Separate substrate provided in each Hapa net consisted of 60 m^2 of plastic shade cloth mesh, considering both sides of the vertical mesh strips, as 60 % of bottom area. No control was attempted for comparison with the Hapa nets of Phase B5. At harvest, a sample of 44 pieces was weighed and measured by ruler to provide individual prawn data, ahead of stocking Phase B6. 200 prawns were randomly selected from the Hapa net population at 35 days post hatchery

as the input to Phase B6, with an average weight of 0.71 g measured for the transferred prawns. No control was attempted for comparison with the triplicate tanks of Phase B6. The Phase B5 harvest sample of 44 pieces was used to obtain the average mass of transferred prawns. The overall survival of Hapa net culture for 100,000 PL was then developed from measurement of the bulk weight of transferred prawns, from Hapa net to commercial grow out pond, and the sample average mass per prawn.

The applied feeding schedule for the nursery in Hapa nets was developed with a target FCR of 1.3 over 180 days, with survival of 50 % and final average mass at 180 days of 45 g BW. Model mass of prawn in culture each day was based on input PL BW and linear progression of these parameters, with 1 g, 2 g, 5 g, and 45 g achieved at 30, 60, 90 and 180 days, respectively, with applied daily feed as percentage of the current modelled mass in culture. Table 3.8 describes the progressive feeding strategy employed. Phase B5 duration was 35 days only, and the population of Phase B5 (200 plus sample for weight and measurement) were extracted from the larger Hapa net population of 100,000. Over the 35-day period of Phase B5, the equivalent feed, molasses addition, and sodium bicarbonate addition, for 200 PL are shown in Table 3.9.

Table 3.8: Feeding strategy for hapa net with calculated feed for extracted Phase B5 population

Culture Period, days	Feed ratio of prawn culture mass	Daily Feed for 200 PL, g	Culture Period, days	Feed ratio of prawn culture mass	Daily Feed for 200 PL, g
1 to 15	7%	0.7 – 7	61 to 90	3%	14 - 23
16 to 30	6%	7 – 11	91 to 120	2.5%	23 - 61
31 to 45	5%	11 - 13	121 to 150	2%	61 - 74
46 to 60	4%	13 – 14	151 to 180	1.5%	56 - 68

Table 3.9: Feeding and additives provided to resultant Phase B5 population

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
27/06/2022	**1**	200 PL input equivalent totals			
28/06/2022	**2**	0.05	0.70	0.35	0.11
29/06/2022	**3**	0.08	1.16	0.58	0.17
30/06/2022	**4**	0.12	1.62	0.81	0.24
01/07/2022	**5**	0.15	2.08	1.04	0.31
02/07/2022	**6**	0.18	2.54	1.27	0.38
03/07/2022	**7**	0.22	2.99	1.50	0.45
04/07/2022	**8**	0.25	3.44	1.72	0.52
05/07/2022	**9**	0.28	3.89	1.94	0.58
06/07/2022	**10**	0.32	4.33	2.17	0.65
07/07/2022	**11**	0.35	4.78	2.39	0.72
08/07/2022	**12**	0.38	5.22	2.61	0.78
09/07/2022	**13**	0.42	5.66	2.83	0.85
10/07/2022	**14**	0.45	6.09	3.05	0.91
11/07/2022	**15**	0.48	6.52	3.26	0.98
12/07/2022	**16**	0.52	6.95	3.48	1.04
13/07/2022	**17**	0.55	6.33	3.16	0.95
14/07/2022	**18**	0.58	6.69	3.34	1.00
15/07/2022	**19**	0.62	7.05	3.53	1.06
16/07/2022	**20**	0.65	7.41	3.71	1.11
17/07/2022	**21**	0.68	7.77	3.88	1.17
18/07/2022	**22**	0.72	8.12	4.06	1.22

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
19/07/2022	**23**	0.75	8.48	4.24	1.27
20/07/2022	**24**	0.78	8.83	4.41	1.32
21/07/2022	**25**	0.82	9.17	4.59	1.38
22/07/2022	**26**	0.85	9.52	4.76	1.43
23/07/2022	**27**	0.88	9.86	4.93	1.48
24/07/2022	**28**	0.92	10.21	5.10	1.53
25/07/2022	**29**	0.95	10.55	5.27	1.58
26/07/2022	**30**	0.98	10.88	5.44	1.63
27/07/2022	**31**	1.02	11.22	5.61	1.68
28/07/2022	**32**	1.05	9.63	4.81	1.44
29/07/2022	**33**	1.08	9.90	4.95	1.49
30/07/2022	**34**	1.12	10.17	5.09	1.53
31/07/2022	**35**	1.15	10.45	5.22	1.57
Total, g			**226.71**	**113.36**	**34.01**

Water quality analysis included T, pH, DO, and TDS using hand-held meter (Hanna multi-meter HI98194, and Horiba DO probe DO210). Test kit analysis was utilized to determine ammonia and nitrite levels [API Freshwater Master Test Kit, MARS Fishcare]. Spectrophotometric measurements of water quality samples were made approximately weekly for alkalinity, NH_4-N, NO_2-N, NO_3-N, and to check calcium and magnesium levels (Hanna IRIS HI801 Spectrophotometer).

3.10 Phases B6 and B7: sixth and seventh phases of experimentation, with all-male *M. rosenbergi*

To conduct Phases B6 and B7, a set of experimental tanks was constructed to IPS design (T1, T2, and T3) following the model of Appendix 1, and with media areas as recorded in Appendix 2 b. 3 units of 230 L tank of 0.33 m^2 plan and 1.4 m^2 submerged tank wall area (total 1.73 m^2), and depth 0.7 m, were equipped with individual aeration, high specific surface area media plates, mesh tubes, and 15 W lighting fixtures operating 12-hours day-1 (7:00 to 19:00). Media in each tank was 600 mm x 500 mm x 40 mm thick with 400 m^2 m^{-3} specific surface area and 94% void space. Outer media surface within the tank was 0.6 m^2 inclusive of both sides, while 4.8 m^2 of biofilm surface area were provided from specific surface area of the media (280 % of the tanks bottom plus side area). No control was attempted for comparison with the triplicate tanks of either Phase B5 or B6. Water was added only to replace evaporation. HDPE mesh tubes were also provided in each tank. Phase B6 was the first phase to utilize these tanks, and fertilization was conducted from 14 July to 31 July, using 4 g of NPK fertilizer, ahead of the addition of prawn population.

For Phase B6 to commence, 66 individuals were transferred from Phase B5 (from Hapa net population) to each of T1, T2, and T3, with resulting density of 200 m^{-2} of tank bottom area of Phase B6. Prawns were cultured for 29 DoC in Phase B6, which made harvested prawns 64 DoC post hatchery.

Phase B7 was started on the same day that Phase B6 was concluded. No fertilization was undertaken for Phase B7, as prawns were transferred direct from Phase B6 harvest, but the media from Phase B6 operation was retained with biomass intact. 60 L of water from the Phase B6 was included in the 230 L for each tank, and made up with fresh, filtered river water. The final harvest from each tank of Phase B6 was distributed at 33 per tank to the same three tanks for commencement of Phase B7, which resulted in Phase B7 density of 100 m^{-2}. Phase B7 was reared for 30 DoC to a total of 95 DoC post hatchery. Residual harvested prawns were measured for length and BW, as samples, and the average BW used in feed model calculation.

The density of prawns applied for the respective durations of Phases B5, B6 and B7 was selected for a relatively good fit in comparison with prior experimental stages using mixed sex *M. rosenbergii*, recorded in Table 3.10.

Table 3.10: Input individuals and density to Phases B5, B6, and B7

Experimental Phase	Individuals m^{-2}	Individuals m^{-3}	Average input mass, g
B5	250	250	0.05
B6	200	287	1.18
B7	100	143	2.62

In each of Phases B5, B6, and B7, supplementary feed, molasses and alkalinity provided were calculated relative to a predicted culture biomass within the IPS systems. Hapa nets of Phase B5 were treated as tanks and fed according to the developed feed table for commercial ponds. Table 3.11 provides the feed calculation for Phase B6 for 38 to 64 DoC. The values did not change greatly over 26 DoC and the average daily quantities of feed, molasses, and sodium bicarbonate added were 4.5 g, 2.24 g, and 0.67 g, respectively, for each tank starting with a population of 66 prawns.

Table 3.11: Feed and additives for Phase B6 for a single tank (66 prawns)

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
01/08/2022	**34**	1.18	3.96	1.98	0.59
02/08/2022	**35**	1.22	4.06	2.03	0.61
03/08/2022	**36**	1.25	4.16	2.08	0.62
04/08/2022	**37**	1.28	4.26	2.13	0.64
05/08/2022	**38**	1.32	4.36	2.18	0.65
06/08/2022	**39**	1.35	4.45	2.23	0.67

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
07/08/2022	**40**	1.38	4.55	2.27	0.68
08/08/2022	**41**	1.42	4.64	2.32	0.70
09/08/2022	**42**	1.45	4.74	2.37	0.71
10/08/2022	**43**	1.48	4.83	2.42	0.72
11/08/2022	**44**	1.52	4.93	2.46	0.74
12/08/2022	**45**	1.55	4.01	2.01	0.60
13/08/2022	**46**	1.58	4.09	2.04	0.61
14/08/2022	**47**	1.62	4.16	2.08	0.62
15/08/2022	**48**	1.65	4.23	2.12	0.63
16/08/2022	**49**	1.68	4.30	2.15	0.65
17/08/2022	**50**	1.72	4.38	2.19	0.66
18/08/2022	**51**	1.75	4.45	2.22	0.67
19/08/2022	**52**	1.78	4.52	2.26	0.68
20/08/2022	**53**	1.82	4.59	2.29	0.69
21/08/2022	**54**	1.85	4.65	2.33	0.70
22/08/2022	**55**	1.88	4.72	2.36	0.71
23/08/2022	**56**	1.92	4.79	2.40	0.72
24/08/2022	**57**	1.95	4.86	2.43	0.73
25/08/2022	**58**	1.98	4.92	2.46	0.74
26/08/2022	**59**	2.02	4.99	2.50	0.75
27/08/2022	**60**	2.12	3.92	1.96	0.59
28/08/2022	**61**	2.22	4.09	2.04	0.61
29/08/2022	**62**	2.32	4.26	2.13	0.64

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
30/08/2022	**63**	2.42	4.43	2.21	0.66
31/08/2022	**64**	2.52	4.59	2.30	0.69
Total, g			**137.90**	**68.95**	**20.69**

Table 3.12 provides the feed calculation for 65 to 95 DoC of Phase B7, for triplicate tanks each starting with 33 prawns. The values do not change greatly over the 30 DoC; however, the weekly values were averaged to ensure relevance while maintaining ease of operations. The weekly average quantities of feed, molasses, and sodium bicarbonate added are recorded in Table 3.13, for each tank starting with a population of 33 prawns.

Table 3.12: Feed and additives for Phase B7 for a single tank (33 prawns)

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
01/09/2022	**65**	2.62	2.57	1.29	0.39
02/09/2022	**66**	2.72	2.66	1.33	0.40
03/09/2022	**67**	2.82	2.75	1.38	0.41
04/09/2022	**68**	2.92	2.84	1.42	0.43
05/09/2022	**69**	3.02	2.93	1.46	0.44
06/09/2022	**70**	3.12	3.01	1.51	0.45
07/09/2022	**71**	3.22	3.10	1.55	0.46
08/09/2022	**72**	3.32	3.18	1.59	0.48
09/09/2022	**73**	3.42	3.27	1.63	0.49
10/09/2022	**74**	3.52	3.35	1.68	0.50
11/09/2022	**75**	3.62	3.44	1.72	0.52

Date	Day	Prawn Individual, g	Feed, g	Molasses, g	Sodium Bicarbonate, g
12/09/2022	**76**	3.72	3.52	1.76	0.53
13/09/2022	**77**	3.82	3.60	1.80	0.54
14/09/2022	**78**	3.92	3.68	1.84	0.55
15/09/2022	**79**	4.02	3.76	1.88	0.56
16/09/2022	**80**	4.12	3.84	1.92	0.58
17/09/2022	**81**	4.22	3.92	1.96	0.59
18/09/2022	**82**	4.32	4.00	2.00	0.60
19/09/2022	**83**	4.42	4.08	2.04	0.61
20/09/2022	**84**	4.52	4.16	2.08	0.62
21/09/2022	**85**	4.62	4.23	2.12	0.63
22/09/2022	**86**	4.72	4.31	2.15	0.65
23/09/2022	**87**	4.82	4.38	2.19	0.66
24/09/2022	**88**	4.92	4.46	2.23	0.67
25/09/2022	**89**	5.02	4.53	2.27	0.68
26/09/2022	**90**	5.46	4.10	2.05	0.61
27/09/2022	**91**	5.91	4.41	2.21	0.66
28/09/2022	**92**	6.35	4.73	2.36	0.71
29/09/2022	**93**	6.79	5.04	2.52	0.76
30/09/2022	**94**	7.24	5.35	2.67	0.80
01/10/2022	**95**	7.68	5.66	2.83	0.85
Total, g			**118.86**	**59.43**	**17.83**

Table 3.13: Operational daily feed and additives for Phase B7 for a single tank

Days post-hatchery	Feed, g	Molasses, g	Sodium Bicarbonate, g
66 to 72	2.84	1.42	0.43
73 to 79	3.43	1.72	0.52
80 to 86	4.00	2.00	0.60
87 to 96	4.70	2.35	0.70

Water quality measurement was similar for Phases B6 and B7. Water quality parameters of temperature, DO, TDS, and pH were measured with a portable handheld meter (Hanna multi-meter HI98194, and Horiba DO probe DO210). Test kit analysis was utilized to determine ammonia and nitrite levels [API Freshwater Master Test Kit, MARS Fishcare]. Spectrophotometric measurements of water quality samples were made approximately weekly for alkalinity, NH_4-N, NO_2-N, NO_3-N, and calcium (Hanna IRIS HI801 Spectrophotometer). Biomass residual within media and tanks was removed by cloth filter at conclusion of Phase B7 only, and oven dried for total mass per tank.

Upon conclusion of each phase, the total number of prawns, and mass removed from the respective tanks were measured and recorded. Excess prawns from Phase B6 harvest, and final harvest of Phase B7 were removed as measurement samples. Each removed prawn was weighed, and ruler measurements were taken.

3.11 Phase C: Commercial phase with all-male *M. rosenbergii*

The commercial phase, Phase C, started with the conversion of a freshwater prawn, *M. rosenbergii*, farm from a conventional, extensive design to an IPS design. The reported results are from the first all-male pond culture in an IPS pond from the period of operation of the farm.

The IPS farm was originally planned with the following main features:

1. Ponds of average 1,200 m^2 each operating in sequence one month apart with 6-month cycles to final harvest.
2. Paddlewheel aerators to maintain DO and current flow within the ponds.
3. IPS media (Appendix 1) parallel to paddlewheel current to minimize loss of flow energy.
4. IPS media added at 30° from horizontal, with area based on ≥ 100% of biofilter model required area (Ponds J4 and J2 of Appendix 2 b).
5. Mesh tubes to facilitate safe refuge during ecdysis (Appendix 1).
6. Typical 50,000 to 100,000 PL stocking to Hapa net nursery with transfer between 30 and 45 days to a single pond.
7. Selective to final harvest of *M. rosenbergii* of above 35 g BW, based on current ex-farm purchase preference for BC males, from about the third to seventh months of a cycle.
8. Automated feeding to facilitate nocturnal feeding since manual nocturnal feeding is difficult to implement, and because *M. rosenbergii* are equipped to feed nocturnally and are generally more active at night (Ahmed *et al.*, 2021).
9. Hapa net nursery within ponds for incoming PL for 30 to 45 DoC at high density (i.e., 250 m^{-2} as in Phase B5). Hapa nursery results in reduced PL energy consumption in sourcing food by reducing domain area, and removed risk of predators, i.e., dragonfly nymphs resident in ponds. Hapa nets residing inside pond provided an additional 30 to 45 DoC without requiring new pond area and are applied only in ponds with young populations, where the additional population density will not impact pond water quality. An effect of increasing total cycles per year for the farm

from 8 to 9 or 10 can result. Phase B5 represents testing of the Hapa net nursery where the Hapa nets are resident within a commercial IPS pond.

10. The model for supplementary feed and additive addition needs to proceed through adaptation to progressively reduce FCR under the selected culture species. Minimization of supplementary feed occurs together with reduction in carbon source (i.e., molasses) amendment for C/N ratio, and for alkalinity (i.e. sodium carbonate) amendment since these additives are supplied proportionately to feed. Feeding ratios continue to evolve but the commercial feeding regime that resulted in Phase C, of FCR = 2.1, is presented in Appendix 3.

The only commercial run data for hatchery and post-nursery grow out was provided in Phase C. Phase C involved stocking of a single nursery pond of 488 m2, pond J4, with all-male *M. rosenbergii*, and transfer at 95 DoC (around 1 May 2022) to a larger grow out pond of 1,800 m2, pond J2, for a further 101 days of culture. 196 DoC of operation of Phase C (27 January to 10 August 2022) were completed up to final harvest. An estimated growth curve was produced at the outset to present the average anticipated prawn mass on each progressive day of culture. A selected survival rate was input to the feed model such that a linear decrease in population resulted in a 70 % SR at 180 DoC, as an initial basis. Feed ratio to prawn mass was modelled as 7 %, 5 %, 4 %, 3%, and 1 % until DoC 60, 90, 120, 180, and 195, respectively. Quantities of supplementary feed were calculated daily. Molasses and sodium bicarbonate additions were calculated as 50 % and 15 % of supplementary feed, respectively. Transfer of prawns from pond J4 to pond J2 at 95 DoC, the end of the Phase C nursery phase, provided data for calculated SR, which was used to adjust the feed model for the subsequent Phase C grow out period. Seven partial harvests occurred from pond J2 over the period from 95 to 195 DoC. Each harvest mass of saleable prawns resulted in estimated 5 % of sale mass as spoilt product. Following each partial harvest, the feed model was recalculated to account for reduced prawn mass in pond J2. The specific

feed model used throughout the culture period appears as Appendix 3 and includes the adjustments made at transfer, and for partial harvests. At final harvest, a small quantity of residual, undersized prawns were transferred into another commercial pond. Equipment applied and dimensions for the two ponds are presented in Table 3.14. The media area recorded represents one side of the media, which was placed at approximately 30 ° from horizontal, and provided habitat area on both sides. Media quantities applied are presented in Appendix 2 b. Habitat areas provided for ponds J4 and J2 were 120 m2 and 288 m2, as 25 % and 16% of bottom area, respectively. This was a much lower relative to additional area provided in tanks, but satisfied the requirement of the biofilter model (Appendix 2 a, and Appendix 2 b)

Table 3.14: Dimensions and equipment for Phase C commercial IPS ponds

Pond	Length, m	Width, m	Water Depth, m	Area, m^{-2}	Vol., m^3	Media area, m^2	kW m^{-2}
J4	37.5	13	1.2	488	585	60	0.0015
J2	75	24	1.2	1,800	2,160	144	0.00125

Water quality analysis, morphometric analysis, and collection and analysis of performance statistics were integrated into the farm operation. Water quality parameters of temperature, DO, TDS, and pH were measured with a portable meter (Hanna multi-meter HI98194, and Horiba DO probe DO210). Test kit analysis was utilized to determine ammonia and nitrite levels (API Freshwater Master Test Kit, MARS Fishcare). Spectrophotometric measurements of water quality samples were made approximately weekly for alkalinity, NH_4-N, NO_2-N, NO_3-N, calcium, and magnesium (Hanna IRIS HI801 Spectrophotometer) according to Siang *et al.* (2013).

3.12 Statistical analysis

Statistical analysis is considered in the design of experiments. The statistical analysis of IPS in the nursery phase considers three factors that can lead to production differences: the density of population, the survival rate (SR), and the body weight (BW) of each produced prawn. SR and BW affect population size and the size of

individual prawns, but together they express the productivity of a particular treatment. Environmental conditions could influence these factors, and in the comparison of two treatments, environmental conditions were maintained as equivalent in each treatment.

In Phases B1 through B3, treatments are carefully expressed with identical systems, and the treatments are distinguished only with respect to the presence of periphyton sufficient to maintain water quality, within the IPS tanks alone. The RAS tanks have equivalent domain areas produced with open mesh and low specific area, which removes the domain area factor from complicating the evaluation. The evaluation compares only the effect of large surface area periphyton within IPS tanks with no such periphyton area within RAS tanks.

Complete analysis of the experimental design meets four common criteria, as follows.

(1.) Is there only one independent variable and why is it an appropriate one for testing the hypothesis?

(2.) Is the dependent variable accurately measured and why is it an appropriate one for testing the hypothesis?

(3.) Are the other potentially important variables controlled (maintained the same in all cases of the independent variable)?

(4.) Is there enough data?

Deciding if the assumptions are reasonable is called justifying the assumptions. To justify an assumption involves indicating (a) why it is important to the experiment and (b) why it probably is or is not true. There are assumptions with respect to statistical analysis, which deal with the validity of statistical analysis.

Use of inferential statistics not only answers the question whether two statistical populations can be regarded as different. Statistics allows evaluation of how different two statistical populations must be, including whether their temporal patterns diverge. Without statistics analysis of a dataset, the patterns in the data are assumed to represent patterns in the sampled statistical populations. Not including inferential statistics ignores the windows into the data available from the latest statistical techniques. Inferential statistics enable distinguishing patterns from scatter and provide an objective estimate for probability.

The essence of the current study experiments is to trigger dynamical response in a biological system at prototype scale. This requires space and special conditions, to isolate the experimental system from contamination. These requirements are not compatible with standard experimental designs. Individual units are interspersed within a common area. Replication is limited to triplicates. As a result, the logic of inferring treatment effects is the same used to interpret either causes of spontaneous events or events triggered by practical manipulations. The observed contrasts can be reasonably interpreted as effects of the treatment only if the magnitudes and the timing make alternative explanations implausible to the reviewer (Oksanen, 2001). The logic of use of inferential statistics is thus explained in the current study. While statistically demonstrated treatment effect is examined, these imperfectly designed experiments are referred to as experimental events, distinguished from perfectly designed experiments.

The statistical analysis of IPS considers that there are several factors that can lead to production differences, which include the stage of growth, density of population, the survival rate (SR), and the body weight (BW) of each produced *M. rosenbergii* prawn. The stage of growth, and the density of population are set by the experiment. The last two factors (SR and BW) result from population size and the size of individual prawns, and together they express the productivity of a particular treatment, relative to starting conditions. Environmental conditions could influence any or all of these. In the comparison of two treatments, environmental conditions were maintained as equivalent in each treatment. The difficulty is in defining how

treatments differ without changing environmental conditions between two compared systems.

In the comparisons of IPS and RAS in Phases B1 through B3, treatments were carefully expressed with identical systems, and the treatments were distinguished only with respect to the presence of periphyton sufficient to maintain water quality, within the IPS tanks alone. The many environmental factors – temperature, amount of food, living space available per prawns, pH and temperature of the water, prawn age and phase of growth, etc. were maintained as equivalent between IPS and RAS. The RAS tanks had equivalent domain areas produced with open mesh and low specific area, which removed the domain area factor from complicating the evaluation. The evaluation compared only the effect of large surface area periphyton within IPS tanks with no such periphyton area within RAS tanks. As a scientific hypothesis, this setup is considered testable; that is, there exists the possibility of disproving it.

Three types of variables are considered in conducting a scientific experiment. The independent variable is an intentionally different factor at the beginning of the experiment. The presence of large surface area periphyton is that variable that identifies two types of treatment: IPS treatment where that area is present, and RAS where that area is not present. IPS and RAS are considered two groups of the independent variable, i.e., where Integrated Periphyton System (IPS) yielded equivalent or better growth performance than RAS. The treatment group is a CATEGORICAL variable.

A second type of variable is the dependent variable. This is the variable that is measured to see if change of the independent variable had an effect. It is the kind of data that is being collected. In the first prediction, the dependent variable is the SR, in the second the BW, and in the third, BW x SR. Since the prawns all began at about the same weight, growth can be compared by looking at the final BW, and performance can be compared by including SR. The dependent variables are ORDINAL variables.

The third type of variable is a controlled variable, and each experiment has several. Controlled variables are the factors that might affect a dependent variable and

that therefore must be consistent for all cases of the independent variable tested. In testing predictions 1, 2, and 3, all tanks used were identical, the amount of oxygen and food provided was the same, the young prawns were of the same species, same originating hatchery, same age, same temperature regime, and same lighting conditions.

M. rosenbergii are subject to Heterogenous Individual Growth (HIG) with extreme differentiation between classes of males with additional differentiation of the female prawn. However, morphological and gender differentiation is hard to identify before 45 DoC of PL, and arguably before 90 DoC. The experiments of Phases B1 and B2 evaluated the nursery stage, which is commonly considered up to 30 DoC, but was extended to 44 DoC. At this age, differentiation of morphology and gender were unreliable or impractical. However, because differentiation was not present, the impacts of BC male claws was considered not to be present. Phases B1 and B2 tested the hypothesis without the complications of HIG or competition for females. Phase B1 was a complete experiment at initial prawn density of 333 m^{-2}, and Phase B2 at initial prawn density of 1,000 m^{-2}. Comparison of two separate experiments, varying the controlled variable of population density, improved the investigation and gave weight to results of the comparison of independent variable groups (IPS and RAS).

The 43 DoC nursery output of Phase B2 showed limited size differentiation, as precursor of more extensive HIG that occurs later in culture age. The output prawns from Phase B2 were considered post-nursery and were nonetheless separated to two size groups. Further culture beyond 43 DoC was considered to require reduced population density. The size differentiation used was based on prawns of approximately less than 1 g BW (Phase B3) and greater than 1 g BW (Phase B4), about 66 % and 34 % of the produced nursery population, respectively. Phase B3 experiment established results from culture of the smaller size post-nursery prawns, cultured from DoC 43 to DoC 104 in the identical tanks of Phase B2, for triplicate IPS versus RAS comparison. Phase B4 experiment established results of the larger size post-nursery prawns over the same period but for a single IPS tank due to equipment constraints. Expressions of HIG over the subsequent 60 DoC (to total 104 DoC of PL) were then observed in both size groups for the individual IPS and RAS tank populations. Once

morphology and gender could be assessed at harvest at DoC 104, HIG expression of SM, OC, and BC males, and gender became dependent variables in the statistical analysis. Assessment revealed the association with BW and BWG of dependent variables in post-nursery prawns, specific to the two experimental size groups.

In Phase B3, stocked with prawns approximately less than 1 g BW, the comparison experiment between IPS and RAS was continued from 43 to 104 DoC of PL, with the six tanks as part of the group of independent variables (Treatment). Sexual differentiation was clear enough to be an additional dependent variable measurable in the harvested prawns.

In Phase B4, stocked with prawns approximately greater than 1 g BW, the total group was raised in a single IPS tank from 43 to 104 DoC of PL. Sexual differentiation was again clear enough that it could become an additional dependent variable measurable in the harvested prawns. This group was no longer strictly part of the original experiment testing the independent variable for IPS versus RAS. It was however part of the original PL stock, of the same age as stock of Phase B3. The dissolved oxygen, and temperature regime were the same. Lighting conditions were similar, and food was provided based on the same relationship of food mass to prawn mass. Phase B4 was treated as part of the independent variable group, and as large nursery stock IPS (IPS_{large}) the results were included in statistical evaluation. It was acknowledged that statistical control was imperfect by some measures, yet statistical evaluation of the experimental events was a useful tool to investigate the results against Phase B3 results, and to investigate the inherent relationships of the independent variable group derived from a common batch of hatchery PL.

3.12.1 Data analysis

It was of primary importance to determine whether the data supported or did not support each hypothesis. If the BW, SR, and BW x SR of the prawns varied significantly for different independent variable groups, then the hypothesis could be evaluated. In these experiments, a secondary hypothesis was expressed as “did not

vary or were considered equivalent" for IPS and RAS. When the secondary hypothesis was supported IPS could be considered "as effective as" RAS.

The statistical analysis: (1) begins with a statement of the hypothesis, (2) indicates the kind of data that would support the hypothesis (the prediction), (3) cites the relevant data that were collected, and (4) draws a conclusion as to whether, or not the hypothesis was supported by the evidence. The main guide used throughout the program was Laerd's online statistical guide for SPSS specific to version 29. Written format was guided by standards of presentation documented in Laerd's guide (Laerd).

Complete analysis of the experimental design met four common criteria:

(1) Is there only one independent variable and why is it an appropriate one for testing the hypothesis?

The independent variable is the treatment type as represented by an individual tank, whether IPS or RAS. The primary advantages of IPS aquaculture are defined by the biofilter being incorporated to the production volume. A comparative study within the independent variable group permits a quantified assessment of IPS against an established alternative aquaculture system, RAS. Population density was changed, with two density groups for nursery (Phases B1 and B2), and post-nursery aquaculture density was reduced for grow out. The densities were selected for commercial evaluation and for performance reasons. The independent variable group of treatment tank was tested with one-Way ANOVA (Laerd), under different population densities to obtain separate sets of data for the same evaluation of hypothesis, against dependent variables BWG and BWG x SR. With comparison of Phases B3 and B4, gender of the harvest was available. A second independent variable, gender (male and female), was applied with two-Way ANOVA, against the same dependent variables, for the mixed sex cultures. The treatment and gender groups are CATEGORICAL variables.

(2) Is the dependent variable accurately measured and why is it an appropriate one for testing the hypothesis?

The dependent variables were BWG, and BWG x SR. The measurements were made on triplicate systems (except for RAS in Phase B1, and IPS_{large}), which is accepted practice in aquaculture evaluations, and with large sample populations. Since the prawns all began at about the same weight, growth was compared by looking at the final BW (and Body Weight Gain, BWG), and performance was compared by including SR (BWG x SR). Accuracy of BW was high with individual measurement of individuals of large harvest populations. The dependent variables are ORDINAL variables.

(3) Are the other potentially important variables controlled (maintained the same in all cases of the independent variable)?

Controlled variables were consistent for all cases of the independent variable tested. In testing predictions 1, 2, and 3, all tanks used were identical (except for Phase B4), the amount of oxygen, and the amount and protein constituents of food provided, were the same, the young prawns were of the same species, same originating hatchery, same age, and same temperature regime, and the same lighting conditions were maintained.

(4) Is there enough data?

Given the representative population size for relevance to commercial scale aquaculture, data was sufficient. The statistical procedure followed was not considered to have been compromised by the quantity of data produced. In a scientific experiment, it is not valuable to overcomplicate the experimental parameters but the opposite, to maintain as direct and straightforward an arrangement as possible. The experiments utilized large populations, representative of commercial scale aquaculture. The large sample populations made the results statistically more robust. Sequencing the experimental events captured changes related to HIG from nursery to post-nursery culture. The sequential experiments with common *M. rosenbergii* stock were considered useful to investigate the consistency of results in different phases. Presenting individual phases separately, i.e., in publication, was extremely challenging

because of the difficulty of expressing the interrelationships between phases when presenting phase outputs separately.

3.12.2 Assumptions of the experiment

Deciding if the assumptions are reasonable is called justifying the assumptions. To justify an assumption involves indicating (a) why it is important to the experiment and (b) why it probably is or is not true.

In the experiment, the following are among the assumptions and justifications:

(1) The responses of *M. rosenbergii* are typical and representative of hatchery produced population of the species and largely of all freshwater prawns. The hypothesis is a generalization meant to apply to freshwater prawns. The study does not claim generalization to marine prawn culture; hence this could be tested in future.

(2) The set of environmental conditions that are optimum for growth do not change as the prawns mature, over the duration of culture phases defined in the experiments. Phases B1 and B2 were both nursery stages approximately up to 43 DoC. Phases B3 and B4 were post nursery, or first stage grow out from 43 DoC up to 104 DoC. Post 104 DoC was assumed better evaluated at commercial scale. The prawns used were young, which is usually a time of rapid growth. Because the conditions that are optimum for prawn growth change as prawns mature, results are valid only for the duration of culture phase of the experiment. In other species, the effects of a particular condition on growth may not change greatly until the organism has reached maturity. In *M. rosenbergii*, HIG is a factor that requires careful differentiation of growth phases. Density needs to be managed as the species individuals become larger, as do feeding levels.

(3) Prawns confined to a tank, at least a tank of similar depth to a pond, demonstrate the same growth patterns as those raised in an aquaculture pond. Nursery culture at higher density is recommended for *M. rosenbergii*. Stock ponds of smaller

dimension could be assumed to function the same as tanks at equivalent densities for nursery rearing. In grow out, post 104 DoC, it was considered that tank rearing of *M. rosenbergii* is not equivalent to ponds. For one reason, typical post 104 DoC grow out densities are very low, hence tank grow out would result in very small populations, with difficulty to reflect community effects that would be expected to be involved in HIG. The prawns used in this investigation are small enough that effects of container size, with the semi-commercial tanks employed, should be negligible. Beyond 104 DoC, *M. rosenbergii* is assumed better evaluated at near commercial, or commercial scale.

3.13 Record keeping and analysis

Table 3.1 provided the timeline for each phase, while the methodology used for collecting and recording experimental data was reported in Table 3.2. Table 3.15 sets out the procedures used for data analysis for each phase of experimentation.

Table 3.15: Procedures for data analysis

Results Originating Phase	Performance based analysis	Statistical Analysis
A	Comparative	Average and standard deviations for a range of observed parameters.
B1 to B7	Standard performance calculations for growth, survival, and efficiency	Average and standard deviations for a range of observed parameters. SPSS, one-way and two-way ANOVA where specific variables were evaluated (number, gender, treatment, BWG, BWG x SR).

Results Originating Phase	Performance based analysis	Statistical Analysis
		Morphology of HIG in males was analyzed by excel spreadsheet (number, gender, treatment, BWG, BWG x SR).
C	Standard performance calculations for growth, survival, and efficiency	Average and standard deviations for a range of observed parameters.

3.14 Ethics

From an ethical standpoint, the only real concern considered was the welfare of the *M. rosenbergii* specimens during experimentation. Culture conditions did not raise any ethical concerns. Harvesting and transferring of species exposed the culture individuals to stresses normally experienced in commercial aquaculture. Samples were anaesthetized with as little as 3 ppm of clove oil (estimated as 4 drops per 20 Litre aquarium unit) prior to sorting, weighing and transfer. Ice was then added to aquariums containing samples for harvest such that temperature drop resulted in stupefaction, following which further ice addition ensured demise of the individuals.

3.15 Limitations

Limitations are recognized in measurement of the contributions from a wide range of variables arising from different substrates, species, operating systems, and environmental conditions representing different ecosystems.

The IPS appears less substantially in literature than other zero discharge systems such as BFT, periphyton substrate addition, and RAS in aquaculture studies

and commercial applications. An attempt has been made to reflect on the fundamental relationships that differentiate and that bind these systems.

Additional new frontiers are predicted where optimized fixed film IPS is commercially invested for live feed production, hatchery, nursery and, intensive grow out aquaculture production across a widening range of species. The current study worked only with *M. rosenbergii*.

Experimentation with mixed sex in *M. rosenbergii* acknowledges the large impact of HIG in males and the distinct growth pattern of females. It is difficult to define how each sex reacts separately to variable applied treatment and control conditions, and studies only acknowledged without analysing the potential impact of sex on results. In the current study, experimentation was carried out with mixed sex *M. rosenbergii*, and with all-male *M. rosenbergii* stocks which were also cultured in the commercial stage. A related issue is the long period for definition of sex to be definitive visually in *M. rosenbergii*, with rapid inspection, i.e., up to 90 DoC. A large proportion of studies of *M. rosenbergii* culture cover up to 60 DoC, but sex ratios in various experiments were not found. The current study examines both all-male, and mixed sex culture with limited assessment of sex ratios. Sex ratio could well be heavily pre-determined by the genetics of broodstock and hatchery operation. Further study can examine comparative performance of culture from different hatcheries beyond the nursery stage. Alternatively, sex ratio could be bound to the biomes carried by hatchery PL, and further by the biomes of the experimental ecosystem. Analysis or control of either biome is out of the scope of the current study.

Experimental Phases B2 through B4 were generated from a single input of PL from a common hatchery mixed sex stock. There was no variable associated with different broodstock. Phases B1 and B2 were supplied from different hatcheries, hence genetic differences could have impacted results. Only B2 nursery was employed as the basis for extended culture experimentation. Phases B5 through B7 were generated from a single input of PL from a common hatchery all-male stock. However, Phases B5 through B7 were supplied from different stock than supplied to the Phase C

commercial operation reported, although from the same all-male *M. rosenbergii* hatchery.

CHAPTER 4

RESULTS

4.1 Phase A: technology establishment

Technology establishment was initiated with tankage of 1.5 m^3 with 1.3 m^3 effective volume as the standard tankage for experimental phases. In addition, production of a high specific surface area media was attempted utilizing HDPE insect mesh and PE shade cloth. The media was formed into plates consisting of multiple layers of the mesh/cloth, with calculated specific surface area upon which biofilm could form, additional to the tank sides and bottom. Aeration was provided by compressor and air stones.

The IPS system was configured and operated over the operational period without prawns, but feed and alkalinity was provided as described in Chapter 3. The results from Phase A were mainly to assess the water quality performance of the IPS under the applied parameters, without culture animals. The results, as measured by portable handheld probe (YSI Professional Plus), are reported in Figure 4.1: Water Quality Results for dual IPS tanks, with temperature, DO and pH shown with error bars.

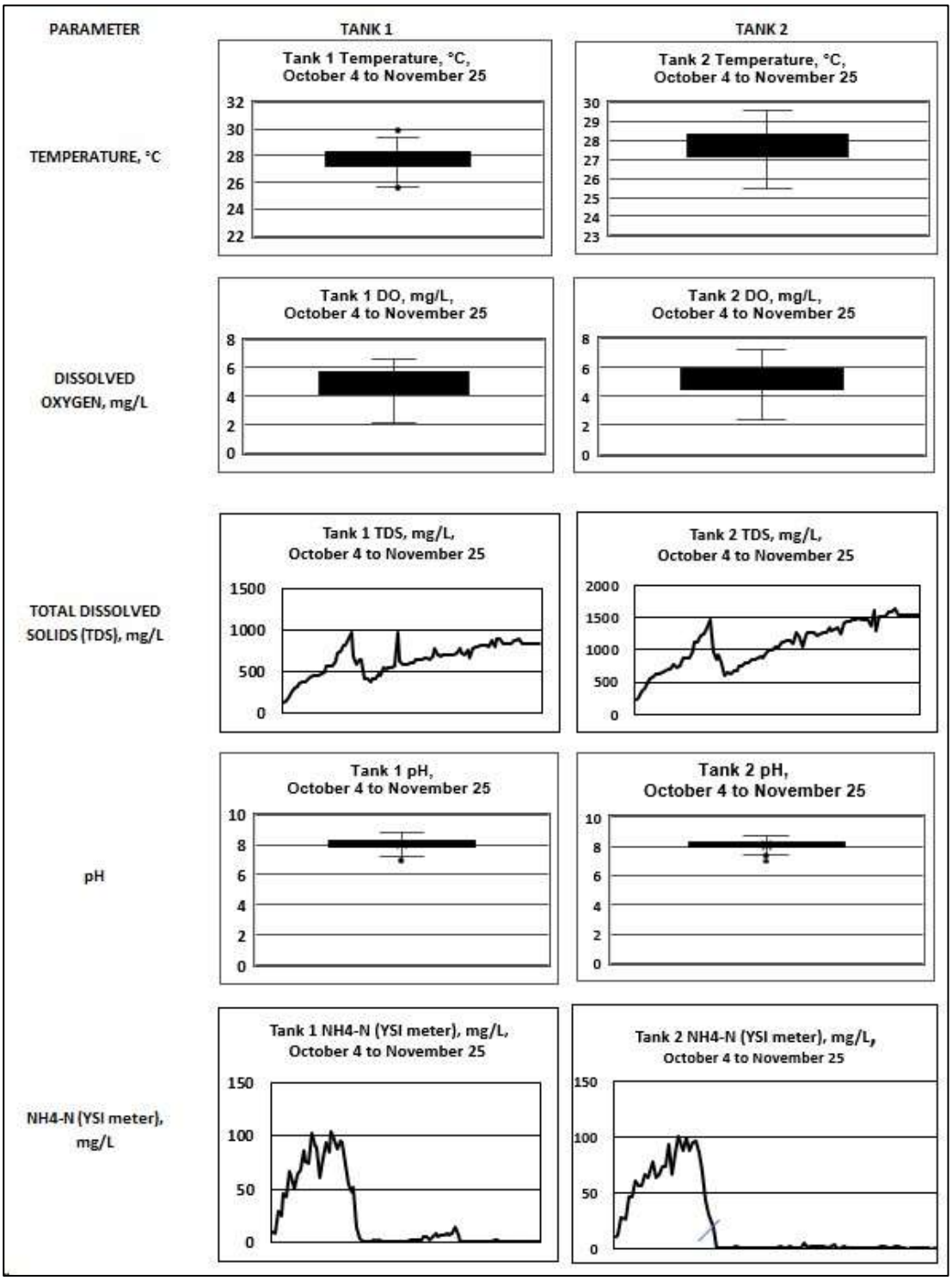

Figure 4.1: Water Quality Results for dual IPS tanks in Phase A

Over the period of operation of both Tank 1 and Tank 2, temperature ranged from about 25.5 °C to about 29.5 °C. Dissolved oxygen (DO) ranged from about 2.0 mg L^{-1} to about 7.0 mg L^{-1}, and pH ranged from about 7 to about 8.75. NH_4-N for both Tank 1 and Tank 2 climbed to a peak of about 100 mg L^{-1}, and fell dramatically at day 18 for both tanks, subsequently remaining at a low level. Laboratory analysis

for NH_4-N was conducted (Shimadzu UV-VIS Spectrophotometer UV-1800), according to Siang *et al.* (2013), on two occasions for comparison with probe results, with the comparison shown in Table 4.1. The probe reading for 10 October on Tank 1 could easily have been higher than the laboratory reading due to meter error.

Table 4.1: Laboratory spectrophotometer NH_4-N results compared to probe results

Date	UV Spectrophotometer, NH_4-N, mg L^{-1}		YSI Meter, NH_4-N, mg L^{-1}	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**
6 Oct 2018	42.9	46.8	44	47
10 Oct 2018	48.9	48.5	-	74

Additional laboratory analysis was performed to gauge the total suspended solids (TSS) levels in the two tanks and to measure alkalinity levels, as reported in Table 4.2.

Table 4.2: Early stage TSS and alkalinity levels in Phase A

Date	TSS, mg L^{-1}		Alkalinity, mg L^{-1}	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**
6 Oct 2018	87	59	4.4	5.2
10 Oct 2018	9	6.8	55	58
12 Oct 2018	-	-	10.1	10.4
14 Oct 2018	2	2	81	83
19 Oct 2018	8.8	5.1	25	25.2

Observationally, the tank volumes were clear from the outset, and this condition was maintained throughout operation. TSS samples were misplaced on 12 October. Although the tanks were operated in a zero-water-exchange mode, with only replacement for evaporation, minimal solids were residual in the tanks at the conclusion of operation.

4.2 Phases B1 and B2: two nursery phases of IPS versus RAS experimentation with mixed sex *M. rosenbergii*

4.2.1 Water quality

Water quality measured for IPS, 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 IPS and RAS for Phase B1 (43 days) and Phase B2 (44 days), was consistent within acceptable aquaculture parameters, with results summarized in Tables 4.3 and 4.4. Laboratory measurements of water quality samples were made for Phase B1, 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).

Table 4.3: Water quality in treatment tanks IPS1, IPS2, and IPS3, and RAS tanks in Phase B1

Phase 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})
B1-IPS1	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
B1-IPS2	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
B1-IPS3	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
B1-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

Table 4.4: Water quality in treatment tanks IPS1, IPS2, and IPS3, and RAS tanks in Phase B2

Phase and Tank	Value	T, (˚C)	DO, (mg L^{-1})	TDS, (mg L^{-1})	pH
B2-IPS1	Average	28.8	7.0	251	7.2
	STDEV	0.95	1.0	78	0.2
B2-IPS2	Average	28.7	7.1	234	7.2
	STDEV	0.96	1.0	72	0.2
B2-IPS3	Average	28.5	7.1	226	7.2
	STDEV	0.87	0.8	78	0.1
B2-RAS1	Average	28.5	8.1	202	7.2
	STDEV	0.69	0.5	62	0.1
B2-RAS2	Average	28.1	8.3	232	7.3
	STDEV	0.61	0.5	81	0.2
B2-RAS3	Average	28.7	8.0	226	7.2
	STDEV	0.60	0.5	68	0.2

Phase B1, unionized ammonia, mg NH_3-N L^{-1} concentration, the toxic species, was determined from total ammonia, mg NH_4^+-N L^{-1} (Table 4.2), as about 2.3 % of NH_4^+-N L^{-1} at 29 °C and pH of 7.5. Total average ammonia of 0.09 mg NH_4^+-N L^{-1} for IPS1, IPS2, and IPS3, 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}.

4.2.2 Performance indicators

The feed model used for each of Phases B1 and B2 was based on assumptions expressed in Table 4.7, below, and determined the total feed supplied as delivered to each run that was used in determination of FCR. The calculated SR, BW, harvest densities (kg m^{-3} and no. m^{-2}), BWG, ADG, and FCR for each of Phases B1 and B2, including standard deviations (S.D.), are reported as averages for all the tanks in each treatment set, in Table 4.5. Standard deviations are provided where individual prawn measurements were available. Input PL and harvested prawns were both counted, from which SR was generated, but the BW data reported for Phase B2 are determined from a subsample.

Table 4.5: Performance indicators for Phases B1 and B2

Metric	Feed Model, Run A	IPS, ave.	IPS (S.D.)	RAS (single)	Feed Model, Run B	IPS, ave.	IPS (S.D.)	RAS, ave.	RAS (S.D.)
	Phase B1				**Phase B2**				
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 (SR), %	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

4.2.3 Residual biomass at conclusion of Phases B1 and B2

Using the procedure described in Chapter 3 of this thesis, residual biomass was extracted from all tanks at conclusion of each of each phase, and dry mass calculated for the three replicate IPS tanks in each Phases B1 and B2. Feed provided, prawn biomass produced, and dry biomass produced over the full Phase DoC for each phase were calculated as average for three IPS replicates in each phase and are recorded in Table 4.6.

Table 4.6: Residual biomass in Phases B1 and B2

Parameter	Phase B1	Phase B2
DoC, days	40	32
Average prawn biomass output per tank, g	330	958
Feed provided per tank, g	1,119	1,935
Range of dry residual biomass in tanks from each Phase, g	22 to 113	53 to 77

4.2.4 Statistical analysis for Phases B1 and B2

4.2.4.1 Input PL for Phases B1 and B2

Data are mean ± standard deviation, unless otherwise stated. Samples of 437 and 450 post larvae (PL) were measured for Phases B1 and B2, respectively. In two runs of independent-sample t-test (SPSS version 29), violation of normality was found in each sample. There were approximately 8 % outliers in both data sets, as assessed by inspection of boxplots. With outliers removed, Phase B1 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). With outliers removed, Phase B2 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).

4.2.4.2 Final harvest of Phases B1 and B2

Data are mean ± standard deviation, 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 IPS (treatment) and RAS (control) groups, for each of Phases B1 and B2, using SPSS version 29. In each case there were outliers (< 5 % for Phase B1 and < 10.5 % for Phase B2), as assessed by boxplot, and the assumption of normal distribution was violated for each treatment group, as assessed by 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 used as a result, which was considered robust with the large sample "n" reported in both Tables 4.9 and 4.10~~, below~~. "n" for Phase B1 was the full population at harvest, while it was a representative random sample of the full population at harvest for Phase B2. A one-way Welch ANOVA was conducted to determine if BW and BW x SR at harvest were significantly different for the IPS and RAS groups for each of Phases B1 and B2, $p < 0.05$. Tables 4.7 and 4.8 summarize means with standard deviations, and rank for BW and BW multiplied by SR (BW x SR), for Phases B1 and B2, respectively.

Table 4.7: Summary of means for Phase B1 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
IPS T1	341	1.2888	1.05829	2	0.8765	0.71953	4
IPS T2	221	1.3160	0.99500	3	0.5788	0.43804	3
IPS T3	194	1.3325	0.88979	4	0.5198	0.34703	2

Table 4.8: Summary of means for Phase B2 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
IPS1	93	1.2394	0.77299	6	0.7662	0.47816	5
IPS2	119	0.7087	0.50243	2	0.5608	0.39743	2
IPS3	101	0.8716	0.66915	3	0.5864	0.45050	3

In Phase B1 the mean BW at harvest increased from the single RAS control (n = 327, 0.52 ± 0.43), to IPS1 (n = 341, 1.29 ± 1.06), to IPS2 (n = 221, 1.32 ± 1.0) to IPS3 (n = 194, 1.33 ± 0.89) tank harvest BW (Table 4.9). The BW at harvest was statistically significantly different for individual treatments and control, Welch's F (3,

471.677) = 113.588, $p < .001$. The mean differences between RAS, and each of the IPS treatment tanks (IPS1, IPS2, and IPS3) were each statistically significant ($p < 0.001$), whereas difference between the IPS treatment tanks was not significant. The group means were statistically significantly different ($p < .001$) and, therefore, we can reject the null hypothesis and accept the alternative hypothesis. It could be observed that the IPS tanks as a distinct group were significantly different and displayed significantly increased BW figures, over the RAS tank as a group. Table 4.9 summarizes Games-Howell analysis for Phase B1 for BW. In this Phase B1 study, IPS established equivalent to improved performance in comparison with RAS.

In the second part of Phase B1, BW was multiplied by Survival Rate (BW x SR), as each tank not only yielded different average BW, but a distinct SR. The mean BW x SR at harvest increased from the RAS control (n = 327, 0.34 ± 0.28), to IPS3 (n = 194, 0.52 ± 0.35), to IPS2 (n = 221, 0.58 ± 0.44), to IPS1 (n = 341, 0.88 ± 0.72) (Table 4.9). The BW x SR at harvest was statistically significantly different for IPS and RAS, Welch's F (3, 521.169) = 67.180, $p < .001$. The mean difference between RAS, and each of the IPS treatment tanks (IPS1, IPS2, and IPS3) were each statistically significant ($p < 0.001$) and, therefore, we can reject the null hypothesis and accept the alternative hypothesis. However, the differences between the IPS treatment tanks IPS1 and IPS2, and IPS1 and IPS3, were also significant. Table 4.9 summarizes Games-Howell analysis for Phase B1 for BW x SR. It could be observed that the IPS tanks as a distinct group were significantly different, and displayed significantly increased BW x SR figures, over the RAS tank as a group. In this Phase B1 study of BW x SR, IPS established improved performance in comparison with RAS.

In Phase B2, the mean BW at harvest increased from the RAS2 (n = 118, 0.70 ± 0.48), to IPS2 (n = 119, 0.71 ± 0.50), to IPS3 (n = 101, 0.87 ± 0.67) to RAS1 (n = 107, 0.93 ± 0.74), to RAS3 (n = 68, 1.13 ± 0.95), to IPS1 (n = 93, 1.24 ± 0.77) (Table 4.10). The BW at harvest was statistically significantly different for IPS and RAS, Welch's F (5, 256.118) = 10.137, $p < .001$, therefore, we can reject the null hypothesis and accept the alternative hypothesis. Table 4.10, below, summarizes Games-Howell analysis for Phase B2 for BW. Within groups, the highest performing tank pairs of IPS and RAS, lowest performing IPS and RAS tanks, and the mid-range performance IPS

and RAS tanks, were each not significantly different as pairs. In this Phase B2 study, IPS was considered able to establish equivalent performance in comparison with RAS.

In Phase B2, BW was multiplied by Survival Rate (BW x SR), as each tank not only yielded different average BW, but a distinct SR. The mean BW x SR at harvest increased from the RAS2 (n = 118, 0.55 ± 0.38), to IPS2 (n = 119, 0.56 ± 0.40), to IPS3 (n = 101, 0.59 ± 0.45) to RAS1 (n = 107, 0.66 ± 0.53), to IPS1 (n = 93, 0.77 ± 0.48), to RAS3 (n = 68, 0.83 ± 0.70) (Table 4.8). The BW at harvest was statistically significantly different for IPS and RAS, Welch's F (5, 258.733) = 4.495, $p < .001$. Table 4.10 summarizes Games-Howell analysis for Phase B2 for BW x SR. Unlike the analysis of BW without SR factor, the differences between the IPS treatment tanks IPS1 and IPS2, were also significant, and in addition, RAS2 differed significantly from RAS3, while RAS2 and RAS3 differed significantly from IPS1 and IPS2, respectively, as shown in Table 4.10, which summarizes Games-Howell analysis for Phase B2 for BW and BW x SR. The group means were statistically significantly different ($p < .05$) as complete groups of IPS and RAS. Therefore, we can accept the null hypothesis and reject the alternative hypothesis. However, it could be observed that the IPS tanks performed equivalently as RAS, pairwise, with IPS2 result close to RAS2, IPS3 close to RAS1, and IPS1 close to RAS3, although RAS3 BW x SR was significantly greater than for IPS1. In this Phase B2 study, IPS was considered able to establish equivalent performance in comparison with RAS.

Comparison of means of BW and BW x SR, of harvested prawns from Phases B1 and B2, are shown in charts presented in Figures 4.2 (top) and (bottom), and 4.3 (top) and (bottom).

Table 4.9: Summary of significant difference by Games-Howell analysis for Phase B1 for BW and BW x SR

Phase	BW			Significance p < 0.005	BW x SR			Significance p < 0.005
	Tank	>/<	Tank		Tank	>/<	Tank	
B1	R	<	IPS3	< 0.001	R	<	IPS1	< 0.001
	R	<	IPS1	< 0.001	R	<	IPS2	< 0.001
	R	<	IPS2	< 0.001	R	<	IPS3	< 0.001
	-		-	-	IPS1	>	IPS3	< 0.001
	-		-	-	IPS1	>	IPS2	< 0.001

Table 4.10: Summary of significant difference by Games-Howell analysis for Phase B2 for BW and BW x SR

Phase	BW			Significance p < 0.005	BW x SR			Significance p < 0.005
	Tank	>/<	Tank		Tank	>/<	Tank	
B2	R1	<	IPS1	0.048	R2	<	R3	0.031
	R2	<	R3	0.008	R2	<	IPS1	0.006
	R2	<	IPS1	< 0.001	R3	>	IPS2	0.046
	R3	>	IPS2	0.012	IPS1	>	IPS2	0.013
	IPS1	>	IPS3	0.007	-	-	-	-

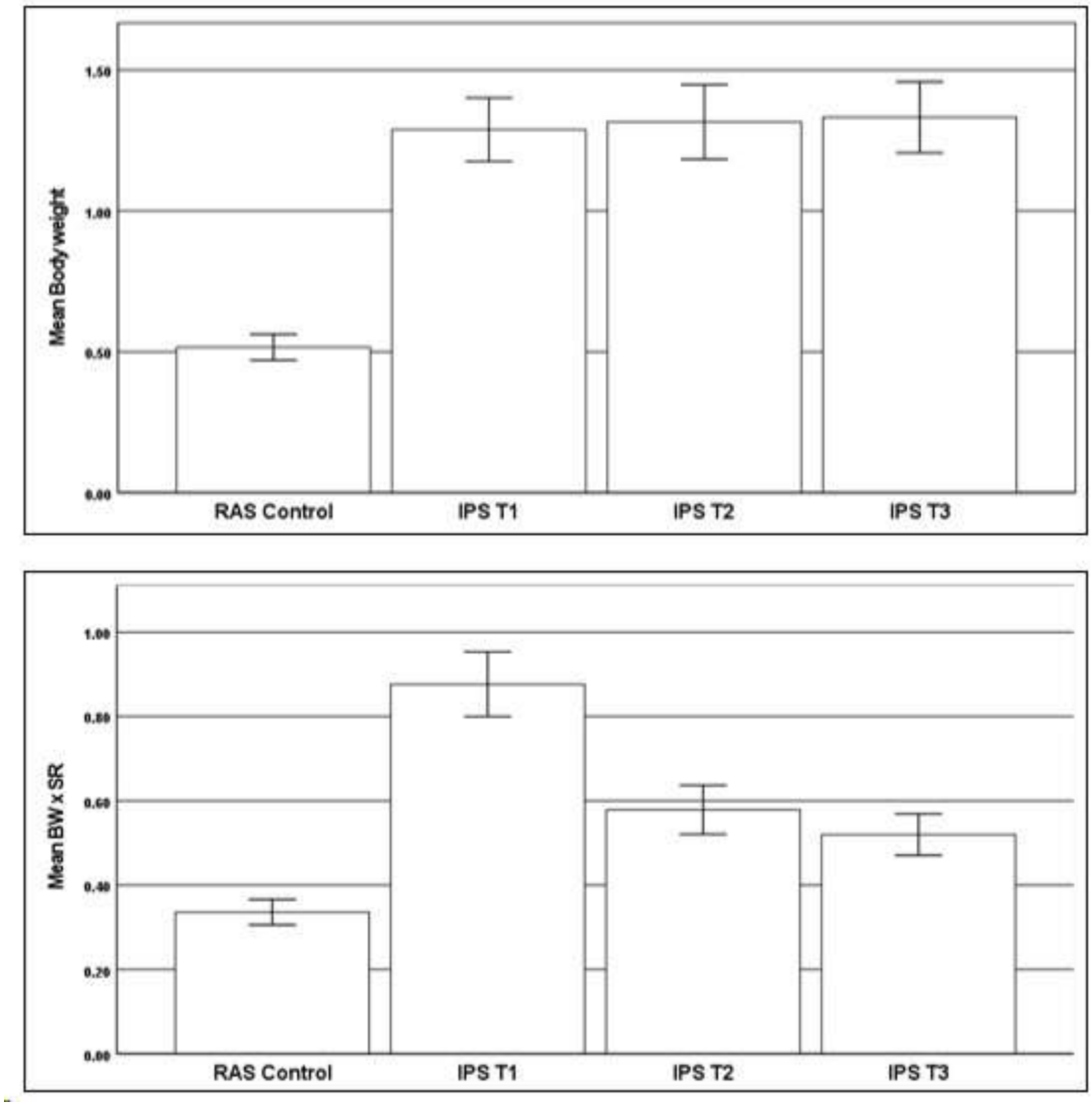

Figure 4.2: Comparison of means of BW (top) and BW x SR (bottom) for Phase B1. The three IPS tanks were significantly different with higher means than RAS.

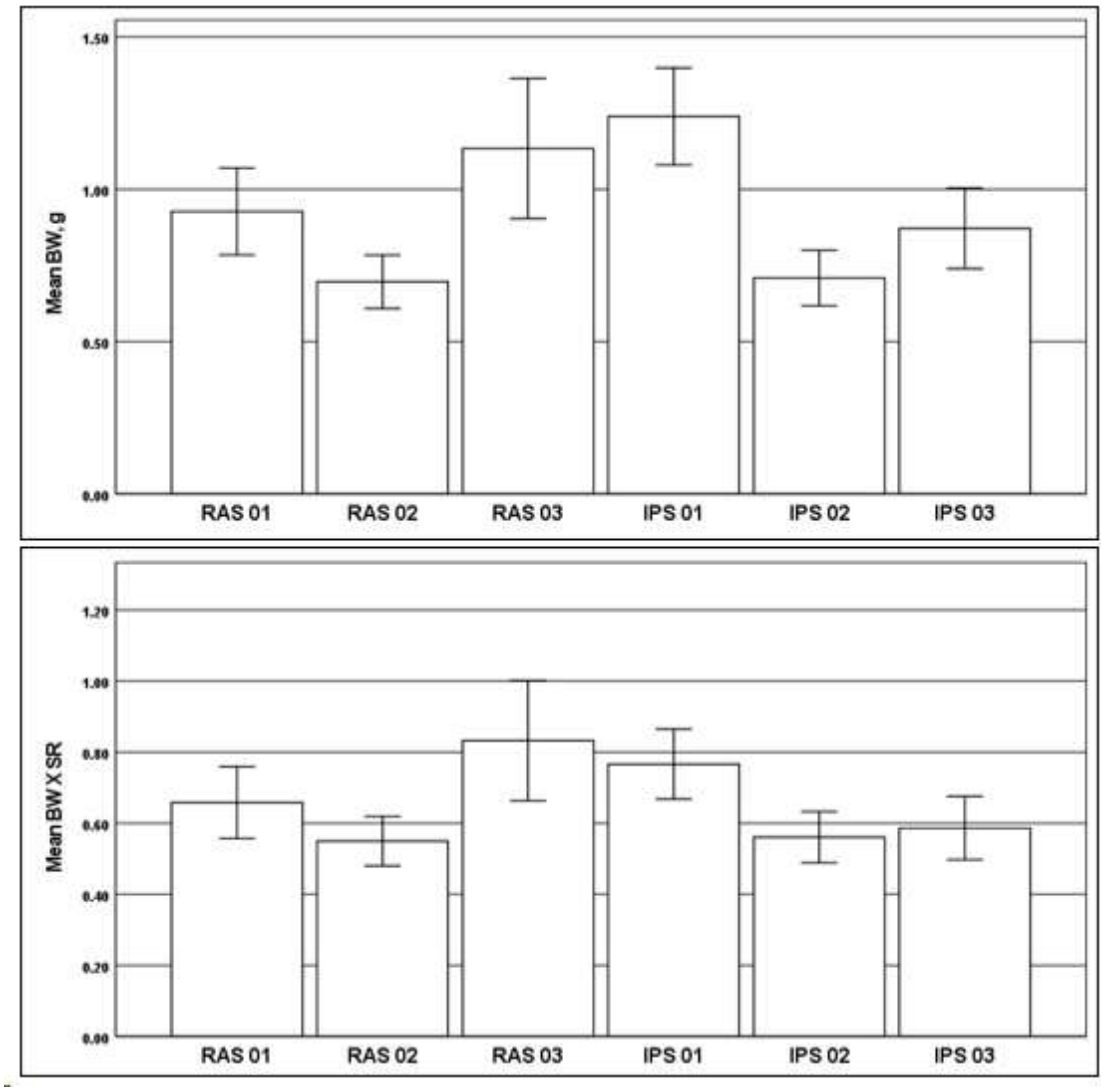

Figure 4.3: Comparison of means of BW (top) and BW x SR (bottom) for Phase B2. Games-Howell analysis showed no significant difference between pairs of IPS and RAS, demonstrating equivalence of the two systems.

4.3 Phase B3: third phase of IPS versus RAS experimentation with post nursery culture of Phase B2 mixed sex *M. rosenbergii* nursery stock

4.3.1 Water quality in Phase B3

Water quality results taken daily for temperature (° C), DO (mg L^{-1}), TDS (mg L^{-1}), and pH were consistently within acceptable aquaculture parameters for both IPS and RAS, with results summarized in Table 4.11.

Table 4.11: Water quality in treatment tanks IPS1, IPS2, and IPS3, and RAS1, RAS2, and RAS3 tanks of Phase B3

Run and Tank	Value	T, (˚C)	DO, (mg L^{-1})	TDS, (mg L^{-1})	pH
IPS1	Average	28.5	6.5	303	7.4
	STDEV	0.7	0.6	73	0.3
IPS2	Average	28.2	6.3	399	7.4
	STDEV	0.7	0.5	98	0.3
IPS3	Average	28.2	6.4	405	7.5
	STDEV	0.7	0.6	82	0.2
RAS1	Average	28.8	6.9	271	7.6
	STDEV	0.8	0.5	48	0.2
RAS2	Average	28.6	7.0	437	7.5
	STDEV	0.7	0.5	69	0.2
RAS3	Average	29.3	7.0	411	7.6
	STDEV	0.6	0.5	80	0.2

4.3.2 Performance indicators in Phase B3

The feed model used was based on assumptions and is expressed in Table 4.12, where equal feed was supplied to each tank based on average input values, survival of 80% over 60 days, average growth of 2 g per survived prawn, fed at 3 % of modelled, cultured prawn mass per day, with total feed provided subsequently used in determination of FCR. The calculated SR, BWG, ADG, and FCR are reported as averages for all the tanks in each group, in Table 4.12.

Table 4.12: Performance indicators for 60-day grow out in Phase B3

Metric	Feed Model	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3
Input PL, No.	580	400	599	519	613	704	647
PL Average BW, g	0.64	0.99	0.53	0.59	0.65	0.54	0.56
Stocking density, No. m^{-2}	387	267	399	346	409	469	431
Harvest, No.	464	258	342	402	259	691	445

Metric	Feed Model	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3
Survival Rate (SR), %	80%	65%	57%	77%	42%	98%	69%
Harvest average BW, g	2.64	3.2 ±2.4	3.4 ±2.7	3.1 ±2.4	3.9 ±3.8	1.8 ±1.9	2.2 ±2.9
Harvest BWG, g	2.00	2.21	2.87	2.51	3.25	1.26	1.64
ADG, g d^{-1}	0.03	0.04	0.05	0.04	0.05	0.02	0.03
Total feed per tank, g	1,530	1,530	1,530	1,530	1,530	1,530	1,530
FCR	1.65	2.68	1.56	1.52	1.82	1.76	2.10

4.3.3 Statistical analysis for Phase B3 as IPS compared with RAS

4.3.3.1 Input prawns to Phase B3

Prawns harvested from IPS1, IPS2, IPS3, RAS1, RAS2, and RAS3 nursery tanks at 43 days were sampled ahead of being transferred to first stage grow out tanks for a further 60 days of culture for the current study. IPS1, IPS2, IPS3, RAS1, RAS2, and RAS3 samples of 53, 78, 71, 83, 89, and 80 individuals, respectively, were weighed from the separated population of smaller prawns of approximately less than 1 g per prawn. Data are mean ± standard deviation, unless otherwise stated. A one-way Welch ANOVA was conducted to determine if BW was significantly different for the IPS (treatment) and RAS (control) groups of the starting populations of each tank, using SPSS version 29. There were 8 outliers in total, and none were extreme, as assessed by boxplot, and the assumption of normal distribution was violated for both groups, as assessed by 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 used as a result to determine if BW of the input prawns for the study was significantly different for the IPS and RAS groups, $p < 0.05$. Table 4.13 summarizes means with standard deviations, and rank.

Table 4.13: Summary of means for BW of input prawns for Phase B3

Tank	N	BW, Mean	BW, STDEV	BW, Rank, Low to High
IPS1	53	1.0011	0.58340	6
IPS2	78	0.5281	0.39300	2
IPS3	71	0.6141	0.40450	3
RAS1	83	0.6690	0.41266	4
RAS2	89	0.5454	0.34707	1
RAS3	38	0.5724	0.42923	5

BW was statistically significantly different between tanks of treatment IPS and RAS. The mean BW increased from IPS2 (n = 78, 0.53 ± 0.39), to RAS2 (n = 89, 0.55 ± 0.35), to RAS3 (n = 38, 0.57 ± 0.43), to IPS3 (n = 71, 0.61 ± 0.40), to RAS1 (n = 83, 0.67 ± 0.41), to IPS1 (n = 53, 1.00 ± 0.58) tank average BW. The BW of input prawns was statistically significantly different for groups, Welch's F (5, 163.215) = 6.353, $p < .001$. The mean differences between RAS (RAS1, RAS2, and RAS3), and IPS1 treatment tank were statistically significant ($p < 0.005$), as derived from Games-Howell post hoc analysis, with results shown in Table 4.14.

Table 4.14: Summary of significant differences defined in Games-Howell post hoc analysis in Phase B3

Tank	Positive (>), or negative (<) difference in means	Tank	Significance of Difference for p < 0.005
RAS1	<	IPS1	0.007
RAS2	<	IPS1	< 0.001
RAS3	<	IPS1	0.002
IPS1	>	IPS2	< 0.001
IPS2	>	IPS3	0.001

4.3.3.2 Final harvest statistical analysis of B3 IPS versus RAS

Data are mean ± standard deviation, unless otherwise stated. Since initial mass of prawns is an average value and different for each tank, BWG was used for statistical

analysis, rather than using harvest BW alone. It was concluded that the following formula (Formula 1) could produce an estimate of harvest BWG of individual prawns from average harvest BW per tank (BW_f), and the average initial BW per tank (BW_i), applied to the individually measured harvest BW (BW_H) of prawns from each tank. The results are reported in Table 4.15, below, and compared with simple calculation of BWG by subtraction of tank average BW at input from tank average BW at harvest.

Table 4.15: Calculated BWG and BWG x SR in Phase B3 using formula 1 in comparison with BWG average from total tank results

Metric	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3
PL Average BW_i, g	0.99	0.53	0.59	0.65	0.54	0.56
Survival Rate (SR), %	65%	57%	77%	42%	98%	69%
Harvest average BW_f, g	3.2 ±2.4	3.4 ±2.7	3.1 ±2.4	3.9 ±3.8	1.8 ±1.9	2.2 ±2.9
Harvest BWG, g	2.21	2.87	2.51	3.25	1.26	1.64
Calculated Harvest BWG, g	2.22 ±1.67	2.85 ±2.10	2.65 ±2.00	3.24 ±3.14	0.96 ±1.07	1.83 ±2.32
Calculated BWG x SR	1.44 ±1.09	1.63 ±1.19	2.04 ±1.54	1.36 ±1.32	0.94 ±1.04	1.26 ±1.60

A one-way Welch ANOVA was conducted to determine if calculated BWG and calculated BWG x SR were significantly different for the IPS (treatment) and RAS (control) groups (Table 4.15). In each case there were outliers, as assessed by boxplot, 1.3 % for BWG and 1 % for BWG x SR. The assumption of normal distribution was violated for each treatment group, as assessed by Shapiro-Wilk test ($p < .001$). Homogeneity of variances was violated for both BWG and BWG x SR, as assessed by Levene's Test of Homogeneity of Variance ($p < 0.001$), and Welch ANOVA was used for BWG and BWG x SR. One-way ANOVA was considered sufficiently robust with large sample sets such that no action was taken to modify sample sets with respect to either violation of normal distribution or of homogeneity of variances. A one-way Welch ANOVA with post hoc Games-Howell analysis was conducted to determine if each of BWG and BWG x SR was significantly different for the IPS and RAS groups, $p < 0.05$, and to further evaluate differences between tanks in each group. Table 4.16

summarizes means with standard deviations, and rank for BWG and BWG x SR, respectively.

Table 4.16: Summary of means for calculated BWG and BWG x SR for 60 days grow out in Phase B3

Tank	N	BWG, Mean	BWG, STDEV	BWG, Rank, Low to High	BWG x SR, Mean	BWG x SR, STDEV	BWG x SR, Rank, Low to High
RAS1	259	3.2375	3.13348	6	1.3581	1.31676	4
RAS2	691	1.1300	1.24535	1	1.1073	1.22020	2
RAS3	445	1.6727	2.18523	2	1.1543	1.50789	6
IPS1	258	2.2162	1.67284	33	1.4402	1.08837	1
IPS2	342	2.8546	2.28871	5	1.6272	1.30484	3
IPS3	402	2.5006	1.92518	4	1.9252	1.48238	5

The mean BWG at harvest was seen to progressively decrease from RAS1 (n = 259, 3.24 ± 3.13), to IPS2 (n = 342, 2.85 ± 2.29), to RAS3 (n = 445, 1.67 ± 2.19), to IPS3 (n = 402, 2.50 ± 1.93), to IPS1 (n = 258, 2.22 ± 1.67), and to RAS2 (n = 691, 1.13 ± 1.25) (Table 4.18). The BWG at harvest was statistically significantly different for treatments and control groups, Welch's F (5, 885.719) = 77.417, $p < .001$. The group means were statistically significantly different ($p < .001$) and, therefore, we can reject the null hypothesis and accept the alternative hypothesis. It could be observed that the IPS tanks as a distinct set within the treatment group were significantly different, but did not all consistently display increased BWG figures, over the RAS tanks as a set within the group. Table 4.17 sets out the significant differences ($p < 0.005$) between individual tanks from Games-Howell post hoc analysis. IPS tank BWG means were significantly greater than RAS tanks, except in the case of mean BWG of RAS1 which was greater than that of both IPS3 and IPS1. In this study, IPS is concluded to have established equivalent performance with respect to BWG in comparison with RAS.

BWG was multiplied by Survival Rate (BWG x SR), as each tank not only yielded different average BWG, but a distinct SR, and the product was considered a statistical measure of productivity. The mean BWG at harvest increased from RAS2

(n = 691, 1.11 ± 1.22), to RAS3 (n = 445, 1.15 ± 1.51), to RAS1 (n = 259, 1.36 ± 1.32), to IPS1 (n = 258, 1.44 ± 1.09), to IPS2 (n = 342, 1.63 ± 1.30), to IPS3 (n = 402, 1.93 ± 1.48) tank harvest BWG x SR (Table 4.18). The BWG x SR at harvest was statistically significantly different for IPS treatment and RAS control groups, Welch's F (5, 952.364) = 22.319, p < .001. The group means were statistically significantly different (p < .001) and, therefore, we can reject the null hypothesis and accept the alternative hypothesis. Table 4.17 sets out the significant differences (p < 0.005) between individual tanks from Games-Howell post hoc analysis. In this study, IPS is concluded to have established improved performance with respect to BWG x SR in comparison with RAS.

Table 4.17: Summary of significant difference by Games-Howell analysis for BWG and BWG x SR in Phase B3

BWG			**Significance p < 0.005**	**BWG x SR**			**Significance p < 0.005**
Tank	**</>**	**Tank**		**Tank**	**</>**	**Tank**	
RAS1	>	RAS2	< 0.001	**RAS1**	<	IPS3	< 0.001
RAS1	>	RAS3	< 0.001	**RAS2**	<	IPS1	< 0.001
RAS1	>	IPS1	< 0.001	**RAS2**	<	IPS2	< 0.001
RAS1	>	IPS3	0.010	**RAS2**	<	IPS3	< 0.001
RAS2	<	IPS1	< 0.001	**RAS3**	<	IPS1	0.044
RAS2	<	RAS3	< 0.001	**RAS3**	<	IPS2	< 0.001
RAS2	<	IPS2	< 0.001	**RAS3**	<	IPS3	< 0.001
RAS2	<	IPS3	< 0.001	**IPS1**	<	IPS3	< 0.001
RAS3	<	IPS1	0.003	**IPS2**	<	IPS3	0.042
RAS3	<	IPS2	< 0.001	-	-	-	-
RAS3	<	IPS3	< 0.001	-	-	-	-
IPS1	<	IPS2	0.001	-	-	-	-

Comparison of means of BWG and BWG x SR, of prawns harvested from individual IPS and RAS tanks are shown in charts presented in Figure 4.4, below.

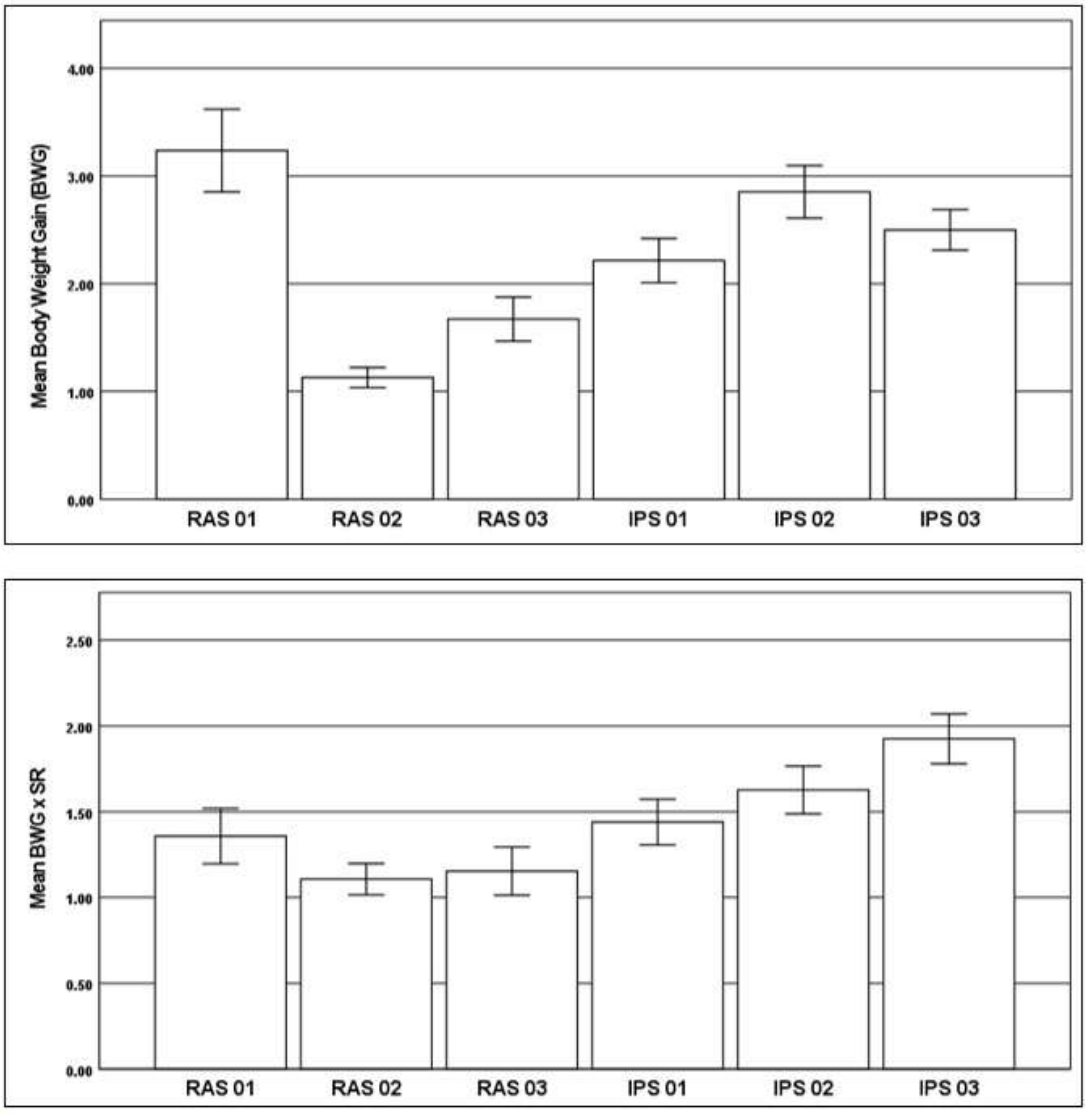

Figure 4.4 Comparison of means of BWG (top) and BWG x SR (bottom) for Phase B3. The IPS group was significantly different with higher means of BWG x SR than RAS.

4.4 Phase B3 in comparison with Phase B4: separate, mass differentiated grow out of Phase B2 nursery stock

In Phase B3, three IPS tanks (IPS1, IPS2, and IPS3), and three RAS tanks (RAS1, RAS2, and RAS3), collectively IPS_{small} and RAS_{small}, were stocked with smaller Phase B2 nursery prawns. Phase B4 was a single, large IPS tank (IPS_{large}) that was populated with the larger size remainder of prawns from Phase B2 nursery run. Methodology of size separation for the two prawn sizes has been detailed in Chapter 3.

4.4.1 Water quality analysis of Phases B3 and B4

Water quality measurements of temperature (° C), DO (mg L^{-1}), TDS (mg L^{-1}), and pH, were consistently within acceptable aquaculture parameters, with results summarized in Table 4.18.

Table 4.18: Water quality in treatment tanks IPS_{small} and RAS_{small} for Phase B3, and the single IPS_{large} tank of Phase B4

Run and Tank	Value	T, (°C)	DO, (mg L^{-1})	TDS, (mg L^{-1})	pH
$IPS1_{small}$	Average	28.5	6.5	303	7.4
	STDEV	0.7	0.6	73	0.3
$IPS2_{small}$	Average	28.2	6.3	399	7.4
	STDEV	0.7	0.5	98	0.3
$IPS3_{small}$	Average	28.2	6.4	405	7.5
	STDEV	0.7	0.6	82	0.2
$RAS1_{small}$	Average	28.8	6.9	271	7.6
	STDEV	0.8	0.5	48	0.2
$RAS2_{small}$	Average	28.6	7.0	437	7.5
	STDEV	0.7	0.5	69	0.2
$RAS3_{small}$	Average	29.3	7.0	411	7.6
	STDEV	0.6	0.5	80	0.2
IP_{large}	Average	27.5	6.5	407	7.3
	STDEV	0.6	0.7	117	0.2

4.4.2 Performance indicators of Phases B3 and B4

The calculated SR, BWG, ADG, and FCR are reported averages for the outputs from individual tanks in each group, in Table 4.19, some of which data is recorded earlier in Table 4.12.

Table 4.19: Performance indicators for 60 days of grow out of Phases B3 and B4

Metric	IPS1 small	IPS2 small	IPS3 small	RAS1 small	RAS2 small	RAS3 small	IPS large
Input PL, No.	400	599	519	613	704	647	1,558
PL Average BW, g	0.99	0.53	0.59	0.65	0.54	0.56	1.46
Stocking density, g m^{-3}	305	244	236	292	292	279	290
Harvest, No.	258	342	402	259	691	445	1,214
Overall Survival Rate (SR), %	65%	57%	77%	42%	98%	69%	78%
Males Harvested, No.	152	123	106	100	455	127	987
Females Harvested, No.	106	219	296	159	236	318	227
Ratio, Male:Female	1.43	0.56	0.36	0.63	1.93	0.40	4.35
Harvest average BW, g	3.2 ±2.4	3.4 ±2.7	3.1 ±2.4	3.9 ±3.8	1.8 ±1.9	2.2 ±2.9	5.7 ±4.4
Male Harvest average BW, g	4.2 ±2.5	6.4 ±2.8	5.8 ±2.2	6.6 ±4.1	2.3 ±1.9	4.5 ±3.7	6.1 ±4,3
Female Harvest average BW, g	1.7 ±1.2	1.9 ±1.3	2.1 ±1.5	2.2 ±2.4	0.8 ±1.6	1.3 ±1.7	3.6 ±3.9
Harvest BWG, g	2.21	2.87	2.51	3.25	1.26	1.64	4.24
ADG, g d^{-1}	0.04	0.05	0.04	0.05	0.02	0.03	0.07
Total feed per tank, g	1,530	1,530	1,530	1,530	1,530	1,530	11,113
FCR	2.68	1.56	1.52	1.82	1.76	2.10	2.16
FCR as group		1.79			1.88		2.16

An FCR of 1.65 was calculated and expressed in Table 4.12. FCR was based on the feed model design assumptions that result in 1,530 g of feed being applied in total over 60 DoC, and total harvest BWG for individual tanks, and averages for IPS and RAS tank groups. The group average FCR realized in IPS_{small} and RAS_{small} as groups was 1.79 and 1.88, respectively. For the single IPS_{large} tank, assumptions, and an anticipated FCR of 1.69 from the feed model, resulted in 11,113 g of feed being applied in total over the 60 DoC, while FCR realized in IPS_{large} was 2.16 over 60 DoC at harvest. The smaller prawn harvests, IPS_{small} and RAS_{small}, achieved lower FCR than the larger prawn harvest from the single tank, IPS_{large}. FCR for all tanks was higher than predicted by the feed model.

4.4.3 Statistical analysis for Phases B3 and B4

4.4.3.1 Input prawns to Phases B3 and B4

Prawns harvested from Phase B2 nursery tanks IPS1, IPS2, IPS3, RAS1, RAS2, and RAS3 at 43 DoC were sampled ahead of being transferred to grow out tanks for a further 60 DoC for Phases B3 and B4. IPS1, IPS2, IPS3, RAS1, RAS2, and RAS3 samples of 53, 78, 71, 83, 89, and 80 individuals, respectively, were weighed from the separated population of smaller prawns of approximately less than 1 g BW, representative of input to Phase B3. A total of 194 prawns were measured as representative of the input to IPS_{large} of Phase B4 with 40, 41, 30, 24, 29, and 30 individuals from the IPS1, IPS2, IPS3, RAS1, RAS2, and RAS3 tanks of B2 harvest, respectively, which were weighed from the separated population of larger prawns of approximately greater than 1 g BW of each tank.

A two-way ANOVA (Laerd) was conducted to examine the effects on BW of size (large size group and small size groups) for six originating treatment tanks (IPS1, IPS2, IPS3, RAS1, RAS2 & RAS3) by analysing the samples extracted from the size separated outputs of nursery harvests from each tank. Data are mean ± standard deviation, unless otherwise stated. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design, and homogeneity of variances was assessed by Levene's test. Outliers were few and ignored. Residuals were not normally distributed ($p < 0.001$) and homogeneity of variances was violated ($p < 0.001$).

There was a statistically significant interaction between the two sizes (size group) and treatment group on BW, $F(5, 594) = 6.6007$, $p < 0.001$, partial $\eta 2 = .053$. Therefore, an analysis of simple main effects for treatment type was performed with statistical significance receiving a Bonferroni adjustment and being accepted at the $p < .05$ level.

The mean BW for the large (> 1 g BW) and small (< 1 g BW) sample sets from each nursery tank of Phase B2 are reported in Table 4.20.

Table 4.20: Mean BW for the large (> 1 g BW) and small (< 1 g BW) sample sets from each nursery tank of Phase B2

Tank	Large Size set, mean BW	Sample number	Small Size set, mean BW	Sample number
IPS1	1.56 ± 0.88	40	1.00 ± .58	53
IPS2	1.05 ± 0.51	41	0.23 ± .39	78
IPS3	1.48 ± 0.78	30	0.61 ± .40	71
RAS1	1.82 ± 0.94	24	0.67 ± .41	83
RAS2	1.16 ± 0.54	29	0.55 ± .35	89
RAS3	1.85 ± 0.96	30	0.57 ± .43	38
Total sample set	1.46 ± 0.72	194	N/A	N/A

There was a statistically significant difference in mean BW scores between larger set and smaller set prawns of all six treatment tanks of Phase B2 harvest, as reported in Table 4.21.

Table 4.21: Statistically significant difference between larger set and smaller set prawn BW by nursery tank of Phase B2

Tank	Statistically significant difference between larger set and smaller set prawn BW by tank
IPS1	$F(1,594) = 22.389$, $p < 0.001$, partial $\eta 2 = .036$
IPS2	$F(1,594) = 23.636$, $p < 0.001$, partial $\eta 2 = .038$
IPS3	$F(1,594) = 50.744$, $p < 0.001$, partial $\eta 2 = .079$
RAS1	$F(1,594) = 79.074$, $p < 0.001$, partial $\eta 2 = .117$
RAS2	$F(1,594) = 26.483$, $p < 0.001$, partial $\eta 2 = .043$
RAS3	$F(1,594) = 86.932$, $p < 0.001$, partial $\eta 2 = .128$

All pairwise comparisons were run for each simple main effect with reported 95% confidence intervals and p-values Bonferroni-adjusted within each simple main effect. Mean BW for prawns in the larger prawn set were larger than for the smaller set, all $p < 0.001$, as reported respectively in Table 4.22.

Table 4.22: Mean BW difference between large (> 1 g BW) and small (< 1 g BW) sets by Phase B2 nursery tank with confidence intervals

Tank	Mean BW difference, large > small, g	95% CI range	
		From low:	To high:
IPS1	0.554	0.324	0.784
IPS2	0.524	0.312	0.736
IPS3	0.867	0.628	1.106
RAS1	1.152	0.897	1.406
RAS2	0.615	0.380	0.850
RAS3	1.273	1.005	1.541

For all nursery treatment tanks, there was a statistically significant difference in BW, between the larger set $F(5, 594) = 11.120$, $p < 0.001$, partial $\eta 2 = .086$, and for the smaller set $F(5, 594) = 5.748$, $p < 0.001$, partial $\eta 2 = .046$. In the larger prawn set RAS1 and RAS3 had significantly higher mean BW than IPS2, by 0.769 ($p < 0.001$) and 0.793 ($p < 0.001$), respectively. In the smaller prawn set, IPS1 had significantly higher mean BW than RAS1, RAS2, and RAS3, by 0.332 ($p = 0.012$), 0.456 ($p < 0.001$), and 0.429 ($p = 0.005$), respectively.

Comparison of means of BW and size separation of Phase B2 nursery treatment sample sets from IPS and RAS nursery tanks are shown in Figure 4.5 and describe the inputs to the Phases B3 and B4 60 DoC grow out study. After extraction of samples for measurement, the entire large set from each tank was transferred to IPS_{large}, while the small sets from each tank were returned to their originating tanks for continuation of culture.

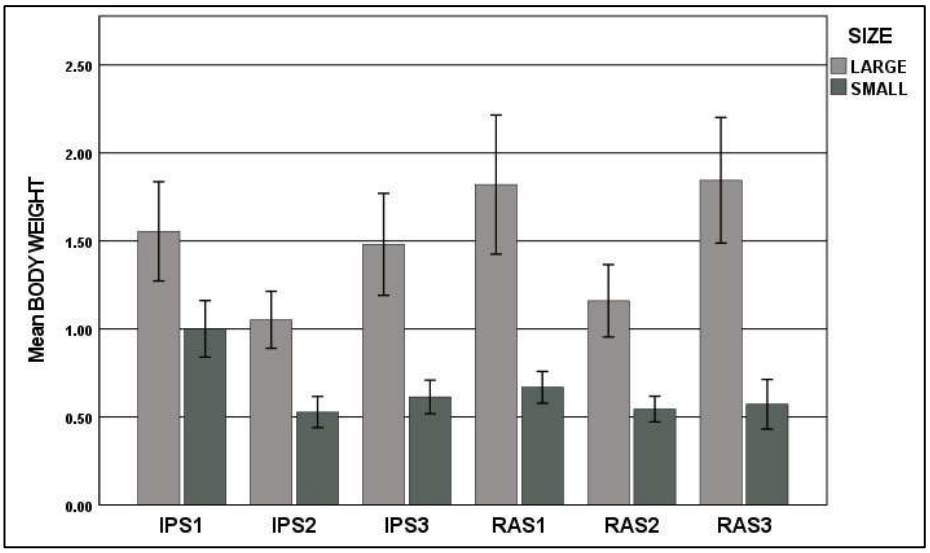

Figure 4.5 Comparison of means of BW for IPS and RAS Phase B2 nursery tanks derived from measurement of large (> 1 g BW) and small (< 1 g BW) sample sets

4.4.3.2 Final harvest of Phases B3 and B4 statistical analysis

A two-way ANOVA was conducted to examine the effects of two groups of independent variables, gender (male, female) and treatment type (IPS_{small}, RAS_{small}, and IPS_{large}), on BWG. Data are mean ± standard deviation, unless otherwise stated. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design, and homogeneity of variances was assessed by Levene's test. There were outliers, with groups similarly skewed and large sample numbers. Residuals were not normally distributed ($p < 0.001$) and homogeneity of variances was violated ($p < 0.001$).

There was a statistically significant interaction between gender and treatment type on BWG, $F(2, 3579) = 30.047$, $p < 0.001$, partial $\eta 2 = .017$. Therefore, an analysis of simple main effects for treatment type was performed with statistical significance receiving a Bonferroni adjustment and being accepted at the $p < .05$ level. There was a statistically significant difference in mean BWG scores between males and females in the treatments IPS_{small}, RAS_{small}, and IPS_{large}, $F(1, 3579) = 284.454$, $p < .001$, partial $\eta 2 = .07412$, $F(1, 3579) = 171.116$, $p < .001$, partial $\eta 2 = .046$, and $F(1, 3579) = 347.460$, $p < .001$, partial $\eta 2 = .088$, respectively.

All pairwise comparisons were run for each simple main effect with reported 95 % confidence intervals and p-values Bonferroni-adjusted within each simple main effect. Mean BWG for IPS_{small} males and females were 4.53 ± 0.15, and 1.32 ± 0.11, respectively. IPS_{small} males had a statistically significantly higher mean BWG than IPS_{small} females, 3.204 (95% CI, 2.83 to 3.58). Mean BWG for RAS_{small} males and females were 2.75 ± 0.11, and 0.76 ± 0.11, respectively. RAS_{small} males had a statistically significantly higher mean BWG than RAS_{small} females, 1.995 (95% CI, 1.70 to 2.29). Mean BWG for IPS_{large} males and females were 6.10 ± 0.09, and 2.19 ± 0.19, respectively. IPS_{large} males had a statistically significantly higher mean BWG than IPS_{large} females, 3.904 (95% CI, 3.49 to 4.31).

There was a statistically significant difference in BWG between the males and between the females reared in the three treatment system groups, $F(2,3579) = 278.716$, $p < .001$, partial $\eta 2 = .135$, and $F(2,3579) = 23.062$, $p < .001$, partial $\eta 2 = .013$, respectively. Mean BWG for IPS_{large} males was 1.57 higher than for IPS_{small} males, (95 % CI, 1.15 to 2.00), and 3.35 higher than for RAS_{small} males, (95 % CI, 3.01 to 3.69). Mean BWG for IPS_{large} females was 0.87 higher than for IPS_{small} females, (95 % CI, 0.34 to 1.40), and 1.44 higher than for RAS_{small} females, (95% CI, 0.92 to 1.96).

There was a statistically significant difference in BWG between the three treatment systems, $F(2,3579) = 174.901$, $p < .001$, partial $\eta 2 = .089$. Data are ± standard error unless otherwise stated. The marginal means for BWG for the three treatments were 2.92 ± 0.10 for IPS_{small}, 1.75 ± 0.08 for RAS_{small}, and 4.15 ± 0.11 for IPS_{large}. Statistically significant mean difference between IPS_{large} and IPS_{small} was 1.22 (95 % CI, 0.88 to 1.46), $p < 0.001$, between IPS_{large} and RAS_{small} was 2.39 (95 % CI, 2.08 to 2.70), $p < 0.001$, and between IPS_{small} and RAS_{small} was 1.17 (95 % CI, 0.88 to 1.46), $p < 0.001$.

Comparison of means of BWG for treatment and sex, of harvested prawns from Phases B3 and B4 are shown in the chart presented in Figure 4.6.

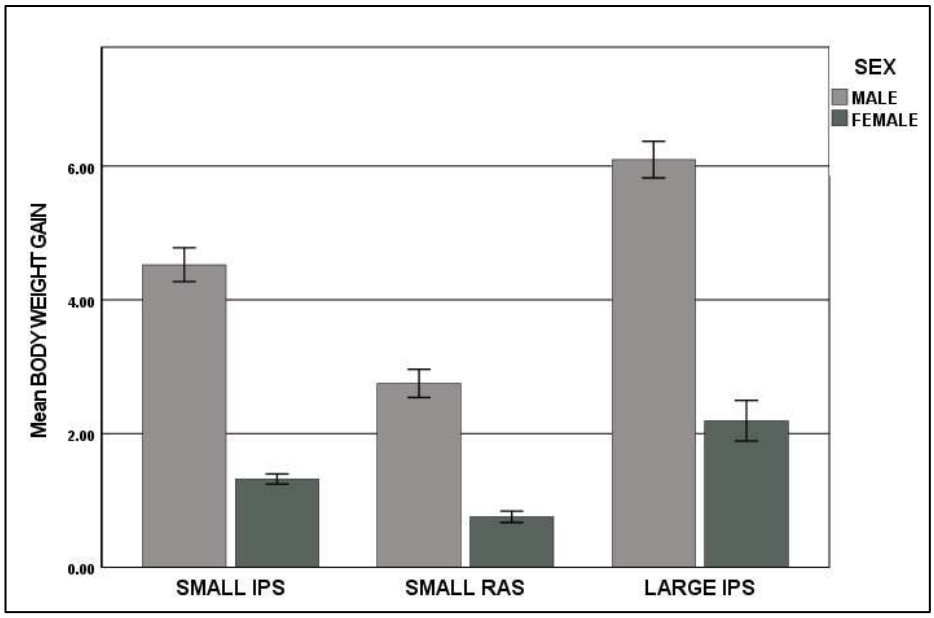

Figure 4.6 Comparison of means of BWG during 60-day grow out for treatment groups and sex of Phases B3 and B4

4.5 Phases B2 to B4: experimental results and morphometric analysis from nursery through post nursery for the mixed sex *M. rosenbergii* population

The overall methodology of the combined phases from Phase B2 to Phase B4 considers that all these phases are part of the culture of a single PL stock, which is presented in Figure 3.1. Water quality results have been discussed in the separate sections of Chapter 4 for Phases B2 to B4.

4.5.1 Performance indicators for Phase B2

The feed model used for Phase B2 nursery stage was based on assumptions expressed in Table 4.5, and determined the total feed supplied that was used in determination of FCR. The tanks used in Phase B2 were designated N-IPS and N-RAS, to reflect the nursery stage. The calculated SR, BWG, harvest density (individuals m^{-2}), BWG, and FCR for Phase B2 were reported as averages, including standard deviations in Table 4.5. Input PL and harvested prawns were both counted from which SR was generated. Harvest from the Phase B2 nursery stage had samples extracted for measurement, after two size groups were formed from the total harvest. The resulting

BW for each treatment and control tank were recorded for both size groups in Figure 4.5.

4.5.2 Performance indicators for Phases B3 and B4 post-nursery phases

Performance Indicators for the post-nursery Phase B3 stocked with the smaller prawns of less than 1 g BW (IPS1, IPS2 and IPS3, and RAS1, RAS2, and RAS3), and Phase B4 stocked with larger prawns of greater than 1 g BW (IPS_{large}), are given in Table 4.23, some of which data is recorded earlier in Table 4.21.

Table 4.23: Performance Indicators for the Phase B3 and Phase B4 post-nursery runs (large and small stock groups).

Metric	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3	IPS large
Phase	**Phase B3**						**Phase B4**
Input PL, No.	400	599	519	613	704	647	1,558
PL Average BW, g	0.99	0.53	0.59	0.65	0.54	0.56	1.46
Stocking density, g m^{-3}	305	244	236	292	292	279	290
Harvest, No.	258	342	402	259	691	445	1,214
Overall Survival Rate (SR), %	65%	57%	77%	42%	98%	69%	78%
Males Harvested, No.	152	123	106	100	455	127	987
Females Harvested, No.	106	219	296	159	236	318	227
Ratio, Male: Female	1.43	0.56	0.36	0.63	1.93	0.40	4.35
Harvest average BW, g	3.2 ±2.4	3.4 ±2.7	3.1 ±2.4	3.9 ±3.8	1.8 ±1.9	2.2 ±2.9	5.7 ±4.4
Male Harvest	4.2 ±2.5	6.4 ±2.8	5.8 ±2.2	6.6 ±4.1	2.3 ±1.9	4.5 ±3.7	6.1 ±4,3

Metric	IPS1	IPS2	IPS3	RAS1	RAS2	RAS3	IPS large
Phase	**Phase B3**						**Phase B4**
average BW, g							
Female Harvest average BW, g	1.7 ±1.2	1.9 ±1.3	2.1 ±1.5	2.2 ±2.4	0.8 ±1.6	1.3 ±1.7	3.6 ±3.9
Harvest BWG, g	2.21	2.87	2.51	3.25	1.26	1.64	4.24
Total feed per tank, g	1,530	1,530	1,530	1,530	1,530	1,530	11,113
FCR	2.68	1.56	1.52	1.82	1.76	2.10	2.16
FCR by group	1.92						2.16

The mean Body Weight Gain (BWG) of harvested prawns from Phase B3, the post-nursery stage stocked with smaller prawns of approximately less than 1 g, is given as “Small IPS” and “Small RAS” in Figure 4.6, above. Mean BWG of both males and females was significantly greater at harvest for IPS, over RAS, and in both cases the BWG of males was significantly higher for males than for females. The Phase B4 “Large IPS” record (rightmost column) indicates BWG of harvested prawns from the single large IPS tank, stocked with larger prawns of approximately greater than 1 g.

Harvested prawns from Phase B4 (large, > 1 g BW) and Phase B3 (small, < 1 g BW) outputs were also classified by morphotype of female prawn, and three male morphotypes (SM, OC, or BC males). The percentages by BW, and by counted number of surviving prawns, for each male morphotype and for female gender, are displayed for each tank, and for the summed total population of all tanks (ALL), in Figure 4.7. The category ALL includes the harvested prawns from both Phases B3 and B4. These prawn populations represent the output at 104 days of the same prawns stocked to the Phase B2 nursery stage, minus approximately 20 % losses incurred during post nursery transfer. Losses occurred as approximately 7 % from mortality losses and 13 % from extracted sample for measurement losses.

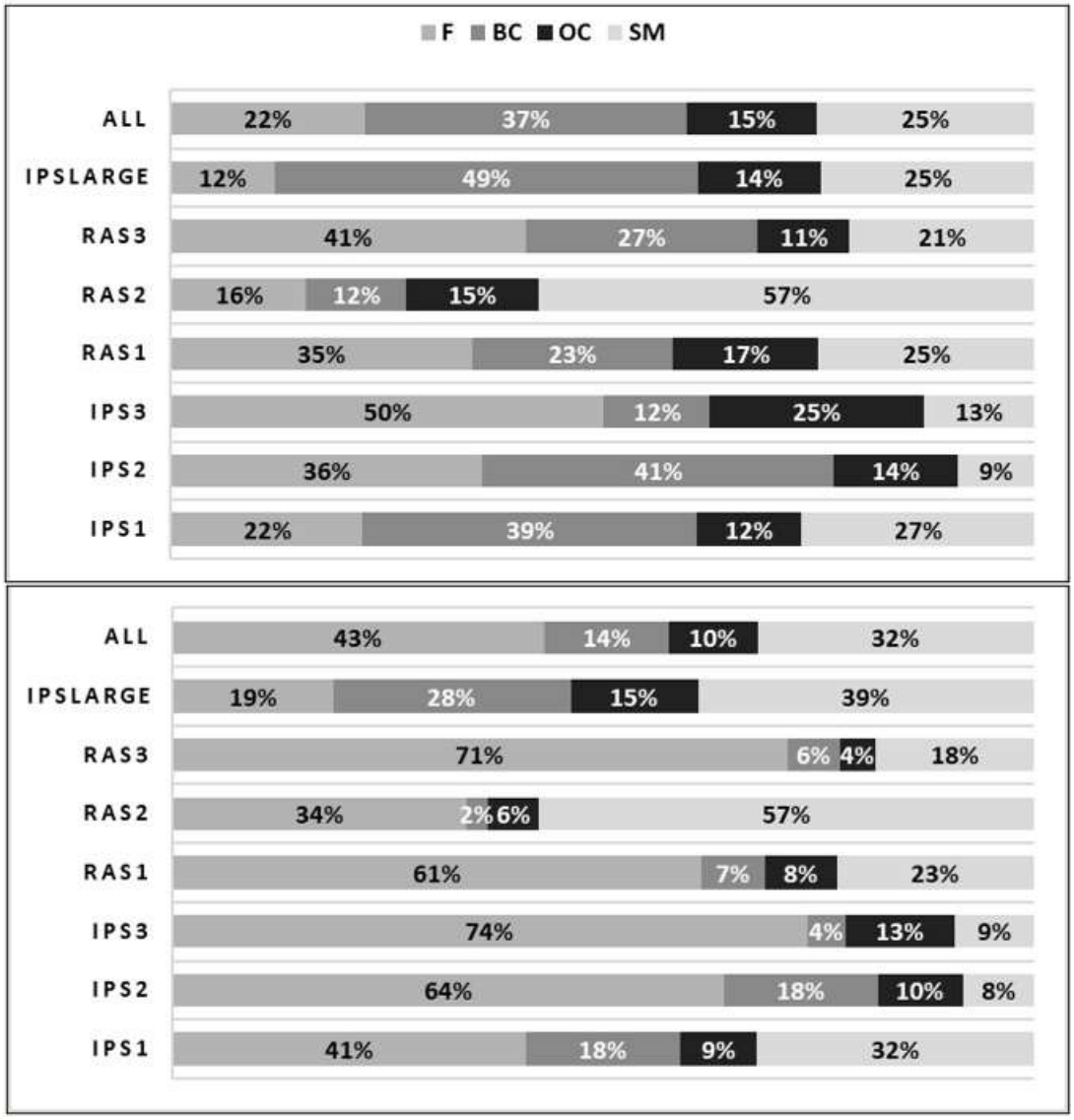

Figure 4.7. Comparison of average percentages of BW in grams (top) and of harvest count (bottom) for triplicate IPS and triplicate RAS post-nursery tanks attributed to Phase B3 small prawns (< 1 g BW) at harvest, to Phase B4 tank IPS_{large} large prawns (> 1 g BW) at harvest, and of the harvest at 104 days as a whole (ALL) including all prawns. Both top and bottom charts sum horizontally to 100 % in each row. HIG morphotypic categories shown include F (female), BC (blue claw male), OC (orange claw male), and SM (small male).

Figure 4.8 provides average BW in grams of surviving prawns, for each male morphotype and female gender for each tank, and for the total harvest population of all tanks (ALL). Figure 4.9 provides the total harvest biomass in grams of surviving prawns, for each male morphotype and female gender for each tank, and for the total harvest population of all tanks (ALL).

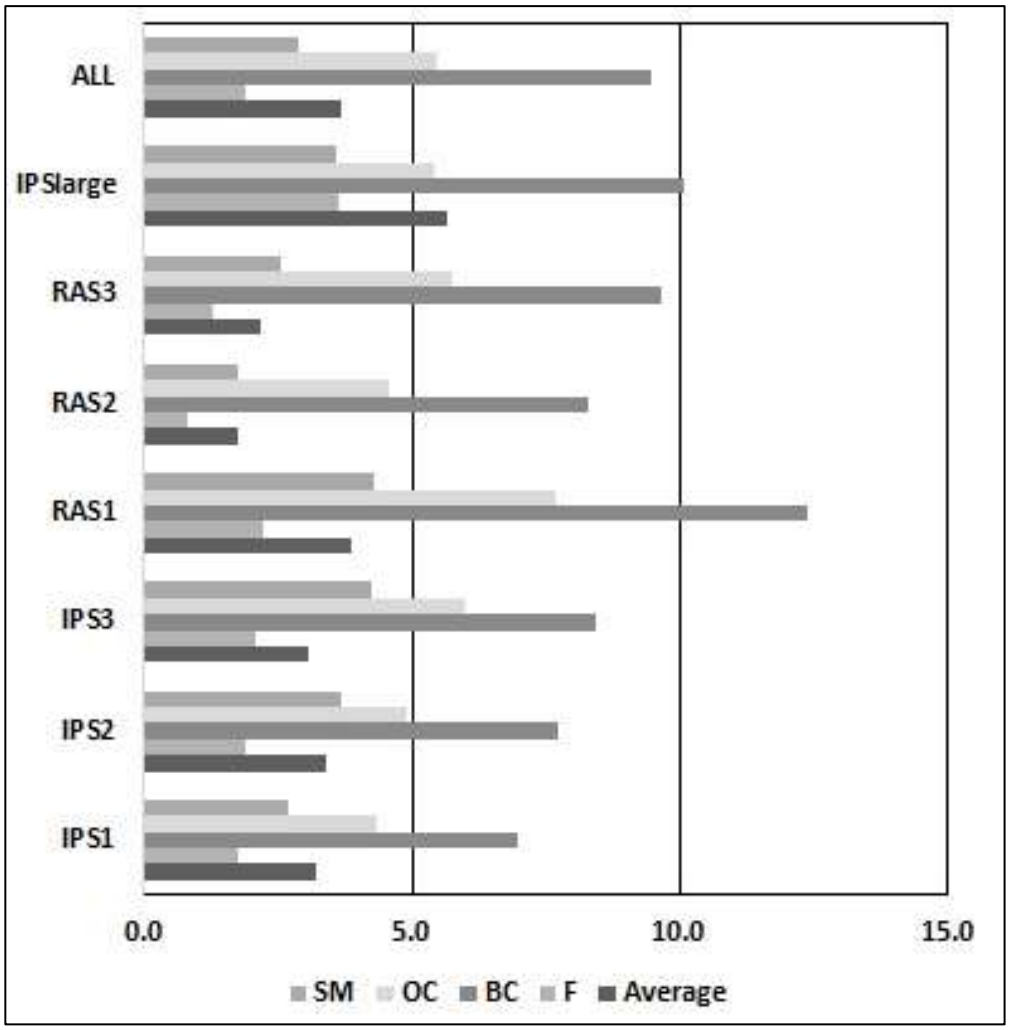

Figure 4.8. Comparison of average BW in grams for IPS and RAS Phase B3 post-nursery tanks derived from measurement of small (< 1 g BW) at harvest, and Phase B4 tank IPS_{large} large (> 1 g BW) at harvest, and of the harvest as a whole (ALL) (ALL). Morphotype average BW shown includes F (female), BC (blue claw male), OC (orange claw male), and SM (small male).

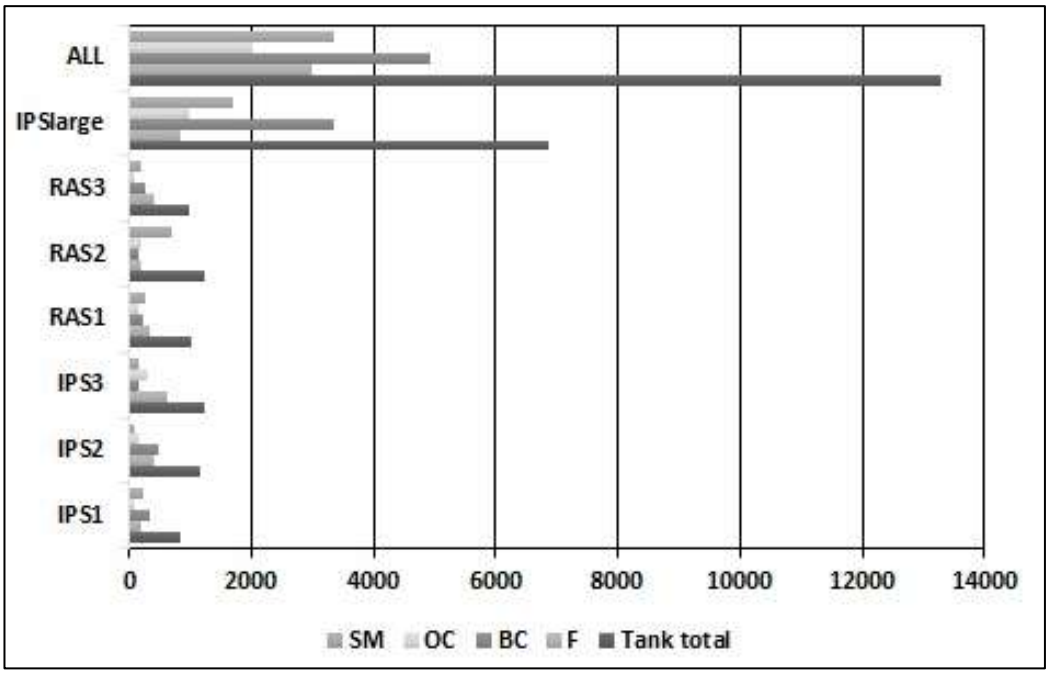

Figure 4.9. Comparison of total harvest biomass (g) for IPS and RAS post-nursery tanks derived from measurement of small (< 1 g BW) of Phase B3 at harvest, and tank IPS_{large} large (> 1 g BW) of Phase B4 at harvest, and of the harvest as a whole (ALL). Average produced biomass by morphotype is shown for F (female), BC (blue claw male), OC (orange claw male), and SM (small male). Left to right of the legend corresponds to top to bottom of the tank records.

Table 4.24 displays the input mass stocked to each tank for the Phase B3 and Phase B4 post-nursery phases, and for the total experimental harvest from 60 days culture of the full nursery phase output (total of 104 days), minus about 20 % losses due to transfer and measurement. SGR was calculated for each tank over the culture duration, and for the total population surviving from Phase B2 (ALL).

Table 4.24: Specific Growth Rate (SGR) calculated for the post-nursery runs (Phase B4 large and Phase B3 small stock) and for the full population (ALL), over the 60 day duration.

Tank(s)	Input Mass, g	Final Mass, g	Mass gain, g	SGR, % d^{-1} (60 days)
IPS1	405	827	422	1.23%
IPS2	317	1,158	841	2.23%
IPS3	306	1,229	923	2.40%
RAS1	401	1,007	606	1.59%
RAS2	379	1,220	841	2.02%
RAS3	361	971	610	1.71%
IPS_{large}	2,263	6,866	4,603	1.91%
ALL	4,432	13,278	8,846	1.89%

4.5.3 Statistical analysis for overall Phases B2 to B4

4.5.3.1 Statistical analysis of final harvest of the Phase B2 nursery experiment

A one-way Welch ANOVA was conducted to determine if BW and BW multiplied by SR (BW x SR) were significantly different for the IPS (treatment) and RAS (control) groups, for Phase B2 results, using SPSS, version 29. Comparison of means of BW and BW x SR, of harvested prawns, is shown in charts presented in Figure 4.3 (top and bottom) in Section 4.2.4.2. In the Phase B2 nursery run, the BW at harvest of the three B2 IPS tanks were significantly different. Games-Howell analysis revealed no significant difference in harvest BW between matched pairs of IPS and RAS from the triplicate sets, demonstrating equivalence of the two systems in the Phase B2 nursery run, shown in Figure 4.3 (top and bottom).

4.5.3.2 Statistical analysis of final harvest of the Phase B3 and B4 post-nursery experiments

A two-way ANOVA was conducted to examine the effects of gender and treatment type (IPS and RAS of small stock in triplicate, and IPS_{large}) on BWG. Data were mean ± standard deviation, unless otherwise stated. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design, and homogeneity of variances was assessed by Levene's test. There were outliers, with groups similarly skewed and large sample numbers. Residuals were not normally distributed ($p < 0.001$) and homogeneity of variances was violated ($p < 0.001$).

There was a statistically significant interaction between gender and treatment type on BWG, $F(2, 3579) = 30.047$, $p < 0.001$, partial $\eta 2 = .017$. Therefore, an analysis of simple main effects for treatment type was performed with statistical significance receiving a Bonferroni adjustment and being accepted at the $p < .05$ level. There was a statistically significant difference in mean BWG scores between males and females in the treatments (IPS and RAS of small stock in triplicate, and IPS_{large}), $F(1, 3579) =$

284.454, p < .001, partial η2 = .07412, F (1, 3579) = 171.116, p < .001, partial η2 = .046, and F (1, 3579) = 347.460, p < .001, partial η2 = .088, respectively. Figure 4.10 displays the post-nursery stage trends of BW for males and females at harvest, averaged for each treatment of smaller and larger prawns.

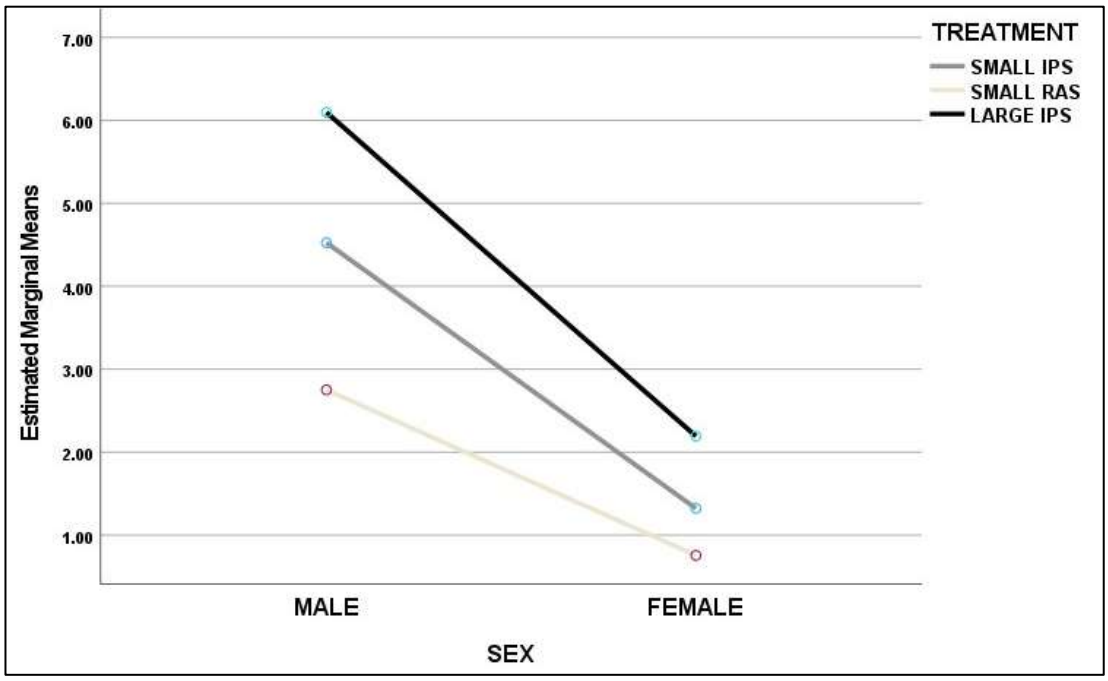

Figure 4.10. Comparison of means of BWG in grams for IPS and RAS of small stock as average of triplicate tanks in Phase B3, and for single Phase B4 tank IPS_{large} for large stock.

4.6 Phase B5: experimentation with nursery of all-male *M. rosenbergii* in Hapa nets maintained within an IPS pond

As described in Chapter 3, the fifth phase of experimentation cultured all-male *M. rosenbergii* in Hapa nets. Prior phases had cultured mixed sex prawns. A sample of PL from a local hatchery was separated for mass measurement, as representative of the 100,000 PL which were input to the Hapa nets, to obtain an average mass for the input PL. 200 prawns were separated from the harvest of the nursery Hapa nets (Phase B5) for transfer to Phase B6. These were separated into three groups of 66 with total measurement. A further, sacrificial sample of 44 prawns was weighed and measured by ruler.

4.6.1 Water quality observation and measurement (Phase B5)

Four (4) Hapa nets, each of 100 m^2, were suspended in IPS pond J2 of 1,800 m^2 surface area. Pond J2 was set up as an IPS grow out pond, with water replacement for evaporation only, following the parameters described for commercial ponds in Chapter 3. As a result, the water quality results within the Hapa nets were very similar to the results in the surrounding water body of pond J2. Table 4.25 describes the handheld meter recorded results and number of readings over the period from 28 June to 3 August (37 Days). The similarity of readings between the two locations establishes the consistency of water quality between the Hapa nets and the surrounding pond.

Table 4.25: Water quality in Phase B5 Hapa nets and the IPS support pond J2

Parameter	DO, mg L^{-1}	Temperature, ° C	pH	TDS, mg L^{-1}
No of readings in period	21	21	19	17
Maximum Hapa	7.5	30.6	9.8	44
Maximum J2	8.0	31.0	10.0	44
Minimum Hapa	3.8	27.5	7.0	31
Minimum J2	3.8	27.6	7.0	26
Average Hapa	5.7	29.0	8.3	35
Average J2	6.1	29.3	8.2	36
STDEV Hapa	1.4	0.9	0.99	4
STDEV J2	1.4	1.1	1.00	6

With respect to evaluation of nitrogen species, the following analysis was performed using freshwater analysis kit, and spectrophotometer (Refer to Chapter 3). Of 14 freshwater analysis kit samples for NH_4-N, all were read as zero except for a single reading of 0.25 mg L^{-1}, on day 15. NH_4-N spectrophotometer analysis of the two locations on day 10, found 0.24 mg L^{-1} in pond J2 and none in the Hapa net sample. Of 10 freshwater analysis kit samples for NO_2-N, all were read as zero except for a single reading of 0.25 mg L^{-1}, on day 28, while three spectrophotometer samples for the parameter registered 0.0 mg L^{-1}. In a single sampling event, spectrophotometer values for alkalinity, were 5 and 6 mg L^{-1} on day 10, and both zero on day 15, for Hapa and the surrounding IPS pond J2, respectively. A single calcium value of 100 mg L^{-1} was recorded on day 15 in pond J2. Visual observation of water quality did not reveal significant suspended solids, although water maintained a green tint throughout.

4.6.2 Production performance observation and measurement (Phase B5)

The 100,000 PL supplied to four Hapa nets representing Phase B5 were transferred to commercial ponds for grow out at conclusion of nursery on day 36 (minus 200 prawns supplied to B6 before harvest). Using the average mass of 0.71 g, and weighed mass of prawns transferred, the number of surviving prawns from Hapa nets 1, 2, 3, and 4, were 15,000, 14,657, 18,142, and 19,000, respectively, for a total of 66,799, indicating survival rate of about 67 %. Transfer of surviving prawns from Hapa nets was considered close to 100%.

Bulk weight measurement of harvest of 200 prawns, harvested from Phase B5, as 66 prawns for each of Tanks 1, 2, and 3, was conducted and is recorded in Table 4.26. The bulk, weighed average mass of prawns harvested from Phase B5, and entering Phase B6, was 0.71 g.

Table 4.26: Mass measurement of 200 prawns from Phase B5, as input to Phase B6

B5 Tanks with 66 prawns each	Total Mass, g	Average prawn mass, g
T1	47.91	0.65
T2	47.13	0.71
T3	49.54	0.75

Results of weight and measurement of a sample of 44 prawns, separate from the population transferred to commercial pond for grow out, are recorded in Table 4.27, with analysis of results in Table 4.28.

Table 4.27: 44 prawn sample mass and length measurement harvested from Phase B5

No.	Length, mm	Mass, g	No.	Length, mm	Mass, g
1	45	0.7	**23**	58	1.29
2	32	0.49	**24**	45	0.78
3	50	0.84	**25**	40	0.43
4	45	0.95	**26**	40	0.46
5	20	0.15	**27**	45	0.6
6	25	0.5	**28**	37	0.4
7	22	0.3	**29**	21	0.12
8	38	0.49	**30**	44	0.53

No.	Length, mm	Mass, g	No.	Length, mm	Mass, g
9	48	0.68	**31**	30	0.22
10	48	0.87	**32**	46	0.47
11	30	0.32	**33**	43	0.57
12	40	0.52	**34**	47	0.69
13	50	0.93	**35**	50	0.76
14	39	0.4	**36**	50	0.81
15	50	0.8	**37**	30	0.29
16	48	0.77	**38**	39	0.4
17	40	0.48	**39**	48	0.54
18	33	0.3	**40**	42	0.63
19	47	0.67	**41**	40	0.52
20	49	0.92	**42**	39	0.4
21	30	0.3	**43**	39	0.5
22	35	0.42	**44**	30	0.22

Table 4.28: Sample mass and length measurement analysis for Phase B5 harvest sample at transfer

Parameter	Length, mm	Mass, g
Maximum	58.0	1.29
Minimum	20.0	0.12
Average	40.2	0.56
STDEV	8.9	0.24

The small sample of 44 prawns yielded a smaller average mass of 0.56 g than the bulk mass measurement of the larger sample size of 200 prawns transferred to Phase B6 (0.71 g). 0.71 g was the average weight used to develop the feed model for the 30-day Phase B6 trial. Prawns input to Phase B6 were quite uniform in appearance.

Derived performance variables for Phase B5 included stocking and harvest densities, related to both area and volume, survival rate, BWG, ADG, and FCR. These values are presented together with the assumed values used in the Phase B5 feed model, applied to Phase B5 Hapa nets, in Table 4.29. The subset of 198 prawns transferred to Phase B6 was treated as residing in a discrete volume within the Phase B5 Hapa nets. One requirement for evaluation of Phase B5 was to estimate the original population that was required for a resultant population of 198 prawns at 35 DoC transferred to Phase B6, by considering survival rate, which was determined as the survival rate found for the whole Hapa net (67 %). Hence, to produce the actual 198

prawn output population, the assumed original population of PL in the Hapa net was calculated as an original population of 299 prawns.

Table 4.29: Phase B5 performance variables over 35-day trial period

Metric	Feed Model	Hapa B5
Input at start (Day 0), number of prawns	203	299
Initial estimated mass per prawn, g	0.05	0.05
Input total mass, g	10	15
Tank volume, m^{-3}	0.8	0.8
Tank plan area, m^{-2}	0.8	0.8
Stocking density, kg m^{-3}	0.01	0.02
Stocking density, kg m^{-2}	0.01	0.02
Stocking density, no. m^{-2}	254	373
Culture duration, days	34	34
Harvest total, number of prawns	200	198
Survival Rate, %	98	67
BW, g	1.15	0.71
Total BW, g	230.0	140.6
Total BWG, g	219.8	125.7
Harvest density, kg m^{-3}	0.29	0.18
Harvest density, kg m^{-2}	0.29	0.18
Harvest density, no. m^{-2}	250	248
Estimated BWG per prawn, g	1.10	0.66
ADG, g d^{-1}	6.47	3.70
ADG per prawn, g d^{-1}	0.03	0.02
Input feed per tank, g	230	230
FCR based on gain	1.05	1.83

4.7 Phase B6: experimentation with post nursery, 30 to 60 DoC, of all-male *M. rosenbergii* in triplicate IPS tanks

The sixth phase of experimentation, Phase B6, was conducted in triplicate, in IPS tanks equipped and operated as described in Chapter 3, and employed in Phase B5, and with the feeding and additive schedule as also described in Chapter 3. Each tank was populated with 66 prawns of average assumed mass of 0.71 g. Days of observation recorded were day 36 (1 August) up to day 66 (30 August).

4.7.1 Water quality observation and measurement (Phase B6)

Table 4.30 describes recorded daily results of handheld meter readings for each tank over the period from 1 August to 30 August (30 Days). The average values recorded for DO, T, pH, and TDS, are consistent between tank readings, as are the standard deviations.

Table 4.30: Water quality measurement by handheld meter in Phase B6

Title	DO, mg L^{-1}	Temperature, ° C	pH	TDS, mg L^{-1}
Maximum, T1	6.03	29.5	9.01	304
Maximum T2,	5.88	29.2	8.84	326
Maximum, T3	6.22	29.2	8.63	312
Minimum, T1	4.23	26.5	8.00	10
Minimum, T2	4.14	26.4	8.00	17
Minimum, T3	4.69	26.2	7.90	23
Average, T1	5.40	27.9	8.32	206
Average, T2	5.35	27.7	8.29	224
Average, T3	5.59	27.6	8.16	214
STDEV, T1	0.50	1.1	0.29	85
STDEV, T2	0.47	1.0	0.28	89
STDEV, T3	0.42	1.0	0.26	84

With respect to evaluation of nitrogen species, the following analysis was performed using freshwater analysis kit, and spectrophotometer (Refer to Chapter 3). Freshwater analysis kit analysis NH_4-N, completed on days 40, 51, 52, and 53 showed zero, while kit readings for tanks T1, T2, and T3, on days 45 and 46, were 1.5, 1.0, and 0.5 mg L^{-1}, and 1.0, 1.0, and 0.5 mg L^{-1}, respectively. Spectrophotometer analysis of NH_4-N completed on days 40, 51, 52, and 53 showed zero, while readings for tanks T1, T2, and T3, on days 54, 57, 58, and 64, are shown in Table 4.31.

Table 4.31: NH_4-N measurement by spectrophotometer in Phase B6

Day	T1, NH_4-N, mg L^{-1}	T2, NH_4-N, mg L^{-1}	T3, NH_4-N, mg L^{-1}
54	0.59	0.66	0.60
57	0.55	0.60	0.56
58	0.71	1.17	0.81
64	0.74	0.81	1.63

Results of 11 days of analysis of NO_2-N, using freshwater analysis kit, are shown in Table 4.32.

Table 4.32: NO_2-N measurement by freshwater analysis kit in Phase B6

Day	T1, NO_2-N, mg L^{-1}	T2, NO_2-N, mg L^{-1}	T3, NO_2-N, mg L^{-1}
40	0.0	0.0	0.0
45	2.0	2.0	2.0
46	1.0	1.0	1.0
50	2.0	2.0	0.3
51	2.0	1.0	0.0
52	0.3	0.5	0.0
53	2.0	0.3	0.0
54	2.0	0.3	0.0
57	0.5	0.1	0.0
59	0.0	0.0	0.0
64	0.0	0.0	0.0

Results of 5 days of analysis of NO_3-N, using spectrophotometer, are shown in Table 4.33.

Table 4.33: NO_3-N measurement by spectrophotometer in Phase B6

Day	T1, NO_3-N, mg L^{-1}	T2, NO_3-N, mg L^{-1}	T3, NO_3-N, mg L^{-1}
52	27.9	10.3	13.6
53	20.7	7.2	19.8
58	10.5	10.2	15.3
59	9.6	14.6	11.8
64	7.0	10.8	6.8

Results of 7 days of alkalinity analysis, as $CaCO_3$, using spectrophotometer, are shown in Table 4.34.

Table 4.34: Alkalinity measurement by spectrophotometer

Day	T1, Alkalinity, mg L^{-1}	T2, Alkalinity, mg L^{-1}	T3, Alkalinity, mg L^{-1}
46	46	54	38
51	70	66	48
52	69	58	43
53	64	50	40
58	77	72	43

Results of 5 days of analysis of calcium, as Ca 2^{+}, using spectrophotometer, are shown in Table 4.35.

Table 4.35: Calcium measurement by spectrophotometer

Day	T1, Calcium, mg L^{-1}	T2, Calcium, mg L^{-1}	T3, Calcium, mg L^{-1}
51	94	128	156
53	109	120	108
58	109	101	140
59	119	101	110
64	116	135	146

In sampling events on days 59 and 64, spectrophotometer values for magnesium for T1, T2, and T3 were 10, 8 and 8 mg L^{-1}, and 9, 9, and 7 mg L^{-1}, respectively. Visual observation of water quality did not reveal significant suspended solids. 12 hours of 15 W artificial light provided supported algal growth on about 50 % of tank walls proximate to lighting, while water remained clear throughout Phase B6.

4.7.2 Production performance and measurement (Phase B6)

The produced prawns of Phase B6 were weighed in two ways. Firstly, the entire production of each separate tank was counted. An attempt to carry out bulk weight measurement was made, although it was not possible to exclude water from the measurement without anticipated damage to the stock prawns for Phase B7. The second measurement of the harvested prawns was conducted only on residual prawns, after subtraction of the 33 prawns per tank required as stock for Phase B7 to establish

density of 200 m^{-2}. Table 4.36 provides the summary Phase B6 harvest data for each of T1, T2, and T3, inclusive of performance metrics such as SR, BWG, ADG, etc.

Table 4.36: Summary harvest measurements from Phase B6

Metric	Feed Model	T1	T2	T3
Input at start (Day 36), number of prawns	66	66	66	66
Initial estimated mass per prawn, g	0.71	0.71	0.71	0.71
Estimated input mass, g	46.86	46.86	46.86	46.86
Tank volume, m^{-3}	0.23	0.23	0.23	0.23
Tank plan area, m^{-2}	0.33	0.33	0.33	0.33
Stocking density, kg m^{-3}	0.20	0.20	0.20	0.20
Stocking density, kg m^{-2}	0.14	0.14	0.14	0.14
Stocking density, no. m^{-2}	200	200	200	200
Culture duration, days	30	30	30	30
Harvest total, number of prawns	61	63	52	34
Survival Rate	92%	95%	79%	52%
Overall survival rate	92%		75%	
Sampled prawns, number	n/a	29	19	1
BW, g	2.52	1.86	1.72	2.08
Overall BW, g	2.52		1.81	
Output mass, g	153.72	114.03	94.12	61.54
Harvest density, kg m^{-3}	0.67	0.50	0.41	0.27
Harvest density, kg m^{-2}	0.47	0.35	0.29	0.19
Harvest density, no. m^{-2}	185	191	158	103
Average length, mm	n/a	58	51.8	53.3
Estimated total BWG per tank, g	106.86	67.17	47.26	14.68
ADG, g d^{-1}	0.058	0.031	0.030	0.014
Input feed per tank, g	137.90	137.90	137.90	137.90
FCR based on gain	1.29	2.05	2.92	9.39

The observed measurements of the residual prawn sample from Phase B6, composed of prawns not transferred into Phase B7, are shown in Table 4.37.

Table 4.37: Sample measurements of Phase B6 harvested prawns

No.	Length, mm	Mass, g	Lengths < 50 mm	Lengths >= 50 mm	Masses < 1.5 g	Masses >= 1.5 g
Count	49	49	19	30	20	29
Maximum	82	4.32	48	82	1.47	4.32
Minimum	32	0.43	32	50	0.43	1.56
Average	52.8	1.8	40.3	60.8	0.8	2.5
STDEV	12.3	1.0	5.3	8.0	0.3	0.8

The above sample measurements were assumed to represent the starting measurements of stock entering Phase B7.

4.7.3 Morphometric observation and measurement (Phase B6)

The average of lengths measured from tip of rostrum to tip of telsun, of the 1, 19, and 29 extracted prawn as samples extracted from T1, T2, and T3, respectively, were 58 (single sample), 51.8 (± 12.5), and 53.3 (± 12.5) mm, respectively.

4.8 Phase B7: experimentation with all-male *M. rosenbergii* in tanks

The seventh phase of experimentation was conducted in triplicate in IPS tanks equipped and operated as described in Chapter 3, and with the feeding and additive schedule as described in Chapter 3. Each tank, previously utilized in Phase B6, was populated with 33 prawns from the harvest of the same tank operated in Phase B6. The average mass was calculated from the total residual sample of 49 prawns as 1.8 g. Recorded days of observation were DoC 65 (31 August) up to DoC 95 (30 September).

4.8.1 Water quality observation and measurement (Phase B7)

Imhoff cone readings were established at Day 7 for each of the three fully mixed tanks, with the Imhoff cone supported in a Styrofoam stand for each test. Figure 4.11 shows that the outcomes were below 1 mL for suspended solids accumulation, which is a good depiction of the IPS clear water throughout the program phases. Table 4.38 and Table 4.39 show water quality results measured by handheld meter and spectrophotometer, respectively.

Table 4.38: Handheld meter water quality readings, Phase B7

Tank	DO, mg L^{-1}	T, ° C	pH	TDS, mg L^{-1}
1	5.1 ± 0.4	27.6 ± 1.2	8.0 ± 0.3	155 ± 27
2	4.9 ± 0.5	27.6 ± 1.0	7.9 ± 0.2	147 ± 13
3	5.1 ± 0.4	27.4 ± 1.1	7.9 ± 0.1	143 ± 20

Eleven NO_2 readings were taken with freshwater aquaculture test kit, with all readings either zero or below 0.5 mg L^{-1}. The NO_2 readings of higher accuracy by spectrophotometer agreed with kit results, as shown in Table 4.39.

Table 4.39: Spectrophotometer water quality readings, Phase B7

Tank	Alkalinity, mg L^{-1}	NH4, mg L^{-1}	NO3, mg L^{-1}	NO2, mg L^{-1}	Ca^{2}+, mg L^{-1}	Mg, mg L^{-1}
1	65 ± 13	0.92 ± 0.41	3.78 ± 2.52	0.08 ± 0.05	105 ± 23	8 ± 1
2	49 ± 11	0.82 ± 0.28	7.71 ± 2.36	0.09 ± 0.03	125 ± 22	9 ± 4
3	48 ± 10	0.79 ± 0.18	8.62 ± 5.73	0.10 ± 0.04	114 ± 28	9 ± 4

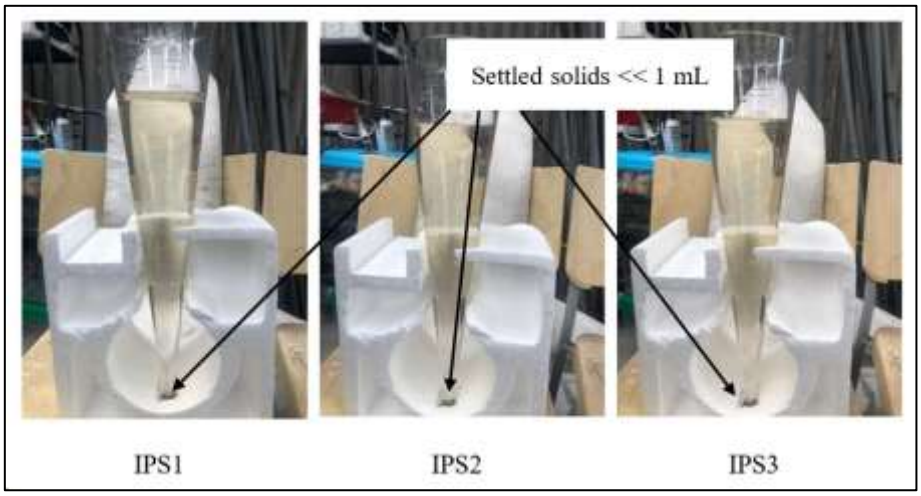

Figure 4.11. Imhoff cone results after 1 hour on Day 7, Phase B7

4.8.2 Production performance observation and measurement (Phase B7)

The produced prawns of Phase B7 were weighed as individuals from each of T1, T2, and T3. Table 4.38 provides the summary harvest data for each of T1, T2, and T3, inclusive of performance metrics such as SR, BWG, and ADG. FCR is calculated from total BWG per tank, and total supplementary feed per tank for Phase B7 of 118.86 g, from Table 3.12, Chapter 3.

Table 4.40: Summary harvest measurements from Phase B7

Metric	Feed Model	T1	T2	T3
Input at start (Day 64), number of prawns	33	33	33	33
Initial estimated mass per prawn, g	2.52	1.8	1.8	1.8
Estimated input mass, g	83.16	59.4	59.4	59.4
Tank volume, m^{-3}	0.23	0.23	0.23	0.23
Tank plan area, m^{-2}	0.33	0.33	0.33	0.33
Stocking density, kg m^{-3}	0.36	0.26	0.26	0.26
Stocking density, kg m^{-2}	0.25	0.18	0.18	0.18
Stocking density, no. m^{-2}	100	100	100	100
Culture duration, days	31	31	31	31

Metric	Feed Model	T1	T2	T3
Harvest total, number of prawns	30	10	27	17
Survival Rate, %	91	30	82	52
Overall survival rate	91%		55%	
Average BW, g	7.24	3.1	3.4	3.9
Overall BW, g	N/A		3.5	
Weighted Average BW, g	N/A		3.5	
Output mass, g	217.2	31	91.8	66.3
Harvest density, kg m^{-3}	0.94	0.13	0.40	0.29
Harvest density, kg m^{-2}	0.66	0.09	0.28	0.20
Harvest density, no. m^{-2}	91	30	82	52
Average length, mm	n/a	65.4	68	72.1
Estimated total BWG per tank, g	134.04	-28.4	32.4	6.9
ADG per prawn, g d^{-1}	0.14	-0.09	0.04	0.01
Total supplementary feed	118.86	118.86	118.86	118.86
FCR based on gain	0.89	N/A	3.67	17.23

4.8.3 Morphometric observation and measurement (Phase B7)

The average of lengths measured from tip of rostrum to tip of telsun, of the 1, 19, and 29 samples extracted from T1, T2, and T3, respectively, were 65.4 (± 14.2), 68 (± 14.9), and 72.1 (± 11.9) mm, respectively.

4.8.4 Residual biomass measurement (Phase B7)

During harvest of Phase B7, each tank volume was drained separately through a cheese cloth filter, yielding a clear filtrate to drain. Solids were retained. Each tank media substrate, and tank walls and bottom, were thoroughly pressure washed, and wash water drained separately through a cheese cloth filter, yielding a clear filtrate to drain. The total mass of retained solids was dried in an oven for 24 hours at 90 ° C,

yielding a dry cake. Table 4.41 records the total mass of dry solids cake retained from each of the three tanks.

Table 4.41: Residual biomass dry cake from Phase B7 tanks

TANK	Dry mass, g
T1	69.01
T2	51.8
T3	68.9

4.9 Phases B1 to B4 and Phases B5 to B7: comparison of mixed sex and all-male phases

Phases B5 through B7 were run with all-male *M. rosenbergii* from PL through to 95 DoC. A comparative analysis of these Phases with mixed sex culture Phases B1 through B4 follows in Table 4.42.

Table 4.42: Comparison of mixed sex Phases B1 to B4 with all-male Phases B5 to B7

Phase & Sex	Start, g	DoC	Culture Range, DoC	Average Intensity, No. m^{-2}	Average SR, %	Average BWG, g	Average ADG, g d^{-1}	Average FCR
B1 mixed	0.05	40	0 to 40	333	50 ± 16	1.26 ± 1.0	0.032	3.39
B2 mixed	0.05	32	0 to 32	1,000	69 ± 9	0.87 ± 0.68	0.027	2.02
B3 mixed	< 1.0	60	33 to 93	337	66 ± 10	2.53 ± 0.34	0.043	1.92
B4 mixed	> 1.0	60	33 to 93	219	78	4.24	0.07	2.16
B5 all-male	0.05	35	0 to 35	250	67	0.66	0.02	1.83
B6 all-male	0.71	30	36 to 64	200	75 ± 22	1.09	0.03 ± 0.015	4.79 ± 4.0

Phase & Sex	Start, g	DoC	Culture Range, DoC	Average Intensity, No. m^{-2}	Average SR, %	Average BWG, g	Average ADG, g d^{-1}	Average FCR
B7 all-male	1.8	30	65 to 95	100	57 ± 23	1.7	0.02 ± 0.02	6.79 ± 8.9

4.10 Phase C: commercial phase with all-male *M. rosenbergii* in IPS ponds

The methodology of the IPS pond establishment and operation is described in Chapter 3. Water quality analysis, and collection and analysis of performance statistics were integrated into the farm operation. Over the period of monitoring and evaluation of ponds J4 and J2, 27 January 2022 to 10 August 2022, that made up Phase C, ponds J1, J3, and J5 were also in operation on different schedules. Monitoring of water quality from all 5 ponds was summarized from 27 June 2022 to 29 September 2022, with description of the population, stage of growth, and prawn biomass in each pond, and is presented in Appendix 4, as supplementary information to the current study. The biofilter model review table provided in Appendix 2 compares biofilter design parameters applied in experimental Phases A, B1 to B7, and the ponds J4 and J2 of the Phase C commercial phase. The model for feed addition provided in Appendix 3 describes the applied feed model which achieved FCR of 2.1 in the Phase C commercial trial, over 195 DoC. The feed models used in the current study have been based on assumed growth curves. There is a need to further refine the growth curve model for mixed sex and all-male *M. rosenbergii*, in nursery, and post nursery including grow out to harvest. If an optimized growth curve leads to an optimized feed model, then an optimized FCR results for minimization of supplementary feed. When supplementary feed is reduced, the molasses amendment for C/N ratio and sodium carbonate addition for alkalinity are also proportionately reduced.

4.10.1 Water quality observation and measurement (Phase C)

Water quality results measured with handheld probe are recorded in Table 4.43.

Table 4.43: Handheld probe results for Phase C, IPS pond J2

Record	DO, mg L^{-1}	T, ° C	pH	TDS, mg L^{-1}
No. of readings for period	22	22	21	18
Maximum value	8.01	31.40	10.4	151
Minimum value	3.78	27.60	6.98	26
Average	5.78 ± 1.23	29.40 ± 0.91	8.96 ± 1.08	62.3 ± 36

Water quality results measured by spectrophotometer are recorded in Table 4.44.

Table 4.44: Spectrophotometer results for Phase C commercial IPS pond, J2

Record	Alkalinity, mg L^{-1} CaCO3	NH4-N, mg L^{-1}	NO2, mg L^{-1}	Ca^{2+}, mg L^{-1}
No. of readings for period	8	8	3	4
Maximum value	30	0.24	0.06	100
Minimum value	0	0.04	0	60
Average	16.4 ± 9.7	0.12 ± 0.09	0.02 ± 0.03	80 ± 21

In addition to the above tabulated measurements, eleven freshwater quality colorimetric kit analyses for NH_4-N were all zero, and 14 NO_3-N kit analyses were all zero except one reading estimated at 0.25 mg L^{-1}. A single magnesium spectrophotometer reading gave an 8 mg L^{-1} value.

4.10.2 Production performance observation and measurement (Phase C)

A total of 50,000 all-male PL *M. rosenbergii* were introduced to a 488 m^2 commercial IPS pond J4 (density of 102 m^{-2}) on 27 January 2022 for the first 95 days, followed by transfer to an 1,800 m^2 commercial IPS pond J2 for the remainder of the culture period (28 m^{-2} based on starting population). Transfer at day 95 was estimated

as 35,500 individuals, which indicated SR of 71% for the nursery phase. Representative samples of over 25 prawns per sample were measured for individual mass, and average sample mass. Total transfer was weighed in four batches: 75 kg of average 16.9 g BW, 46 kg of average 5.7 g BW, 63.5 kg of average 10.1 g BW, and 53 kg of average 4.0 g BW. Average mass of 35,500 prawns transferred to pond J2 was assumed to be 18.6 g according to the feed model (Appendix 3), but the size measurement of many groups composing transfer led to a smaller average mass estimate of 11.5 g, which is evidence for modification of the feed model to reduce supplementary feed. Revision of the model requires an incremental approach towards commercial optimization, and modification towards FCR of 1.0, to improve economics.

The first through the seventh harvest counts of pond J2 are recorded in Table 4.45, using the measured harvest mass and the estimated average prawn mass of each harvest of 35 g at point of sale. Although the purchaser was responsible for accepting or rejecting prawns based on perceived size, the perceived selection cut-off mass was 35 g based on the purchaser experience in the estimation. First sorting took place in the ponds with manual selection of larger prawns, targeting BC males. The sorted prawns were transferred to a holding tank and the purchaser then manually sorted the prawns by eye. The producer targeted BC males, hence, the purchased 25,921 total number were assumed to be above 80 % BC males supplemented by some larger OC males and females. Samples were taken with measurement of mass at transfer and typically showed average mass of about 20 g. After producer sorting, subsequent sample measurement was typically in close agreement with an average of 35 g and only the first partial harvest showed a lower average mass of 32 g. Prawns not selected by the purchaser were returned to the originating pond. An estimated 5 % loss during each harvest, based on sale mass, was assessed. The small quantity of undersized prawns remaining after final harvest were transferred to another commercial pond and the quantity considered insignificant. Table 4.45 shows the calculation of total estimated produced prawn output of 31,330 prawns, under IPS pond J2 production. This total with the estimated input population to the pond (35,500) indicates that 88 % survival was experienced over the grow out period which took place in pond J2.

Table 4.45: Production results for Phase C, IPS pond J2

Harvest	Harvest Date	Days of Culture, DoC	Produced prawn mass, kg	Estimated average prawn mass, g	Estimated prawn, nos.
1	7/5/2022	101	62.3	32	1,947
2	27/5/2022	121	51.7	35	1,477
3	15/6/2022	140	181.5	35	5,186
4	22/6/2022	147	282.3	35	8,066
5	1/7/2022	156	40.1	35	1,146
6	3/8/2022	188	219.5	35	6,271
7	10/8/2022	196	64	35	1,829
Sub-Totals			901.4		25,921
Losses at Harvest			45.1	32	1,408
Transferred			60	15	4,000
Produced			1,006		31,330

The intensity of prawns transferred from pond J4 into pond J2 was approximately 28 m^{-2}. The overall SR over 196 DoC was approximately 62 %, estimated as the product of 71 % SR in the nursery 95-day period in pond J4 and 88 % SR of the grow out 101-day period in pond J2. Feed applied over the 196 DoC was approximately 2,070 kg (Appendix 3) and produced prawn mass was 1,006 kg (Table 4.45), which resulted in a calculated FCR for the full cycle of about 2.1.

4.11 Phases B2 to B4 and Phase C: comparison of mixed sex and all-male phases

Phases B2 through B4 were run with mixed sex *M. rosenbergii* from PL through to 104 DoC. The first phase of Phase C in commercial IPS pond J4 was conducted at 102 m^{-2} for 95 DoC with all-male *M. rosenbergii*. A comparative analysis of the mixed sex nursery Phases with nursery output from 95 DoC of all-male culture in IPS pond J4 is presented in Table 4.46. SR for IPS tanks in Phase B2 was 69 %. Phases B3 and B4 represented 34 % and 66 % of population, respectively. SR for IPS tanks in Phases B3 and B4 was 66.3 % and 78 %, respectively. The combined SR for

Phase B3 and B4 IPS tanks was estimated as 74 %. SR for IPS tanks from Phase B2 through to harvest of Phases B3 and B4 was then estimated as 51 %.

Table 4.46: Comparison of performance between mixed sex Phases and all-male Phase C nursery pond J4

Phase	**Start, g**	**Culture Range, DoC**	**Initial Intensity, No. m^{-2}**	**Average SR, %**	**Average BWG, g**	**Average ADG, g d^{-1}**	**Ave. FCR**
C, IPS Pond J4	0.05	0 to 95	100	71	7.35	0.08	2.14
B2 IPS	0.05	0 to 44	1000				
B3 IPS		45 to 104	581	51	3.38	0.04	1.66
B4 IPS		45to 104					

CHAPTER 5

DISCUSSION

Results generated through this study are analysed against the following three study objectives.

1. to compare the production efficiency of IPS with RAS in nursery and post-nursery to 104 DoC with mixed sex *M. rosenbergii*.
2. to evaluate performance of IPS for mixed sex and all-male *M. rosenbergii* up to post hatchery above 90 DoC, and all-male in IPS commercial production from hatchery to 195 DoC.
3. to develop useful culture system design and operational parameters, and feed model for culture of the target species, *M. rosenbergii* in IPS tanks and ponds.

Meeting the three objectives serves the general objective in demonstrating that the characteristics of IPS are serviceable to advance sustainable aquaculture. Discussion proceeds in view of the experimental program with reference to the study objectives, the findings from respective Phases, and the referenced literature, to develop substantial theory as to the innovation, relevance, and utility of the study outcomes.

In developing the innovative IPS technology, objective 1 has focused on engineering of the system, and subsequent testing against more established technology, i.e., RAS. Comparative study was used to be able to report results despite the high complexity of semi-commercial scale experimental setups. The current study has elaborated and provided relevance to the complex factors that lead to IPS efficiency. Further investigation is required into factors of operation with the aim to define how such factors arise, how they may be controlled, and how they may permit IPS and the culture of *M. rosenbergii* to be improved upon. Factors that were outside the scope of the current study included the diversity and interrelationships of biota resident in three-dimensional IPS media with biota resident in the production volume and biota resident within the gut biome of the culture species. Evaluation of health benefits derived from quorum sensing and direct transfer of compounds from biomass to culture species was also out of current study scope. Objective 1 is served by answering hypothesis 1, with respect to predictions 1a, 1b, and 1c.

Objective 2 was served by the evaluation of performance of IPS in culture of *M. rosenbergii* in comparison with culture in RAS, of nursery versus grow out stages for mixed sex versus all-male *M. rosenbergii*, and of mixed sex versus all-male *M. rosenbergii* in IPS culture. In this regard, objective 2 is served by answering hypothesis 2, with respect to predictions 2a, and 2b.

Objective 3 is served by answering hypothesis 3, with respect to prediction 3, through the evaluation of study results in gaining insight into FCR optimization. FCR optimization requires consideration of BWG, SR, ADG, SDG, and areal yield (i.e., mt ha^{-1} $year^{-1}$), and the range of experimentation of all Phases contributed to the understanding of this interaction. Prediction 3 also considers how FCR is optimized with respect to the growth curve predicted for *M. rosenbergii*, on which the feed model for any culture activity will be based. Objective 3 is also partially served by both hypotheses 1 and 2 and their predictions, and fulfilment of objectives 1 and 2, because the contributions of the system design, and the growth curve and performance of *M. rosenbergii* support evaluation of achievement of optimized FCR.

5.1 Objective 1

1. to compare the production efficiency of IPS with RAS in nursery and post-nursery to 104 DoC with mixed sex *M. rosenbergii*.

With respect to objective 1, throughout Phases B1 to B7, and the commercial Phase C, the IPS system was able to manage water quality parameters, with minimum residual waste, and no water exchange. Mixed sex Phases B1 through B4 water quality results were discussed and have been analysed in Chapter 4. The performance of IPS systems was shown to be equivalent or better than RAS in terms of aquaculture water quality management. Results for IPS all-male Phases B5 to B7, and the all-male commercial IPS pond in Phase C, were reported within Chapter 4, and acceptable aquaculture conditions were also maintained. In all cases, minimal water input only was provided to account for evaporation and losses from the tanks and from the unlined commercial ponds. Zero discharge from both IPS and RAS over the 60-day grow out programs, did not result in increase of TDS above 500 mg L^{-1}, while TDS for IPS ponds reported for Phase C, and in Appendix 4 showed TDS maintained below the maximum of 151 mg L^{-1}. Further research into the mass balance inputs and outputs from the biological processes in IPS with zero water exchange is required to explain the low residual solids and low dissolved solids observed. It is concluded that these low dissolved and residual solids are indicative of an efficient ecosystem at work.

Water quality analytical parameters bore out that periphyton is effective with respect to maintenance of low ammonia and nitrite levels, while low TSS established the advantage of periphyton in maintaining clear water conditions. Water quality was maintained without requirement to impart mixing energy to tank volumes for suspension of floc. NH_4^+-N mg L^{-1} were extremely low, as were NO_2-N concentrations, as measured in Phase B1, which supported the conclusion that periphyton biomass effectively utilized both excretory, and waste feed TAN. In IPS pond operation, spectrophotometric analysis of NH_4^+-N peaked at about 0.35 mg L^{-1} for three months of operation of five IPS ponds, which supported the extension of the effect to both IPS tanks and IPS ponds.

The performance of IPS systems was compared with and shown to be equivalent or better than RAS in terms of *M. rosenbergii* aquaculture performance in each of Phases B1 through B3. The factors responsible for significant difference tank-wise in BWG and BWG x SR, between treatment (IPS) and control (RAS), may be explained by the access of prawns in IPS to periphyton biomass. This supported acceptance of the null hypotheses for predictions 1a, 1b, and 1c. The same result has relevance to and is further discussed under Objective 2.

5.1.1 Phase A

IPS relocates the technology of submerged biofilter to within the planned production volumes. The program started with a biofilter model in Phase A. The biofilter model was reviewed in Chapter 3. Appendix 2 reviews the application of biofilter media in the different experimental phases. The experimental philosophy considered that the capital cost of biofilter was small, given the advantages it proffered, and that its cost is spread out over more than 20 production cycles.

Beginning with Phase A, the current study has demonstrated that nitrogen species are well-managed with both the IPS treatment and RAS control treatment. The conversion of nitrogen and the cycling of organics and inorganics between photoautotrophic, autotrophic, and heterotrophic microbes, and the movement of waste nitrogen from dissolved feed and animal excretions into microbial protein are fundamental to integrated biomass systems. Studies utilizing Aquamat™ media have demonstrated that nitrogen species are typically maintained within boundaries of adequate conditions for farming of aquaculture species, with marine (Audelo-Naranjo *et al.*, 2010, Audelo-Naranjo *et al.*, 2011, Audelo-Naranjo *et al.*, 2012a, Audelo-Naranjo *et al.*, 2012b, Huang *et al.*, 2013), and freshwater species (Arndt *et al.*, 2002, Yu *et al.*, 2016, Li *et al.*, 2019). The other water quality variables which were monitored during the experiment were maintained in adequate condition.

The two, Phase A tanks were provided supplementary feed as if a maximum production population was under culture. The biofilter responded very well under this

intense loading. Observationally, the tanks were visually clear from the outset and this condition was maintained throughout operation. Water quality analysis showed that a period of 18 days was required for biomass to populate the submerged media, such that ammonia levels (NH_4-N) climbed for about 9 days then receded to a relatively low level in both tanks by the 18th day, which remained relatively stable thereafter. The tanks were operated in a zero-water-exchange mode with only replacement for evaporation, hence there was no physical outlet for solids from the tanks during the experiment. Minimal solids were residual in the tanks at the conclusion of operation, and it was concluded that supplementary feed uptake by the periphyton system was highly efficient, while biomass did not accumulate. While measurement of residual solids was included in subsequent zero water exchange experiments, residuals remained very low.

Food conversion ratio evaluates the utilization of feed by the culture species and is not affected by distinctions between types of aquaculture systems employed. In the evaluation of zero discharge, biofilter inclusive systems such as IPS or RAS, or suspended biofloc BFT systems, the way excess feed and fish excretions feed biomass within the media substrate requires further research. The complete ecosystem represented by biomass supported on and within three-dimensional media substrate, or biofloc, results in more complete reduction of waste feed into fresh biomass, respired gas, and inert substances, with greatly reduced waste per cycle. There is a need for future research into the efficiency of digestion of supplementary feed in IPS to understand how the microbial ecosystem functions in IPS. The further phases of experimentation explore one aspect of this through comparison with RAS, where the biofilter is external to the production volume and is not directly exposed to supplementary feed.

Under intense loading of the submerged biofilter in Phase A, it was observed that the biomass responsible for the observed treatment was resident on and within the media, on media fibre surface area, and not in the water column. The biofilter successfully maintained water quality, residuals were reduced or removed, and a clear aquaculture production volume was maintained. In Phase A, the media substrate responded well to very high loading. What this meant going forward was that no matter

what feed ratio of supplementary feed to prawn biomass was provided in the aquaculture system, it was reasonable to assume that any excess feed would be processed by the biofilter. These results supported the continuation of the subsequent experimental phases.

Phase A only utilized duplicate IPS tanks as an observation of the technology without the complication of a target species. The 490 g of feed per day resulted in about 0.01 kg d^{-1} of TAN added to the tanks, thus simulating a high production population. Within 18 days, TAN was removed in a steady state, while TSS fell to below 10 mg L^{-1} and maintained the low level of a clear water system. At the conclusion of the experiment, insignificant amounts of residual solids were retained. The features demonstrated were capacity for high TAN loading, while the periphyton biomass was able to reduce residual solids to very low levels and maintain a clear water system. These characteristics were consistently observed throughout the experimental tank phases.

5.1.2 Phases B1 and B2

The current study investigated comparative features of IPS versus RAS with external biofilter, as part of a series of experiments to present evidence directly bearing on commercial potential of the integrated periphyton approach. The two data sets were compared for consistent performance of the prototype, not to infer a perfect statistical experiment, but rather as a statistical event.

Nursery culture is receiving increased attention for multiple species under aquaculture due to its ability to control the initial phase of growth within highly controlled conditions at higher densities than are employed for grow out. Coyle *et al.* (2010) assembled a convincing case that implementation of a nursery stage increases resistance to predation, cannibalism, and environmental conditions, improves survival rate, and generally results in higher individual weight, production, and harvest value. Nursery ADG in IPS exceeded RAS at both densities. Operation of a nursery period at higher density reduces the net volume required for the full culture cycle. An efficient

nursery culture has implications for the efficiency of the full farm production cycle. However, even if BW is reduced in high nursery density, compensatory growth should yield production advantage in grow out (Marques *et al.*, 2010; Marques & Lombardi, 2011; Marques *et al.*, 2012). Productivity as the product of average BW and SR at harvest was calculated. BW x SR for nursery Phases B1 and B2 were 0.065 and 0.063 g d^{-1} for IPS tanks, and 0.034 and 0.065 g d^{-1} for RAS tanks, respectively, indicating productivity achieved by IPS was equivalent or better than for RAS.

The performance of IPS systems was compared with and shown to be equivalent or better than RAS in terms of *M. rosenbergii* aquaculture performance in Phases B1 and B2, and supported acceptance of the null hypotheses for predictions 1a, 1b, and 1c.

5.1.3 Phase B3

When evaluating a new aquaculture system, both nursery and grow out should be examined. Phase B3 was a post nursery continuation from the earlier Phase B2 nursery investigation. Tank-based freshwater IPS, and RAS were compared in Phase B3, and the performance of IPS systems was shown to be equivalent or better than RAS in terms of *M. rosenbergii* aquaculture performance in Phase B3, which supported acceptance of the null hypotheses for predictions 1a, 1b, and 1c.

5.1.4 Performance of commercial phase (Phase C)

The commercial phase started with the conversion of a freshwater prawn, *M. rosenbergii*, farm from a conventional, extensive design to an IPS design. Water quality and performance results were reported from operation of an all-male *M. rosenbergii* pond culture over 195 days at the site. The media surface area provided to ponds J2 and J4 are described in Chapter 3 and Appendix 2b. The specific surface area provided achieved 100 % of the biofilter model calculated requirement. Installation of the near vertical media (30 ° from vertical) provided 25 % and 16 % of the pond bottom

area in additional habitat area to ponds J4 and J2, respectively. Water quality was reported from a set of five IPS ponds over a 3-month period of operation (Appendix 4).

The performance of commercial IPS systems was shown to meet commercial RAS achievement of water quality suitable for aquaculture, i.e., of *M. rosenbergii*, which supported acceptance of the null hypotheses for predictions 1a, 1b, and 1c.

5.1.5 Effective SGR of IPS and RAS

Specific growth rate (SGR) for triplicate tanks with smaller prawns, IPS_{large} with larger prawns, and the overall population (ALL) was observed. IPS_{large} was populated by a subset of the largest nursery prawns and yielded a marginally higher SGR (1.91 % d^{-1}) than for the overall population (1.89 % d^{-1}). However, three tanks rearing smaller nursery prawn subsets displayed higher SGR than ALL. Asaduzzaman *et al.* (2008) reported SGR for various C/N ratios and application of periphyton substrate as ranging from 1.56 to 1.75 % d^{-1} harvest after 120 days of culture. Ballester *et al.* (2017) reported SGR of 1.39 % d^{-1} for a RAS and 1.21 % d^{-1} for a periphyton based trial. SGR achieved by IPS and RAS using Matala™ media displayed more effective SGR, than these prior studies. The improved SGR result was consistent for the range of nursery prawn subsets generated in the experiment. This result could support the selection of nursery prawns by size, to produce a higher SGR, but the result could also be attributed to the adoption of IPS in the experiment.

5.2 Objective 2

2. to evaluate performance of IPS for mixed sex and all-male *M. rosenbergii* up to post hatchery above 90 DoC, and all-male in IPS commercial production from hatchery to 195 DoC.

5.2.1 Phase B3: Post nursery comparison of IPS with RAS rearing of mixed sex *M. rosenbergii*

To transition from nursery Phase B2 to post nursery Phase B3, it was necessary to measure and to establish a lower density for the post nursery phase. The technique of size separation was applied to the output from triplicate IPS and RAS tanks which reduced population per tank, and 34 % overall. The decision was taken to return the size separated populations from each tank back into their original tanks. Effectively, each Phase B2 nursery tank was partially harvested to remove larger prawn population of about 34 %. Whatever hierarchy of growth had developed in the nursery phase was preserved in Phase B3 post nursery with 66 % of the prawn population.

Tank populations were selected from prawns reared in identical nursery tank systems for the preceding 43 days in Phase B2, determining Feed Conversion Ratio (FCR), Body Weight Gain (BWG, g), Survival Rate (SR, %), and Average Daily Gain (ADG, g d^{-1}). Following Phase B2, larger prawns of approximately more than 1 g BW were removed to a separate grow out. The 66 % of the nursery population that were approximately below 1 g BW were retained in the original nursery tanks as Phase B3 with resulting lower tank population densities for both IPS and RAS. An applied feed schedule was applied to manage supplementary feed, alkalinity adjustment, and adjustment of C/N ratio through addition of molasses.

Water quality parameters for both IPS and RAS remained within the suitable range. Average SR for IPS and RAS were 66 % and 72 %, respectively. BWG x SR was examined by Welch ANOVA with Games-Howell post hoc testing, to evaluate performance and productivity. BWG x SR yielded a number relevant to statistical evaluation of productivity of each tank. Significance was assigned at the 0.05 % level. Results were examined for comparisons of averages and significance of difference in BWG x SR, for triplicates of each of the two groups IPS and RAS, to evaluate the outcome on the hypothesis that IPS yields at least equivalent performance to RAS in the application of *M. rosenbergii* intensive culture. System results for BWG x SR were significantly different. Triplicate IPS tanks were ranked as the top three with respect to BWG x SR, with triplicate RAS tanks ranked as the bottom three. It was concluded that IPS demonstrated improvement over RAS in terms of productivity.

Survival from nursery in RAS was higher than survival in IPS, hence RAS densities were higher at the outset of Phase B3 in RAS tanks. Compensatory growth was considered to effectively balance experimental effects in Phase B3. Within the IPS triplicate tanks, SR correlated as higher for higher density, which was also the case for RAS triplicate tanks. Density of population at conclusion of Phase B3 averaged 339 and 436 individuals m^{-2} for IPS and RAS, respectively. Aquaculture of *M. rosenbergii*, is typically conducted at lower densities than applied to marine prawns, both in nursery and grow out. The highest grow out densities of *M. rosenbergii* globally have appeared in Chinese aquaculture up to 65 m^{-2}, and consistent Malaysian densities of 15 m^{-2} have been reported (New & Kutty, 2010), while grow out of marine Whiteleg shrimp, *L. vannamei* has been variously reported between about 250 and 500 m^{-2} (Ray *et al.*, 2011; Suantika *et al.*, 2018; Schveitzer *et al.*, 2012; Zhang *et al.*, 2010a; Zhang *et al.*, 2010b;, Zhang, 2010; Zhang *et al.*, 2020). Consequently, both IPS and RAS grow out in Phase B3 were conducted at high population density compared with global practice for *M. rosenbergii* aquaculture, and yet accomplished SR of 66 % and 72 % for IPS and RAS, respectively, at densities within the range of those employed for Whiteleg shrimp. It was concluded from this result that there could be benefit obtainable through size separation, since the smaller size group demonstrated effective SR. Hence the null hypothesis 2 was rejected and prediction 2a was found indicative of the result.

5.2.2 B2 through B4 morphological and gender performance evaluation of rearing of mixed sex *M. rosenbergii*

Mixed sex *M. rosenbergii* aquaculture encounters a growth-impacting hierarchy amongst males (HIG), and strong differentiation of females in terms of growth over the culture cycle. Post nursery size selection can remove heterogeneity that appears within the nursery phase, making grow out utilization more accurate, with improved product uniformity (Coyle *et al.*, 2010). Heterogenous individual growth (HIG) has been identified as having major impact on successful aquaculture of the species, mainly due to lack of uniformity required for effective marketing of production (Ranjeet & Kurup, 2002).

Size separation of nursery prawns was applied ahead of the post nursery trial. Daniels and D'Abramo (1994) and Daniels *et al.* (1995) conducted similar experiments, separating nursery *M. rosenbergii* using size selecting grid mesh. At 104 days total DoC, females and the three male morphotypes from size-separated groups were harvested and visually identified, to gain insight into performance characteristics of split size groups, and of the complete prawn stock population. Harvested prawns were weighed and assessed for male morphological features distinguishing between BC, OC, and SM, and gender. A 2-way ANOVA was performed, permitting comparative observation of system, gender, and split size group growth performance. Gender, morphological, and performance differences were used to evaluate the potential of separation technique for commercial application.

Complications to evaluation included that the process of separation by size served to reduce density of population after the nursery period through distribution to greater tank area of the harvested population. In addition, sacrificial samples were taken from nursery stock averaging 13% of stocks, and significant mortalities occurred during sampling averaging 7% of stocks, which together amounted to a loss of about 20 % of the population count between the nursery and post-nursery stages.

Average SR for IPS_{small}, RAS_{small}, and IPS_{large} were 66 %, 71 % and 78 %, respectively. RAS and IPS nursery survival rates were similar, both for rearing smaller nursery prawn subsets. The Phase B4 larger prawn set of nursery stock was reared in a single IPS tank and achieved a SR of 78 %. The full range of difference was not found to be significant. From PL introduction to the Phase B2 nursery stage, to the harvest of prawns from Phases B3 and B4 post-nursery tanks at 90 days, the overall survival for both size groups together was 40 %, before adjustment for the 20 % losses, and an with adjustment was estimated to be about 74 %. Marques and Lombardi (2011) observed survival rates in *M. rosenbergii* ranging from about 65 to 70 %, in net-pen systems stocked at densities from 50 to 1,200 m^{-2}. Crab *et al.* (2010), applied BFT with different carbon sources and found a 75 % survival rate. Asaduzzaman *et al.* (2008) recorded survival ranging from about 63 to 72 %, while evaluating increments in C/N ratio with application of periphyton substrates. Pérez-Fuentes *et al.* (2013) reported survival values of 85% rearing *M. rosenbergii* in both BFT and traditional ponds. The

SR found in the current study are broadly like those reported in these prior studies selected to represent the higher range of SR previously achieved in comparable systems. It was concluded based on SR that there could have been benefit demonstrated because of size separation. Hence the null hypothesis 2 was rejected and prediction 2a was found indicative of the result.

RAS harvest biomass, in the triplicate post-nursery of Phase B3, for the smaller prawn set, was 95 % of IPS biomass. Phase B3 IPS_{small} performance was superior to RAS_{small} for BWG. Both male and female BW in triplicate IPS were significantly greater than the corresponding gender BW in triplicate RAS, of Phase B3. Phase B4 IPS_{large} was stocked with larger prawns and both male and female harvested prawns had greater BW than those produced from the smaller set stocked to Phase B3 triplicate IPS and RAS.

With respect to harvest count of female, SM, OC males, and BC males, over the whole harvest (ALL), the percentage distribution was 43 %, 32 %, 10 % and 14 %, respectively. Karplus (2005) and Karplus and Sagi (2010) extensively reviewed evaluations of proportion of male morphotypes and females in experimental populations. Proportions vary significantly with density. At low densities BC males has been reported to reach 20 % with small males decreasing to 33 %. BC males are the desired market product yet decrease in proportion at higher densities. IPS_{large} showed an increase count of BC to 28 % with a concurrent decrease of female prawns to 19 %. This result pointed to an effective BC population for the 40% group of larger prawns separated from Phase B2 harvest. The smaller prawns in Phase B3 harvest displayed ranges of 4 to 18 % (IPS) and 2 to 7 % (RAS). There is an important indication in this result that before Phase B2 DoC 44 the gender and morphology are likely set in terms of ratios. By extracting larger prawns, BC males were disproportionately transferred and over 60 DoC, the smaller set of prawns did not demonstrate a morphological advancement in males from SM and OC males into BC males. It is concluded from this result that there is little benefit obtainable through size separation. Hence the null hypothesis 2 is accepted and predictions 2a and 2b were not indicative of the result.

The average BW attributed to females, and to SM, OC, and BC males, followed a similar hierarchy for all post-nursery tanks, and for the total of all tanks (ALL) in Phases B2 through B4. Female prawns ranged from about 1.0 to 3.5 g (IPS_{large}), up to 2.0 g for all tanks except for IPS_{large} wherein female BW ranged as high as 3.5 g. Ranges in BW over post-nursery for SM, OC males, and BC males were about 1.4 to 5.5 g, 4 to 7.5 g, and 7 to above 12 g, respectively. At 104 DoC, females performed no better than SM. This result supports the use of all-male stock since the female population is lagging in growth, yet consuming feed, and plays a role in stimulation of BC male conflicts. To an extent, this result validates prediction 2c, for the anticipated advantage of all-male *M. rosenbergii* culture.

Total prawn biomass at harvest, after allowing for losses in transition (Figure 4.11), designated ALL, was 13,434 g. There were about 5 kg of BC males, and almost 3.5 kg of those BC males (70 %) arose in Phase B4 IPS_{large}, which also had a total harvest mass of almost 7 kg. The average BW of BC males in IPS_{large} was about 10 g, which was exceeded by tank RAS1 (rearing the smaller prawn set) wherein BW of BC males averaged above 12 g. The BW of BC males could not be said to be enhanced in any way by the separation of small and large nursery prawns. It is further concluded from this result that there is little benefit obtainable through size separation, the null hypothesis 2 is accepted and predictions 2a and 2b were not indicative of the result.

5.2.3 Comparative performance between mixed sex Phases B1 to B4 and all-male Phases B5 to B7

With respect to nursery culture of *M. rosenbergii*, all-male Phase B5 (35 DoC) can be compared to mixed sex Phases B1 (43 DoC) and B2 (44 DoC). Phase B5 density was lowest (250 m^{-2}), followed by Phase B1 (333 m^{-2}) and Phase B2 (1,000 m^{-2}). FCR for Phase B5 (1.83) was better than for Phase B2 (2.02) or Phase B1 (3.39). SR for Phase B2 (69 %) was better than for Phase B5 (67 %) and Phase B1 (50 %). ADG was better for Phase B1 (0.032 g d^{-1}) than for Phase B2 (0.027 g d^{-1}) and Phase B5 (0.02 g d^{-1}). Average BW for Phase B1 (1.31 ± 1.0 g) was better than for Phase B2 (0.92 ± 0.68 g) and Phase B5 (0.71 g). FCR, SR, ADG, and BW results were not aligned with differences in population densities. With lowest FCR and highest survival rate, Phase

B5 all-male may be the most commercially viable in the nursery phase. However, Phase B5 had the lowest ADG, hence there is no clear advantage established for all-male versus mixed sex *M. rosenbergii* in the nursery phase. These results do not clearly validate prediction 2c, for the anticipated advantage of all-male *M. rosenbergii* nursery culture. This is not in agreement with results presented by several researchers comparing all-male and mixed sex *M. rosenbergii*, such as Tidwell *et al.* (2015), and more recently Phanasawat *et al.* (2022).

With respect to first stage grow out of all-male *M. rosenbergii*, from about 35 DoC up to about 90 DoC, culture Phases B6 (36 to 64 DoC), and B7 (65 to 95 DoC) can be compared to mixed sex Phases B3 (44 to 104 DoC) and B4 (44 to 104 DoC). Culture densities were somewhat different between Phases B6 (200 m^{-2}), B7 (100 m^{-2}), B3 (337 m^{-2}), and B4 (219 m^{-2}), but comparison can reflect on the impact of applied densities. All-male culture of Phases B6 and B7 did not perform as well as the mixed sex culture of Phases B3 and B4 in tank systems, despite lower densities. Phases B6 and B7 exhibited performance issues that may have stemmed from unrecorded power failure. Alternatively, the performance of these two all-male phases may point to all-male *M. rosenbergii* being less well suited than mixed sex culture to close environments, i.e., tanks, which may be investigated in future research. Another possible explanation could be that the feed models applied to the all-male Phases B6 and B7 were based on low FCR values that provided less supplementary feed than to the mixed-sex phases, and either or both IPS biomass or the prawn population may have been impacted. Likely, poor performance was due to a combination of factors. These results clearly do not validate prediction 2c, for the anticipated advantage of all-male *M. rosenbergii* nursery culture, again not in agreement with prior studies (Tidwell *et al.*, 2015; Phanasawat *et al.*, 2022).

Phases B3 and B4 represented a trial splitting of nursery production by size differentiation, with Phase B3 composed of nursery stock approximately less than 1 g BW, and Phase B4 composed of nursery stock approximately greater than 1 g BW. In grow out, lower density gives advantage in performance, hence mixed sex grow out was at a disadvantage to all-male Phases B6 and B7. The population weighted BWG of Phase B3 combined with Phase B4 was about 3.21 g over 104 DoC. From input BW

of 0.71 g, Phase B6 followed by Phase B7 achieved a BWG of approximately 2.79 g over 90 DoC. All-male did not perform quite as well as mixed sex in BWG and performed much worse with respect to ADG. FCR for both Phases B3 (1.92) and B4 (2.16) were far better than FCR for Phases B6 (4.79) or B7 (6.79). These results clearly do not validate prediction 2c, for the anticipated advantage of all-male *M. rosenbergii* nursery culture.

In comparison with globally reported performance statistics, only Phase B7 all-male grow out performance was sub-optimal. It is possible that splitting of nursery populations by size for separated grow out could be effective in culture of all-male. Future research should consider trial comparison between size-split all-male and mixed sex populations. Apart from performance, a primary target is to overcome lack of uniformity in market output resulting from HIG. Both the use of all-male stock, and the technique of splitting nursery populations, are potential technologies to address HIG. However, the relatively poor performance of all-male in tanks in the current study did not support investigation of HIG morphotypes, nor did it validate prediction 2c, for the anticipated advantage of all-male *M. rosenbergii* nursery culture. Further research is required into HIG within all-male culture in combination with partial harvesting over the full production cycle.

5.3 Objective 3

3. to develop useful culture system design and operational parameters, and feed model for culture of the target species, *M. rosenbergii* in IPS tanks and ponds.

5.3.1 Feed model development for optimum FCR in IPS culture of *M. rosenbergii*

One focus over the course of the program was on refining a feed model for intensive culture of *M. rosenbergii*, inclusive of gaining insight into how low an FCR could be achieved with IPS culture of the species. Taking Phase B2 as nursery (at population density 1,000 m^{-2}) and Phases B3 plus B4 combined as grow out up to DoC

93, the combined FCR was 1.66. FCR for the IPS pond preliminary commercial grow out in Phase C was also low at 2.1. Both results were low relative to results reported globally, hence indicative of commercial validity of the IPS system.

For all-male *M. rosenbergii*, FCR for each of Phases B5 (37 DoC), B6 (26 DoC), and B7 (29 DoC), were 1.83, 1.53, and 0.87, respectively. Phase B7 was not considered successful due to a variety of factors. Based on Phases B5 and B6 alone, FCR of 1.83 and 1.53 were low relative to results reported globally, hence supporting the applied feed model in application to the IPS system, for all-male culture. It seems unlikely that the continuation of the same feed model for Phase B7 could be attributed to the poor performance for DoC 65 to 94. Performance of the commercial pond system, Phase C, with FCR of 2.1, for 195 DoC, was low relative to results reported globally, stocked with all-male *M. rosenbergii*, although the commercial pond system will need to be replicated in future.

FCR was calculated based on the days of feeding in culture. The Phase B2 nursery stage IPS tanks in triplicate achieved FCR of 2.02. In the Phase B3 post-nursery stage, for the smaller set of prawns over 60 days post-nursery culture, the triplicate IPS tanks average FCR ranged from 1.52 to 2.68. The Phase B4 larger set of prawns reared in a single IPS tank (IPS_{large}) achieved an FCR of 2.16. FCR for the entire population raised in IPS tanks, from PL introduction to harvest of prawns from post-nursery tanks at 104 days, resulted in an FCR of about 2.3. FCR were improved over those reported by Asaduzzaman *et al.* (2008) with values between 2.40 to 2.97, with controlled C/N ratios, and integrated periphyton. Valenti and Moraes-Riodades (2004) observed FCR of 2.50 for conventional clear water nursery systems. In the present study, there was no clear leader in terms of FCR between culture in the IPS or RAS triplicates of Phases B2 or B3. Future research is needed to elaborate the applied growth curve used to design supplementary feed and additive addition over the experimental duration.

Crab *et al.* (2007) noted that periphyton in IPS utilized available TAN efficiently, the produced biomass could be utilized by prawns, which would permit reduction in feed provided. However, the current study is limited to comparative

evaluation with RAS, rather than achieving a quantitative measure of the type of biomass consumed, or the extent to which supplementary feed requirement was reduced in the nursery phase. There is insufficient evidence in other studies to provide quantitative comparison. In the current study, the coprophagous nature of prawns (D'Abramo & New, 2010) was the likely explanation for the relative absence of residual biomass in all tank systems at harvest, inclusive of filtration of the clear water of the production tanks. The absence of residual solids is a noted operational advantage. The apparent complete consumption of supplementary feed should be investigated further in future with mass balance that includes analysis of the resident microbiota.

The commercial IPS pond (Phase C) performed well in comparison to the tank-based systems, but at much lower density. Difference was observed in FCR, where Phase C was 29 % higher than the tank phase combined FCR. Some of this difference might have been attributed to the feed models applied rather than being indicative of performance. This indicates that the feed model of Appendix 3, applied to Phase C used a growth curve for all-male *M. rosenbergii*, which over-estimated prawn growth in the nursery stage. FCR for both experimental and commercial phases was on a par with the lowest reported values globally. for *M. rosenbergii* aquaculture. These observations support prediction 2 c in that all-male demonstrated higher ADG. More importantly, the need for an accurate growth curve matching growth in the nursery phase, as well as post-nursery, is a requirement for optimum FCR, hence validating prediction 3. Further research is recommended to pursue optimization of all-male and mixed sex *M. rosenbergii* in ponds to permit efficient allocation of supplementary feed which constitutes a large proportion of farm operating cost

Development of the feed model that controls supplementary feed addition requires an accurate growth curve. Growth curves need to respond to phases of growth since growth characteristics in nursery stage differ from grow out. SR also needs to be registered within the feed model as per Appendix 3. While the peak average prawn mass in the Phase C feed model was 60 g, and resulted in excessive feed application, the estimated SR of about 55 % at 195 DoC was quite close to the actual SR achieved. A SR of 71 % was estimated for the 35,500 population at transfer from the nursery

stage IPS pond J4 to IPS pond J2. Partial harvests of the commercial pond J2 during DoC 96 to 196 of Phase C progressively reduced the density of population, and SR of 88 % was estimated. Resultant SR of 62 % calculated for the full 196-day period of Phase C was on a par with global performance for *M. rosenbergii* aquaculture. That higher SR occurred in grow out than in the nursery stage is a useful economic result since higher costs occur during the grow out than nursery portion of the culture period. It also means that the growth curve in the feed model should reflect this change in SR to manage supplementary feed accordingly. The density of the nursery population in pond J4 was about 102 m^{-2}. After transfer to the larger grow out pond J2 the population density reduced to about 20 m^{-2}, based on input of 35,500 post nursery prawns into pond J2. Applied density of 20 m^{-2} was higher than reported commercial averages with few exceptions. Considering the input population of 50,000 PL to the nursery stage, and the pond area of pond J2 of 1,800 m^2, overall full cycle culture could be said to have operated at 28 m^{-2}. The output of about 8.1 mt ha^{-1} $year^{-1}$ compares well with output of 9.4 mt ha^{-1} $year^{-1}$ reported for all-male *M. rosenbergii* by Sagi *et al.* (1985). FCR of 2.1 was calculated from total feed applied (Appendix 3) and total measured prawn production for the Phase C ponds. FCR was generally lower than those reported in prior studies at similar densities (Asaduzzaman *et al.*, 2008; Valenti & Moraes-Riodades, 2004). The results of Phase C demonstrated that IPS can achieve performance considered to be higher than global average at lower FCR. The discrepancies in matching the growth curve experienced in Phase C to the curve employed in the feed model can be rectified through further research. This conclusion supports prediction 3 in that the importance of the feed model and its underlying assumptions is verified and supports prediction 2 c in the improved performance of all-male in comparison with global statistics.

5.3.2 Influence of size separation on optimization of IPS culture of *M. rosenbergii*

The current study did not test impact of size separation for all-male culture, where female differentiation has been removed. At the nursery stage, size separation of mixed sex prawns to two groups is expected to find a greater number of females in one group than in the other, which should be the group of smaller prawns. Females do

not achieve a size above about 35 g, hence are not expected to exhibit advanced growth in early stages. Males on the other hand pursue a hierarchy (HIG) that leads to distinctively higher growth amongst a sub-set of males that would be expected to continue until they reach a Blue Claw (BC) morphology. Hence, the group of larger prawns is expected to obtain a higher male, and a higher BC ratio. After separation, males in the group of smaller prawns are expected to evolve a sub-set of males expected to continue until they reach a Blue Claw (BC) morphology, and this means an overall increase in the concurrent population of BC males, which are the most marketable product. Figure 4.9 shows that BC harvest biomass from the larger prawn set in an IPS tank was over twice the harvest biomass of females. The figure also shows that female biomass exceeded BC biomass in all triplicate RAS tanks, but BC biomass exceeded female biomass in two of three IPS tanks. This is an indication that IPS tanks may favour BC development, which has been considered likely in the current study because of the added domain provided to prawns, which was expected to reduce mortality and stress in combative males.

Techniques of size separation of mixed sex prawns after nursery may permit farms to feed size-separated populations with more accurate feed schedules to match different growth rates. Partial harvesting from two offset populations could achieve product of higher uniformity, than from one total population, which would improve marketability as well as improving FCR. It is recognized that the concept of size separation requires separated grow out facilities, and that a separation operation adds complexity to farm operations. Size separation also comes with costs, and in the current study there was an approximate 7 % loss in transfer and measuring operations, which could potentially be reduced through experience.

The technique involves fast and low-cost operation with size screening, counting, and weighing as applied in previous studies. Karplus (2005) reviewed the history of evidence for HIG being a social mechanism, therefore modifiable by changing the social structure in the production volume. In the current study, however, it was noted that the ADG of the two groups of size separated stock did not differ significantly over the subsequent culture period. Both the large- and small-sized groups of stock would be expected to experience a compensatory spurt in growth after

the nursery stage, once density was reduced. Regardless of compensatory growth from one or the other of the two groups, their harvest would clearly be on different schedules, which might be an advantage in commercial operation. Figure 4.10 shows that female average BW was significantly less than male BW in all tank outputs, which was strong evidence in support of future investigation of all-male culture. Female differentiation is not conducive to uniform product for sale, and BC male are the preferred product in the marketplace. Feeding females as a sub-group may be perceived as leading to low return on applied supplementary feed. Overall, the current study showed that splitting nursery output at about 30 to 45 DoC had the potential to be effective in improving the overall production performance of grow out in mixed sex prawns. Further study can seek to formalize the commercial application of size separation, and to investigate how size separation, for all-male and mixed sex culture, may improve operational size assessment, which could achieve better match of supplementary feed to prawn mass, improve survival and FCR, and generate uniform market sized prawns in a predictable manner.

IPS may provide a large, healthy prawn, at a high nursery density. Achieving a higher survival rate, producing larger prawns at high densities, requires further study of design changes. An example of improved results achievable from substrate design adjustment was given by McCallum, *et al.* (2018) with studies showing increased production performance with media angles to 60 degrees from horizontal. Such a change may reduce cannibalism, through better utilization of media surface area as safe resting area during ecdysis. An additional measure of concealment for moulting prawns might have an additional positive impact. In the experimental phases and in the commercial pond trial, Phase C, net mesh corrugation tubes, constructed like those described in the IPS of Appendix 1, were provided for this purpose. However, there was no means to quantify their impact. Future study could devise an experiment to compare performance of culture with tubes, with culture without the tubes.

Phase A and subsequent phases demonstrated that nitrogen species are well-managed with both the IPS treatment and RAS control. The conversion of nitrogen and the cycling of organics and inorganics between photoautotrophic, autotrophic, and heterotrophic microbes, and the movement of waste nitrogen from dissolved feed and

animal excretions into microbial protein are fundamental to integrated biomass systems, namely BFT and IPS. Abundant studies utilizing Aquamat™ media have demonstrated that nitrogen species are typically maintained within boundaries of adequate conditions for farming of aquaculture species, with both marine (Audelo-Naranjo *et al.*, 2010; Audelo-Naranjo *et al.*, 2011; Audelo-Naranjo *et al.*, 2012a; Audelo-Naranjo *et al.*, 2012b; Huang *et al.*, 2013), and freshwater species (Arndt *et al.*, 2002; Yu *et al.*, 2016; Li *et al.*, 2019). The other water quality variables which were monitored during the experiment were also maintained in adequate condition.

BW at harvest of the Phase C, all-male commercial ponds over 195 DoC was guided by the market size at which partial harvest removed prawns from production, which was at or above the ex-farm purchase minimum size of around 35 g BW. Purchasers set this minimum size in the expectation of purchasing above 80 % BC males. SGR of the all-male commercial ponds over 195 DoC could be calculated from final weight of 1,006 kg and initial weight of 2.5 kg, as 3.1 % d^{-1}. Asaduzzaman *et al.* (2008) reported SGR for various C/N ratios and application of periphyton substrate as ranging from 1.56 to 1.75 % d^{-1} for harvest after 120 days of culture, achieving 0.48 mt ha^{-1} per cycle, or 1 mt $year^{-1}$ for 3 cycles harvesting at 40 g. 195 days for 35 g prawn output was broadly like commercial cycle times reported in the literature. The output of about 8.1 mt ha^{-1} $year^{-1}$ compared well with output of 9.4 mt ha^{-1} $year^{-1}$ reported for all-male *M. rosenbergii* by Sagi *et al.* (1985). SR found in the current study were broadly like those reported in prior studies, while the FCR of 2.1 for the Phase C ponds was generally lower than those reported in prior studies at similar densities.

5.3.3 Summary of financial efficiency of IPS pond culture of *M. rosenbergii*

Tank-based experiments were not continued to commercial harvest size. The mean body weight gain at 60 days for males and females shown in Figure 4.6 reflects the lack of growth achieved in females. This was strong evidence in favour of all-male culture, considering that shortest cycle times to reach 35 g is the route to improved financial efficiency in farming of *M. rosenbergii*. IPS did exhibit significant improvement in terms of achieving larger males.

The output per hectare resultant from a single IPS pond cycle of 8 mt ha^{-1} $year^{-1}$ forms the basis for any recommendation of IPS for pond farming of *M. rosenbergii*, hence further investigation is required. Appendix 2 b provides the basis of design for IPS applied to the nursery pond J4 and the grow out pond J2, of 488 m^2 and 1,800 m^2, respectively. Required biofilter periphyton surface area for the two ponds was calculated using the biofilter model developed and resulted in 849 m^2 and 2,059 m^2 of periphyton surface area for J4 and J2, respectively. At the specific surface area of 400 m^2 m^{-3} for the applied Matala™ media, with media thickness of 40 mm, 53 m^2 and 129 m^2 of Matala media were required for J4 and J2, respectively. The media outer surface area provided by hanging the media in the ponds was 120 m^2 and 288 m^2, for J4 and J2, respectively.

Table 5.1 describes the derivation of a cost for installation of the IPS to both ponds J4 and J2, for Phase C. The total cost of RM12,416.00 (USD$2,901.00) can be distributed over the production of 1,006 kg of *M. rosenbergii* sold at RM65.00 kg^{-1}, at about 35 g per piece, which results in RM12.34 (USD$2.88) per kg of the single cycle. As the life span of the equipment, which has no moving parts, can be estimated as at least 5 years with minimum 2 cycles per year, the cost per kg over 5 years would be RM1.23 (USD$0.29), or about 1.9% of the sale price.

Table 5.1: Financial results for Phase C, IPS ponds J4 and J2

Description	**Nursery Pond, J4**	**Grow out Pond, J2**
Matala media required, m^2	52	129
Matala media applied, m^2	60	144
Media cost, RM m^{-2}	50.00	50.00
Media cost, RM	3,000.00	7,200.00
Rope cost, RM	100.00	250.00
Chain weight cost, RM	300.00	750.00
Floats cost, RM	240.00	576.00
Sub-total cost, RM	3,640.00	8,776.00
Total cost, RM	12,416.00	
Prawn harvest for single cycle, kg	1,006	
Cost per single cycle harvest, RM kg-1	12.34	

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

IPS is an intensive aquaculture system which provides an efficient way of overcoming water and land scarcity and decreasing environmental impact from aquaculture. These experimental events defined and reinforced IPS as a promising new technological approach to *M. rosenbergii* culture with advantages in nursery and grow out; no external effluent treatment system requirement, zero water discharge, reduced waste generation, and potential for improved FCR. Maintaining biomass sufficient to manage water quality throughout the culture period, as periphyton on IPS media, without requirement for mixing energy for suspension of biofloc, while the IPS effectively replaces external biofilter required by RAS to maintain water quality, are demonstrated benefits of the IPS.

The current experiments compared the effects of the periphyton media (biomass growing on high specific surface area fixed film media) integrated into the production volume, with the effects of an identical system minus periphyton but with external RAS biofilter and filtration clarifier. Some low surface area screening substrate provided in the RAS system production volume which removed bias from RAS not having additional resting substrate within the production volume. In the RAS treatment, screens provided separation of individuals and additional resting area, but insignificant added periphyton area, hence the advantages of periphyton were distinguished from domain improvement. Under these constraints, IPS effectively replaced the external biofilter required by RAS, with respect to maintenance of water quality, thus reducing both footprint of the overall system, as well as relative

operational complexity and cost. For freshwater prawn culture, the study demonstrated equivalence to a RAS system approach.

The key feature in IPS is the periphyton media. The literature displays a lack of comparison of performance of IPS, RAS, and BFT. Performance evaluation was the central pillar of analytical procedure. Overall SR, FCR, gross yield, net production, SGR, and AGR were compared in the two systems. Comparative analysis was considered an effective means to observe and obtain meaningful data from highly complex systems that are present in all semi-commercial and commercial scale trials. The current research study has contributed to understanding of the important distinctions between the biomass systems, IPS and RAS, that nonetheless share significant characteristics, notably low to no water exchange. The methodology of employing comparative analysis of two systems is recommended for future research, and for future comparison of IPS and BFT as types of zero discharge, integrated biomass system.

In *M. rosenbergii*, the HIG of males and decoupled growth of females make production of uniform output for market extremely difficult. Claw development in males enhancing cannibalism, HIG, and female differentiation also make accurate prediction of a growth curve difficult. Commercial operation must anticipate fine tuning of growth curves to match applied hatchery stock, and other site-specific culture conditions. Accurate delivery of supplementary feed is difficult without an accurate growth curve, and predictable SR, which is a major commercial impact since both over feeding and under feeding are undesirable. The current study revealed the importance of and provided insight into matching a feed schedule to the growth of the culture species.

Development and implementation of all-male culture of *M. rosenbergii* supports better targeting of supplementary feed to culture population requirements. Further investigation is needed to evaluate minimization of FCR, and uniformity derived from partial harvesting of desirable BC males from size-separated populations, of all-male *M. rosenbergii* culture.

IPS has some relative benefits when compared with BFT. BFT requires mixing energy to maintain flocs in suspension, whereas IPS only requires diffusion of oxygen sufficient to maintain pond life, and general circulation to be maintained across media surfaces. The two systems require equivalent aeration energy to maintain dissolved oxygen, but IPS requires less mixing energy. Low-cost surface aerators, which are common in pond rearing of *M. rosenbergii*, may not produce sufficient mixing to prevent settlement of biofloc if applied to BFT. Periphyton fixed film has greater robustness to changing conditions than does suspended biofloc, due to more permanent and diverse biota better able to process changing nutrients and wastes. Periphyton has less propensity to support pathogenic populations than do biofloc. IPS biomass is retainable between crop cycles without separation because it retains viability on the fixed media. Further research is needed to identify the practicality of IPS media transfer from one cycle to the next.

Periphyton is preferred as a source of feed by grazing species to suspended biofloc. Although IPS requires a fixed media component to be provided that BFT does not, constituting a major capital cost, the cost has been demonstrated to be recoverable in a few cycles and equipment life is expected to be above 20 cycles or 10 years. Fixed media in an aquaculture volume requires management such as its removal during stock transfer or harvesting. However, moving media with attached biomass may be preferable to settlement of biofloc to pond bottom during similar management events for BFT systems.

In the past, aquaculture has relied on extensive and low capital cost systems, which consumed large amounts of land and water. The present trend is towards more technologically complex systems that exert control over production in an industrial approach, with means to respond to monitoring results. Capital expenditure in modern systems is relies on economic analysis, with technological approaches capable of controlling risk. The current study contributes to the basis for such analysis for IPS applied to the target species, *M. rosenbergii*.

In males, the development of the three male morphotypes, SM, OC, and BC males is referred to as HIG, and the female also has heterogenous individual growth,

as a fourth set within the population. HIG makes farm output of *M. rosenbergii* less profitable without aquaculture techniques to manage it. Providing additional surface area other than the pond bottom where *M. rosenbergii* typically reside, provides prawn individuals a safer location for ecdysis. The logic is that bottom feeders engage in cannibalism through feeding engagement when ecdysis occurs on the bottom. The additional domain provided on substrate surface, with periphyton for grazing, manages hierarchical stress. There may be impetus for larger BC populations with additional domain. Traditional, extensive ponds rely on low population densities distributed across bottom area. The advantages of combining biofilter layers, and other substrate into production volumes provides more and diverse surface area or domain. The current study was unable to conclusively define how additional surface area might reduce HIG, or bring about more uniform harvest product, with fewer partial harvests.

Selective harvesting, principally of BC males, or OC males if above 35 g, was facilitated by use of an innovative net harvesting technique to achieve the gains discussed by Sagi and Aflalo (2005), and supported by Rahman *et al.* (2010), whereby selective harvesting brings about compensatory growth in the remaining OC and SM males. Net harvesting technology was developed to facilitate partial harvesting with minimal manpower and disruption of the culture pond. Another outcome was the development of an improved media design to package the media for insertion to the pond, making it easily moved and removed during harvests and during other pond maintenance activities. Facilitation of partial harvesting is an important contribution to *M. rosenbergii* farming, as partial harvesting produces uniform market product.

The current study investigated aquaculture of both all-male and mixed sex *M. rosenbergii*. IPS is not expected to completely prevent inhibition of SM male growth nor to consistently stimulate metamorphosis of OC males into BC males. SM likely remain small through dietary suppression to avoid confrontation, and evade larger prawns (Karplus, 2005). IPS improves the potential for growth through hierarchical stress reduction and additional, periphyton grazing area, and reduces cannibalism by providing safer habitat for ecdysis.

All-male culture is expected to produce significantly higher uniformity amongst male morphotypes, i.e., through the lack of female presence reducing competition responses, but HIG is still active. Future research may evaluate mixed sex versus all-male culture in BFT compared with IPS, to determine whether significant positive impacts on performance and product uniformity can be achieved. While comparison of IPS performance with mixed sex and all-male populations was undertaken, a considerable amount of further research is needed to discover and validate available potential. The current study increased understanding of what can be accomplished with IPS and other intensive aquaculture systems, and the avenues of research that can lead to economic improvement with intensive production of *M. rosenbergii*.

Methods of incorporation of IPS substrate to commercial scale, intensive pond aquaculture will require further investigation and refinement. Operational and economic comparison with BFT should be considered for future research. Comparative examination of the microbial populations in BFT and IPS is also suggested to further evaluate disease resistance, health, water quality, and performance benefits of the two integrated biomass systems. There is a current global research focus on biome importance to animal health and culture of species. Future research will focus on interaction of culture species gut biome with the pond microbiological ecosystem, inclusive of pond sediment and water column, and with technological elements. Both IPS and BFT employ culture volume or biofilter volume biomes to achieve benefits of water quality, health, and nutrition improvement for culture species. The features of bacteria within biomass may influence the availability and transfer of useful compounds such as PHB, or of bacterial enzymes for reuse by culture species. The importance of these technologies in aquaculture will be well-served by future intensive investigation of system and species biomes, and their interdependencies. It is concluded that a way forward for research will investigate microbiological species involved in the IPS biome. Selective seeding of IPS media holds promise due to the robustness of biomass retention through the production cycle. Conducting trials with bacterial seeding could investigate the potential of influencing species selection within the IPS biome to positively impact performance of the culture species, i.e., *M. rosenbergii*.

Understanding of IPS as a distinct system of the same class as BFT is important to guide further development along the lines of what has taken place with BFT development. However, BFT has faced challenges over 20 years of development that persist today, which are principally high energy costs and management of residual biofloc solids (Emerciano *et al.*, 2021). IPS has demonstrated that it deals with both of those critical issues. The general objective of this study was to prepare, operate, and characterize a representative IPS as a sustainable means of culture of *M. rosenbergii* at high intensity. The IPS explored by experimental and commercial trial has demonstrated the required performance characteristics that are commercially viable by reference to global statistics for culture of *M. rosenbergii*. BFT continues to be identified as a sustainable aquaculture system (El-Sayed, 2020), and by extension IPS deserves the same identification and more attention to its further development. IPS shows the desirable features of clear water operation at low energy consumption, with low residual solids generation.

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APPENDICES

Appendix 1

Patent Articles for Applied Patent

(Figures 2 to 5 omitted by request)

TITLE OF THE INVENTION:

PERIPHYTON AND MOULTING-PROTECTION MODULE -

Module for delivery of an Integrated Periphyton System (IPS)

TECHNICAL FIELD

The present invention generally relates to a device to permit improvements in aquaculture systems and more particularly to a module of biological media with innovative functions together with attached module of moulting tubes with innovative design.

BACKGROUND ART

There is known a suspended media in aquaculture which is made of various materials, including synthetic resin fibre, that has the purpose of providing abundant surface area upon which bacteria and other microorganisms may grow. Additionally, there is known a suspension of physical structure or substrate, within an aquaculture production volume, that has the purpose of providing separation of domain for aquaculture species. Additionally, there is known an inclusion of open-ended vessels that have the purpose of providing protection for aquaculture species during mating, during sensitive activities associated with birth and nurture, and during moulting. The method of forming large numbers of open-ended vessel is similar to the known production of light structural sheets by inclusion of interior corrugated sheet, whereas the structure is adapted to the production of a compact set of a large number of vessels.

The advantages of each of these components has not been optimized in a single device capable of imparting all the advantages. However, the coincident use of media and vessels has so far developed such that the perceived cost of implementation of the measures has appeared too great to support implementation in aquaculture, with a resulting loss of potential performance of certain classes of aquaculture systems.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a suspended media that provides numerous advantages; support for microorganisms, concealment for aquaculture species, positive health impact, water quality improvement, increased grazing area, supplementary feed replacement, and support for moulting, breeding, and nurturing devices within the aquaculture water volume.

The suspended media is selected for high specific surface area (area per unit volume), and open structure to permit diffusion of gases, and transfer of nutrients, waste products and microorganisms from the external to internal surfaces. The microorganisms are capable of processing culture species wastes within the production volume, and also utilizing waste feeds, such that the need for external biofilter is reduced or removed, and little or no waste is discharged from the production volume. The media is suspended at an angle 0 from the horizontal. Angle 0 is selected such that waste solids will be removed by gravity from the upper face, or less than the angle of repose for waste solids. Angle 0 also places the upper side of the media in sun exposure relative to the underside, which preferentially promotes algae formation on the upper side. The underside, where the open-ended vessels are located, is protected from solids deposition, and is a lower light environment.

The attached, so-called moulting (including breeding and nurturing) tubes are constructed to maximize individual compartments per unit area employing shared

walls to minimize material consumption, and with flexibility of material selection. The moulting tubes are formed in the manner of corrugated cardboard (but with different purpose) using material safe in the aquatic and aquaculture environment, and with corrugations sized to suit both the species and anticipated utilization of the vessels.

The combined set of media and vessels serves to provide the advantages to aquaculture within production volumes of water through suspension of the modules on buoys, racks or cables, whereas the individual components may also be applied separately, or affixed to walls or bottom of aquaculture tanks, ponds or raceways.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an exploded view illustrating the major components of media and a single row of vessels, and Angle 0 in its relationship to the vertical;

FIG. 2 is a view of a combined module with three rows of vessels showing a typical separation of vessel rows, on preferred embodiment of a combined module according to the present invention, with Angle 0 = 0; **[Omitted by request]**

FIG. 3 is an exploded perspective view of the vessel construction showing relevant dimensions; **[Omitted by request]**

FIG. 4 is a top view of a typical array of combined modules in the center of a raceway tank; **[Omitted by request]**

FIG. 5 is an elevation perspective view of a raceway tank, with one tank side cut away, showing an array of modules at an inclination angle 0. **[Omitted by request]**

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A frame suspended media sheet, 1, of L-1 length and W-1 width, is open to the water volume on both sides. The media sheet is held at an inclination of angle 0. The thickness and specific surface area of 1 determine the capability to sustain microbial biomass variety and quantity.

A multiple folded or constructed element, the zigzag element, 2, up to L-1 length and W-2 in width, forming the internal division of open-ended vessels, is attached to the underside of the media sheet 1. The underside forms the inner surface of corrugated vessels.

A strip of material, 3, of identical length and width to element 2, is attached to form the outside wall of the corrugated vessels. The zig-zag sheet of stiff material 2 is attached to media 1 and flat sheet of material, 3, i.e., by rivets, at contact points. This forms one row of corrugated vessels between 1 and 2, and a second interlocked set of vessels between 2 and 3. The dimension L-2 is the height, and W-3 is the width of the corrugation employed. L-2 and W-3 are selected to produce the desired opening dimension of the resulting corrugated tube that is suitable to the aquaculture species under culture, for identified moulting, nurturing, or mating activities. The dimension W- 2 is also selected according to the length of vessel suitable to the aquaculture species for the identified activities.

The means of attachment of 1, 2, and 3 together depends on the thickness and properties of 1, 2 and 3. They may be rivetted, sewn, or otherwise attached together. The requirement for attachment is to keep the two components in approximate contact, with loose tolerance requirement. Forces on the components are low in the aquatic environment. If multiple rows of corrugations 2 and 3 are attached to the media sheet 1, the openings of the corrugated vessels need to be distant by W-4 from the vessel openings in the adjacent row, which distance is determined according to the aquaculture species and application.

CLAIMS:

1. A module with an active biological media comprising:

a media sheet 1 with surface exposed to aerated water volume; a frame (not shown) as required to support suspension from racks, cables or floats, and a set of corrugated vessels formed from 2 and 3 attached to 1, characterized in that the module is suspended in the water volume. It provides benefits arising from waste conversion, water quality improvement, concealment, increased grazing area, and protected vessels for sensitive activities such as moulting, nurturing, and breeding.

2. The media sheet in claim 1 characterized in that the media sheet 1 surface area is a property of the fibres making up the media. An example media specification utilizes synthetic fibre mat, e.g., high specific surface area fibre mat with over 90% free space within the mat, and surface area of individual fibres summing to up to 400 square meters per cubic meter of material. The media sheet is typically < 50 mm thick so that oxygen from the water volume may diffuse into the media. At the centre of the media, with formation of biomass, oxygen will be depleted and the innermost depth from outer surfaces will be populated by anaerobic bacteria. The outer layer including the media outer surface will be dominated by aerobic bacteria, and the intermediate layer will contain facultative bacteria able to function in both environments. The microbial ecosystem can complete the conversion of nutrients from waste feed and excretions into biomass and harmless by-products. A further beneficial effect of treating waste excretions and feed within the production volume is that the need for an external recirculating biofilter is reduced or removed, and little or no waste need be discharged or treated.

3. The media sheet in claim 1 characterized in that the aerobic layer and microorganisms feeding within the ecosystem become available to the aquaculture species under culture as live feed of continuously refreshed biomass of microorganisms that provide safe nutrition to aquaculture species, which has the effect of replacing part of supplementary feed requirement. The same biomass exerts a quorum sensing effect which deters pathogens and may supply further health support in acting as probiotic and supplying digestive enzymes.

4. The media sheet in claim 1 characterized in that the presence of this ecosystem with its high concentration of harmless bacteria, exerts negative competitive pressure on pathogens referred to as quorum sensing.

5. The media sheet in claim 1 characterized in that some crustaceans and fish are detritivores, herbivores, or omnivores which consume live feed as nutritious microorganisms growing on detritus, or live biofilm. These species can graze on the microorganisms on the surface of the media.

6. The media sheet in claim 1 characterized in that some crustaceans and fish perceive media sheet surface area as increased domain, which may reduce stress.

7. The media sheet in claim 1 characterized in that some crustaceans and fish perceive the underside of the media sheet as concealment within the water volume, which is also known to reduce stress in some aquaculture species.

8. The module of corrugated vessels in claim 1 comprising:

a. a zig-zag sheet 3 and flat sheet 2 forming the internal and outside separator for tubular vessels with shared walls;

b. utilizing the suspended media sheet 1 as one inside wall of the vessels;

c. able to be constructed of a variety of materials to optimize cost and effects in the aquatic environment; and,

d. able to be constructed of mesh material to provide permeability, to lower material consumption and cost, while maintaining concealment.

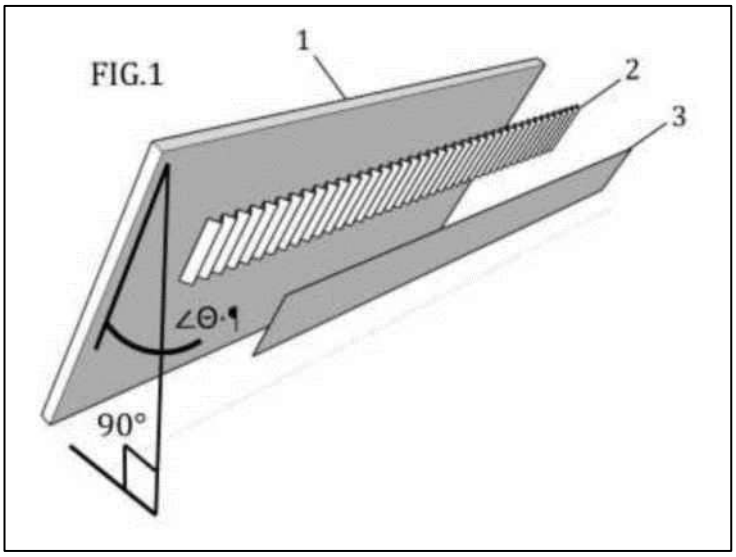
9. The module of corrugated vessels in claim 4 characterized in that it provides many protected vessels per unit area employed for sensitive activities such as moulting, nurturing and breeding, which is necessary for high density rearing of crustaceans and fish in aquaculture production volumes, especially where the species has a higher temporal frequency of the activities during rearing.

10. The combined module changes the apprehension of the aquaculture species under culture and permits higher density rearing in an identical water volume.

ABSTRACT

PERIPHYTON AND MOULTING-PROTECTION MODULE

The present invention generally relates to improvements in aquaculture systems, whether ponds, raceways, or tanks for the culture of certain aquaculture species, which are known to benefit from the characteristics provided by the invention when emplaced in the water volume. The present invention discloses a media sheet (1), frame (unspecified), and a module or set of corrugated vessels (2, 3). The media sheet forms the residence of a microbial ecosystem is responsible for waste conversion, positive health impact, water quality improvement, concealment, increased grazing area, and supplementary feed replacement. The media sheet is employed such that its support frame is shared by the corrugated vessels, and one side of the media sheet, typically the underside resulting from inclination at an angle, forms one side of the corrugated vessels. The corrugated vessels provide protected environment for sensitive activities such as moulting, nurturing, and breeding. Together the module changes the apprehension of the aquaculture species under culture and permits higher density rearing in an identical water volume.



Appendix 2

2(a): BIOFILTER MODEL APPLIED IN PHASE A, and
2(b): BIOFILTER MEDIA OF SUBSEQUENT PHASES

2(a) Biofilter model applied in Phase A:

Parameter	Quantity	Unit	Origin, Calculation, or Description
Tank effective volume	1.3	m^3	Actual tank capacity. 870 mm water depth used
Peak culture density	30	kg m^{-3}	Used to calculate feed addition at peak culture density
Culture mass (prawns)	39.2	kg	
Daily feed ratio	1.25	%	
Daily feed at peak culture density	490	g	Feed per day determines the TAN loading applied.
Crude Protein (CP)	32	%	CP content of feed
Conversion to TAN from CP	6.5	%	TAN from protein
Daily TAN from feed	0.01	kg day^{-1}	TAN from applied feed to be removed by the biofilter component of IPS
Conversion to TAN from bulk feed	2.08	%	TAN from protein in bulk feed
Target residual TAN	1	mg L^{-1}	(Or lower) Calculation purpose
Passive nitrification (PN)	10	%	Non-biofilter nitrification allowance
Daily TAN from feed after PN	0.009	kg day^{-1}	TAN to biofilter
Alkalinity requirement as sodium bicarbonate	73.5	g/day	TAN kg d^{-1} x 7; 15 % of feed
Biofilter area for TAN removal	0.45	gTAN m^{-2} d^{-1}	
Required biofilter area	20.4	m^2	
Media specific area	200	m^2 m^{-3}	
Oxygen required by feed added	0.147	kg d^{-1}	30 % of daily feed

Parameter	Quantity	Unit	Origin, Calculation, or Description
O_2 for passive nitrification	0.005	kg d^{-1}	TAN removed in passive nitrification x 4.57
O_2 for biofilter nitrification	0.042	kg d^{-1}	TAN removed in biofilter x 4.57
Total O_2 required	0.193	kg d^{-1}	
Specific Oxygen Transfer Efficiency (SOTE)	2.45	%	Estimated from average dirty water using fine pore diffuser SOTE at 0.7 m x 3.5 % m^{-1}
Total O2 required for SOTE	7.9	kg d^{-1} oxygen	
Air required at 21% O2	37.6	kg d^{-1} air	
Air volumetric requirement	20.4	m^3 day^{-1}	1.84 kg m^{-3}
	0.9	m^3 h^{-1}	15 L min^{-1}
Factor of Safety (FOS)	1.5		Air supply margin
Air at FOS	21.3	L min^{-1}	

2(b) Biofilter media applied in experimental Phases:

Parameter	Unit	Phase A	Phase B1	Phase B2	Phase B3	Phase B4	Phase B5	Phase B6	Phase B7	Phase C, Pond J4	Phase C, Pond J2
Tank effective volume	m^{-3}	1.3	1.3	1.3	1.3	8.0	1.2	0.23	0.23	585	2,160
Tank plan area	m^2	1.5	1.5	1.5	1.5	7.1	1.0	0.33	0.33	488	1,800
Peak culture density (max. predicted in feed model)	kg m^{-3}	30	0.62	1.49	0.72	0.79	0.13	0.65	1.00	1.16	0.77
Peak Culture mass (prawns)	kg	39.2	0.78	1.94	0.93	6.3	0.15	0.15	0.23	680	1,650
Daily feed at peak culture density	g	490	56	135	28	188	10.5	4.6	5.0	20,400	49,500
Crude Protein (CP)	%	32	32	32	32	32	32	32	32	32	32
Conversion to TAN from CP	%	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Daily TAN from feed	kg/day	0.0102	0.0012	0.0028	0.0006	0.0039	0.0002	0.0001	0.0001	0.4243	1.0296
Conversion to TAN from bulk feed	%	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
Target residual TAN	mg/L	1	1	1	1	1	1	1	1	1	1
Passive nitrification (PN)	%	10	10	10	10	10	10	10	10	10	10
Daily TAN from feed after PN	kg/day	0.0092	0.0010	0.0025	0.0005	0.0035	0.0002	0.0001	0.0001	0.3819	0.9266
Biofilter specific area for TAN removal	gTAN m^{-2} d^{-1}	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Required biofilter area	m^2	20.4	2.3	5.6	1.2	7.8	0.4	0.2	0.2	849	2059
Media specific area	m^2 m^{-3}	300	300	300	300	400	400	400	400	400	400
Media volume required	m^3	0.068	0.008	0.019	0.004	0.020	0.001	0.000	0.001	2.122	5.148
Media thickness	mm	30	30	30	30	30	40	40	40	40	40
Media area required	m^2	2.3	0.3	0.6	0.1	0.7	0.027	0.012	0.013	53.0	128.7
Media area provided	m^2	3.0	3.0	3.0	3.0	8.0	13	0.3	0.3	60	144
2 sides habitat area	m^2	6.0	6.0	6.0	6.0	16.0	26.7	0.6	0.6	120.0	288.0
Media outer surface area provided	m^2 m^{-2}	2.0	2.0	2.0	2.0	1.1	13.3	0.9	0.9	0.1	0.1

Appendix 3

EXPERIMENTAL FEEDING CHART FOR COMMERCIAL PHASE
FEED TABLE INTEGRATED WITH TRANSER AND HARVEST

POND J4: 27 JANUARY 2022 TO 5 FEBRUARY 2022 - NURSERY
POND J2: 6 FEBRUARY 2022 TO 10 AUGUST 2022 - GROWOUT WITH PARTIAL HARVESTS

GO	**=**	**Growout**
T	**=**	**Transfer**
N	**=**	**Nursery**

Date	DAY	Mass, g	SR, %	Event	Popu-lation	Feed Ratio, %	Prawn Feed, kg	Mol-asses, kg	Na-HCO_3, kg
5000		J4		J2					
27/1	1	0.05	100	N	50,00	7	0.2	0.1	0
28/1	2	0.08	99.83	N	49,91	7	0.3	0.1	0
29/1	3	0.12	99.67	N	49,83	7	0.4	0.2	0.1
30/1	4	0.15	99.5	N	49,75	7	0.5	0.3	0.1
31/1	5	0.18	99.33	N	49,66	7	0.6	0.3	0.1
1/2/	6	0.22	99.17	N	49,58	7	0.8	0.4	0.1
2/2/	7	0.25	99	N	49,50	7	0.9	0.4	0.1
3/2/	8	0.28	98.83	N	49,41	7	1	0.5	0.1
4/2/	9	0.32	98.67	N	49,33	7	1.1	0.5	0.2
5/2/	10	0.35	98.5	N	49,25	7	1.2	0.6	0.2
6/2/	11	0.38	98.33	N	49,16	7	1.3	0.7	0.2
7/2/	12	0.42	98.17	N	49,08	7	1.4	0.7	0.2
8/2/	13	0.45	98	N	49,00	7	1.5	0.8	0.2
9/2/	14	0.48	97.83	N	48,91	7	1.7	0.8	0.2
10/2	15	0.52	97.67	N	48,83	7	1.8	0.9	0.3
11/2	16	0.55	97.5	N	48,75	7	1.9	0.9	0.3
12/2	17	0.58	97.33	N	48,66	7	2	1	0.3
13/2	18	0.62	97.17	N	48,58	7	2.1	1	0.3
14/2	19	0.65	97	N	48,50	7	2.2	1.1	0.3
15/2	20	0.68	96.83	N	48,41	7	2.3	1.2	0.3
16/2	21	0.72	96.67	N	48,33	7	2.4	1.2	0.4
17/2	22	0.75	96.5	N	48,25	7	2.5	1.3	0.4
18/2	23	0.78	96.33	N	48,16	7	2.6	1.3	0.4
19/2	24	0.82	96.17	N	48,08	7	2.7	1.4	0.4
20/2	25	0.85	96	N	48,00	7	2.9	1.4	0.4
21/2	26	0.88	95.83	N	47,91	7	3	1.5	0.4
22/2	27	0.92	95.67	N	47,83	7	3.1	1.5	0.5
23/2	28	0.95	95.5	N	47,75	7	3.2	1.6	0.5
24/2	29	0.98	95.33	N	47,66	7	3.3	1.6	0.5
25/2	30	1.02	95.17	N	47,58	7	3.4	1.7	0.5
26/2	31	1.05	95	N	47,50	5	2.5	1.2	0.4
27/2	32	1.08	94.83	N	47,41	5	2.6	1.3	0.4
28/2	33	1.12	94.67	N	47,33	5	2.6	1.3	0.4
1/3/	34	1.15	94.5	N	47,25	5	2.7	1.4	0.4
2/3/	35	1.18	94.33	N	47,16	5	2.8	1.4	0.4
3/3/	36	1.22	94.17	N	47,08	5	2.9	1.4	0.4
4/3/	37	1.25	94	N	47,00	5	2.9	1.5	0.4
5/3/	38	1.28	93.83	N	46,91	5	3	1.5	0.5
6/3/	39	1.32	93.67	N	46,83	5	3.1	1.5	0.5

Date	DAY	Mass, g	SR, %	Event	Popu-lation	Feed Ratio, %	Prawn Feed, kg	Mol-asses, kg	Na-HCO_3, kg
7/3/	40	1.35	93.5	N	46,75	5	3.2	1.6	0.5
8/3/	41	1.38	93.33	N	46,66	5	3.2	1.6	0.5
9/3/	42	1.42	93.17	N	46,58	5	3.3	1.6	0.5
10/3	43	1.45	93	N	46,50	5	3.4	1.7	0.5
11/3	44	1.48	92.83	N	46,41	5	3.4	1.7	0.5
12/3	45	1.52	92.67	N	46,33	5	3.5	1.8	0.5
13/3	46	1.55	92.5	N	46,25	5	3.6	1.8	0.5
14/3	47	1.58	92.33	N	46,16	5	3.7	1.8	0.5
15/3	48	1.62	92.17	N	46,08	5	3.7	1.9	0.6
16/3	49	1.65	92	N	46,00	5	3.8	1.9	0.6
17/3	50	1.68	91.83	N	45,91	5	3.9	1.9	0.6
18/3	51	1.72	91.67	N	45,83	5	3.9	2	0.6
19/3	52	1.75	91.5	N	45,75	5	4	2	0.6
20/3	53	1.78	91.33	N	45,66	5	4.1	2	0.6
21/3	54	1.82	91.17	N	45,58	5	4.1	2.1	0.6
22/3	55	1.85	91	N	45,50	5	4.2	2.1	0.6
23/3	56	1.88	90.83	N	45,41	5	4.3	2.1	0.6
24/3	57	1.92	90.67	N	45,33	5	4.3	2.2	0.7
25/3	58	1.95	90.5	N	45,25	5	4.4	2.2	0.7
26/3	59	1.98	90.33	N	45,16	5	4.5	2.2	0.7
27/3	60	2	90.17	N	45,08	5	4.5	2.3	0.7
28/3	61	2.46	90	N	45,00	3	3.3	1.7	0.5
29/3	62	2.92	89.83	N	44,91	3	3.9	2	0.6
30/3	63	3.38	89.67	N	44,83	3	4.6	2.3	0.7
31/3	64	3.85	89.5	N	44,75	3	5.2	2.6	0.8
1/4/	65	4.31	89.33	N	44,66	3	5.8	2.9	0.9
2/4/	66	4.77	89.17	N	44,58	3	6.4	3.2	1
3/4/	67	5.23	89	N	44,50	3	7	3.5	1
4/4/	68	5.69	88.83	N	44,41	3	7.6	3.8	1.1
5/4/	69	6.15	88.67	N	44,33	3	8.2	4.1	1.2
6/4/	70	6.62	88.5	N	44,25	3	8.8	4.4	1.3
7/4/	71	7.08	88.33	N	44,16	3	9.4	4.7	1.4
8/4/	72	7.54	88.17	N	44,08	3	10	5	1.5
9/4/	73	8	88	N	44,00	3	10.6	5.3	1.6
10/4	74	8.46	87.83	N	43,91	3	11.1	5.6	1.7
11/4	75	8.92	87.67	N	43,83	3	11.7	5.9	1.8
12/4	76	9.38	87.5	N	43,75	3	12.3	6.2	1.8
13/4	77	9.85	87.33	N	43,66	3	12.9	6.4	1.9
14/4	78	10.31	87.17	N	43,58	3	13.5	6.7	2
15/4	79	10.77	87	N	43,50	3	14.1	7	2.1

Date	DAY	Mass, g	SR, %	Event	Popu-lation	Feed Ratio, %	Prawn Feed, kg	Mol-asses, kg	Na-HCO_3, kg
16/4	80	11.23	86.83	N	43,41	3	14.6	7.3	2.2
17/4	81	11.69	86.67	N	43,33	3	15.2	7.6	2.3
18/4	82	12.15	86.5	N	43,25	3	15.8	7.9	2.4
19/4	83	12.62	86.33	N	43,16	3	16.3	8.2	2.5
20/4	84	13.08	86.17	N	43,08	3	16.9	8.5	2.5
21/4	85	13.54	86	N	43,00	3	17.5	8.7	2.6
22/4	86	14	85.83	N	42,91	3	18	9	2.7
23/4	87	14.46	85.67	N	42,83	3	18.6	9.3	2.8
24/4	88	14.92	85.5	N	42,75	3	19.1	9.6	2.9
25/4	89	15.38	85.33	N	42,66	3	19.7	9.8	3
26/4	90	15.85	85.17	N	42,58	3	20.2	10.1	3
27/4	91	16.31	85	N	42,50	2	13.9	6.9	2.1
28/4	92	16.77	84.83	N	42,41	2	14.2	7.1	2.1
29/4	93	17.23	84.67	N	42,33	2	14.6	7.3	2.2
30/4	94	17.69	84.5	N	42,25	2	14.9	7.5	2.2
1/5/	95	18.15	84.33	N	42,16	2	15.3	7.7	2.3
2/5/	96	18.62	71	Tr	35,50	2	13.2	6.6	2
3/5/	97	19.08	70.83	GO	35,41	2	13.5	6.8	2
4/5/	98	19.54	70.67	GO	35,33	2	13.8	6.9	2.1
5/5/	99	20	70.5	GO	35,25	2	14.1	7	2.1
6/5/	10	20.46	70.33	GO	35,16	2	14.4	7.2	2.2
7/5/	10	20.92	70.17	**2,044**	33,03	2	14.7	7.3	2.2
8/5/	10	21.38	70	GO	32,95	2	15	7.5	2.2
9/5/	10	21.85	69.83	GO	32,87	2	15.3	7.6	2.3
10/5	10	22.31	69.67	GO	32,78	2	15.5	7.8	2.3
11/5	10	22.77	69.5	GO	32,70	2	15.8	7.9	2.4
12/5	10	23.23	69.33	GO	32,62	2	16.1	8.1	2.4
13/5	10	23.69	69.17	GO	32,53	2	16.4	8.2	2.5
14/5	10	24.15	69	GO	32,45	2	16.7	8.3	2.5
15/5	10	24.62	68.83	GO	32,37	2	16.9	8.5	2.5
16/5	11	25.08	68.67	GO	32,28	2	17.2	8.6	2.6
17/5	11	25.54	68.5	GO	32,20	2	17.5	8.7	2.6
18/5	11	26	68.33	GO	32,12	2	17.8	8.9	2.7
19/5	11	26.46	68.17	GO	32,03	2	18	9	2.7
20/5	11	26.92	68	GO	31,95	2	18.3	9.2	2.7
21/5	11	27.38	67.83	GO	31,87	2	18.6	9.3	2.8
22/5	11	27.85	67.67	GO	31,78	2	18.8	9.4	2.8
23/5	11	28.31	67.5	GO	31,70	2	19.1	9.6	2.9
24/5	11	28.77	67.33	GO	31,62	2	19.4	9.7	2.9
25/5	11	29.23	67.17	GO	31,53	2	19.6	9.8	2.9

Date	DAY	Mass, g	SR, %	Event	Popu-lation	Feed Ratio, %	Prawn Feed, kg	Mol-asses, kg	Na-HCO_3, kg
26/5	12	30	67	GO	31,45	2	20.1	10.1	3
27/5	12	30.46	66.83	**1,551**	29,82	1	10.2	5.1	1.5
28/5	12	30.92	66.67	GO	29,73	1	10.3	5.2	1.5
29/5	12	31.38	66.5	GO	29,65	1	10.4	5.2	1.6
30/5	12	31.85	66.33	GO	29,57	1	10.6	5.3	1.6
31/5	12	32.31	66.17	GO	29,48	1	10.7	5.3	1.6
1/6/	12	32.77	66	GO	29,40	1	10.8	5.4	1.6
2/6/	12	33.23	65.83	GO	29,32	1	10.9	5.5	1.6
3/6/	12	33.69	65.67	GO	29,23	1	11.1	5.5	1.7
4/6/	12	34.15	65.5	GO	29,15	1	11.2	5.6	1.7
5/6/	13	34.62	65.33	GO	29,07	1	11.3	5.7	1.7
6/6/	13	35.08	65.17	GO	28,98	1	11.4	5.7	1.7
7/6/	13	35.54	65	GO	28,90	1	11.6	5.8	1.7
8/6/	13	36	64.83	GO	28,82	1	11.7	5.8	1.8
9/6/	13	36.46	64.67	GO	28,73	1	11.8	5.9	1.8
10/6	13	36.92	64.5	GO	28,65	1	11.9	6	1.8
11/6	13	37.38	64.33	GO	28,57	1	12	6	1.8
12/6	13	37.85	64.17	GO	28,48	1	12.1	6.1	1.8
13/6	13	38.31	64	GO	28,40	1	12.3	6.1	1.8
14/6	13	38.77	63.83	GO	28,32	1	12.4	6.2	1.9
15/6	14	39.23	63.67	**5,445**	22,79	1	12.5	6.2	1.9
16/6	14	39.69	63.5	GO	22,70	1	12.6	6.3	1.9
17/6	14	40.15	63.33	GO	22,62	1	12.7	6.4	1.9
18/6	14	40.62	63.17	GO	22,54	1	12.8	6.4	1.9
19/6	14	41.08	63	GO	22,45	1	12.9	6.5	1.9
20/6	14	41.54	62.83	GO	22,37	1	13.1	6.5	2
21/6	14	42	62.67	GO	22,29	1	13.2	6.6	2
22/6	14	42.46	62.5	**8,469**	13,74	1	13.3	6.6	2
23/6	14	42.92	62.33	GO	13,65	1	13.4	6.7	2
24/6	14	43.38	62.17	GO	13,57	1	13.5	6.7	2
25/6	15	43.85	62	GO	13,49	1	13.6	6.8	2
26/6	15	44.31	61.83	GO	13,40	1	13.7	6.8	2.1
27/6	15	44.77	61.67	GO	13,32	1	13.8	6.9	2.1
28/6	15	45.23	61.5	GO	13,24	1	13.9	7	2.1
29/6	15	45.69	61.33	GO	13,15	1	14	7	2.1
30/6	15	46.15	61.17	GO	13,07	1	14.1	7.1	2.1
1/7/	15	46.62	61	**1,203**	11,78	1	14.2	7.1	2.1
2/7/	15	47.08	60.83	GO	11,70	1	14.3	7.2	2.1
3/7/	15	47.54	60.67	GO	11,62	1	14.4	7.2	2.2
4/7/	15	48	60.5	GO	11,53	1	14.5	7.3	2.2

Date	DAY	Mass, g	SR, %	Event	Popu-lation	Feed Ratio, %	Prawn Feed, kg	Mol-asses, kg	Na-HCO_3, kg
5/7/	16	48.46	60.33	GO	11,45	1	14.6	7.3	2.2
6/7/	16	48.92	60.17	GO	11,37	1	14.7	7.4	2.2
7/7/	16	49.38	60	GO	11,28	1	14.8	7.4	2.2
8/7/	16	49.85	59.83	GO	11,20	1	14.9	7.5	2.2
9/7/	16	50.31	59.67	GO	11,12	1	15	7.5	2.3
10/7	16	50.77	59.5	GO	11,03	1	15.1	7.6	2.3
11/7	16	51.23	59.33	GO	10,95	1	15.2	7.6	2.3
12/7	16	51.69	59.17	GO	10,87	1	15.3	7.6	2.3
13/7	16	52.15	59	GO	10,78	1	15.4	7.7	2.3
14/7	16	52.62	58.83	GO	10,70	1	15.5	7.7	2.3
15/7	17	53.08	58.67	GO	10,62	1	15.6	7.8	2.3
16/7	17	53.54	58.5	GO	10,53	1	15.7	7.8	2.3
17/7	17	54	58.33	GO	10,45	1	15.7	7.9	2.4
18/7	17	54.46	58.17	GO	10,37	1	15.8	7.9	2.4
19/7	17	54.92	58	GO	10,28	1	15.9	8	2.4
20/7	17	55.38	57.83	GO	10,20	1	16	8	2.4
21/7	17	55.85	57.67	GO	10,12	1	16.1	8.1	2.4
22/7	17	56.31	57.5	GO	10,03	1	16.2	8.1	2.4
23/7	17	56.77	57.33	GO	9,954	1	16.3	8.1	2.4
24/7	17	57.23	57.17	GO	9,870	1	16.4	8.2	2.5
25/7	18	60	57	GO	9,787	1	17.1	8.5	2.6
26/7	18	60	56.83	GO	9,704	1	17.1	8.5	2.6
27/7	18	60	56.67	GO	9,620	1	17	8.5	2.6
28/7	18	60	56.5	GO	9,537	1	17	8.5	2.5
29/7	18	60	56.33	GO	9,454	1	16.9	8.4	2.5
30/7	18	60	56.17	GO	9,370	1	16.9	8.4	2.5
31/7	18	60	56	GO	9,287	1	16.8	8.4	2.5
1/8/	18	60	55.83	GO	9,204	1	16.8	8.4	2.5
2/8/	18	60	55.67	GO	9,120	1	16.7	8.3	2.5
3/8/	18	60	55.5	**6,585**	2,452	1	16.7	8.3	2.5
4/8/	19	60	55.33	GO	2,369	1	16.6	8.3	2.5
5/8/	19	60	55.17	GO	2,286	1	16.6	8.3	2.5
6/8/	19	60	55	GO	2,202	1	16.5	8.2	2.5
7/8/	19	60	54.83	GO	2,119	1	16.5	8.2	2.5
8/8/	19	60	54.67	GO	2,036	1	16.4	8.2	2.5
9/8/	19	60	54.5	**1,920**	32	1	16.4	8.2	2.5
TOTAL							**2,070**	**1,035**	**311**

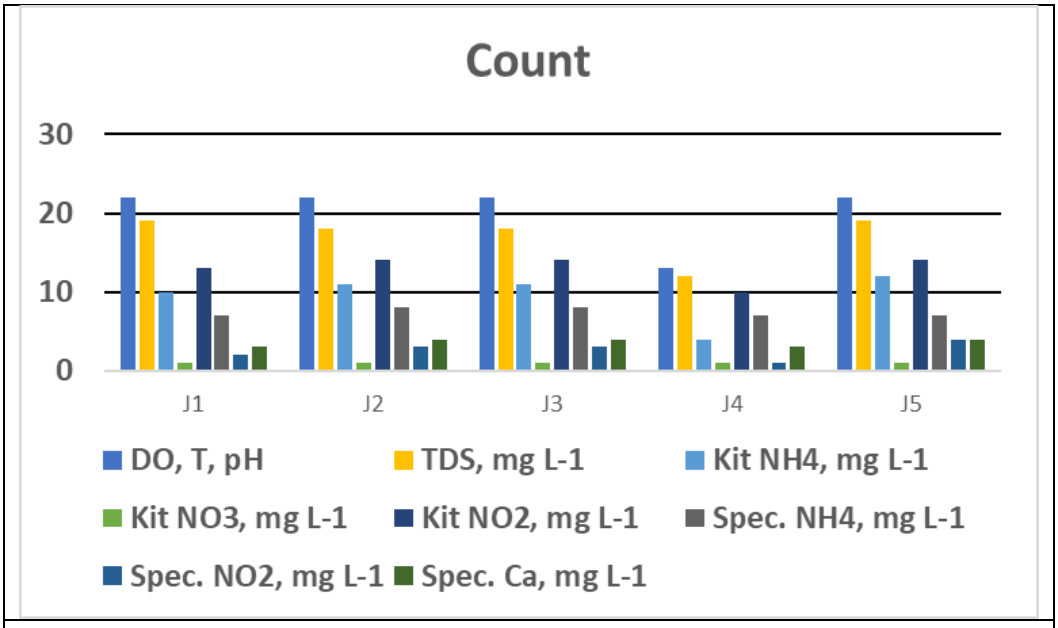
Actual total prawn production: 1,006 kg

Calculated FCR: 2.1

Approximately 32 prawns transferred to other ponds continuing grow out.

Appendix 4

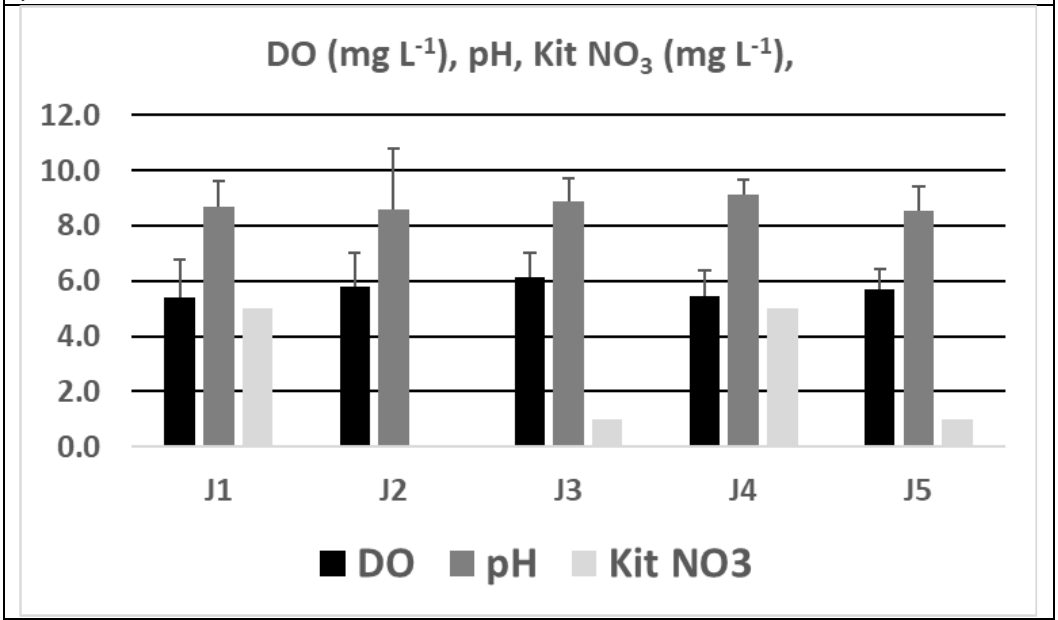
Summary Water Quality from Commercial IPS Ponds J1 to J5 over 27 June 2022 to 29 September 2022



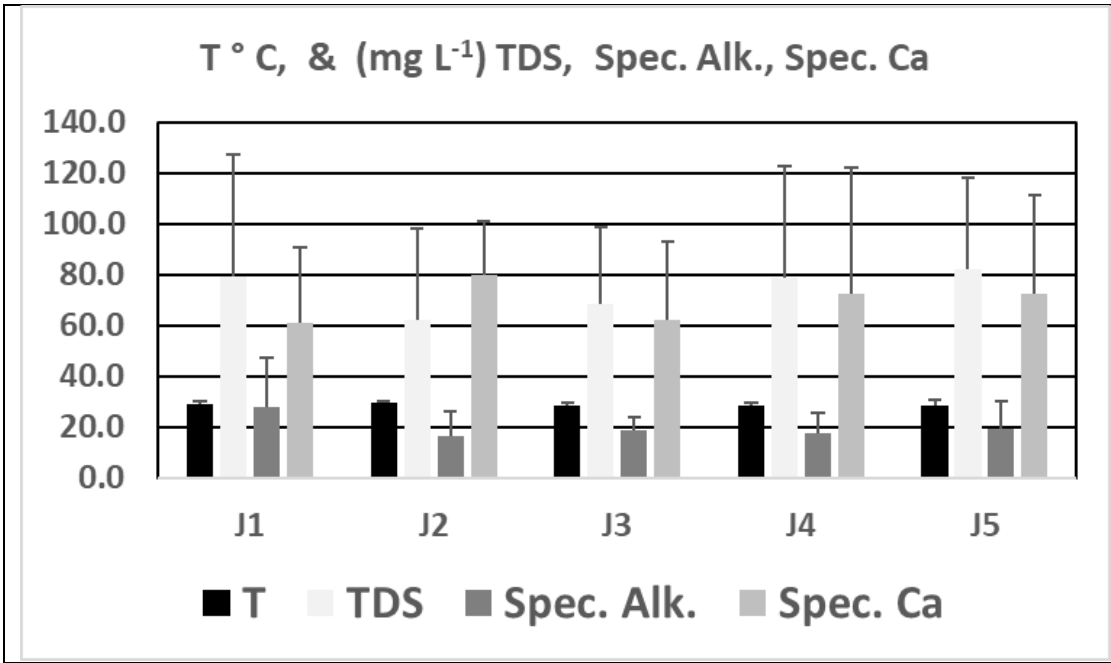
Count of water quality analysis events by test and parameter for each of 5 IPS ponds, J1 to J5 over the period of 27 January 2022 to 10 August 2022.

POND	27/6/2022 to 27/7/2022	28/7/2022 to 27/8/2022	28/8/2022 to 29/9/2022
J1	Empty	Nursery	Nursery
J2	P 1	P 2 & 3	P4
J3	Nursery	Nursery	Nursery
J4	Empty	Nursery	Nursery
J5	Grow out	P 1 & 2	P 3 & 4

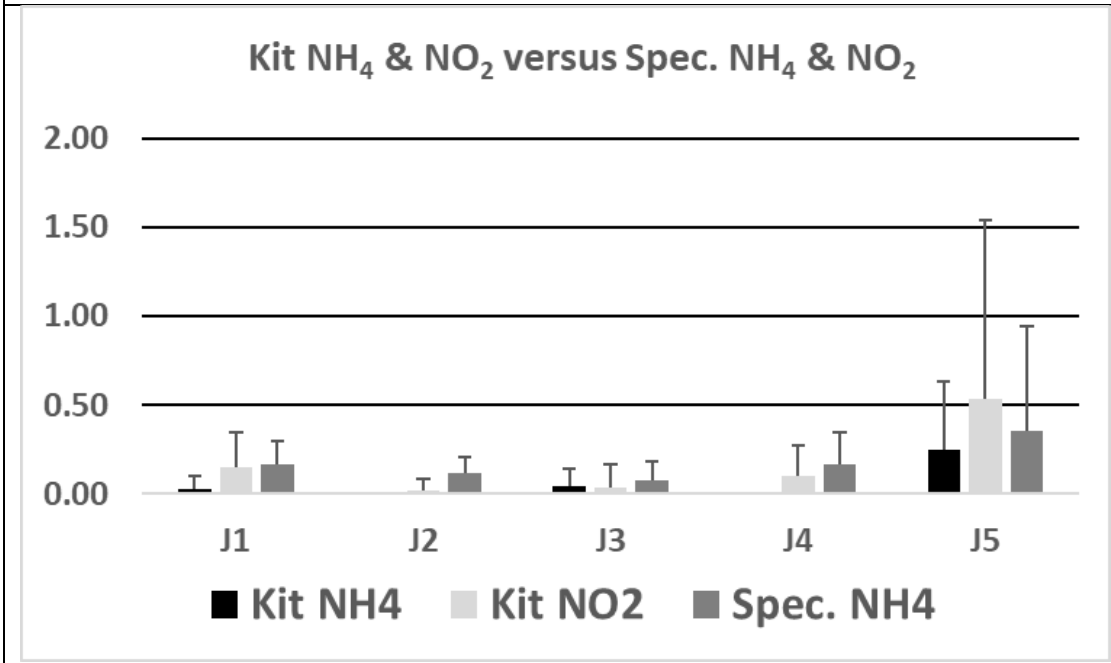
Activity record for IPS ponds over the period of 27 January 2022 to 10 August 2022. Nursery indicates PL stocked. Grow out indicates post-nursery. P indicates partial harvests.



Average values for the period. DO and pH by handheld meter. NO3 by freshwater photometric kit analysis.



Average values for the period. Temperature and TDS by handheld meter. Alkalinity and Calcium by Hannah Spectrophotometer.



Average values for the period. NH4 and NO2 by freshwater photometric kit analysis

BIODATA OF AUTHOR

Mr. Marioni has completed Bachelor of Applied Science in Civil Engineering, University of British Columbia, Vancouver, Canada (1989), and Master of Environment, Faculty of Science and Environmental Studies, UPM, Malaysia (2001) ahead of his enrolment to PhD by research in Aquaculture, Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengannu (2017 to present). His presentation of papers at conferences over a long career is represented in his ORCID ID: 0000-0001-8108-8208, and presented papers related to his current studies are recorded above. Mr. Marioni practiced environmental engineering following graduation from UBC in 1989, B.App.Sc. in Civil Engineering, and continues in consultancy to date. His expertise in water and wastewater treatment supported his passion for aquaculture, which has led him to form several aquaculture companies and to engage in aquaculture farming in Malaysia, where he is resident.

Presentations

Marioni, D., Mhd Ikhwanuddin (2023). Evaluation of Integrated Periphyton System (IPS) aquaculture of Mixed-sex Giant Freshwater Prawn (*Macrobrachium rosenbergii*). 10th National and 6th International Symposium on Marine Science & Fisheris 2023, June 10th - 11th, 2023 at Faculty of Marine Science and Fisheries, Universitas Hasanuddin (UNHAS), Makassar, Indonesia. (**Oral presentation**).

Marioni, D., Mhd Ikhwanuddin (2022). Experimental analysis evaluating a new integrated periphyton system for intensive *Macrobrachium rosenbergii* aquaculture. International Fisheries Symposium 2022 "Adapt to changes", December 5th – 7th, 2022 at Nha Trang University, Nha Trang City, Vietnam. **(Oral presentation)**.

Marioni, D., Mohd Kamruzzaman, Mhd Ikhwanuddin (2021). Nursery Rearing of Macrobrachium rosenbergii with a C/N Modified Periphyton Integrated Biofilter RAS Alternative. 4th KRIPIK-SCiFiMaS, Innovation and Reinforcement for Fisheries and Marine Sustainability in the Covid-19 Pandemic Era, November 12th - 13th, 2021 in Purwokerto, Central Java, Indonesia (via online). **(Oral presentation)**.

Marioni, D., Mohd Kamruzzaman, Mhd Ikhwanuddin (2019). Morphometric comparison of periphyton and non-periphyton nursery culture of Giant Freshwater Prawn (*Macrobrachium rosenbergii*). 2019 ASEAN-FEN 9th International Fisheries Symposium, 18-21 November 2019 – Seri Pacific Hotel, Kuala Lumpur, Malaysia. **(Oral presentation)**.

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A. (2018). Review of Attached and Suspended Biomass Applications Integrated to Recirculating Aquaculture Systems. 8th International Fisheries Symposium 2018. **(Oral presentation)**.

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A. (2018). The use of substrates for aquaculture species' domain improvement and biofiltration. Asian-Pacific Aquaculture (APA) 2018, Taipei, Taiwan. www.was.org. **(Oral presentation)**.

Publications

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A. **(2020)**. Review of Attached and Suspended Biomass Applications Integrated to Recirculating Aquaculture Systems. 8th International Fisheries Symposium 2018. *IOP Conference Series: Earth and Environmental Science* 416 (2020) 012004. doi:10.1088/1755-1315/416/1/012004

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A. **(2025)**. Metanalysis of Integrated Periphyton Technology (IPT) in Freshwater and Marine Aquaculture. Currently in preparation.

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A., Rosas, V.T., Vethamony, P., Al-Khayat, J.A. **(2025)**. Grow Out Analysis by Gender with Size Split in Integrated Periphyton System (IPS) and Recirculating Aquaculture System (RAS) for Intensive Giant Freshwater Prawn, *Macrobrachium rosenbergii*, Aquaculture. Currently accepted in preparation for publication.

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A., Rosas, V.T., Vethamony, P., Al-Khayat, J.A. **(2025)**. Innovative Integrated Periphyton Technology for Sustainable and Intensive Nursery Culture of Giant Freshwater Prawn, *Macrobrachium rosenbergii*. *Journal of Sustainability Science and Management, Volume 20 Number 8, August 2025: 1651-1666. eISSN: 2672-7226. © UMT Press*

Marioni, D., Ihkwannudin, Mhd, Kasan, M.A., Rosas, V.T., Vethamony, P., Al-Khayat, J.A. **(2025)**. Validation of Integrated Periphyton Technology in Mixed Sex Culture of Giant Freshwater Prawn, *Macrobrachium Rosenbergii*: Insights into Impact of Heterogenous Independent Differentiation and Gender on Growth Dynamics in Grow Out. *Tropical Life Sciences Research*. In process of publication as of July 2025.

Patent Applications

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Application number	:	PI 2021002361
Filing Date	:	28 April 2021
Country	:	Malaysia
Inventor	:	David Marioni; Mhd Ikhwanuddin Abdullah; Institute of Tropical Aquaculture, Universiti Malaysia Terengganu
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Status	:	PI 2021002361; 28 April 2021