UNIVERSITI PUTRA MALAYSIA

OPTIMIZING RED CLAW CRAYFISH, Cherax Quadricarinatus REARING TANK'S WATER QUALITY USING PHOTOSYNTHETIC BACTERIA FOR ENHANCED AQUACULTURE CONDITIONS

HANNAH HEROH ANYIE

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HANNAH HEROH ANYIE

BACHELOR OF SCIENCE AQUACULTURE (HONOURS)

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HANNAH HEROH ANYIE

FACULTY OF AGRICULTURAL AND FORESTRY SCIENCES
UNIVERSITI PUTRA MALAYSIA BINTULU SARAWAK CAMPUS

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By

Hannah Heroh Anyie

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural and Forestry Sciences Universiti Putra Malaysia Bintulu Sarawak Campus.

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ABSTRACT

The treatment of wastewater is an important aspect of environmental management that aims to reduce the negative impact of contaminants discharged into water systems. This study investigates the efficacy of utilizing photosynthetic bacteria for wastewater treatment in the culture of red claw crayfish, *Cherax quadricarinatus*. Photosynthetic bacteria (PSB) have been proven to purify water and enhance the growth of aquatic organisms as this bacterium are capable of fixing atmospheric nitrogen and converting it into ammonia or related compounds. Photosynthetic bacteria are microorganisms capable of harnessing light energy to facilitate the synthesis of organic compounds through the process of photosynthesis. This process is known as nitrogen fixation. These bacteria play a crucial role in the nitrogen cycle, contributing to the availability of nitrogen compounds for other organisms. Five individuals of red claw crayfish, Cherax quadricarinatus were placed in each treatment and reared for 63 days. The result shows that the there is no significant ($p>0.05$) between the average ammonia and nitrite distribution for PSB1 (1.9ppm, 0.45ppm), PSB2 (1.7ppm, 0.5ppm), PSB3 (1.7ppm, 1.1ppm) and positive control (1.8ppm, 0.45ppm) but was significantly different ($p<0.05$) with negative control (4.0ppm, 2.1ppm). The reading was gradually decrease from day 15 and constantly low until the end of the experiment days except for negative. The nitrate distribution shows significantly different between PSB1 (13.75ppm), PSB2 (9.02ppm), PSB3 (13.80ppm), positive (6.1ppm) and negative (20.08ppm). Based on this result, it demonstrated that how PSB can be use as alternative treatments to treat and maintain the water quality in fish rearing tanks. The utilization of cultured PSB in culture systems has the potential to expedite the nitrogen cycle, hence offering a sustainable approach to wastewater treatment. This finding suggests that the PSB treatment demonstrated the bacteria's capacity to use and absorb

nutrients, preserving and enhancing the quality of the water. It also demonstrated how alternate treatments, such as cultivated PSB, can be employed to maintain and treat the water quality in fish rearing tanks. The utilization of commercial and cultured bacteria in culture systems has the potential to expedite the nitrogen cycle, hence offering a sustainable approach to wastewater treatment.

ABSTRAK

Rawatan air sis merupakan aspek penting dalam pengurusan alam sekitar yang bertujuan untuk mengurangkan impak negatif bahan pencemar yang dibuang ke dalam air. Kajian ini menyiasat keberkesanan penggunaan bakteria fotosintetik (PSB) untuk rawatan air sisa dalam pemeliharaan udang krai, *Cherax quadricarinatus*. Bakteria fotosintetik (PSB) terbukti dapat membersihkan air dan meningkatkan pertumbuhan organisma akuatik kerana bakteria ini mampu membaiki nitrogen atmosfera dan mengubahkannya menjadi ammonia atau sebatian berkaitan. Bakteria fotosintetik adalah mikroorganisma yang mampu menggunakan tenaga cahaya untuk memudahkan sintesis sebatian organik melalui proses fotosintesis. Proses ini dikenali sebagai fiksasi nitrogen. Bakteria ini memainkan peranan penting dalam kitaran nitrogen, menyumbang kepada ketersediaan sebatian nitrogen untuk organisma lain. Lima ekor udang krai, *Cherax quadricarinatus* ditempatkan dalam setiap rawatan dan dipelihara selama 63 hari. Keputusan menunjukkan bahawa tidak terdapat perbezaan signifikan ($p>0.05$) antara taburan purata amonia dan nitrit untuk PSB1 (1.9ppm, 0.45ppm), PSB2 (1.7ppm, 0.5ppm), PSB3 (1.7ppm, 1.1ppm), dan kawalan positif (1.8ppm, 0.45ppm), tetapi terdapat perbezaan yang signifikan ($p<0.05$) dengan kawalan negatif (4.0ppm, 2.1ppm). Bacaan ini secara beransur-ansur menurun dari hari ke-15 dan kekal rendah sehingga akhir hari eksperimen kecuali untuk kawalan negatif. Taburan nitrat menunjukkan perbezaan yang signifikan antara PSB1 (13.75ppm), PSB2 (9.02ppm), PSB3 (13.80ppm), positif (6.1ppm), dan negatif (20.08ppm). Berdasarkan hasil ini, ianya membuktikan bagaimana PSB boleh digunakan sebagai rawatan alternatif untuk merawat dan mengekalkan kualiti air dalam tangki pemeliharaan ikan. Penggunaan PSB yang terkultur dalam sistem pemeliharaan mempunyai potensi untuk mempercepatkan kitaran nitrogen, oleh itu menawarkan pendekatan yang mampan

untuk rawatan air sisa. Penemuan ini menunjukkan bahawa rawatan PSB menunjukkan keupayaan bakteria ini untuk menggunakan dan menyerap nutrien, mengekalkan serta meningkatkan kualiti air. Ia juga menunjukkan bagaimana rawatan alternatif, seperti PSB yang dikultur, boleh digunakan untuk mengekalkan dan merawat kualiti air dalam tangki pemeliharaan ikan. Penggunaan bakteria komersial dan terkultur dalam sistem pemeliharaan mempunyai potensi untuk mempercepatkan kitaran nitrogen, oleh itu menawarkan pendekatan yang mampan untuk rawatan air sisa.

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APPROVAL SHEETS

I certify that this research project report entitled "Optimizing Red Claw Crayfish, *Cherax Quadricarinatus* Rearing Tank's Water Quality Using Photosynthetic Bacteria for Enhanced Aquaculture Conditions" has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus.

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Ts. Dr. Suhaili Mustafa

Faculty of Agricultural Science and Forestry

Universiti Putra Malaysia Bintulu Campus Sarawak

(Supervisor)

_____

Dr. Zamri Bin Rosli

Faculty of Agricultural Science and Forestry

Universiti Putra Malaysia Bintulu Sarawak Campus

Dean

Date:

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LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
PSB	Photosynthetic bacteria
VMP	Veterinary medicinal products
GAqP	Code of Good Aquaculture Practices
DO	Dissolved oxygen
PVC	Polyvinyl chloride
AU	Absorbance unit
RPM	Revolutions per minute
mL	Milliliter
TSS	Total suspended solids
COD	Chemical oxygen demand
TDS	Total dissolved solids
$HgSO_4$	Mercuric sulfate
$K_2Cr_2O_7$	Potassium dichromate
SGR	Specific growth rate
FCR	Feed conversion ratio
SAS	Statistical Analysis System
ANOVA	Analysis of Variance
RAS	Recirculating Aquaculture System
BOD	Biochemical Oxygen Demand

CHAPTER 1

INTRODUCTION

1.1 Background

Aquaculture plays a pivotal role in global food security, contributing substantially to the world's seafood supply. Aquaculture, the cultivation of aquatic organisms under controlled conditions, has become a vital component of global food production, providing a significant source of protein for a growing population. As the aquaculture industry expands to meet increasing demand, concerns regarding environmental sustainability and water quality management have emerged. The industry has witnessed exponential growth over the past few decades, with a diverse range of aquatic species being cultivated for human consumption. Global aquaculture statistics reveal the immense scale of the industry, highlighting its economic significance and the need for sustainable practices to ensure long-term viability. Aquaculture stands out as the most varied form of farming globally, considering the multitude of species, farming techniques, and environments employed in its practices (Métian et al., 2019). In the intricate web of global aquaculture, an industry of diverse facets is flourishing to satisfy the rising demand for seafood while freshwater fish make up approximately 70% of global edible aquaculture volume due to their efficient conversion from live to edible weight, which is more favorable compared to mollusks and crustaceans with higher shell weights (Edwards et al., 2019, as cited in Naylor et al., 2021) although concurrently addressing concerns associated with maintaining a sustainable aquaculture. Spanning varied geographic regions and encompassing a broad array of aquatic species, cultivation techniques, and technological innovations, aquaculture has evolved into a linchpin of global food security. Food security is the primary means of improving socioeconomic condition in each nation to combat hunger (Pradeepkiran,

2019). The dynamic expansion of aquaculture is propelled by continuous technological breakthroughs, refinement of management strategies, and an escalating awareness of its potential to alleviate pressure on strained marine and freshwater ecosystem as a whole. Nevertheless, the journey towards sustainable aquaculture is not devoid of challenges. Effective management strategies should be developed through a comprehensive assessment of the present condition of sustainable aquaculture production systems (Jiang et al., 2022). Hence, this thesis addresses these concerns by exploring the application of photosynthetic bacteria to improve water quality in tank culture systems, with a specific focus on mitigating the impact of aquaculture wastewater.

Aquaculture in Asia is a multifaceted and crucial sector, serving as a linchpin for the region's food security, economic growth, and societal structure. Asia dominates the list of countries with the highest aquaculture species diversity, with China holding a substantial lead among the top-ten ranked nations (Naylor et al., 2021). According to Food and Agriculture Organization (FAO) (2018), as cited in Ahmed and Thompson (2019), Asia accounted for 89% of global aquaculture production, with China ranking first at 61.5%, followed by India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, Chile, Myanmar, and Thailand in 2016. With these nations taking the lead, Asia stands as a global force in the production of aquaculture (Naylor et al., 2021). Due to the rapid expansion of the industry, the region has shown significant advancements in aquaculture research, marked by an upswing in both investment and research capabilities (Silva & Yuan, 2022). Despite its benefits, aquaculture generates wastewater rich in nutrients, organic matter, and other pollutants. The region's aquaculture industry faces unique challenges related to water quality, environmental sustainability, and the intensification of production systems. Investigating innovative

methods, such as the application of photosynthetic bacteria, becomes crucial for addressing these challenges and promoting sustainable aquaculture practices in Asia. The release of untreated wastewater into natural water bodies can lead to ecological imbalances, negatively impacting aquatic ecosystems. Effective wastewater management strategies are imperative to minimize environmental degradation associated with aquaculture activities. Thus, this thesis explores the potential of photosynthetic bacteria as a novel and eco-friendly approach to improve the quality of aquaculture wastewater within tank culture systems.

Aquaculture is a key priority for Malaysia's economic development, drawing widespread participation due to progress across the nation. It offers employment, business, and investment prospects, boasting over 18,000 aquafarmers and a total farm size exceeding 34,000 hectares as of 2017 (Jumatli & Ismail). In 2018, Malaysia achieved a fish production of 1.84 million tons, with 79% attributed to capture fisheries and 21% to aquaculture (Samah, 2018). Malaysia's tropical climate and abundant water resources make it conducive for aquaculture practices. Several techniques of culture are currently used, and many species are cultured to fulfill the demand for aquaculture products (Kurniawan et al., 2021). Malaysia is a key player in the global aquaculture market, exporting a variety of seafood products. According to Harun, et al. (2023), aquaculture in Malaysia is seen as a key driver for economic progress, positioned strategically for future significance. The nation consistently prioritizes this industry in its development initiatives, recognizing its favorable location abundant in surface and groundwater resources, crucial for successful aquaculture implementation. Efforts are ongoing to address challenges such as environmental sustainability, disease management, and market competitiveness, ensuring the continued growth and sustainability of aquaculture in Malaysia. This country acts as a key player in the Asian

aquaculture landscape, provides a specific case study within this thesis. Analyzing the status of aquaculture in Malaysia involves understanding the industry's growth, challenges, and environmental implications. By examining the potential application of photosynthetic bacteria in Malaysian tank culture systems, the thesis aims to contribute valuable insights and practical solutions to enhance water quality and promote sustainable aquaculture practices in the country. Therefore, in this study, the isolated photosynthetic bacteria was evaluated against commercially available PSB as an alternative treatment to study the efficacy status of photosynthetic bacteria in maintaining water quality for rearing red claw crayfish, *Cherax quadricarinatus*.

1.2 Problem statement

Despite the advancements in aquaculture practices, the persistent issue of maintaining ideal water quality parameters remains a significant obstacle in aquaculture tank culture systems. Fluctuations in pH, dissolved oxygen levels, and the accumulation of harmful substances such as ammonia pose threats to the well-being of the species, affecting growth rates and overall productivity. Conventional water treatment methods have limitations, necessitating exploration into innovative approaches. The viability and efficacy of utilizing photosynthetic bacteria in mitigating these challenges warrant in-depth investigation and analysis.

1.3 Research Questions

1. What is the impact of introducing photosynthetic bacteria on nutrient levels in a tank culture system, specifically focusing on nitrogen cycle and water quality?
2. How do the mechanisms associated with photosynthetic bacteria impact key water quality parameters in tank culture systems, and what are the implications for sustainable water management in aquaculture?

1.3 Objectives of the study

1. To study the efficacy status of photosynthetic bacteria in maintaining water quality for rearing red claw crayfish, *Cherax quadricarinatus*.
2. To record the growth rate of red claw crayfish, *Cherax quadricarinatus* in the rearing system.

1.4 Research Hypothesis

Null Hypothesis, H_0: The application of photosynthetic bacteria in red claw crayfish, *Cherax quadricarinatus* tank culture systems will lead to a significant improvement in water quality parameters.

Alternative hypothesis, H_A: The application of photosynthetic bacteria in red claw crayfish, *Cherax quadricarinatus* tank culture systems will not lead to a significant improvement in water quality parameters.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Aquaculture and Tank Culture Systems

2.1.1 Background of Aquaculture

Aquaculture can be defined as the cultivation of aquatic organisms in controlled or semi-controlled environments (Stickney & Gatlin, 2022) to enact various purposes, such as food production, conservation, research, or recreation. Aquaculture is presently and anticipated to remain a progressively vital and essential element of the global food system, supplying the world with affordable, high-quality aquatic food for human consumption (Bartley, 2022). According to Food and Agriculture Organization (FAO), 2020, the FAO has observed a 31.8% rise in the total count of commercially farmed aquaculture species items, escalating from 472 in 2006 to 622 in 2018. Moreover, recent research indicates that around 250 additional species are being cultivated, but these are not included in FAO reports during the routine data reporting of aquaculture production (as cited in Bartley, 2022). The fundamental objective of aquaculture is to optimize conditions for growth, reproduction, and harvest, ensuring a sustainable and efficient production system.

2.1.2 Importance of Water Quality in Tank Culture Systems

A specific aspect of aquaculture is tank culture systems, where aquatic organisms are raised in confined tanks or containers. According to Lucas et al. (2019), tanks, following ponds, are frequently utilized structures in aquaculture. Their advantage lies in enabling the utilization of land typically deemed unsuitable for aquaculture, as the water is enclosed within the structures, preventing contact with the surrounding soils. This method allows for precise control of environmental variables such as water

quality, temperature, and feeding regimes as tanks are designed to mimic natural habitats, providing a controlled environment that promotes optimal growth and health of the cultivated species.

In a tank culture system, practitioners can carefully manage and monitor the conditions to prevent diseases, optimize feed conversion, and control water quality parameters. The quality of water is a crucial factor in domestic, agricultural and industrial water supply, fisheries, aquaculture production, aquatic recreation, and the well-being of ecosystems (Boyd, 2020). The effects of water quality are pivotal in a tank culture system, directly influencing the health and growth of aquatic organisms. Elements that have a detrimental impact on the water quality of a fish culture system include the feeding rate, the composition of the feed, the metabolic rate of the fish, and the quantity of uneaten feed and fecal solids produced in fish tanks daily (Aquaculture in the Tropics, 2021). Poor water quality can lead to stress, diseases, and stunted growth, negatively impacting the overall productivity and success of the tank culture system. Regular monitoring and appropriate management of water quality are critical aspects of maintaining a thriving and sustainable aquaculture operation within a tank culture system.

2.1.3 Challenges in Maintaining Water Quality

Water, being a universal solvent, is never found in its pure form in the natural environment, and it inherently contains contaminants. If the concentrations of these contaminants in fish tanks surpass a specific threshold, it leads to both water pollution and the mortality of fish (Aquaculture in the Tropics, 2021). Ensuring and managing water quality in tank culture systems present a spectrum of challenges that demand careful consideration for the health and success of aquatic organisms. One primary

challenge involves the accumulation of ammonia and nitrites, metabolic byproducts of fish, requiring constant monitoring and management to prevent toxicity. Organic wastes contribute to a substantial oxygen demand, frequently resulting in decreased concentrations of dissolved oxygen, while nitrogen and phosphorus in effluents contribute to the process of eutrophication (Boyd, 2020). According to Alltech (2022), high population density in farming environments result in diminished water quality due to reduced levels of dissolved oxygen and the buildup of harmful metabolic wastes, notably ammonia. Ammonia, recognized as one of the most toxic elements in water quality, can induce stress and damage to gills, even at low concentrations. The author also mentioned that aquatic organisms subjected to prolonged exposure to low levels of ammonia display increased vulnerability to bacterial infections, stunted growth, and reduced tolerance to routine handling. At elevated concentrations, ammonia becomes lethal, and numerous unexplained declines in production have been associated with this hazardous substance. To tackle these multifaceted challenges, a comprehensive approach is essential, involving routine water testing, meticulous system design, effective filtration methods, and unwavering adherence to best management practices, thereby ensuring the sustained optimal conditions required for the flourishing of aquatic life in tank culture systems.

2.2 Overview of Red Claw Crayfish, *Cherax quadricarinatus*

2.2.1 Biological Characteristics

Figure 1. Red claw crayfish, *Cherax quadricarinatus*

Red claw crayfish, *Cherax quadricarinatus* is just like any other decapods, where the males grow faster and bigger than the females (Zheng et al., 2020). According to Oficialdegui (2022), the males will have carapace ranging from 43 to 45mm and will have wider chelae and chelipeds if there are larger males while females will have a wider abdomen. The soft and red patches on the propodus of the males are the obvious traits to distinguish between a male and female crayfish. These patches indicate the fighting capability which will use the appendages in aggressive interactions. The females attach their eggs to the fine hairs that are called 'setae' which located on the margins of the pleopods. Haubrock et al. (2021) stated that the females are highly prolific because females may lay more than a thousand eggs in a single clutch, where they will mature very fast. The females will also curl up their tail and create a chamber to protect the eggs (Oficialdegui 2022). Eprilurahman et al. (2021) stated an observation on the *Cherax quadricarinatus* found in two different places have similar morphological characteristics that match the identification of the species. The characteristics include having large first pereopod and males have a red patch, three pairs of rostrum spines, transparent membrane on the tail, the male genitalia are more

complex and characterized by the opening of the genital organs at the base of the fifth pereopod while females are at the third pereopod.

Table 1: Taxonomy of red claw crayfish, *Cherax quadricarinatus*

Classification	Name
Kingdom	Animalia
Phylum	Arthropoda
Class	Malacostraca
Subclass	Eumalacostraca
Order	Decapoda
Family	Parastacidae
Genus	Cherax
Species	*Cherax quadricarinatus*

2.2.2 Economic Significance in Aquaculture

Red claw crayfish is a freshwater crustacean that is originally distributed in Australia and Papua New Guinea (Oficialdegui, 2022). Known for being a hardy species (Patoka et al., 2016, as cited in Hassan et al., 2023), it is introduced into Malaysia and has been cultivated commercially since 2003 (Alimon et al.,2003, as cited in Hassan et al., 2023) in Peninsular Malaysia, Johor (Chang 2001; Naqiuddin et al. 2016, as cited in Ismail et al., 2021) This crayfish species is easy to cultivate and has a high fecundity (Haubrock et al., 2021) in which it contributes to a significant essence in aquaculture (Samad et al., 2022). Red claw crayfish is also known to have remarkable advantages because it can adapt well in a wide range of weather conditions, can eat anything including plant-based food as it has an omnivorous feeding habit, high growth rate and can be handled easily (Méndez-Martínez et al., 2021, as cited in Martínez et al. 2022). Cannibalism starts since it is still young when the population density is high and it will prey on other crayfish that are sick and moulting (Abinawanto et al., 2018; Wiyanto and Hartono, 2003, as cited in Widyasari et al., 2021). This crustacean is not the species

that is favored to be consumed by the locals in Malaysia (Tee et al., 2022) but it is used as ornamental organisms due to its unique physique and color (Samad et al., 2022). Despite having a good potential in aquaculture due to its high tolerance in extreme conditions and high growth rate (Naranjo-Páramo et al., 2004, as cited in Yin et al., 2022), it also has some challenges in maintaining a good health.

2.2.3 Sensitivity to Water Quality Parameters

Red claw crayfish requires specific water quality conditions for successful rearing. This species is able to adapt in a robust condition but it is compulsory to ensure optimal health, growth, and reproduction. The pH levels should be kept within the range of 6.5 to 8.0, maintaining a neutral to slightly alkaline environment (*How To Farm Red Claw Crayfish*, 2023). It is essential to maintain water temperatures between 25 to 30 degrees Celsius. Adequate dissolved oxygen levels, preferably above 5 mg/L (Guide Printing | Business and Industry | Queensland Government, 2018), are crucial for the respiratory needs of Red Claw crayfish, especially in systems with high stocking densities. Monitoring and controlling ammonia levels, aiming for values as close to zero as possible, as crayfish are sensitive to these toxic substances. Implementing efficient mechanical and biological filtration is essential to remove uneaten food, debris, and metabolic waste, preventing the accumulation of harmful substances. Avoiding overstocking is crucial, as high stocking densities can lead to increased waste production and stress on the crayfish, negatively impacting overall health and growth. Proactive monitoring, regular water testing, and responsible management practices are integral for maintaining the balanced and stable aquatic environment necessary for successful *Cherax quadricarinatus* aquaculture.

2.3 Photosynthetic Bacteria: Characteristics and Functions

2.3.1 Definition and Classification of Photosynthetic Bacteria

Photosynthetic bacteria (PSB) is a type of bacteria that can undergo photosynthesis where it uses solar energy to produce food. Sigee (2005) stated that there are three groups of PSB-green sulphur bacteria (Chlorobacteriaceae), purple sulphur bacteria (Thiorhodaceae) and purple non-sulphur bacteria (Athiorhodaceae) where each group comes in different shapes and sizes. They can be in form of spherical, elliptical, rod-shaped or vibrioid. This author also mentioned that PSB vary in their motility as some have flagellates to move around (p. 312). Bryant & Liu (2013) explained that green sulfur bacteria, also known as Chlorobacteriaceae, are a type of photosynthetic bacteria found in water environments like freshwater and marine sediments. These bacteria have a unique way of carrying out photosynthesis called anoxygenic photosynthesis, which doesn't produce oxygen. Instead, they use specialized structures called chlorosomes to capture light energy and pigments called bacteriochlorophylls to absorb specific wavelengths of light. They make use of sulfur compounds like hydrogen sulfide as a source of electrons during photosynthesis. Green sulfur bacteria prefer habitats with low light levels, such as deep sediments or layered water bodies. They thrive in environments without oxygen and have adapted to use sulfur compounds for energy, giving them an advantage in their habitats. Scientists have been studying green sulfur bacteria to understand their photosynthetic mechanisms, how they metabolize different substances, and their ecological importance. These bacteria play a significant role in the cycling of sulfur in aquatic ecosystems. Purple sulphur bacteria is in the Chromatiaceae family, or previously known as Thiorhodaceae (Molisch, 1907, as cited in Kushkevych et al., 2021). According to Kushkevych, Bosáková, et al. (2021), this bacterium is an anoxygenic bacteria, where it can carry

out photosynthesis that does not produce oxygen as a byproduct. Unlike green sulphur bacteria, purple sulphur bacteria does not contain chlorosomes to capture light energy, hence, they live above green sulphur bacteria as they require higher light intensity and lower hydrogen sulfide concentrations for photosynthesis. Purple non-sulphur bacteria can tolerate in wide range of conditions, this includes in marine sediment and mangrove environment (Al Azad & Mohamad Lal, 2023). This bacterium utilizes different metabolic modules in order to adapt in different environments (McKinlay, 2014). Similar to purple sulphur bacteria, purple non-sulphur bacteria can carry out anoxygenic photosynthesis. This bacterium uses organic acids as electron donor during anoxygenic photosynthesis (Petushkova et al., 2021). However, purple non-sulphur bacteria differs from purple sulphur bacteria because purple non-sulphur bacteria are classified in alpha and beta division while purple sulphur bacteria are in gamma division of proteobacteria (Spanoghe et al., 2022). Purple non-sulphur bacteria is widely known to treat wastewater because of its characteristics to use organic compound for growth. Hence, PSB are able to maintain water quality in red claw crayfish aquaculture system.

2.3.2 Role of Photosynthetic Bacteria in Aquatic Environments

According to Miyasaka et al. (2021), the author concluded that one of the species of PSB, *Rhodovulum sulfidophilum*, a marine purple nonsulfur PSB, can increase the chances of survival of kuruma shrimp, *Marsupenaeus japonicus* as it acts as probiotics. Based on the previous study conducted in the microbiology and parasitology laboratory in University Putra Malaysia Bintulu Sarawak Campus, it was shown promising result of PSB in treating water quality. Based on the study, the amount of ammonia and nitrite reduced significantly at the treatment. Applying PSB in shrimp

culture will also help save cost for fish farmers as they are economically feasible (Miyasaka et al., 2021) and thus help to increase shrimp production in the country.

2.3.3 Potential Benefits in Improving Water Quality

PSB is also known for its role as bioremediators as it treats water in man-made ecosystem where PSB is used to purify water. Apart from treating water, PSB is also known to assist the growth performance of aquaculture animals (Shapawi et. al., 2012, as cited in Chumpol, 2017) where one of the types of PSB which is purple non-sulphur bacteria are able to protect shrimps from diseases (Chumpol, 2017). (Chang et al. (2019) also suggested that the addition of PSB in multitrophic recirculating aquaculture system (MRAS) can help to inhibit cyanobacteria, potential pathogenic bacteria and algae growth whereas anoxygenic PSB will increase more in order to maintain an optimum water quality. Another study has shown that PSB, *R. sulfidophilum* can sustain the bacterial communities and this will be beneficial in accomplishing a sustainable marine aquaculture farm (Ying et al., 2020).

2.4 Water Quality Parameters in Aquaculture

2.4.1 Physicochemical Parameters

Water physicochemical parameters serve as indicators utilized to assess the quality of water (Bilewu et al., 2022). Testing water with various physicochemical parameters is essential and fundamental before its utilization for domestic, agricultural, drinking, and industrial purposes (Beniwal et al., 2021). Physicochemical parameters, including pH, temperature, turbidity, conductivity, total dissolved solids, total suspended solids, total alkalinity, biological oxygen demand, chemical oxygen demand, dissolved oxygen, total organic carbon, sulfate, nitrate, and phosphate, were assessed and measured (Ma et al., 2020). Monitoring these parameters is crucial in understanding

the health and suitability of aquatic ecosystems for sustaining life and in determining the success of rearing aquatic organisms, as deviations from optimal ranges can impact growth, reproduction, and overall well-being. Regular assessment and management of water quality parameters are fundamental in environmental conservation, fisheries management, and maintaining the balance of aquatic ecosystems.

2.4.1.1 pH Levels

pH is an essential factor when assessing water quality by influencing a range of biological and chemical processes in water bodies (Bilewu et al., 2022). pH measures the acidity or alkalinity of a solution, indicating the concentration of hydrogen ions. This parameter indicates the concentration of hydrogen ions (H+) in the water. The scale used to assess acidity levels is termed the pH scale, spanning from 1 to 14. At a temperature of 25 degrees Celsius, a pH of 7.0 is regarded as neutral, meaning neither acidic nor basic. Values below 7.0 are considered acidic, while those above 7.0 are considered basic (Philminaq, 2022). In aquatic environments, pH influences the solubility of minerals and nutrients, as well as the metabolic processes of aquatic organisms.

2.4.1.2 Temperature

Temperature is a crucial factor influencing the growth and survival of all organisms. Yet, it holds particular significance for the growth and survival of aquaculture animals such as shrimp and fish, as they are poikilothermic or cold-blooded. These animals lack the ability to regulate their body temperature and instead adjust to the temperature of the surrounding water (Global Seafood Alliance, 2018). A study in Water Temperature - Environmental Measurement Systems (2019) claimed that the temperature of water can influence the metabolic rates and biological activity of

aquatic organisms. Fluctuations outside these optimal ranges can stress or harm aquatic life. Monitoring water temperature is essential for assessing the suitability of aquatic environments for various purposes, including fisheries management, drinking water supply, and ecosystem conservation.

2.4.1.3 Dissolved Oxygen

The concentration of dissolved oxygen in a water body is influenced by its physical, chemical, and biological factors. Notably, the concentration of DO exhibits an inverse relationship with temperature, meaning that as temperature rises, the concentration of DO decreases correspondingly (Mishra et al., 2023). Fish rely on dissolved oxygen for respiration and metabolic activities. Consequently, low levels of dissolved oxygen are frequently associated with incidents of fish mortality. Conversely, maintaining optimal levels can lead to favorable growth, ultimately resulting in high production yields (Philminaq, 2022).

2.4.2 Nutrient Levels

2.4.2.1 Ammonia and Nitrite

Ammonia and nitrite are nitrogen compounds crucial in aquatic environments. Ammonia, originating from organic matter decay and fish waste, can be toxic to aquatic organisms, causing stress and health issues. Fish release ammonia and smaller quantities of urea into the water as byproducts of their waste (Towers, 2023). Nitrite, a byproduct of ammonia breakdown, is also harmful in elevated concentrations, impacting the ability of fish to transport oxygen. The harmful effects of nitrite are influenced by chemical factors like the reduction of calcium, chloride, bromide, and bicarbonate ions, as well as the levels of pH, dissolved oxygen, and ammonia (Philminaq, 2022). Both ammonia and nitrite levels are closely monitored in

aquaculture, as their presence can adversely affect the well-being and growth of aquatic species.

2.4.2.2 Phosphates

Phosphate is a water quality parameter that measures the concentration of phosphorus compounds in aquatic environments. The primary origins of phosphate in the water are the waste products of organisms, decomposition of food substances, and the use of phosphate fertilizers (Ghosh et al., 2019). Phosphates pose no harm to humans or animals unless they are found in extremely elevated concentrations. If excess phosphate is present, it can lead to eutrophication (Ma et al., 2020). Controlling phosphate levels is essential for maintaining the ecological balance and overall well-being of aquatic environments.

2.4.3 Microbial Load

Bacteria in freshwater systems, specifically in aquatic environments, have been utilized as an indicator of the abundance of the microbial community (Banu, 2001). This microbial community can influence the overall health and performance of aquaculture systems. While some microorganisms are beneficial and play roles in nutrient cycling and waste breakdown, others can be harmful, causing diseases in aquatic organisms. Biofilters play a crucial role in managing water quality by harboring microorganisms that transform generated ammonium into nitrate nitrogen through the process of nitrification (Suurnäkki et al., 2020).

2.5 Application of Photosynthetic Bacteria in Aquaculture Systems

2.5.1 Case Studies on the Use of Photosynthetic Bacteria

Ying et al. (2019) concluded in their resesrch on the introduction of the photosynthetic purple bacterium, *Rhodovulum sulfidophilum* in a marine integrated multitrophic aquaculture system in southwestern Taiwan cultivating *Chanos chanos*, where it had notable effects. The supplementation with *R. sulfidophilum* resulted in a decrease in chemical oxygen demand, nitrate levels, and sulfonamide-resistant bacteria in the fishpond water compared to the control group after rearing. Saejung et al. (2021) also concluded that the study of the potential of anoxygenic photosynthetic bacteria for fairy shrimp cultivation had a very promising survival and growth performances in one of the treatments with *R. faecalis PA2*. The concentrations of dissolved oxygen (DO), ammonia, nitrite, and nitrate in treatments containing photosynthetic bacteria were within acceptable levels. Next, the method of biodegradation by purple sulfur bacteria (PSB) offers a favorable alternative for a novel wastewater treatment approach (Chen et al., 2020). Chang et al. (2019), suggested that the introduction of *Rhodovulum sulfidophilum*, a photosynthetic bacteria led to a reduction in ammonia levels, stability in total phosphorous levels in the recycled water of the marine recirculating aquaculture system (MRAS). Moreover, the communities of cyanobacteria, algae, Vibrio, Escherichia, and other potentially harmful bacteria were suppressed in the (MRAS). Meng et al. (2021) illustrated that utilizing purple sulfur bacteria (PSB) for the purification of heavily polluted rivers and immobilizing microorganisms, such as PSB, could offer a promising strategy to improve their effectiveness in water bodies characterized by low carbon, low dissolved oxygen (DO), high nitrogen, and high phosphorus levels.

2.5.2 Mechanisms of Action in Water Quality Improvement

Purple sulfur bacteria serve as bioremediation agents for sulfide by utilizing anoxygenic photosynthetic activity, wherein H2S is employed as a source of electrons to assimilate CO2. This process results in the production of non-toxic elemental sulfur instead of oxygen at the conclusion of photosynthesis (Friedrich et al., 2001; Triani et al., 2005, as cited in Sadi & Firmansyah, 2020). Certain strains can even oxidize taurine in the presence of carbon dioxide (as cited in J. Chen et al., 2020). The capacity to utilize various organic carbon sources, particularly cellulosic residues, carbohydrates, and organic acids, would be advantageous in fostering the growth of purple non-sulfur bacteria (PNSB) involved in the bioremediation of polluted environments (Dhar et al., 2023).

2.5.3 Challenges and Limitations

The cultivation of purple sulfur bacteria (PSB) poses challenges and is costly due to the complexities in growing them without contamination, demanding rigorous quality control measures (Cho et al., 2006, as cited in Cho & Kim, 2022). Next, it remains uncertain whether certain micropollutants, prohibited by production standards in domestic wastewater, might exert a concealed impact on the subsequent reuse of biomass by purple sulfur bacteria (PSB). Further research is necessary to verify the safety of PSB cultured in wastewater substrates, a determination that may hinge on the judicious selection of wastewater substrates (J. Chen et al., 2020). Y. Chen et al. (2019) noted that the utilization of photosynthetic bacteria in wastewater treatment showed the effectiveness of treatment and the yield of high-value substances constrain the potential of this technology.

2.6 Integration of Photosynthetic Bacteria in Red Claw Crayfish, *Cherax quadricarinatus* Culture

2.6.1 Adaptability of Photosynthetic Bacteria to Tank Systems

The adaptability of photosynthetic bacteria to tank systems is a significant aspect of their utilization in various applications, including aquaculture and wastewater treatment. Photosynthetic bacteria demonstrate versatility in thriving within controlled tank environments, showcasing resilience to fluctuations in conditions such as light, temperature, and nutrient availability. This adaptability is crucial for their sustained performance in tank systems, ensuring their effectiveness in processes like nutrient cycling, organic matter degradation, and the production of valuable substances. Moreover, their ability to function in tank systems allows for the optimization of their application in diverse settings, from enhancing water quality in aquaculture to facilitating the treatment of wastewater. The adaptability of photosynthetic bacteria contributes to their potential as sustainable and versatile solutions in various tank-based applications.

2.6.2 Impact on Red Claw Crayfish, *Cherax quadricarinatus* Growth and Health

Photosynthetic bacteria, particularly those capable of nitrogen fixation, contribute significantly to the nitrogen cycle, converting atmospheric nitrogen into ammonia and other compounds. These compounds, serving as essential nutrients, promote the growth and vitality of red claw crayfish, *Cherax quadricarinatus*. Moreover, the presence of photosynthetic bacteria has a positive influence on water quality by participating in biological processes that break down organic matter and recycle nutrients. There are also uses of photosynthetic bacteria in crayfish culture which are proven to be able to effectively enhance the immunity, survival rate and improve the

water quality (Wang et al., 2019). Clean and well-balanced water parameters are essential for the crayfish's growth and health, and the activities of these bacteria contribute to creating a favorable aquatic environment. Additionally, photosynthetic bacteria can establish beneficial interactions with other microorganisms in the water, potentially enhancing the crayfish's digestive processes and immune system. This microbial community dynamics may contribute to a reduced risk of diseases and improved overall health for red claw *Cherax quadricarinatus*.

2.6.3 Interaction with Other Components of the Ecosystem

PSB exhibit phototrophic growth rather than photosynthetic growth. Their most notable feature lies in their ability to utilize light as an energy source, employing organic carbon compounds, sulfides, or hydrogen as hydrogen donors to assimilate carbon dioxide during anoxygenic photosynthesis (J. Chen et al., 2020).

2.7 Comparative Analysis with Other Water Quality Management Strategies

2.7.1 Conventional Filtration Methods

Regarding traditional filtration techniques for water tanks treated with photosynthetic bacteria, a number of relevant sources can offer insightful information about sustainable aquaculture approaches. An example of a paper that provides information on the application of biological aerated filters for the removal of contaminants from aquaculture wastewater is "The Treatment of Aquaculture Wastewater with Biological Aerated Filters: From the Treatment Process to the Microbial Mechanism" by Ding et al. (2023). Furthermore, Huang et al.'s paper from 2021, "Diatomite Dynamic Membrane Fouling Behavior during Dewatering of Chlorella pyrenoidosa in Aquaculture Wastewater," offers details on the application of dynamic membrane filtration to the purification of aquaculture wastewater. The use of beneficial bacteria

is also examined in "Investigation of a Farm-scale Multitrophic Recirculating Aquaculture System with the Addition of *Rhodovulum sulfidophilum* for Milkfish (*Chanos chanos*) Coastal Aquaculture" by Chang et al. (2019). This study can be helpful in comprehending the possible uses of photosynthetic bacteria in aquaculture systems.

2.7.2 Chemical Treatments

It is crucial to take sustainability, aquatic life, and water quality into account when contrasting chemically treated with photosynthetic bacterially treated water tanks. Water quality indicators as dissolved oxygen, nutrient levels, and microbial activity can be affected by the use of photosynthetic bacteria (Nordstedt & Jones 2020; Ko et al., 2018). Chemical treatments can change the amount of nutrients in the water and remove toxins, among other specific effects on water quality (Chang et al., 2019; Laktuka, 2023). One way to gain insight into the different effects of various interventions on the aquatic environment is to compare how they affect water quality metrics (ISMAIL et al., 2022; Ding et al., 2023). Fish and other aquatic invertebrates may experience changes in growth, survival, and overall health as a result of applying photosynthetic bacteria (Wang et al., 2021; Morni, 2023). Chemical treatments may affect fish health and the ecosystem as a whole, among other direct and indirect effects on aquatic life (Silva, 2021; Batubara et al., 2022). Assessing the possible advantages and disadvantages of these treatments in aquaculture systems can be done by contrasting how they affect aquatic life (Higuchi-Takeuchi et al., 2019; Mujeeb et al., 2022). It is imperative to assess the sustainability of these therapies, taking into account variables including resource consumption, ecological consequences, and extended feasibility (Lu et al., 2022; Suriyadin et al., 2023). Evaluation of photosynthetic bacteria's ecological and economic sustainability as well as chemical

treatments' overall suitability for aquaculture techniques can yield important insights (Reis et al., 2022; Idi, 2023). Decision-making and best practices for aquaculture management can be influenced by comparing the sustainability implications of different treatments (Laktuka, 2023; Zhao, 2021). Analyzing the effectiveness of chemical and photosynthetic bacterial treatments can reveal information on how well each approach works to maintain and improve water quality for aquatic life (Wang et al., 2021; Wong et al., 2021). Finding the best strategy for aquaculture systems can be aided by comparing how well various treatments work to solve different water quality issues (Liu et al., 2017; Saejung et al., 2018). The creation of integrated and sustainable water treatment methods can be influenced by assessing the possible trade-offs or synergies between various treatments (Mykhalchyshyna & Sinenok, 2020; Sutisna et al., 2020).

2.7.3 Biological Control Agents

It is crucial to take into account the possible effects on water quality, aquatic life, and sustainability when contrasting water tanks treated with photosynthetic bacteria and those treated with biological control agents. Water quality indicators as dissolved oxygen, nutrient levels, and microbial activity can be influenced by photosynthetic bacteria and biological control agents (Foysal et al., 2019; Cifuentes-Torres et al., 2021). Understanding the different effects of these treatments on the aquatic environment can be gained by comparing how they affect different water quality metrics (Foysal et al., 2019; Cifuentes-Torres et al., 2021). The growth, survival, and general health of aquatic creatures, such as fish and invertebrates, can be impacted by the application of photosynthetic bacteria and biological control agents (Foysal et al., 2019; HALLA et al., 2023).

2.8 Regulatory and Environmental Considerations

2.8.1 Compliance with Aquaculture Standards

It is critical to evaluate the effects of numerous aspects on sustainable aquaculture operations while thinking about environmental and regulatory issues that adhere to aquaculture standards. Aquaculture's use of circular economy concepts must be in line with the legal framework to guarantee adherence to strict environmental protection and social benefits requirements. Regueiro et al. (2021) in order to maintain community resilience and sustainable aquaculture practices, governance and regulatory effects are essential (Engle & Senten, 2022). Ensuring work safety in rural and aquaculture settings requires adherence to Occupational Health and Safety laws (Silva et al., 2021). To guarantee adherence to environmental quality standards and regulatory procedures, an integrated approach is necessary for the environmental risk assessment of veterinary medicinal products (VMPs) in aquaculture (Rico et al., 2018). Improved environmental performance in the aquaculture sector can be ensured by using a capacity approach to evaluate compliance with aquaculture sustainability standards (Samerwong et al., 2020). Sustainable aquaculture practices are crucial, as demonstrated by the significant environmental advantages of seaweed farming in China (Zheng et al., 2019). Achieving water quality criteria and controlling nutrient load in aquaculture operations depend on adherence to the Code of Good Aquaculture Practices (GAqP) (Baldoza et al., 2020).

2.8.2 Environmental Impacts of Photosynthetic Bacteria Application

The possible environmental effects of using photosynthetic microorganisms have attracted a lot of attention. The capacity of photosynthetic bacteria, such cyanobacteria and purple bacteria, to use light and different environmental elements for growth and

high-value product production has been the subject of much research (Touloupakis, 2023; Foong et al., 2020; Farrokh et al., 2019). According to Foong et al. (2020), these bacteria have shown promise in the sustainable production of bioenergy, biochemicals, and biopolymers. They have also been investigated for their potential in plastic biodegradation and biofuel production, highlighting their environmentally beneficial uses (Farrokh et al., 2019; Barone et al., 2020). Furthermore, through enhancing photosynthetic responses, the usage of photosynthetic bacteria has been linked to the improvement of environmental challenges in crops, such as water stress (Song et al., 2021). This implies that the use of photosynthetic bacteria in agricultural contexts may be able to lessen environmental pressures. But because the methods and ramifications of using engineered nanomaterials to increase photosynthetic efficiency are still unclear, it is imperative to take the potential environmental effects into account (Liu et al., 2019). Additionally, it has been demonstrated how photosynthetic bacteria interact with other microorganisms in a variety of environments, highlighting their significance for both the equilibrium of aquatic planktonic ecosystems and global primary production (Gopalakrishnappa et al., 2023; Deng et al., 2022). According to Gopalakrishnappa et al. (2023), these findings highlight the significance of comprehending the environmental modulators of algae-bacteria interactions and the possible ramifications for ecosystem activities.

2.9 Future Prospects and Research Directions

2.9.1 Potential Innovations in Photosynthetic Bacteria Application

Biotechnology, environmental remediation, and sustainable production are just a few of the many sectors in which photosynthetic bacteria use has great promise for innovation. Potential uses for photosymbiosis in the biomedical domain could result in creative solutions for a range of biotechnological and medical uses. Chávez and

others (2020). Therapeutic and diagnostic advances may be made possible by the use of photosynthetic bacteria in biological applications. One novel way to make use of the quick and plentiful reduction potential produced by photosynthetic bacteria is to engineer photosynthetic bioprocesses for sustainable chemical production (Stephens et al., 2021). This creative use could aid in the creation of environmentally friendly and sustainable chemical production methods. Innovative methods for enhancing crop growth and addressing environmental issues include the use of photosynthetic bacteria in agriculture and environmental remediation, such as light-triggered photosynthetic engineered bacteria and boron nanofertilizers (FRANCO-LAGOS et al., 2023; Yin et al., 2023). These cutting-edge applications might support environmentally responsible behavior and sustainable farming methods. Novel methods to bioproduction and biosensing applications include the manufacture of pinene in purple non-sulfur photosynthetic bacteria and the creation of intact photosynthetic bacteria-based electrochemical biosensors (Wu et al., 2021; Grattieri et al., 2022). The development of cutting-edge biosensing technology and the production of biofuels may be impacted by these creative uses.

2.9.2 Unexplored Areas for Further Research

It is still unknown how photosynthetic bacteria might be used for bioremediation and bioenergy applications, especially in the co-generation of biofuels in conjunction with mining activities. The Banerjee group (2017). Research on the function of photosynthetic bacteria in therapeutic probiotics and their possible effects on public health is still lacking (Das et al., 2023). To evaluate the efficiency of photosynthetic bacteria for cleaning aquaculture wastewater and comprehend the microbial mechanisms involved, more study is required, especially in biological aerated filters (Ding et al., 2023). Research on the possible invasive methods of hybrid species,

taking into account information from soil microbial communities and chemical characteristics, is still lacking (Sun et al., 2019). Bacteriological domains like bioremediation, aquaculture, biofuel production, and medicine should investigate the potential uses of anoxygenic photosynthetic bacteria further (Batubara et al., 2022). Purple non-sulfur photosynthetic bacteria have the ability to synthesize pinene, but their biosynthesis has not been thoroughly studied (Wu et al., 2021). To fully comprehend how photosynthetic bacteria, like *Rhodovulum sulfidophilum*, might suppress the growth of pathogenic bacterial communities in aquaculture systems, more research is required (Chang et al., 2019). To ascertain their commercial-scale applicability, more research is needed on the operational parameters and removal capacities of waste phosphorus removal devices, notably in aquaculture (Luo, 2022). More research is required to examine the possible applications of promising marine bacteria in the aquaculture business, as noted by Mujeeb et al. (2022).

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Materials

The previously isolated stock culture of PSB was obtained from Parasitology Laboratory, Kompleks Akademik Pusat, -Universiti Putra Malaysia Bintulu Sarawak Campus. A number of 75 Red claw crayfish, *Cherax quadricarinatus*, was obtained from Aquatic Biotechnology Laboratory and River around Universiti Putra Malaysia Bintulu Sarawak Campus. 15 units of large round basin (D58cm X 27cm), commercial PSB bought from local Aquarium shop, Bintulu, water quality test kit (API Fresh Water Master Test Kit for Aquarium & Pond pH, Ammonia, Nitrite and Nitrate, country of origin: USA), multiparameter, turbidimeter, DO meter, aerator (SOBO Aquarium Fish Tank Air Pump Double Output 2X4L), aquarium air stone, 15 units, silicone hose oxygen tube, 10m 12. Aquarium fish net, 15 units of PVC pipe for the housing of the crayfish (20cm long),

3.2 Study Area

The sampling was undertaken at the river in UPMKB and the experiment was conducted at Parasitology, Aquatic Biotechnology and Post-mortem laboratory of the faculty of Agricultural and Forestry Sciences, University Putra Malaysia Bintulu Sarawak Campus (Figure 2)

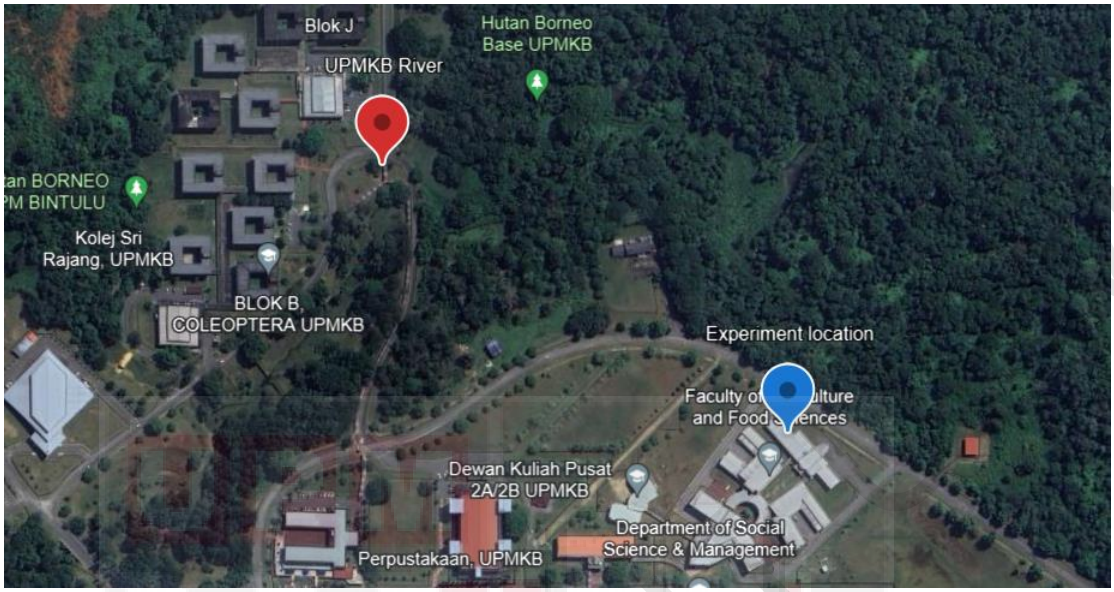

Figure 2. Map showing the study area of the project. The experiment was conducted at Parasitology, Aquatic Biotechnology and Post-mortem laboratory UPMKB (3°12'24"N 113°05'44"E) and UPMKB river (3°12'30"N 113°05'33"E) was the sampling location.

The crayfish were caught in UPMKB river and were reared in Post-mortem laboratory UPMKB for grading. 75 crayfish were graded with the initial length within range of six centimetres to eight centimetres. Five crayfish were selected randomly and were added in each five treatments with triplicates; PSB1, PSB2, PSB3, Positive and Negative.

3.3 Preparation and Cultivation of Photosynthetic Bacteria

A total of 200 mL of peptone water was meticulously prepared by dissolving 30 g of peptone powder (Spectrum Chemical) in 200 mL of distilled water. Concurrently, 350 mL of nutrient agar was meticulously prepared by dissolving 9.8 g of nutrient agar powder (Himedia Nutrient Agar Powder) in 350 mL of distilled water. The peptone water and nutrient agar solutions were subsequently autoclaved in an autoclave machine (Hirayama HVE-50) for an approximate duration of two hours.

The nutrient agar, in its liquid state and while still hot, was carefully poured into sterile petri dishes. At least ¾ of the agar was poured into each petri dish and allowed to solidify at room temperature in a fume cupboard. The agar plates were methodically labeled from 10-1 to 10-8, with triplicates designated as A, B, and C.

Eight Falcon tubes were meticulously prepared by dispensing 9 mL of peptone water into each tube to facilitate serial dilution. The tubes were methodically labeled as 10-1, 10-2, 10-3, 10^{-4}, 10-5, 10-6, 10-7, and 10-8. Subsequently, 1 mL of cultured photosynthetic bacteria (PSB) was pipetted into the Falcon tube labeled 10-1 and thoroughly agitated. The serial dilution process was then iterated from the 10-2 tube to the 10-8 tube.

To execute the spread plate technique, 0.1 mL of the 10-1 dilution was pipetted onto a nutrient agar plate and evenly spread until no liquid remained on the agar surface, utilizing a sterilized hockey stick. This procedure was then repeated for the 3 replicates and dilutions from 10-2 to 10-8. The completed spread plates were subsequently incubated in 38 degrees Celsius for a minimum of 24 hours.

Following a 24-hour incubation period, the agar plates were carefully removed from the incubator for bacterial colony enumeration. CFUs were quantified by calculating the average of selected nutrient agar plates, multiplied by the corresponding serial dilution factor, and divided by 0.1 (the volume of dilution pipetted onto nutrient agar plates). The enumeration of CFU for each plated was counted using the following formula:

A volume of 200 mL of nutrient broth underwent autoclaving at 121 degrees Celsius for a minimum duration of two hours to ensure sterility. Following the autoclaving process, the nutrient broth was allowed to cool to room temperature. Subsequently, the

cooled nutrient broth was dispensed into six universal bottles, with each bottle receiving 10 mL of the nutrient broth.

3.3.1 Isolation of Photosynthetic bacteria

For each replication, two single colonies were meticulously isolated using an inoculation loop, resulting in a total of six single colonies. Subsequently, each single colony was introduced into individual universal bottles, and the bottles were incubated for approximately 24 hours to allow for bacterial growth.

After the 24-hour incubation period, the absorbance of the colonies was assessed using a spectrophotometer (D-LAB SP-UV1000 Spectrophotometer) at a wavelength of 600 nm. A volume of 0.5 mL of the colony solution from each of the six universal bottles was placed inside the spectrophotometer. Centrifugation ensued once the absorbance of the colonies in each bottle exceeded 1.0 AU.

A volume of 2 mL from each of the PSB colonies was pipetted and transferred into individual microcentrifuge tubes. This process was repeated for five microcentrifuge tubes. The tubes were subjected to centrifugation at 10,000 RPM for two minutes by using a centrifuge machine (Benchtop High Speed Centrifuge MSLHC22). The supernatant was then discarded from each microcentrifuge tube. Subsequently, 2 mL of PSB was added again, and centrifugation was repeated until a pellet was formed in each of the six microcentrifuge tubes.

Four out of the six pellets were individually stored, each in a solution comprising 0.5 mL of nutrient broth and 0.5 mL of pure glycerol stock. The pure glycerol had undergone autoclaving beforehand for sterilization. The pellets inside the nutrient broth and glycerol stock solutions were vortexed to ensure proper mixing. The tubes

were then placed in a 4°C refrigerator for one hour and subsequently transferred to a -20°C storage freezer.

3.3.2 Cultivation of Photosynthetic bacteria

A specific and carefully selected set of ingredients were incorporated into the medium for PSB culturing with final volume 100 ml per bottle. Once the media ready, 1.0 ml of the pure culture was inoculated into each of the medium and shake vigorously. The culture was left outside with exposure to direct sunlight for 2 weeks. Once the starter cultured matured, it was further upscale into 1500 ml of culture media and exposed to sunlight for further incubation approximately 2 to 4 more weeks. Five bottles, each with a capacity of 1.5 liters, were meticulously prepared for use in this experiment for cultivating photosynthetic bacteria (PSB). Throughout this duration, the bottles were also regularly agitated, and the caps were opened every two to three days to release excess gas. The medium's coloration changes were systematically observed, particularly after approximately twenty days of culture. The medium exhibited a transition to a dark red color, signifying the maturity of the photosynthetic bacteria. The matured medium containing photosynthetic bacteria was carefully stored for future use after reaching maturity. This stored medium would be utilized in subsequent phases of the experiment.

3.4 Preparation of red claw crayfish (*Cherax quadricarinatus*)

Seventy-five red claw crayfish (*Cherax quadricarinatus*) were captured from the river adjacent to the University Putra Malaysia Bintulu Campus Sarawak, as depicted in Figure 2. For each of the five treatments, five crayfish were reared, with three replications implemented within each treatment.

The red claw crayfish were allowed to acclimate to their new environment for a minimum of one to two days before being introduced into the treatment tanks. This acclimatization period aimed to ensure the adaptation of crayfish to their surroundings, promoting better conditions for subsequent experimentation.

A daily feeding regimen was established, providing an approximate amount of three percent of the red claw crayfish body weight in feed. Leftover feed was meticulously observed each day, facilitating necessary adjustments to the daily feeding amount to optimize nutrition and promote the well-being of the crayfish.

3.5 Red claw crayfish tank water treatment.

The experiment comprised five distinct treatments, each replicated three times to ensure robust data analysis. Within the experiment, three treatment groups involved the addition of cultured photosynthetic bacteria (PSB), with varying concentrations: PSB1, supplemented with two milliliters of PSB, PSB2, supplemented with four milliliters of PSB, PSB3, supplemented with eight milliliters of PSB. Additionally, two control groups were included for comparison: Positive, received four milliliters of commercial PSB and Negative, no bacteria were added to this tank, serving as the baseline reference.).

3.6 Water Quality Measurement

Throughout the two-month experimental period (from 30/8/2023 to 31/10/2023), water parameters in each tank were meticulously observed at three-day intervals. Various instruments, including the API Test Kit for Ammonia, Nitrite, and Nitrate, a multiparameter device, a turbidimeter, and a Dissolved Oxygen (DO) meter, were employed for data collection.

Water samples were collected from each treatment every fifteen days (from 30/8/2023 to 29/10/2023) for further analysis of total suspended solids (TSS) and chemical oxygen demand (COD). Notably, no water exchanges were performed, and additional water was introduced only when losses occurred due to evaporation and sampling activities.

Readings for various parameters, including pH, temperature, turbidity, conductivity, dissolved oxygen (DO), total dissolved solids (TDS), ammonia, nitrite, nitrate, chemical oxygen demand (COD), and total suspended solids (TSS), were systematically recorded. These data were subsequently employed in statistical analyses to assess and compare the water quality among the different treatments.

3.6.1 API Test Kit

Water samples were taken from each treatment. In the ammonia testing procedure, a clean test tube was filled with 5 mL of the water sample, ensuring precise measurement up to the designated line on the tube. Subsequently, 8 drops from Ammonia Test Solution Bottle #1 were added to the test tube, with the dropper bottle held upside down in a completely vertical position to guarantee uniform drop distribution. Following this, 8 drops from Ammonia Test Solution Bottle #2 were introduced in a similar manner. The test tube was then securely capped, subjected to vigorous shaking for 5 seconds, and left undisturbed for 5 minutes to allow for color development. The test results were interpreted by comparing the color of the solution to the corresponding values on the Ammonia Color Card.

In the nitrite testing protocol, a clean test tube was employed, and it was filled with 5 mL of the water sample, ensuring precise measurement up to the designated line on the tube. Subsequently, 10 drops from the Nitrite Test Solution Bottle were added, with

the dropper bottle held upside down in a completely vertical position to guarantee uniform drop distribution. The test tube was securely capped and subjected to vigorous shaking for 5 seconds. Following this, the test tube was left undisturbed for 5 minutes to facilitate color development. After the designated time, the color of the solution was compared with the corresponding values on the Nitrite Color Card to interpret the test results.

In the nitrate testing procedure, a clean test tube was employed and filled with 5 mL of water sample, ensuring accuracy up to the specified line on the tube. Subsequently, 10 drops from the Nitrate Test Solution Bottle were added, holding the dropper bottle upside down in a completely vertical position to ensure uniform drop dispensing. The test tube was securely capped and shaken vigorously for 5 seconds. Subsequent to this, the test tube was left undisturbed for 5 minutes to allow for color development. Following the waiting period, the color of the solution was compared with the appropriate values on the Nitrate Color Card for the interpretation of results. These procedures were done thrice, in each replication for the treatment groups. API tests were taken every three days for sixty-three days.

3.6.2 Multiparameter

Multi-Parameter Water Quality Meter Model Wqc-24 were used to monitor pH, conductivity, temperature and total dissolved solids (TDS). The calibration of the instrument was undertaken in adherence to the manufacturer's guidelines, especially if calibration had not been performed recently. The electrodes were immersed in the water sample, allowing for stabilization before recording the observed value. Systematic documentation of all readings, including pH, conductivity, temperature,

and TDS, was undertaken. Probes were rinsed with distilled water between measurements to mitigate the risk of cross-contamination.

In the monitoring of dissolved oxygen (DO) levels using the Eutech Basic Portable DO Meter DO 6+, the initial step involved ensuring the calibration procedures were rigorously followed. Upon immersing the DO sensor into the water in each treatment tank, adequate time was allowed for the DO reading to stabilize, typically within a few minutes, before recording the measurement. After each measurement, the DO sensor was diligently rinsed with distilled water to prevent any potential cross-contamination.

In the monitoring of turbidity levels utilizing the HACH 2100P Portable Turbidimeter, the initial step involved ensuring the calibration procedures were diligently conducted. Upon securing the calibration and confirming the cleanliness of the turbidimeter's optical components, water samples were carefully transferred into the turbidimeter's optical cell. Following the insertion of each water sample into the turbidimeter's optical cell, a stabilization period was observed to allow the turbidity readings to achieve equilibrium. Once stabilized, turbidity measurements were systematically recorded.

3.6.3 Chemical Oxygen Demand

Initially, fifty milliliters of distilled water were meticulously dispensed into a conical flask designated for blank preparation. Concurrently, fifty milliliters of water sample for each treatment were prepared. Approximately one tablespoon of anti-bumping agent was introduced into the conical flasks, accompanied by the addition of one gram of mercuric sulfate ($HgSO_4$). Next, five milliliters of sulfuric acid reagent were incorporated into the flasks, along with twenty-five milliliters of potassium dichromate ($K_2Cr_2O_7$) and seventy milliliters of sulfuric acid reagent. The resulting solutions were subjected to controlled heating for approximately two hours, employing FAVORIT

Stirring Hotplate HS070V2. Following the two-hour heating period, both the water samples and blank were allowed to cool to room temperature. To each flask containing the water sample and blank, one hundred and fifty milliliters of distilled water were added. Subsequently, two milliliters of Ferroin Indicator were precisely aliquoted into the flasks of each treatment and blank. The ensuing step involved titration, wherein the treatments and blanks were titrated against standard Ferrous Ammonium Sulfate. During titration, the coloration of the water in the water samples and blank was keenly observed, with the endpoint marked by the transition to a green hue.

3.6.4 Total Suspended Solid

Aluminum foils were meticulously prepared to serve as holders for filter paper, which underwent a preliminary treatment involving washing with fifty milliliters of distilled water. Following this, the filter papers were subjected to a drying process in an oven, lasting approximately fifteen minutes. Subsequently, the dried filter papers were allowed to cool within a desiccator for approximately ten minutes. The initial weights of the filter papers were precisely measured using a digital balance (Kern & Sohn Analytical Weighing Balance). These filter papers were then carefully positioned within the cylinders of a multi-branch filtration system. Fifty milliliters of water sample from each treatment were methodically poured into the respective cylinders containing the pre-positioned filter papers. The filtration process was diligently carried out until each cylinder no longer retained any water. Post-filtration, the filter papers were subjected to a thorough drying process in the oven, lasting approximately one hour. The final weights of each filter paper were meticulously recorded. Both the initial and final weights were documented, as they play a crucial role in the subsequent calculation of Total Suspended Solids (TSS) using the pertinent formula. This comprehensive procedure adhered to established methodologies and protocols,

ensuring precision and accuracy in the determination of suspended solids in the water samples under investigation. The recorded weights of the filter papers served as essential data points for the calculation of TSS, contributing to the analytical rigor and reliability of the research findings.

3.7 Red Claw Crayfish Growth Rate Measurement

Over the two-month period spanning from 30/8/2023 to 31/10/2023, the weight and length of crayfish within each treatment tank were systematically measured on a weekly basis by measuring the total length. This consistent monitoring aimed to capture any changes in the biometric parameters of the crayfish populations.

In addition to the biometric measurements, the mortality in each treatment was diligently recorded to facilitate the computation of the survival rate. Key metrics such as specific growth rate (SGR) and feed conversion ratio (FCR) were also calculated based on the collected data.

Recorded parameters, including weight, length, specific growth rate (SGR), survival rate, and feed conversion ratio (FCR), were meticulously documented. These recorded metrics served as crucial inputs for subsequent statistical analyses, enabling a comprehensive assessment of the impact of different treatments on crayfish growth, survival, and overall performance.

3.8 Statistical Analysis

The acquired results will be subjected to statistical analyses utilizing the Statistical Analysis System (SAS) 9.4 For Windows. The analytical procedures employed encompassed the application of the Tukey test and Analysis of Variance (ANOVA). These statistical tests were chosen to rigorously assess and compare the data obtained across different treatment conditions.

CHAPTER 4

RESULTS

4.1 Analysis of Water Quality Parameters

A comprehensive assessment of water quality parameters was conducted, encompassing the monitoring of pH, temperature, turbidity, conductivity, dissolved oxygen, total dissolved solids (TDS), ammonia, nitrite, nitrate, chemical oxygen demand (COD), and total suspended solids (TSS). Notably, the in-situ measurement approach was employed for all parameters except COD and TSS, where water samples for analysis were systematically collected every fifteen days. Subsequent analysis of COD and TSS was performed at the Water Quality Laboratory within the Department of Animal Science and Fisheries Directory, UPMKB. The pH level for PSB1 (Table 2.1) showed an increasing trend starting from day 1 to day 15, where fluctuation started to show on day 18 to day 57 but remain stable on day 60 to day 63. PSB2 (Table 2.2) displayed the highest pH on day 21 while the lowest was on day 1. The pH level in PSB3 (Table 2.3) peaked at day 48 but had the lowest pH on day 1. Negative treatment (Table 2.4) illustrated a rising trend of pH from day 1 to day 48 but gradually decreasing from day 48 to day 63. The pH level in treatment Positive (Table 2.5) increased from day 1 to day 18 while showing a decreasing result on day 21 to day 36 and fluctuation was showed on from day 39 to day 48, which later plummeted on starting from day 51 until day 63. The temperature for every five treatments depicted a rise and fall trend during the entire project in which the temperature ranges approximately from 26 to 28 degrees Celsius. As for nitrate, ammonia and nitrite level, every treatment showed the highest concentrations on the first day but dropped dramatically after day 1 until day 63. The level of total dissolved solids (TDS) in PSB1

climbed from day 1 to day 57 which later gradually decreased on day 60 to day 63. TDS in PSB2 presented a rising trend from the start until the end of the project while PSB3 and Negative had climbed from day 1 to day 54 and decreased after. The TDS in Positive increased from day 1 to day 39 but fluctuated on day 42 to day 48 which later increased until day 63. The turbidity in PSB1 decreased from day 1 to day 15 but went up and down after day 15 until day 63. PSB2 showed an increased turbidity level on day 1 to day 24 but rise and fall trend occurred until day 63. PSB3 also showed a rising and fluctuating trend; rising on day 1 until day30 and fluctuated on day 36 to day 63. Negative had shown an increasing level of turbidity on day 1 until day 39 but decreased on day 42 to day 48 which later fluctuated until day 63. Positive only illustrated a rising and falling trend during the experiment. The conductivity level in all treatments increased from day 1 to day 63. The dissolved oxygen (DO) in every treatment presented a rising and falling trend during the entire experiment was conducted.

4.1.1 Tables

Table 2.1, Table 2.2, Table 2.3, Table, 2.4 and Table 2.5 illustrated the water quality parameters in each treatment, PSB1, PSB2, PSB3, Negative and Positive. The water quality were monitored for sixty-three days and were recorded in Windows Excel Microsoft 365 and the results were expressed in average±standard error.

4.1.1.1 The effect of photosynthetic bacteria on PSB1 treatment

Table 2.1. The effect of photosynthetic bacteria on PSB1 treatment

Treatment	Day	Parameter								
		pH	Temperature (ºC)	Nitrate (NO3)	Nitrite (NO2)	Ammonia (NH3)	Total Dissolved Solid (TDS)	Turbidity (NTU)	Conductivity (S/M)	Dissolved Oxygen (mg/L)
PSB1	1	6.33±0.01	28.13±0.07	160.00±0.00	0.00±0.00	3.33±0.67	0.13±0.03	0.20±0.06	15.50±0.06	6.26±0.01
	3	7.42±0.01	27.9±0.06	13.33±3.33	0.00±0.00	4.33±2.03	0.17±0.07	0.10±0.06	24.63±0.09	5.65±0.01
	6	7.46±0.01	28.13±0.03	13.33±6.67	0.00±0.00	8.00±0.00	0.30±0.06	0.10±0.06	35.57±0.07	3.09±0.01
	9	7.71±0.01	27.77±0.09	16.67±3.33	2.67±2.67	5.33±2.67	0.37±0.03	0.13±0.07	46.83±0.09	1.43±0.09
	12	7.86±0.00	27.07±0.03	11.67±4.41	0.00±0.00	6.67±1.33	0.50±0.06	0.13±0.09	53.30±0.06	2.47±0.01
	15	7.86±0.01	27.8±0.06	11.67±4.41	0.00±0.00	6.67±1.33	0.40±0.06	0.17±0.09	52.70±0.06	1.93±0.01
	18	7.34±0.25	28.17±0.07	15.00±5.00	0.25±0.00	1.00±0.00	0.33±0.03	222.33±0.88	87.60±0.06	1.50±0.06
	21	7.93±0.04	27.87±0.03	8.33±1.67	0.67±0.17	1.00±0.00	0.47±0.03	71.27±0.37	56.70±0.06	5.36±1.00
	24	7.84±0.00	27.07±0.03	5.00±0.00	0.83±0.17	0.83±0.17	0.67±0.03	236.33±1.20	75.50±0.06	4.14±0.01
	27	7.84±0.01	27.57±0.03	3.33±1.67	0.33±0.08	0.75±0.25	0.70±0.06	30.70±0.06	86.2±30.03	1.89±0.01
	30	7.76±0.01	27.73±0.03	0.33±0.33	0.33±0.08	0.42±0.08	0.63±0.03	183.00±0.58	72.97±23.98	1.45±0.01
	33	7.86±0.01	27.230.03	5.00±0.00	0.08±0.08	0.83±0.17	0.66±0.03	19.00±0.06	85.47±0.09	1.45±0.00
	36	7.5±0.00	28.6±0.00	3.00±1.73	0.33±0.08	1.33±0.33	0.73±0.09	472.33±0.33	82.50±0.06	1.25±0.01
	39	7.72±0.01	27.7±0.06	1.33±0.67	0.00±0.00	1.33±0.33	0.73±0.03	102.33±0.33	96.13±0.03	2.32±0.00
	42	7.81±0.01	27.93±0.03	0.00±0.00	0.00±0.00	4.00±0.00	0.63±0.03	16.97±0.03	93.23±0.03	4.33±0.00
	45	7.55±0.00	27.67±0.03	3.00±1.73	0.33±0.08	0.67±0.17	0.73±0.03	23.47±0.07	0.10±0.00	4.20±0.00
	48	7.07±0.00	27.67±0.03	3.00±1.73	0.25±0.00	0.67±0.17	0.93±0.03	17.00±0.00	0.12±0.00	3.13±0.00
	51	7.35±0.01	27.27±0.03	5.00±0.00	0.50±0.00	0.67±0.17	0.97±0.03	23.37±0.03	0.11±0.00	7.07±0.01
	54	7.23±0.03	27.07±0.03	3.00±1.73	0.50±0.00	0.50±0.00	1.00±0.00	11.07±0.07	0.11±0.00	7.17±0.03
	57	7.08±0.00	27.03±0.03	3.00±1.73	0.25±0.00	0.67±0.17	1.03±0.03	10.33±0.33	0.14±0.00	6.83±0.03
	60	7.43±0.03	27.53±0.03	0.00±0.00	0.00±0.00	1.33±0.33	0.97±0.03	9.33±0.00	0.11±0.00	8.54±0.00
	63	7.43±0.03	27.63±0.03	1.67±1.67	1.00±0.50	1.33±0.33	0.93±0.03	19.60±0.40	0.13±0.00	7.80±0.00

4.1.1.2 The effect of photosynthetic bacteria on PSB2 treatment

Table 2.2. The effect of photosynthetic bacteria on PSB2 treatment

Treatment	Day	Parameter								
		pH	Temperature (ºC)	Nitrate (NO3)	Nitrite (NO2)	Ammonia (NH3)	Total Dissolved Solid (TDS)	Turbidity (NTU)	Conductivity (S/M)	Dissolved Oxygen (mg/L)
PSB2	1	5.34±0.07	28.17±0.03	40.00±0.00	0.00±0.00	6.00±2.00	0.07±0.03	0.70±0.06	13.47±0.03	6.50±0.06
	3	7.28±0.01	27.23±0.03	13.33±3.33	0.00±0.00	2.00±1.00	0.07±0.03	0.10±0.06	26.00±0.58	6.17±0.03
	6	7.37±0.03	28.10±0.06	10.00±0.00	0.00±0.00	5.33±1.33	0.17±0.03	0.03±0.03	32.77±0.03	4.37±0.32
	9	7.53±0.04	27.73±0.03	5.00±0.00	0.00±0.00	0.83±0.17	0.20±0.00	0.07±0.03	34.43±0.03	1.32±0.01
	12	7.66±0.03	27.03±0.03	6.67±1.67	0.00±0.00	4.00±0.00	0.40±0.06	0.10±0.06	48.43±0.03	2.32±0.01
	15	7.73±0.00	27.83±0.03	6.67±1.67	0.00±0.00	1.00±0.00	0.43±0.03	0.17±0.09	53.20±0.06	2.09±0.01
	18	7.91±0.00	28.23±0.03	5.00±0.00	0.25±0.00	0.83±0.17	0.47±0.03	13.30±0.35	64.63±0.68	2.45±0.01
	21	7.94±0.03	27.93±0.03	6.67±1.67	0.50±0.00	0.83±0.17	0.47±0.03	14.23±0.03	53.23±0.03	6.17±0.04
	24	7.57±0.00	27.10±0.06	0.00±0.00	0.08±0.08	0.83±0.17	0.60±0.06	308.67±0.33	70.37±0.03	1.74±0.01
	27	7.74±0.01	27.47±0.03	0.00±0.00	0.50±0.00	1.00±0.00	0.63±0.03	32.00±0.56	83.43±0.03	1.74±0.00
	30	7.65±0.00	27.93±0.03	1.67±1.67	0.17±0.17	0.83±0.17	0.40±0.06	126.00±0.58	66.73±0.03	1.85±0.00
	33	7.73±0.00	27.30±0.00	5.00±0.00	0.00±0.00	1.67±0.33	0.57±0.03	73.27±0.03	82.30±0.35	1.27±0.00
	36	7.75±0.00	28.67±0.03	5.00±0.00	0.25±0.00	1.33±0.33	0.70±0.06	86.67±0.03	80.57±0.03	3.02±0.00
	39	7.74±0.00	27.63±0.03	1.67±1.67	0.25±0.00	1.17±0.44	0.80±0.06	122.33±0.33	90.00±0.58	3.24±0.01
	42	7.77±0.04	27.77±0.03	3.33±1.67	0.58±0.22	1.17±0.44	0.67±0.03	33.00±0.58	93.70±0.06	4.94±0.00
	45	7.77±0.01	27.73±0.03	8.33±1.67	1.17±0.44	0.67±0.17	0.57±0.03	34.30±0.35	90.20±0.06	5.04±0.01
	48	7.70±0.00	27.53±0.03	8.33±1.67	0.50±0.00	0.33±0.08	0.97±0.03	34.3±0.35	0.11±0.00	3.74±0.00
	51	7.53±0.00	27.10±0.06	5.00±0.00	0.25±0.14	0.33±0.08	0.77±0.03	18.37±0.32	0.11±0.00	7.07±0.00
	54	7.70±0.06	27.17±0.12	26.67±12.02	1.17±0.44	0.50±0.00	0.90±0.06	24.73±0.09	0.15±0.00	6.35±0.01
	57	7.37±0.14	27.07±0.09	8.33±1.67	3.67±1.33	0.33±0.83	0.97±0.03	18.93±0.09	0.12±0.00	6.93±0.04
	60	7.22±0.00	27.73±0.03	5.00±0.00	0.25±0.00	0.75±0.25	0.77±0.09	28.53±0.07	0.11±0.01	8.44±0.00
	63	7.54±0.00	27.00±0.00	5.00±0.00	0.67±0.17	0.67±0.17	0.97±0.03	15.50±0.06	0.12±0.00	7.77±0.00

4.1.1.3 The effect of photosynthetic bacteria on PSB3 treatment

Table 2.3. The effect of photosynthetic bacteria on PSB3 treatment

Treatment	Day	Parameter								
		pH	Temperature (ºC)	Nitrate (NO3)	Nitrite (NO2)	Ammonia (NH3)	Total Dissolved Solid (TDS)	Turbidity (NTU)	Conductivity (S/M)	Dissolved Oxygen (mg/L)
PSB3	1	6.12±0.01	28.33±0.07	106.67±26.67	0.00±0.00	5.33±1.33	0.07±0.03	0.10±0.06	10.35±0.05	6.52±0.01
	3	7.32±0.00	27.37±0.03	10.00±0.00	0.00±0.00	2.67±0.67	0.07±0.03	0.10±0.06	17.73±0.09	6.25±0.01
	6	7.34±0.00	28.03±0.03	6.67±1.67	0.00±0.00	8.00±0.00	0.17±0.03	0.00±0.00	27.70±0.06	4.15±0.00
	9	7.55±0.01	27.37±0.09	10.00±0.00	0.00±0.00	6.67±1.33	0.17±0.03	0.10±0.06	31.83±0.34	1.26±0.03
	12	7.50±0.00	27.13±0.03	16.67±3.33	0.00±0.00	4.00±0.00	0.23±0.07	0.10±0.06	37.53±0.03	2.34±0.01
	15	7.51±0.01	27.93±0.03	5.00±0.00	0.00±0.00	0.42±0.08	0.27±0.03	0.10±0.06	40.63±0.03	2.21±0.11
	18	7.62±0.01	28.17±0.03	8.33±1.67	0.42±0.08	0.67±0.17	0.40±0.06	170.00±0.58	51.60±0.06	2.13±0.00
	21	7.73±0.03	28.83±0.03	6.67±3.33	0.42±0.08	0.42±0.08	0.20±0.06	250.00±0.58	24.67±0.33	6.16±0.01
	24	7.37±0.01	27.33±0.03	8.33±1.67	1.67±0.33	0.33±0.08	0.50±0.06	336.00±0.58	52.60±0.06	3.22±0.00
	27	6.52±0.01	28.10±0.06	5.00±0.00	0.86±0.17	0.58±0.22	0.40±0.06	356.00±0.58	31.60±5.72	1.03±0.00
	30	7.33±0.01	28.03±0.09	13.33±3.33	5.00±0.00	0.42±0.08	0.60±0.06	332.00±0.58	62.63±0.03	1.67±0.00
	33	7.560.01	27.40±0.06	8.33±1.67	5.00±0.00	0.00±0.00	0.50±0.06	28.77±0.09	61.50±0.06	1.50±0.00
	36	7.52±0.01	27.03±0.03	6.67±1.67	1.67±0.33	0.25±0.00	0.30±0.06	73.63±0.03	29.13±0.03	3.39±0.01
	39	7.65±0.01	27.80±0.06	11.67±4.41	2.67±1.20	0.33±0.08	0.60±0.06	109.33±0.33	66.70±0.06	4.50±0.00
	42	7.65±0.01	27.97±0.03	5.00±0.00	1.33±0.33	0.50±0.00	0.60±0.06	15.40±0.06	74.30±0.06	5.45±0.00
	45	7.74±0.01	27.73±0.09	5.00±0.00	0.17±0.17	0.25±0.00	0.73±0.03	9.89±0.01	89.87±0.03	4.13±0.04
	48	7.76±0.00	27.47±0.03	11.67±4.41	1.17±0.44	0.17±0.08	0.73±0.03	12.30±0.06	87.30±0.06	4.86±0.03
	51	7.43±0.03	27.23±0.03	5.00±0.00	1.33±0.33	0.25±0.00	0.67±0.03	34.00±0.58	88.43±0.03	7.02±0.00
	54	7.66±0.03	28.10±0.06	6.67±1.67	0.42±0.08	0.42±0.08	1.10±0.06	76.67±0.33	0.11±0.00	6.81±0.01
	57	7.15±0.00	27.20±0.06	8.33±1.67	0.42±0.08	1.00±0.00	0.93±0.09	126.67±0.33	0.11±0.00	6.80±0.00
	60	6.93±0.00	27.73±0.03	16.67±3.33	5.00±0.00	0.25±0.00	0.83±0.03	50.77±0.03	0.13±0.03	8.00±0.00
	63	7.14±0.00	27.70±0.00	10.00±0.00	0.25±0.00	0.25±0.00	0.83±0.03	19.33±0.33	0.10±0.00	7.77±0.00

4.1.1.4 The effect of photosynthetic bacteria on Negative treatment

Table 2.4. The effect of photosynthetic bacteria on Negative treatment

Treatment	Day	Parameter								
		pH	Temperature (ºC)	Nitrate (NO3)	Nitrite (NO2)	Ammonia (NH3)	Total Dissolved Solid (TDS)	Turbidity (NTU)	Conductivity (S/M)	Dissolved Oxygen (mg/L)
NEGATIVE	1	6.82±0.01	28.4±0.00	160.00±0.00	0.00±0.00	0.75±0.25	0.13±0.03	0.23±0.03	15.50±0.06	6.06±0.03
	3	7.30±0.12	27.47±0.03	5.00±0.00	0.00±0.00	1.00±0.00	0.13±0.03	0.10±0.06	25.37±0.09	5.88±0.00
	6	7.45±0.00	27.97±0.03	6.67±1.67	0.00±0.00	5.33±1.33	0.27±0.03	0.10±0.06	41.70±0.06	2.43±0.01
	9	7.60±0.01	27.30±0.35	8.33±1.67	0.00±0.00	8.00±0.00	0.23±0.03	0.23±0.12	43.43±0.03	1.28±0.00
	12	7.65±0.01	27.40±0.06	8.33±1.67	0.00±0.00	8.00±0.00	0.50±0.06	0.10±0.06	58.70±0.06	2.17±0.01
	15	7.64±0.03	27.83±0.03	6.67±1.67	0.00±0.00	6.67±1.33	0.43±0.03	0.10±0.06	54.63±0.32	1.72±0.00
	18	7.73±0.01	28.70±0.06	20.00±0.00	5.00±0.00	4.33±2.03	0.60±0.06	71.43±0.03	70.47±0.03	2.00±0.06
	21	7.77±0.03	28.20±0.06	13.33±3.33	3.00±1.00	4.00±0.00	0.47±0.03	81.77±0.03	65.57±0.03	6.14±0.00
	24	7.53±0.03	27.40±0.06	13.33±3.33	6.67±1.67	6.67±1.33	0.57±0.03	197.00±0.58	77.23±0.12	1.67±0.01
	27	7.66±0.00	27.70±0.06	5.00±2.89	1.12±0.44	5.33±1.33	0.60±0.06	242.00±0.58	99.70±0.06	1.62±0.00
	30	7.46±0.03	27.87±0.03	5.00±0.00	0.75±0.25	5.33±1.33	0.63±0.03	334.33±0.33	79.63±0.09	0.52±0.04
	33	7.65±0.00	27.23±0.03	6.67±1.67	1.33±0.67	3.33±0.67	0.73±0.03	389.67±0.88	93.97±0.03	0.87±0.01
	36	7.76±0.03	28.43±0.03	6.67±1.67	0.83±0.17	5.33±1.33	0.47±0.09	421.67±0.88	52.33±0.33	1.56±0.01
	39	7.77±0.01	27.90±0.06	10.00±0.00	1.08±0.51	1.67±0.33	0.70±0.06	413.00±0.58	0.14±0.03	0.67±0.03
	42	7.76±0.03	27.93±0.03	11.67±4.41	0.75±0.25	5.33±1.33	0.97±0.03	132.00±0.58	0.12±0.00	5.44±0.01
	45	7.92±0.00	27.87±0.03	6.67±1.67	1.17±0.44	1.67±0.33	0.93±0.03	99.60±0.06	0.12±0.00	5.27±0.04
	48	7.81±0.01	27.47±0.03	40.00±0.00	4.00±1.00	2.17±1.01	0.97±0.03	189.00±0.58	0.12±0.00	4.77±0.00
	51	7.43±0.04	27.27±0.03	6.67±1.67	0.83±0.17	1.50±0.50	1.10±0.10	265.00±0.58	0.11±0.00	6.96±0.01
	54	7.38±0.00	27.47±0.03	20.00±0.00	4.00±1.00	3.00±1.00	1.20±0.10	254.67±0.33	0.12±0.00	5.50±0.06
	57	7.36±0.01	27.30±0.06	16.67±3.33	5.00±0.00	1.08±0.51	0.67±0.03	266.67±0.33	0.13±0.00	6.87±0.00
	60	7.23±0.00	27.87±0.03	16.67±3.33	4.00±1.00	0.25±0.00	0.73±0.03	190.00±0.58	0.12±0.00	7.96±0.00
	63	7.33±0.04	27.63±0.03	16.67±3.33	4.00±1.00	2.67±0.67	0.87±0.09	146.00±0.33	0.12±0.00	7.82±0.00

4.1.1.5 The effect of photosynthetic bacteria on Positive treatment

Table 2.5. The effect of photosynthetic bacteria on Positive treatment

Treatment	Day	Parameter								
		pH	Temperature (ºC)	Nitrate (NO3)	Nitrite (NO2)	Ammonia (NH3)	Total Dissolved Solid (TDS)	Turbidity (NTU)	Conductivity (S/M)	Dissolved Oxygen (mg/L)
POSITIVE	1	5.59±0.01	28.23±0.03	5.00±0.00	0.00±0.00	2.00±0.00	0.07±0.03	0.50±0.06	11.63±0.32	6.65±0.01
	3	7.11±0.00	27.00±0.06	4.00±1.00	0.00±0.00	1.33±0.33	0.20±0.06	0.17±0.03	24.40±0.06	5.54±0.01
	6	7.27±0.00	28.17±0.03	1.33±0.67	0.00±0.00	4.00±0.00	0.40±0.06	0.10±0.06	39.20±0.06	2.70±0.06
	9	7.55±0.01	27.73±0.03	2.33±1.45	0.00±0.00	6.67±1.33	0.40±0.06	0.17±0.09	45.37±0.03	1.23±0.00
	12	7.52±0.00	27.07±0.03	1.33±0.67	0.00±0.00	4.67±1.76	0.40±0.06	0.10±0.06	48.20±0.06	2.53±0.01
	15	7.22±0.01	27.43±0.09	5.00±0.00	0.00±0.00	6.67±1.33	0.60±0.06	0.13±0.09	64.40±0.06	1.63±0.04
	18	7.82±0.00	28.20±0.06	5.00±0.00	1.33±0.33	2.33±0.88	0.63±0.07	5.52±0.01	73.40±0.06	2.07±0.00
	21	7.81±0.00	27.83±0.09	0.67±0.67	0.00±0.00	0.42±0.08	0.53±0.03	11.30±0.35	63.30±0.06	6.56±0.03
	24	7.65±0.00	27.07±0.03	5.00±0.00	0.83±0.17	0.75±0.25	0.40±0.06	49.17±0.09	65.63±0.32	4.28±0.00
	27	7.43±0.01	27.17±0.03	2.33±1.45	0.50±0.29	1.08±0.51	0.13±0.09	11.03±6.54	44.80±0.06	1.12±0.00
	30	7.65±0.01	27.70±0.00	5.00±0.00	0.58±0.22	0.50±0.00	0.60±0.06	138.00±0.58	64.40±0.06	1.66±0.03
	33	7.87±0.01	27.30±0.06	10.00±0.00	0.42±0.08	1.17±0.44	0.40±0.06	9.05±0.01	47.76±0.03	1.47±0.03
	36	7.79±0.00	28.37±0.03	5.00±0.00	0.33±0.08	1.33±0.33	0.37±0.03	32.6±0.70	45.37±0.03	3.05±0.00
	39	7.64±0.00	27.57±0.03	2.33±1.45	0.17±0.08	1.17±0.44	0.83±0.09	8.54±0.01	90.83±0.09	2.73±0.04
	42	7.91±0.01	27.83±0.03	3.00±1.00	0.50±0.00	1.00±0.00	0.67±0.03	4.43±0.01	93.73±0.03	2.44±0.03
	45	7.86±0.03	27.43±0.03	5.00±0.00	0.75±0.25	1.00±0.00	0.73±0.03	3.97±0.04	0.13±0.03	4.75±0.01
	48	7.87±0.00	27.63±0.03	3.00±1.00	0.67±0.33	0.83±0.17	0.77±0.03	6.65±0.01	0.10±0.00	0.44±0.01
	51	7.63±0.03	27.37±0.32	4.00±1.00	0.58±0.22	0.25±0.14	0.70±0.06	15.27±0.03	0.10±0.00	7.13±0.00
	54	7.54±0.00	27.20±0.06	5.67±2.33	1.17±0.44	0.33±0.08	0.70±0.06	14.47±0.03	0.10±0.00	6.46±0.01
	57	7.40±0.00	27.20±0.06	2.67±1.20	0.33±0.08	0.33±0.08	0.80±0.06	10.60±0.06	0.10±0.00	7.24±0.01
	60	7.46±0.03	27.90±0.06	0.00±0.00	0.25±0.00	1.17±0.44	0.97±0.03	10.30±0.06	0.11±0.00	9.44±0.03
	63	7.37±0.03	27.60±0.00	3.00±1.00	1.33±0.33	0.50±0.25	0.93±0.03	28.73±0.37	0.12±0.00	7.61±0.01

4.1.2 Distribution of Water Quality Parameters

Figure 3.1 presented the distribution of pH. The lowest level of pH are in treatment PSB3.The lowest level of pH is also in the optimum range which is 7.35. Figure 3.2 showed the distribution of temperature. The result indicated the temperature in all treatments are significantly equivalent ($p<0.05$) in which the temperature ranges in 27.6 to 28 degrees Celsius. Figure 3.3 depicted the distribution of ammonia in the water of crayfish rearing tank. Result shown that Negative had the highest level of ammonia while PSB3 had the lowest level of ammonia. Low level of ammonia indicates that the efficient microbial activity of PSB are in PSB3 tank because the reduction of ammonia are due to the nitrogen cycle by the PSB. Figure 3.4 depicted the distribution of nitrite in five treatments. Result shown that Negative had the highest amount of nitrite concentration among the treatments. This indicates that the amount of organic wastes such as the feed and wastes from crayfish in the tank are high. High concentration led to high mortality in the treatment because the crayfish would get stressed. Figure 3.5 showed the distribution of nitrate in the water tank. Among the treatments, Negative was also had the highest ammonia concentration. High ammonia could result stress in fish and poor water quality. Figure 3.6 showed the distribution of total dissolved solids (TDS). PSB3 had the lowest level of TDS while PSB1 had the highest level of TDS among the treatments. High amount of TDS indicates the high among of solid wastes found in the water. Figure 3.7 presented the distribution of turbidity. Positive were shown to have the lowest level of turbidity while Negative had the highest. This indicates that the water of Negative were murky while the water of Positive were clear. Figure 3.8 showed the distribution of conductivity in the treatments. Highest conductivity can be found in Negative while the lowest were in

PSB3. High conductivity indicate high level of dissolved solids (DO) while Figure 3.9 showed the amount of DO were relatively withing a close rage in all treatments.

4.1.2.1 Distribution of pH in five treatments

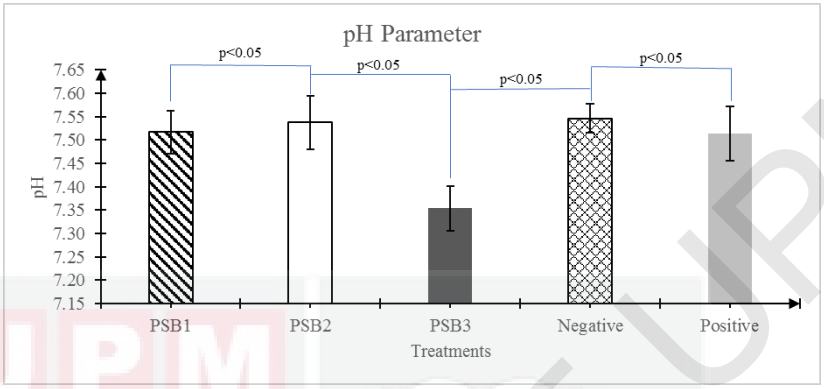

Figure 3.1 Distribution of pH

4.1.2.2 Distribution of temperature in five treatments

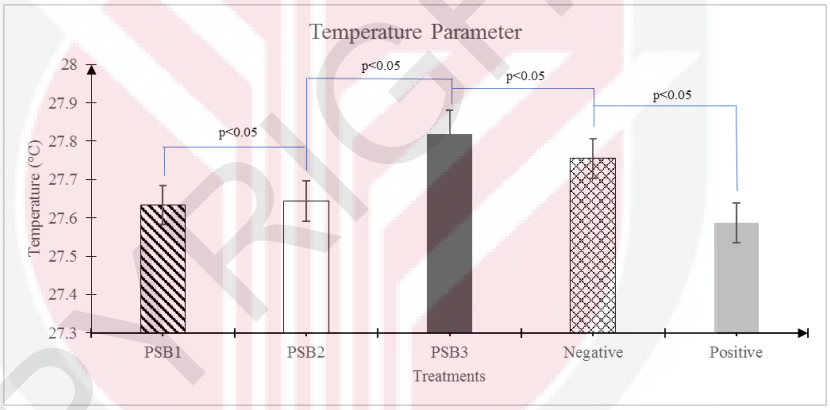

Figure 3.2 Distribution of temperature

4.1.2.3 Distribution of ammonia in five treatments

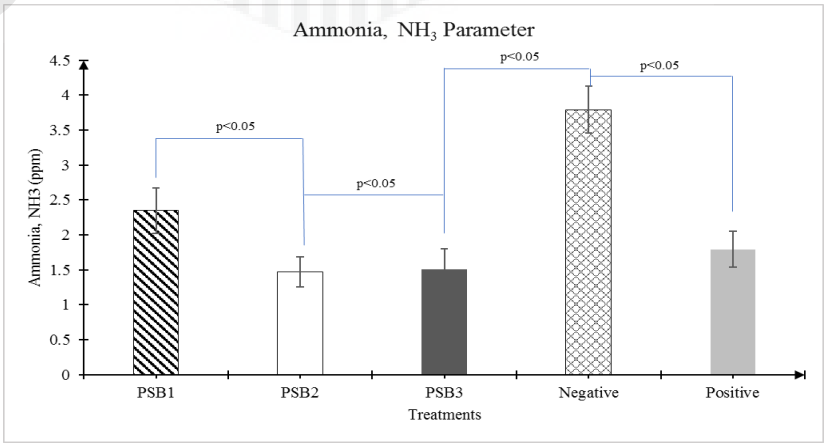

Figure 3.3 Distribution of ammonia, NH_3

4.1.2.4 Distribution of nitrite in five treatments

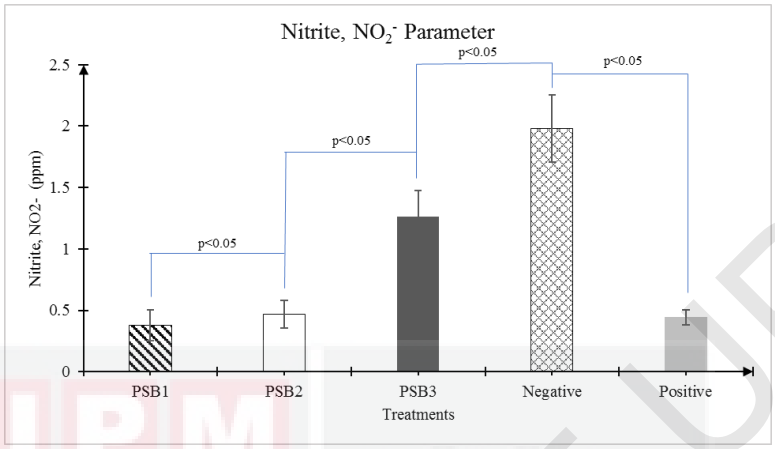

Figure 3.4 Distribution of nitrite, NO_2^-

4.1.2.5 Distribution of nitrate in five treatments

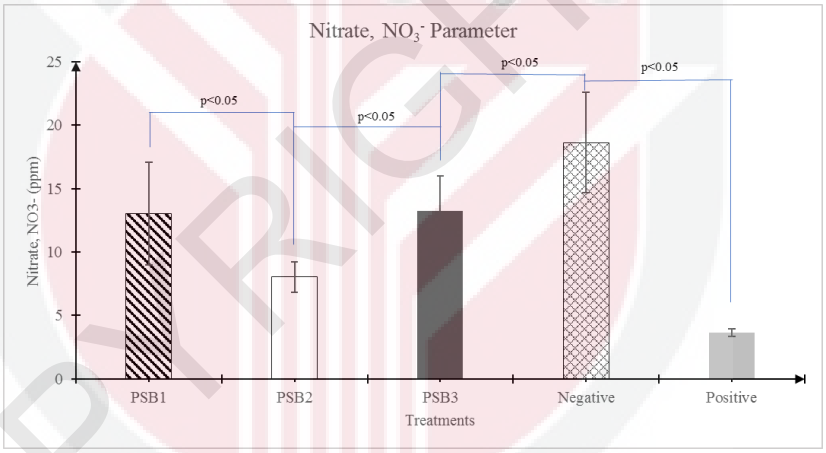

Figure 3.5 Distribution of nitrate, NO_3^-

4.1.2.6 Distribution of total dissolved solids (TDS) in five treatments

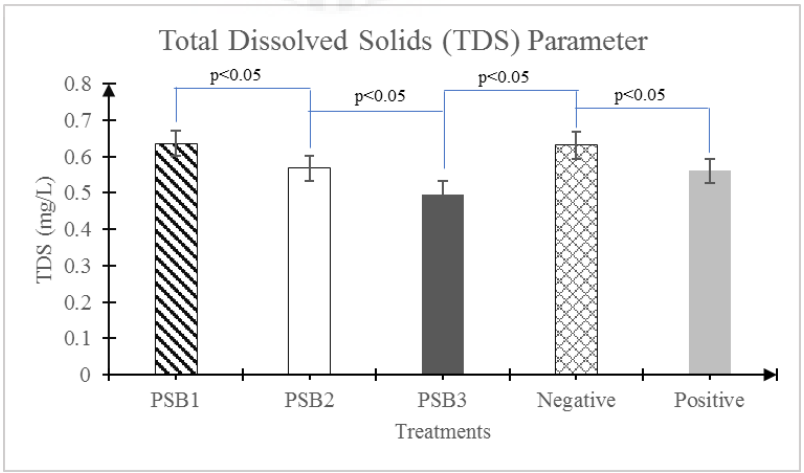

Figure 3.6 Distribution of Total Dissolved Solids (TDS)

4.1.2.7 Distribution of turbidity in five treatments

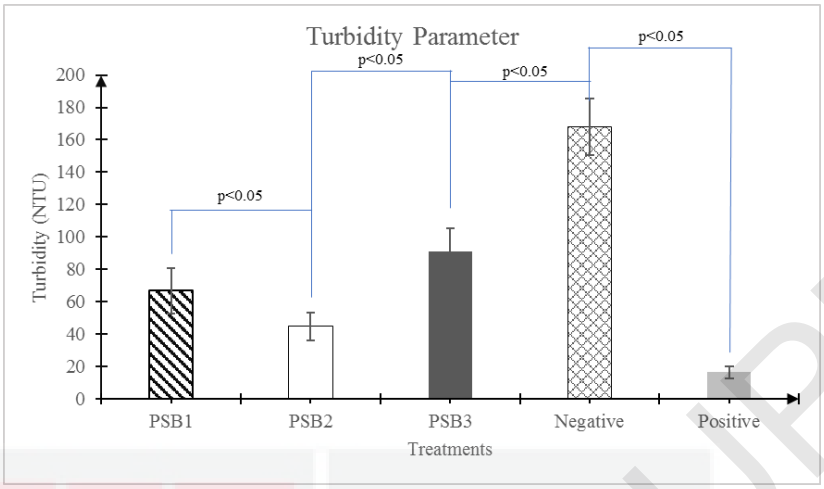

Figure 3.7 Distribution of turbidity

4.1.2.8 Distribution of conductivity in five treatments

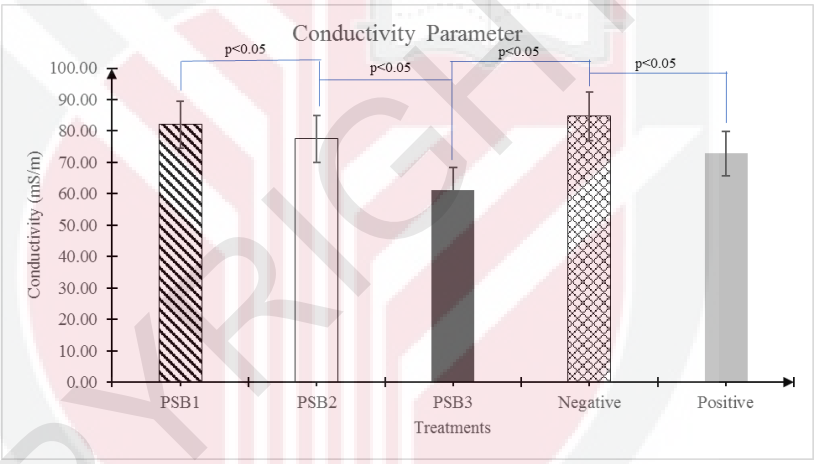

Figure 3.8 Distribution of conductivity

4.1.2.9 Distribution of dissolved oxygen (DO) in five treatments

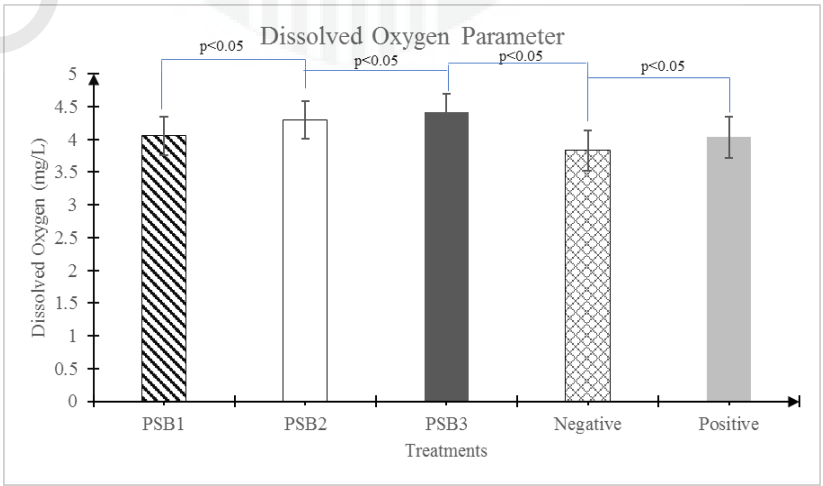

Figure 3.9 Distribution of dissolved oxygen (DO)

4.1.2.10 Water Parameter Against Days

Figure 3.10 indicated the distribution of pH against days for five treatments. The first day showed the lowest level of pH in all treatments. The pH level were approximately the same in all PSB-treated treatments after day-1 because of the application of PSB. Figure 3.11 showed the distribution of temperature for sixty-three days in which the result portrayed fluctuations of temperature. Figure 3.12 indicated the ammonia level in all treatments for sixty-three days. In the period of day-6 to day-12, the level of ammonia were high in all treatments but the treatments with isolated PSB and Positive had reduced gradually while Negative remained the highest until the end of the experiment day. In Figure 3.13, the distribution of nitrite were shown. There were fluctuations of the nitrite level in all treatments but on the last day of the experiment, PSB3 were shown to have the lowest nitrite level while Negative have the highest concentration of nitrite. Low level of nitrite is due to the efficient nitrogen cycle by PSB in PSB3. Figure 3.14 presented the distribution of nitrate against days. Only on the first day of the experiment showed a high level of nitrate in both PSB1 and Negative. Figure 3.15 showed the distribution of total dissolved solids (TDS) in which the result shown were gradually increasing from day-1 to day-63. Figure 3.16 depicted the result of turbidity where the highest turbidity from PSB1 peaked on day-36 and were gradually decreasing until the end of the experiment. Figure 3.17 and Figure 3.18 showed the distribution results of conductivity and dissolved oxygen (DO) respectively. The conductivity showed an increasing trend while the dissolved oxygen in all treatments showed fluctuations; DO were decreasing from day-1 to day-18 and increased until day-21, in which the DO decreased from day-21 to day-33 and rise again until day-63.

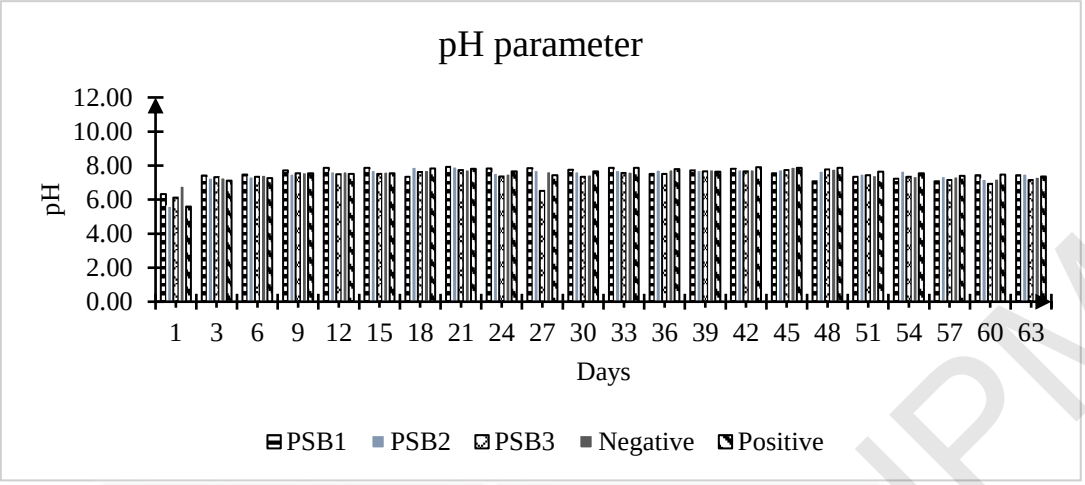

Figure 3.10. Distribution of pH against day of the treatment

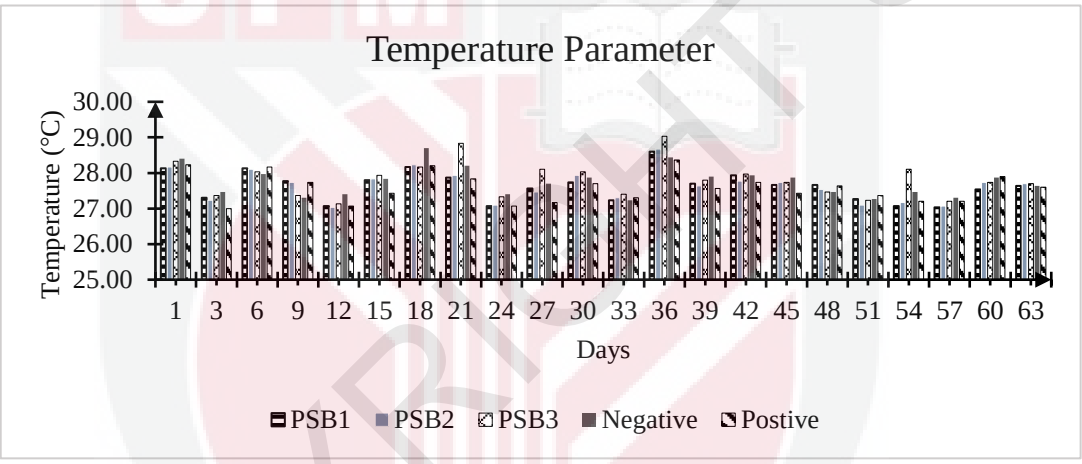

Figure 3.11. Distribution of temperature against day of the treatment

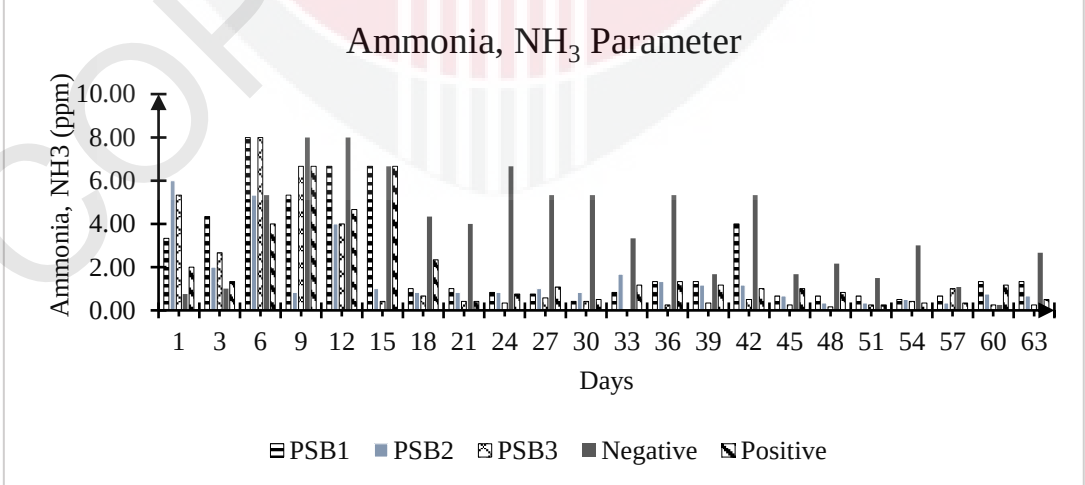

Figure 3.12. Distribution of ammonia, NH_3 against day of the treatment

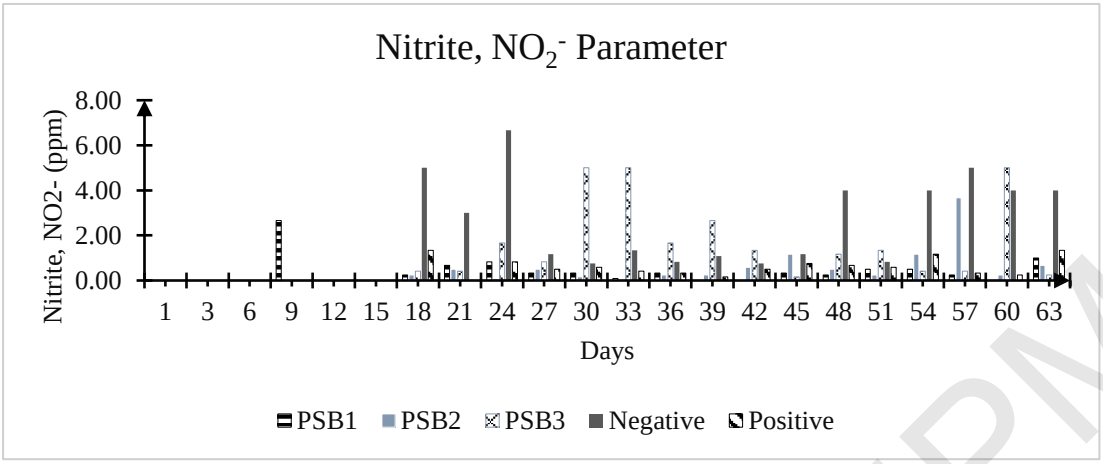

Figure 3.13. Distribution of nitrite, NO_2^- against day of the treatment

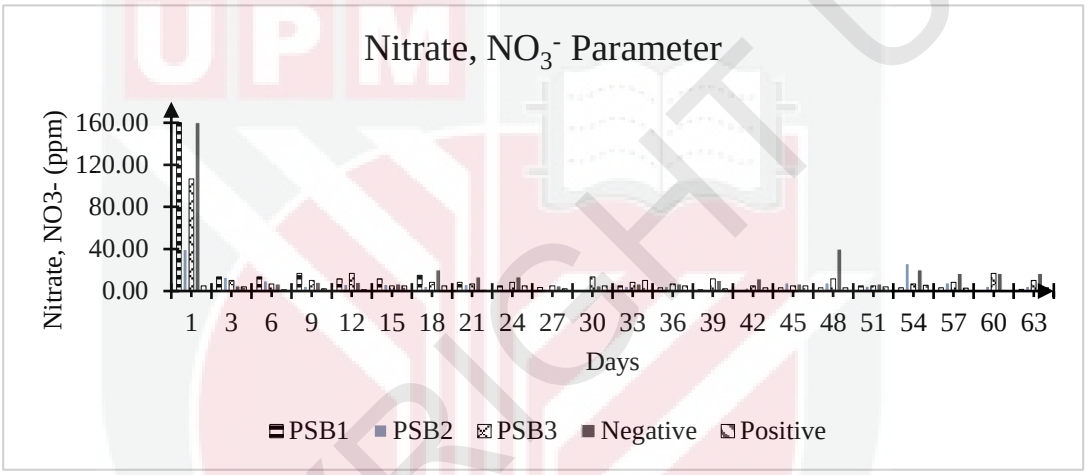

Figure 3.14. Distribution of nitrate, NO_3^- against day of the treatment

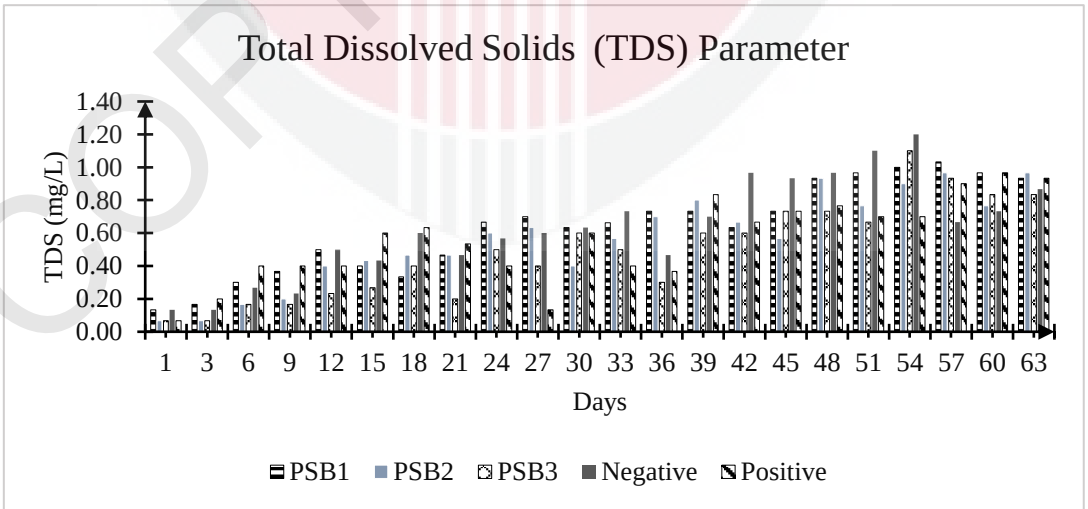

Figure 3.15. Distribution of total dissolved solids (TDS) against day of the treatment

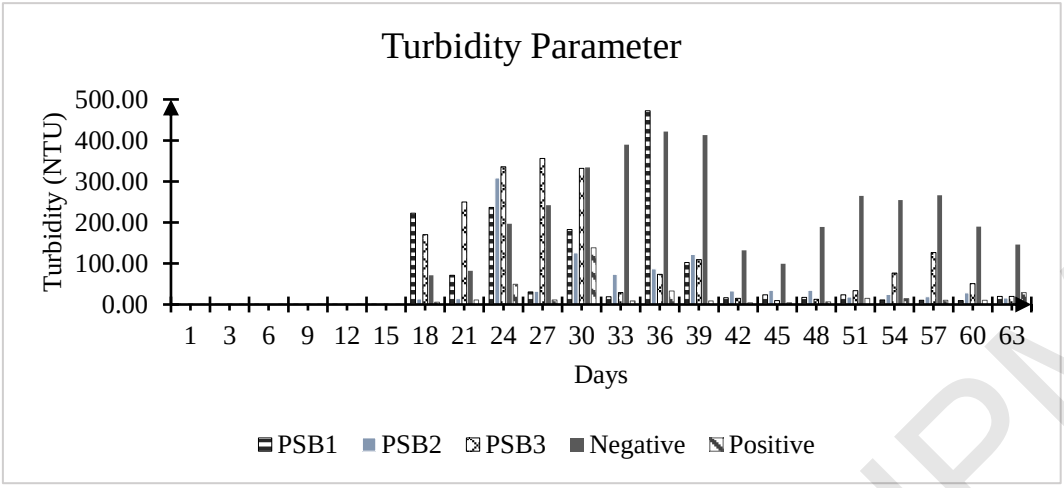

Figure 3.16. Distribution of turbidity against day of the treatment

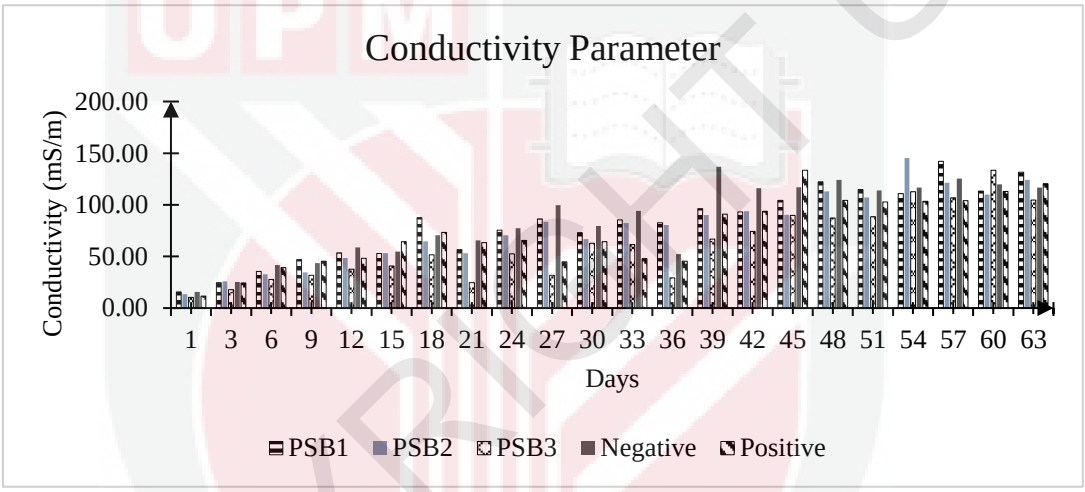

Figure 3.17. Distribution of conductivity against day of the treatment

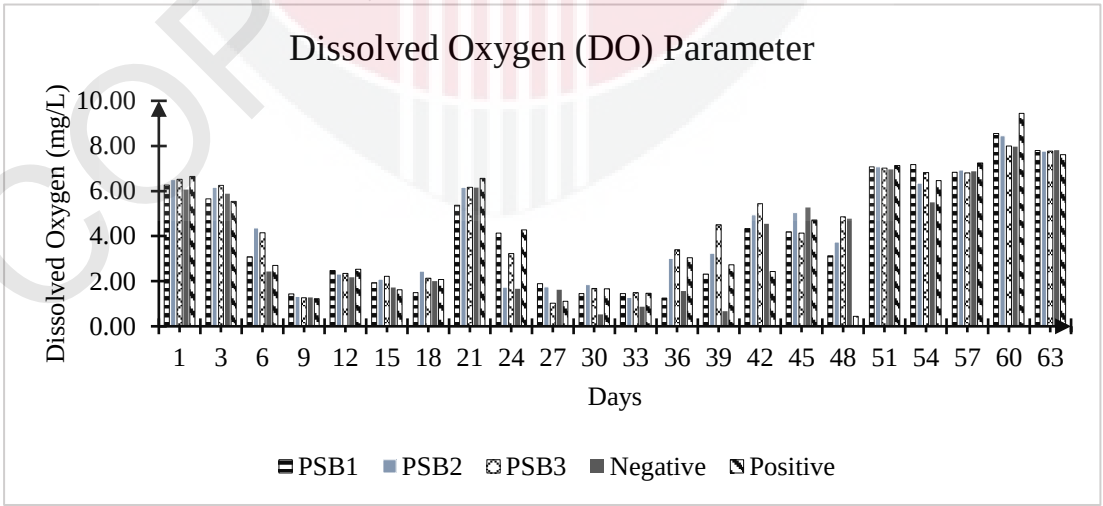

Figure 3.18. Distribution of dissolved oxygen (DO) against day of the treatment

4.2 Analysis of Chemical Dissolved Oxygen (COD)

Table 3 indicated the amount of chemical oxygen demand in the rearing water of the crayfish tank. All treatments experienced a decreasing trend during the sixty days in which Negative portrayed a lowest COD among the treatment contrast to PSB2 which had the highest COD. The interpretation of the COD level can be looked clearly in Figure 3.19 which the concentration level of COD was in treatment Negative while Positive had the highest.

Table 3. Analysis of chemical oxygen demand (COD) for sixty days

Days	Chemical Oxygen Demand, COD (mg 0^2/L)				
	PSB1	PSB2	PSB3	Negative	Positive
0	21.98	22.05	21.03	20.75	22.43
15	6.86	15.52	12.09	12.16	18.39
30	10.74	10.5	11.05	5.27	12.23
45	12.53	12.42	12.42	8.91	12.51
60	13.23	14.75	13.8	10.12	13.74

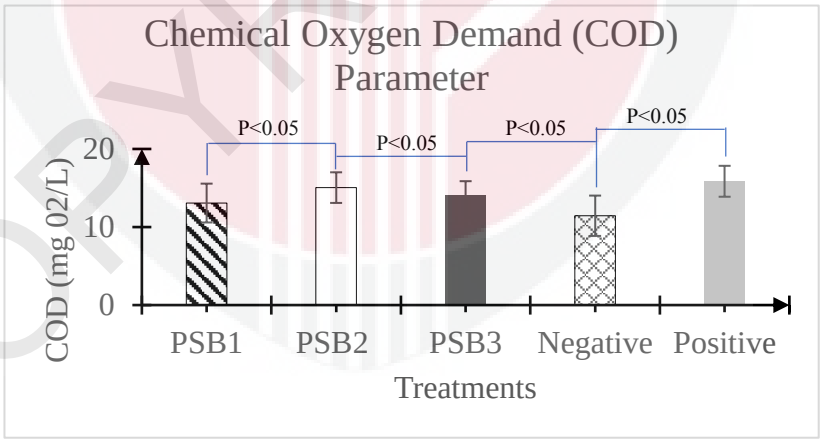

Figure 3.19. The distribution of chemical oxygen demand (COD)

4.3 Analysis of Total Suspended Solid (TSS)

Based on the Table 4 above, the level of total suspended solids (TSS) in PSB1 increased from day 1 to day 30 which later declined until day 60. PSB2 depicted to have an increasing trend from day 1 to day 30 and decreased from day 45 to day 60. The TSS in PSB3 depicted an increased amount from day 1 to day 30 but decreased from day 45 to day 60. The TSS level in Negative had climbed until day 30 but showed a decreased amount of TSS on day 45 and peaked on day 60. The TSS level in Positive was seen to be rising from day 1 to day 45 which later decreased on day 60. The Figure 3.20 was portrayed to show that the lowest level of TSS among the treatments was in PSB2 while PSB3 had the highest TSS followed by PSB1, Negative and Positive.

Table 4. Analysis of total suspended solids (TSS) for sixty days

Days	Total Suspended Solids, TSS (mg/L)				
	PSB1	PSB2	PSB3	Negative	Positive
0	0.024	0.036	0.002	0.002	0
15	0.395	0.231	0.014	0.038	0.01
30	0.394	0.41	0.218	0.331	0.014
45	0.101	0.019	0.096	0.148	0.388
60	0.125	0.088	0.886	0.493	0.347

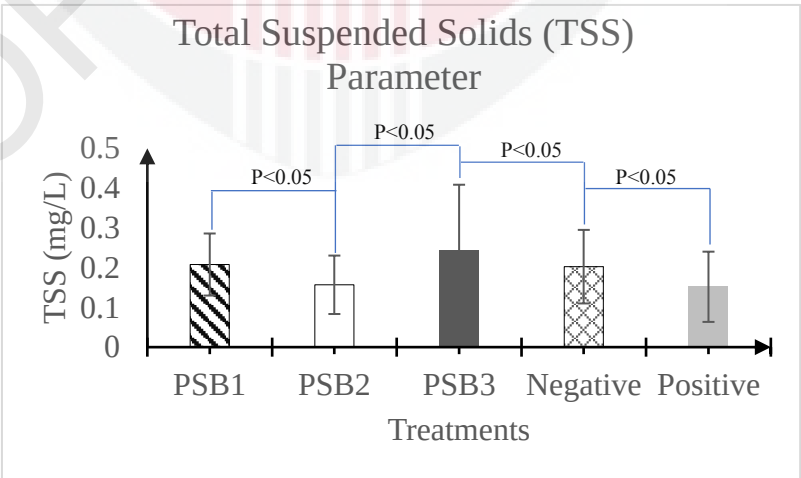

Figure 3.20. Distribution of total suspended solids (TSS)

4.4 Analysis of Red Claw Crayfish Growth Performance

4.4.1 Weight and length of Red Claw Crayfish, *Cherax quadricarinatus*

Based on Table 5, all treatments had shown an increasing length and weight except for Negative which had a fluctuated measurement of length and weight. *Cherax quadricarinatus,* exhibit fluctuations in weight and length influenced by multifactorial determinants including age, sex dimorphism, environmental variables, dietary factors, and genetic predispositions (Naguib et al. 2021). The growth trajectory of these crustaceans tends to be more rapid in early developmental stages, with a subsequent deceleration as individuals approach sexual maturity. Furthermore, variations in size among individuals within a population are noteworthy, emphasizing the need for continual monitoring and assessment of these parameters in aquaculture practices. (Dali et al., 2023).

Table 5. Weight and length analysis for five treatments

Days	PSB1		PSB2		PSB3		Negative		Positive	
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Length	Weight
0	8.10±0.12	12.53±0.66	8.18±0.14	12.47±0.58	7.89±0.28	9.73±0.56	8.19±0.23	13.53±1.29	8.25±0.13	13.00±0.57
7	8.17±0.14	12.62±0.74	8.15±0.14	12.26±0.57	7.65±0.23	9.69±0.56	8.35±0.16	13.64±0.77	8.13±0.30	13.47±1.31
14	8.06±0.16	12.79±0.73	8.07±0.13	12.47±0.59	7.63±0.27	9.91±0.58	8.12±0.17	13.78±0.75	8.02±0.29	13.43±1.28
21	8.20±0.14	12.58±0.69	8.29±0.15	12.81±0.79	7.84±0.25	9.87±0.58	8.70±0.18	15.12±0.95	8.20±0.32	13.19±1.26
28	8.34±0.15	13.12±0.73	8.39±0.17	13.46±0.93	7.94±0.25	10.33±0.71	9.06±0.24	16.91±1.34	8.38±0.30	13.75±1.28
35	8.41±0.15	13.46±0.82	8.46±0.16	12.85±1.00	7.72±0.20	10.43±0.77	9.15±0.26	17.22±1.40	8.37±0.30	14.11±1.34
42	8.51±0.15	13.26±0.69	8.39±0.13	13.41±0.84	8.05±0.23	10.53±0.78	9.45±0.24	19.29±1.38	8.40±0.29	14.31±1.29
49	8.51±0.15	13.57±0.74	8.46±0.13	13.21±0.86	7.95±0.27	10.29±0.84	9.65±0.24	19.54±1.58	8.36±0.29	13.94±1.29
56	8.55±0.13	14.69±0.61	8.40±0.17	13.93±0.88	7.91±0.24	11.12±0.87	9.63±0.26	22.83±1.55	8.36±0.29	14.45±1.33
63	8.69±0.09	12.58±0.94	8.26±0.13	13.23±0.86	7.68±0.21	10.32±0.83	9.60±0.23	22.15±1.60	8.44±0.28	13.92±1.22

4.4.2 Growth performance of Red Claw Crayfish, *Cherax quadricarinatus*

Based on Table 6 below, it has shown the specific growth rate (SGR), survival rate and feed conversion ratio (FCR) for all treatments. Due to the factor of moulting, there were inconsistency of the weight and length which contributed to the fluctuation of the SGR and FCR in every treatment such as treatment Positive. The lowest survival rate would be in Negative because of the high mortality of crayfish in the tank. In contrast, the highest survival rate would be in Positive and PSB3 which had no mortality followed by PSB1 and PSB2.

Table 6. The growth performance of red claw crayfish, *Cherax quadricarinatus*

Treatment	Replication	Initial weight (g)	Final weight (g)	Weight gained (g)	Specific growth rate (%)	Survival rate (%)	Feed conversion ratio
PSB1	1	14	16.93	2.93	0.301635	98	7.0
	2	11.6	12.45	0.85	0.112247	100	18.8
	3	12	14.53	2.53	0.303665	100	6.7
PSB2	1	11.8	12.34	0.54	0.071026	98	29.8
	2	13	15.98	2.98	0.327601	98	6.7
	3	12.6	11.37	-1.23	-0.163045	98	-13.6
PSB3	1	9.4	10.09	0.69	0.112437	100	19.4
	2	9.2	8.78	-0.42	-0.07417	100	-29.6
	3	10.6	12.1	1.5	0.210082	100	9.2
Negative	1	13.4	16.98	3.58	0.375844	98	6.1
	2	10.6	22.98	12.38	1.228207	96	1.9
	3	16.6	26.5	9.9	0.742448	96	2.3
Positive	1	15	13.66	-1.34	-0.148537	100	-13.7
	2	12.8	11.17	-1.63	-0.216212	100	-9.0
	3	11.2	16.93	5.73	0.655831	100	4.0

4.4.3 Analysis of covariance

Figure 4.1 illustrated the growth performance of the red claw crayfish, *Cherax quadricarinatus*, represented in terms of covariance. The dispersion of dots in Figure 14 serves as an indicator of the covariance strength within each treatment. A greater dispersion implies a weaker covariance, while dots closer to the line signify a stronger linear relationship. Consequently, among the treatments, T4 (Negative) and T3 (PSB3) exhibit the weakest covariance, indicating suboptimal crayfish growth in these treatments. In contrast, T1 (PSB1), T2 (PSB2), and T5 (Positive) demonstrate efficient growth performance, evidenced by the close alignment of dots along the line.

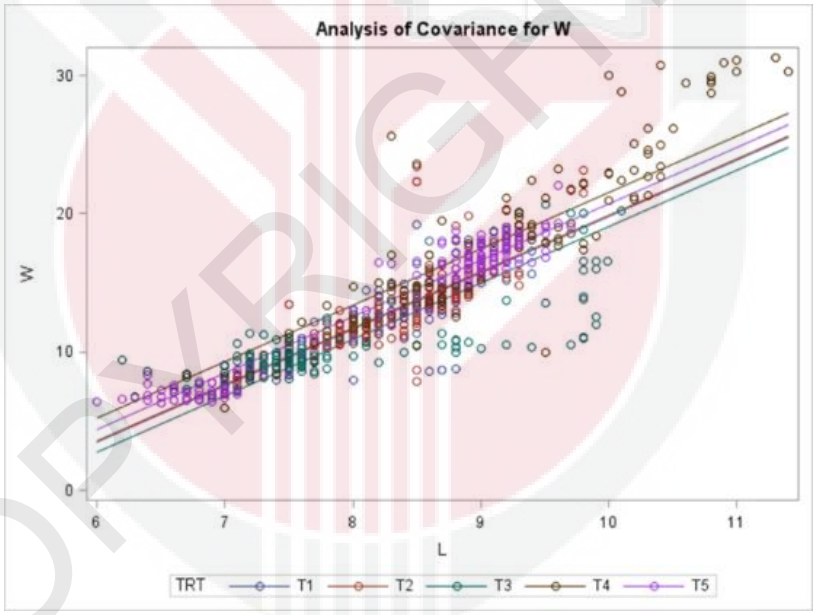

Figure 4.1. Distribution of the covariance in different treatments

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13070.54655	2614.10931	643.45	<.0001
Error	744	3022.59650	4.06263		

Figure 4.2 showed the covariance of weight against length for PSB1. From this result, it is shown that there are some dots representing the growth performance of crayfish are farther from the line; suggests a weaker linear relationship. The increased distance of data points from the regression line in the SAS analysis indicates a weaker linear association in the growth performance of crayfish. This suggests that the observed values are less closely aligned with the predicted values, implying a potentially more scattered or variable relationship in the data.

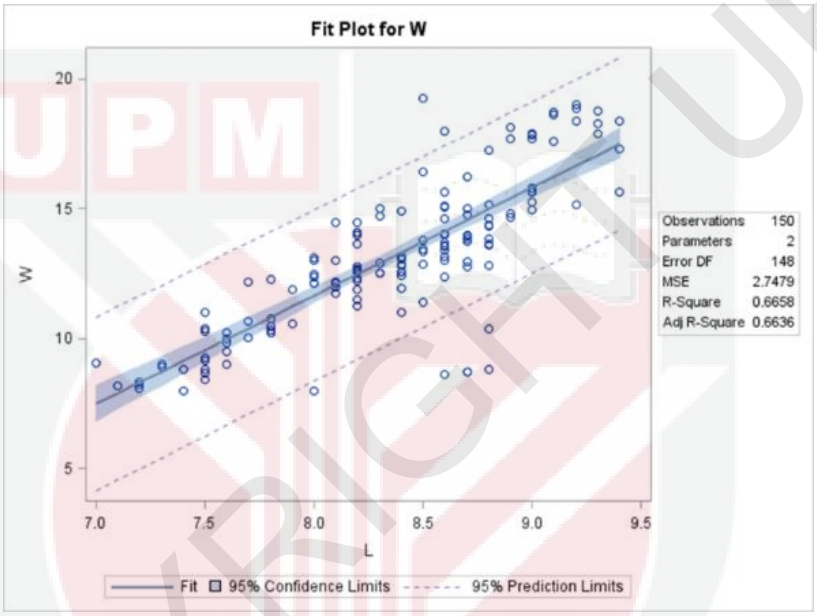

Figure 4.2. Distribution of the covariance in PSB1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	810.31320	810.31320	294.88	<.0001
Error	148	406.69362	2.74793		

The dots representing the growth performance of crayfish in PSB2 (Figure 4.3) are closer to the regression line in SAS analysis which suggests a stronger linear relationship. The proximity of data points to the regression line in the SAS analysis indicates a stronger linear association in the growth performance of crayfish. Although, there were dots that spred further away from the line but mostly had a tight grip along the line. This suggests that the observed values closely align with the predicted values, indicating a more consistent and predictable relationship in the data.

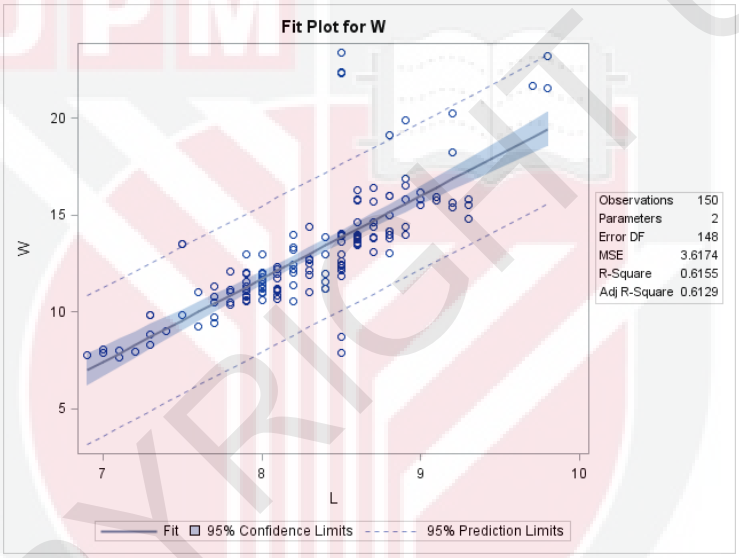

Figure 4.3. Distribution of the covariance in PSB2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	857.10017	857.10017	236.94	<.0001
Error	148	535.37985	3.61743		

This result of PSB3 (Figure 4.4) indicated a weaker linear relationship because the dots representing the crayfish growth performance are further from the line. A weaker linear association in the growth performance of crayfish is indicated by the greater distance of data points from the regression line in the SAS analysis. This shows that there may be a more erratic or dispersed relationship in the data since the observed values are not as closely linked with the expected values.

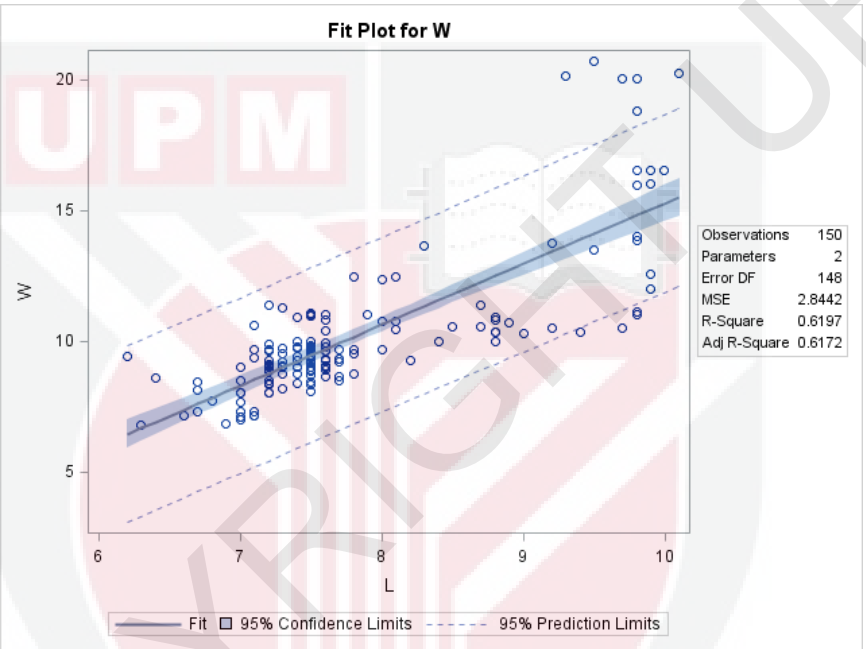

Figure 4.4. Distribution of the covariance in PSB3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	686.06910	686.06910	241.21	<.0001
Error	148	420.94894	2.84425		

A stronger linear association is suggested by the fact that the dots in Figure 4.5 that reflect the growth performance of crayfish in Negative are closer to the regression line in the SAS analysis. There is a greater linear correlation between the growth performance of crayfish and the proximity of data points to the regression line in the SAS analysis. Even yet, most of the dots had a firm grasp along the line, with a few spreading beyond from it. This implies that there is a more consistent and predictable link in the data since the observed values closely match the projected values.

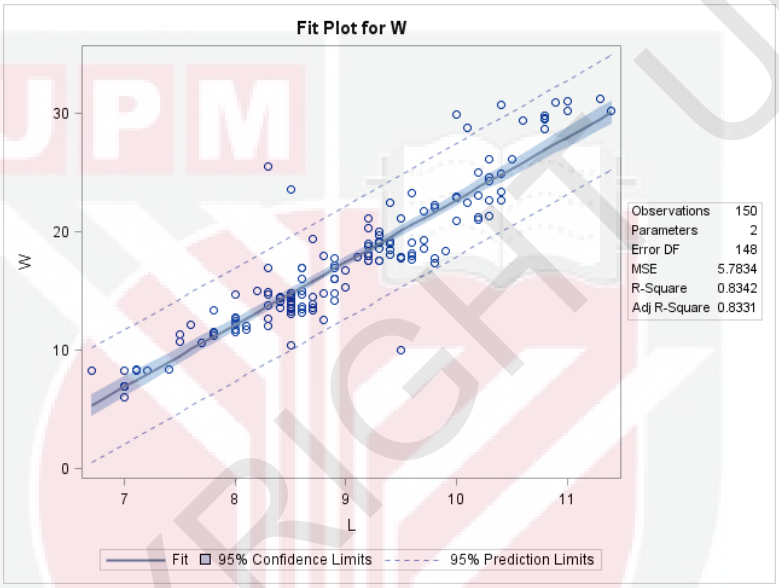

Figure 4.5. Distribution of the covariance in Negative

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4307.90406	4307.90406	744.88	<.0001
Error	148	855.93841	5.78337		

The SAS analysis indicates a stronger linear link between the growth performance of crayfish in Positive, as seen by the tight grip the dots had along the regression line (Figure 4.6). Greater growth performance of crayfish in comparison to other treatments is indicated by the SAS analysis's data points' proximity to the regression line, which suggests a stronger linear correlation. This implies that there is a more consistent and predictable link in the data since the observed values closely match the projected values.

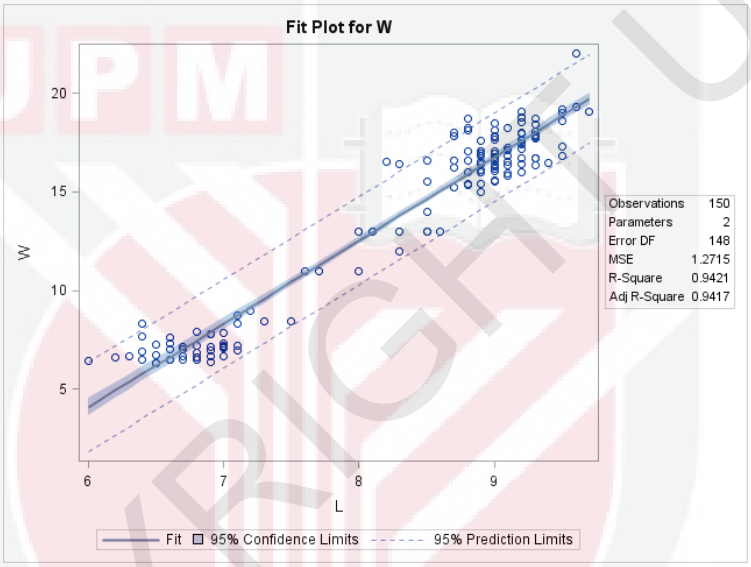

Figure 4.6. Distribution of the covariance in Positive

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3063.12562	3063.12562	2409.01	<.0001
Error	148	188.18596	1.27153		

CHAPTER 5

DISCUSSION

5.1 Photosynthetic Bacteria Impact on Nutrient Cycling

The impact of photosynthetic bacteria on water's ammonia, nitrate, and nitrite concentrations is a crucial component of the dynamics of aquatic ecosystems. In water environments, photosynthetic bacteria are essential to the cycling of nutrients. According to Archer et al. (2017), these bacteria have an impact on nutrient processing since they show a much higher abundance of anticipated genes related to nitrogen metabolism, carbon fixation, and photosynthetic processes than ambient water and sediment. It has also been demonstrated that photosynthetic bacteria influence the microbiota and water quality in aquatic environments, resulting in a reduction in the amount of anaerobic and nitrite-reducing bacteria (Zhu et al., 2014). An essential aspect of the dynamics of aquatic ecosystems is the effect of photosynthetic bacteria on the concentrations of ammonia, nitrate, and nitrite in water. Photosynthetic bacteria are necessary for the cycling of nutrients in aquatic environments. Since these bacteria exhibit a far higher abundance of expected genes related to nitrogen metabolism, carbon fixation, and photosynthetic processes than ambient water and sediment, Archer et al. (2017) claim that these bacteria have an impact on nutrient processing. Moreover, photosynthetic bacteria have been shown to affect the microbiota and water quality in aquatic environments, which lowers the quantity of anaerobic and nitrite-reducing bacteria (Zhu et al., 2014). Moreover, it has been extensively documented that photosynthetic bacteria can be found in the water columns of rice fields, activated sludge systems, wastewater environments, and aquatic sediments. These reports highlight the widespread presence of these bacteria and their potential influence on the

cycling of nutrients in different types of water ecosystems (Mae et al., 2013). The capacity of photosynthetic bacteria to produce high-molecular-weight polyhydroxyalkanoates (PHA) in seawater supplemented with acetate lends additional credence to their involvement in nutrient cycling and utilization (Higuchi-Takeuchi et al., 2016). Furthermore, the significance of photosynthetic bacteria in nutrient cycling in aquatic ecosystems is further highlighted by their capacity to regulate organic contaminants in wastewater and improve water quality in aquaculture systems (Luo et al., 2012).

5.1.1 Isolation and Cultivation Efficiency

For a number of industrial and environmental applications, photosynthetic bacterial isolation and cultivation efficiency are essential. For uses including single-cell protein synthesis, wastewater treatment, and the production of bioenergy, photosynthetic bacteria must be successfully cultivated. The presence of pollutants, the inefficiency of using light as an energy source, and the requirement for intensive cultivation systems with high biomass concentration and productivity are some of the technical difficulties associated with the cultivation of photosynthetic bacteria (Pruvost et al., 2008; Shipman et al., 1975; Bertling et al., 2006). Furthermore, for photosynthetic bacteria to be used in bioproduction processes, it is essential that they are successfully isolated from natural settings like saltwater (Higuchi-Takeuchi et al., 2016). The use of artificial light sources, such as lasers, to provide radiation appropriate for the culture of photosynthetic bacteria has been investigated as a means of improving the process (Bertling et al., 2006). Additionally, in order to make it easier for photosynthetic bacteria to be used in bioproduction processes, screening techniques for isolating them from naturally occurring seawater have been developed (Higuchi-Takeuchi et al., 2016). Research has demonstrated that co-cultivating photosynthetic bacteria with

marine heterotrophs might improve photosynthetic growth. This suggests that diverse microbial species in culture systems may interact synergistically (Beliaev et al., 2014; Stevenson et al., 2011). Additionally, research has been done on the use of non-traditional substrates, like distillery effluent, for the growth of green photosynthetic bacteria. This suggests that waste streams could be used as growth substrates for photosynthetic bacteria (Segin et al., 2020). Furthermore, the creation of soil diffusion systems and diffusion bioreactors has been suggested as a means of facilitating the culture of a variety of bacterial taxa that were previously uncultivated; this approach may also be used to produce photosynthetic bacteria (Chaudhary & Kim, 2019; Kakumanu & Williams, 2012).

5.1.2 Nutrient Reduction Patterns

A number of variables affect the decrease patterns of ammonia, nitrite, and nitrate in photosynthetic bacteria treatment in a crayfish tank system. Several enzymes, such as nitrate reductases, nitrite reductases, nitric oxide reductases, and nitrous oxide reductases, catalyze the process of denitrification, which involves the reduction of nitrate to gaseous nitrogen (The Martineau group, 2015). According to earlier research, nitrite is eliminated through a variety of pathways, such as endogenous nitrite-to-nitrate conversion, excretion in the urine, and outflow through the gills (Knudsen & Frank, 1997). Furthermore, it has been discovered that seaweed absorbs nitrite and nitrate, indicating that the system has completely removed the nitrogen (Tran et al., 2019). Environmental factors and crayfish behavior also affect the effects of ammonia, nitrite, and nitrate reduction. For example, it has been discovered that the presence of shelter in culture systems decreases exposure to light and increases effective bottom area, which has an impact on the growth and survival of juvenile crayfish (Savolainen et al., 2003). Additionally, it has been demonstrated that different invasive crayfish

species have different effects on stream populations, thereby lowering the densities of different taxa and altering algal biomass (Klose & Cooper, 2011). Furthermore, it has been shown that crayfish exposed to suspended silt have a reduced aerobic scope; native species are more vulnerable than invading species (Rosewarne et al., 2013). Studies have also been conducted on the physiological reactions of aquatic creatures to nitrite exposure. In juvenile hybrid groupers, nitrite exposure has been demonstrated to significantly lower hemoglobin and hematocrit levels, suggesting possible stress reactions (Kim et al., 2022). Furthermore, it has been discovered that nitrate decreases the aerobic scope of freshwater organisms, underscoring the detrimental effects of excessive nitrate on physiological function (Isaza et al., 2018). The control of water quality in aquaculture systems depends critically on the effectiveness of nitrogen reduction. Research has indicated that pulse aeration can lower nitrate levels in secondary clarifiers, which is advantageous for managing growing sludge (Lemma et al., 2009). Moreover, the difficulties and advancements in ammonia storage for nitrogen removal are highlighted by the utilization of metal ammine complexes as ammonia sources for the selective catalytic reduction of NOx in automobiles (Johannessen et al., 2009).

5.2 Water Quality Enhancement in Tank Culture Systems

5.2.1 API Test Kit Results

Research has been done on the use of water-test kits, including the API test kit, to evaluate the water quality in aquaculture systems Naigaga et al. (2016). These studies have shown how water-analysis kits may be used practically in aquaculture and have shed light on the efficiency and dependability of these kits for monitoring the microbiological water quality (Tandlich, 2020). Furthermore, the API test and other

phenotypic identification kits are frequently used to identify particular bacteria in fish farms, demonstrating their usefulness in evaluating the microbiological water quality in aquatic environments (BALCI et al., 2023). Accurate evaluation of water quality in aquaculture systems depends on the dependability and effectiveness of water-test kits, such as the API test kit. These kits are essential for detecting specific bacteria, tracking compliance in aquatic habitats, and monitoring the microbiological water quality. As a result, using API test kits to measure the quality of the water in treatment groups containing varying amounts of photosynthetic bacteria in tank culture systems can be beneficial.

5.2.2 Multiparameter Analysis

A thorough grasp of how these bacteria affect water quality and the rearing environment is necessary for the multiparameter analysis of groups treated with various measurements of photosynthetic bacteria in crayfish rearing tanks. A multiparameter aquaculture water quality tester with a decision support system was also presented by Lazo et al. (2021), and it may prove useful for examining the water quality parameters in crayfish rearing tanks treated with various doses of photosynthetic bacteria.

5.2.3 Chemical Oxygen Demand Assessment

A substantial amount of organic pollutants or contaminants is indicated by a high chemical oxygen demand (COD) in the water, which can cause dissolved oxygen levels in aquatic environments to drop. Because of the high concentration of organic materials, more oxygen is needed for its breakdown, which could cause oxygen depletion and harm aquatic life (Naidua et al., 2021; Cardia et al., 2023; Nur & Isworo, 2020). Furthermore, poor biodegradability, resistant organic matter, and a rise in

pollution from organic sources can all be indicated by high COD levels (Naidua et al., 2021; Cardia et al., 2023; Nur & Isworo, 2020).

Conversely, low COD levels in water indicate a lower organic pollutant concentration, improved water quality, and a less oxygen requirement for breakdown processes. According to Zaghloul et al. (2019) and Nur & Isworo (2020), this may be a sign of a healthier aquatic environment with less pollution and a decreased danger of oxygen depletion.

5.2.4 Total Suspended Solid Influence

Elevated amounts of total suspended solids (TSS) in water can affect the aquatic ecosystem in a number of ways. Reduced water clarity, increased turbidity, and light penetration are frequently caused by elevated TSS levels (Boyd, 2019; Godwin & Oborakpororo, 2019). By impeding photosynthesis, lowering primary productivity, and changing the habitat for aquatic creatures, this can have a detrimental effect on aquatic ecosystems (Boyd, 2019; Godwin & Oborakpororo, 2019). Furthermore, eutrophication and deteriorated water quality may result from the buildup of organic matter, nutrients, and contaminants brought on by elevated TSS levels (Kurek et al., 2019; Situmorang et al., 2021). On the other hand, low TSS levels in the water typically signify reduced turbidity and greater clarity, which is advantageous for aquatic ecosystems. Better light penetration in water with lower TSS levels promotes photosynthesis and the development of aquatic plants (Boyd, 2019). Additionally, decreased TSS levels can lessen contaminants and organic matter deposition, improving water quality and creating a healthy aquatic habitat (Kurek et al., 2019; Situmorang et al., 2021).

5.3 Distribution of Water Quality Parameters

5.3.1 NO2, NO3, NH3 Trends

In the presence of photosynthetic bacteria, the efficient removal of ammonia, nitrate, and nitrite concentrations occurs through various mechanisms. These bacteria incorporate ammonia and nitrite into their cellular biomass during growth, actively participating in nutrient assimilation. Additionally, certain species of photosynthetic bacteria engage in denitrification, a process that converts nitrate into nitrogen gas, effectively eliminating it from the aquatic environment. The Chang et al. (2019) study is very pertinent to look into how well photosynthetic bacteria reduce ammonia, nitrite, and nitrate in water. The addition of *Rhodovulum sulfidophilum* to a farm-scale multitrophic recirculating aquaculture system for milkfish is investigated in this work. The findings show that ammonia levels have decreased, indicating that photosynthetic bacteria have the ability to reduce ammonia in aquaculture systems. Furthermore, in a recirculating aquaculture system, the work by Ilma et al. (2022) focuses on the isolation and identification of bacteria that can remove ammonia, nitrite, and nitrate from bioball filters. This research sheds light on certain bacteria's capacity to break down nitrogenous chemicals, which is pertinent to the current project. Additionally, the Saidu et al. (2021) study looks into the possibility of removing nitrate through the isolation of photosynthetic bacteria from a coal mining site. The efficiency of photosynthetic bacteria for nitrate removal is specifically addressed in this work, offering important new information on their possible use in water treatment.

5.3.2 pH Variations

Photosynthetic bacteria play a pivotal and multifaceted role in upholding optimal water quality, making substantial contributions to crucial parameters vital for the well-being

of crayfish. As elucidated by Chen et al. (2020), these bacteria, leveraging their capacity for harnessing sunlight through photosynthesis, exhibit notable efficacy in the regulation of pH levels within water bodies. The observed photosynthetic bacteria growth range, situated between 6.8 and 8.5, aligns with the findings presented in Figure 3.1, where the pH levels across treatments fell within the range of 7.35 to 7.54. This evidence signifies the abundance of bacteria during this period, consequently leading to a reduction in ammonia, nitrite, and nitrate concentrations. Throughout the photosynthetic process, these bacteria actively absorb carbon dioxide, resulting in a decrease in its concentration within the water. This mechanism contributes to the stabilization and elevation of pH levels, creating an environment conducive to the health of aquatic organisms. The pH level has the potential to impact the functioning of cell membrane proteins and extracellular enzymes, influence cell membrane permeability, and affect the dissociation and absorption processes of nutrients and electron acceptors, such as carbon dioxide (CO_2). Consequently, these factors collectively contribute to influencing the growth of photosynthetic bacteria (PSB) (Cao et al., 2020). Beyond their role in pH regulation, photosynthetic bacteria significantly contribute to nutrient cycling within aquatic environments. The accumulation of excess nutrients, including ammonia, nitrite, and nitrate, can precipitate eutrophication—an occurrence characterized by algae overgrowth and subsequent oxygen depletion. Photosynthetic bacteria engage in nutrient assimilation, incorporating ammonia and nitrite into their cellular biomass during growth. Additionally, certain species partake in denitrification, a transformative process converting nitrate into nitrogen gas, effectively eliminating it from the water. Through the regulation of nutrient levels, photosynthetic bacteria serve as a safeguard against

the detrimental impacts of eutrophication, ensuring the preservation of a balanced and healthy aquatic ecosystem.

5.3.3 Temperature Fluctuations

Temperature is another crucial parameter influenced by photosynthetic bacteria. These bacteria thrive in environments with moderate temperatures, and their metabolic activities are often influenced by changes in temperature. The optimal temperature for photosynthetic bacteria to effectively reduce concentrations of ammonia, nitrite, and nitrate resulting from biomass accumulation falls within the range of 25 to 35 degrees Celsius, as indicated by Gonzalez et al. (2017). The findings from a 63-day observation period reveal that there were no substantial differences in temperature levels among all groups, with temperatures consistently ranging between 27 and 28 degrees Celsius. By participating in photosynthesis, these bacteria indirectly contribute to temperature regulation by influencing the balance of heat and energy in aquatic ecosystems. In this way, photosynthetic bacteria contribute to maintaining the thermal stability necessary for the survival and growth of the red claw crayfish.

5.3.4 Turbidity Impact

The influence of photosynthetic bacteria on the turbidity of water in a crayfish tank system is a multifaceted problem. It has been demonstrated that the presence of photosynthetic bacteria in aquarium systems purifies the water (Jeong et al., 2009). According to Jeong et al. (2009), these bacteria have been effectively employed in pond cultures to reduce the impact of viruses and cleanse water. Nonetheless, it is impossible to ignore how crayfish affect the quality of the water. Through their bioturbation practices, like as burrowing and tail-flipping, crayfish have been shown to increase turbidity (Dunoyer et al., 2019). Furthermore, it has been demonstrated that

crayfish alter water quality, particularly in terms of turbidity, and can impact rice seedlings in addition to causing direct devastation (Anastácio et al., 2005). Moreover, environmental elements that can influence the quantity of environmental DNA released into the water, such as temperature and flood occurrences, have an impact on crayfish behavior (Dunn et al., 2017; Robinson et al., 2000). The growth of photosynthetic bacteria and biological traits can be significantly impacted by turbidity in water. It has been demonstrated that in estuary environments, high water column turbidity promotes fast light attenuation, which leads to ideal photosynthetic conditions (Gameiro et al., 2011). Furthermore, it has been discovered that changes in environmental elements, like as turbidity, have an impact on photosynthetic bacterial development in meromictic lakes (Mikami et al., 2002). Research has also examined the correlation between turbidity and the distribution of native and alien crayfish, suggesting that water pollution may have an impact on crayfish species (Svobodová et al., 2012; Holdich & Reeve, 1991). Apart from the biological issues, the effect of turbidity on disinfection efficacy and water quality is an important factor to take into account. In disinfection systems, high turbidity levels have been linked to less success in inactivating germs during brief retention periods (Tomás-Callejas et al., 2012). Moreover, statistical models have been created to forecast how turbidity would affect the quality of drinking water, highlighting the significance of turbidity level monitoring and control (LeChevallier et al., 1981). This is demonstrated by the turbidity result in Commercial PSB, which is low in this treatment and hence encourages improved crayfish growth (Figure 1(g)). Better light penetration in clearer water promotes the growth of aquatic plants, which in turn can give crayfish a place to live and food. Furthermore, clearer water can help crayfish find and catch prey more easily, which will speed up their feeding process.

5.3.5 Conductivity Patterns

A crucial factor in determining the quality of water is conductivity, and measuring it can reveal important details about the make-up and properties of the water. Different situations and ramifications might arise from high or low conductivity in water quality. Elevated amounts of dissolved salts, minerals, and other ions can be a sign of pollution, especially from industrial or agricultural sources, as shown by high conductivity in water (Elhag et al. 2019; Zhang et al., 2023). It may also indicate the existence of contaminants such heavy metals, which can be harmful to human health and aquatic ecosystems (Bihn et al., 2021; Sadeghi et al., 2019). Poor water quality may be linked to high conductivity levels, particularly in places where anthropogenic contamination or particular land use patterns are present (Jóźwiakowski et al., 2021). Water with low conductivity may be free of dissolved ions and minerals, which is a sign of pure, unpolluted natural water sources (Wu et al., 2020). Additionally, it may indicate lower dissolved salt and nutrient levels, which could have an effect on the general health and productivity of aquatic ecosystems (Tian et al., 2020). In other situations, low conductivity levels might be linked to particular water sources, including rainwater or particular kinds of groundwater, which may have low mineral contents by nature (Rojano et al., 2020). Low conductivity can also be a sign of relatively pure environmental conditions and water sources with little human influence (Ahmed et al., 2021).

5.3.6 Total Dissolved Solid Dynamics

The total amount of dissolved metals, minerals, and inorganic salts in a liquid is known as total dissolved solids, or TDS in water. The TDS levels can reveal important details about the make-up and properties of water. Elevated concentrations of dissolved salts, minerals, and metals can be a sign of pollution, especially from natural, agricultural, or industrial sources (Braga et al. 2022; Sajeesh & Pulikkal, 2022). High TDS levels in water can be a sign of this contamination. Additionally, it may indicate the existence of contaminants such heavy metals, which have a negative impact on human health and aquatic ecosystems (Jena et al., 2022; Piffer et al., 2022). Poor water quality may be linked to high TDS levels, particularly in regions impacted by geological features, particular land use patterns, or anthropogenic pollution (Hamid et al., 2023; Emurotu & Habib, 2020). High TDS levels may occasionally be associated with salinity, which may affect the water's appropriateness for drinking and other applications (Akita et al., 2021; Al-Hamdani & Kaplan, 2022). Water with low TDS levels may be free of dissolved salts and minerals, which is a sign of pure, unpolluted natural water sources (Shamim, 2022; Farooqui et al., 2020). Additionally, it may indicate lower dissolved ion and nutrient levels, which could have an effect on the general health and productivity of aquatic ecosystems (Akita et al., 2021; Bhutiani et al., 2019). Low TDS levels can occasionally be linked to particular water sources, such rainwater or particular kinds of groundwater, which may naturally contain low mineral contents (Imagwuike & Chibundo, 2021; Godwin & Oborakpororo, 2019). Low TDS can also be a sign of generally unspoiled environmental conditions and water sources with little human influence (Godwin & Oborakpororo, 2019).

5.4 Chemical Dissolved Oxygen Patterns

As photosynthetic bacteria are added to tank water, high dissolved oxygen (DO) is usually a sign of good water quality. A healthy and well-oxygenated aquatic environment is facilitated by fish and other creatures' ability to breathe, which is made possible by elevated DO levels (Munirah et al., 2021; Wu et al., 2017). Elevated DO levels are frequently linked to better water quality, which supports aquatic ecosystem production and general health (Munirah et al., 2021; Wu et al., 2017). On the other hand, low dissolved oxygen levels in tank water treated with photosynthetic bacteria may be harmful to aquatic life and the overall health of the environment. Fish and other aquatic creatures' ability to survive and thrive can be severely impacted by hypoxia or anoxia caused by insufficient DO (Munirah et al., 2021; Wu et al., 2017). According to Munirah et al. (2021) and Wu et al. (2017), low DO levels are frequently linked to poor water quality, which may have negative ecological effects and diminish biodiversity in aquatic habitats.

5.4.1 Oxygen Levels and Bacterial Activity

A vital component in maintaining the activity of photosynthetic bacteria and other microbial communities in water is the dissolved oxygen (DO) content. The general quality of water and the amount of bacterial activity can be greatly impacted by high or low DO levels. High DO levels help photosynthetic bacteria and other aerobic microbes respire aerobically, which supports their metabolic processes and aids in the decomposition of organic matter in water (Holmes et al., 2019; Fan et al., 2019). High DO levels are necessary to promote the overall ecological balance, encourage the growth of beneficial bacteria, and preserve the productivity and health of aquatic ecosystems (Holmes et al., 2019; Fan et al., 2019). According to Holmes et al. (2019)

and Fan et al. (2019), high DO levels are a sign of good water quality conditions, which encourage the aerobic activity of photosynthetic bacteria and other microorganisms participating in biogeochemical processes. Reduced metabolic activity and a poorer ability to break down organic materials in water are two possible outcomes of low DO levels, which might inhibit the aerobic respiration of photosynthetic bacteria and other aerobic microorganisms (Holmes et al., 2019; Fan et al., 2019). The survival and growth of photosynthetic bacteria and other aerobic microorganisms, as well as the general health of aquatic ecosystems, can be severely impacted by hypoxia or anoxia caused by insufficient DO (Holmes et al., 2019; Fan et al., 2019). According to Holmes et al. (2019) and Fan et al. (2019), low DO levels are frequently linked to poor water quality, which may have negative ecological effects and diminish biodiversity in aquatic habitats.

5.5 Total Suspended Solid Analysis

5.5.1 Clarity and Bacterial Influence

Water bodies' general look, light penetration, and water clarity can all be affected by total suspended solids (TSS) (Akhrianti et al., 2023; Kim et al., 2020). Increased TSS levels have the ability to change aquatic habitat conditions and restrict light penetration by reducing water clarity (Akhrianti et al., 2023). By offering substrates for microbial colonization and altering the availability of nutrients and organic matter, high TSS levels can change bacterial activity (Rodríguez-Martínez et al., 2021; Cui et al., 2017). TSS, phytoplankton, and other elements all have an impact on water clarity, which is essential for maintaining aquatic life and the health of ecosystems (Rose et al., 2017; Kim et al., 2020). High TSS levels can cause reduced water clarity, which can change light availability and disrupt photosynthetic activities as well as the dynamics of

microbial communities. This can have an effect on bacterial activity (Kim et al., 2020). Because light is necessary for photosynthetic bacteria and other microbial functions, water clarity can have an impact on bacterial activity (Kim et al., 2020). TSS and water clarity have the potential to impact bacterial activity because they can alter the availability of organic matter, light, and nutrients for microbial processes (Cui et al., 2017; Kim et al., 2020). Elevated Total Suspended Solids (TSS) levels have the potential to affect bacterial activity and nutrient cycling in aquatic environments by influencing the availability of organic matter and providing substrates for bacterial colonization (Rodríguez-Martínez et al., 2021; Cui et al., 2017). Because light is necessary for photosynthetic bacteria and other microbial functions, water clarity can have an impact on bacterial activity (Kim et al., 2020; Cui et al., 2017).

5.6 Red Claw Crayfish Growth Performance

5.6.1 Correlation with Water Quality Parameters

The growth performance of crayfish in the presence of photosynthetic bacteria can be influenced by various water quality parameters. The addition of photosynthetic bacteria may impact the growth, survival, and overall health of crayfish. The correlation of water quality parameters with the growth performance of crayfish can provide valuable insights into the factors influencing their development.

5.6.2 Implications for Aquaculture Practices

The sustainability of aquaculture operations is greatly affected by the incorporation of photosynthetic bacteria into rearing tank systems. Numerous possible advantages are presented in this application, all of which support aquaculture's overall ecological and financial sustainability. Thanks to their ability to assimilate and transform necessary substances, including atmospheric nitrogen, into forms that aquatic species may easily

access, photosynthetic bacteria are crucial to the cycle of nutrients. The rearing tank's aquatic habitat is healthier overall as a result of this nutrient cycling. Additionally, through a variety of metabolic activities, photosynthetic bacteria actively participate in the removal of contaminants like ammonia, which helps to control the quality of water. Their function in nutrient assimilation and nitrogen fixation aids in the preservation of ideal water quality, lowering the possibility of environmental deterioration and fostering an environment that is favorable for aquaculture species. A biologically balanced system is promoted by the introduction of photosynthetic bacteria, which have an impact on things like pH regulation and oxygen production. The respiratory health of aquaculture species is positively impacted by their contribution to dissolved oxygen levels. Apart from these advantages, certain bacteria that photosynthesize have the ability to outcompete algae in the competition for nutrients, which helps regulate algal blooms. This improves the quality of the water while also lessening the possible harm that excessive algal development could do to aquaculture operations. Using photosynthetic bacteria could help lessen reliance on outside resources, including chemical additions for water treatment. This is in line with sustainable aquaculture methods, which reduce the environmental impact of operations. In the end, adding photosynthetic bacteria can improve aquaculture systems' total output by creating a nutrient-rich, well-balanced habitat. The growth and well-being of cultured organisms are positively impacted by enhanced water quality and nutrient availability. Overall, by establishing robust and ecologically sound systems, the use of photosynthetic bacteria is consistent with the more general objectives of sustainable aquaculture methods.

5.7 Comparative Analysis with Previous Studies

The present study's results are consistent with those of the earlier examination, which supports the effectiveness of photosynthetic bacteria (PSB) in water cleaning and the improvement of crayfish development performance. These findings are reported in the research conducted by Wang et al. (2019). Although the concentrations of PSB used in the previous trial varied, the outcomes consistently supported PSB's efficacy. Notably, high PSB concentrations can have negative impacts on crayfish as well as water quality, which emphasizes how crucial it is to carefully choose the right PSB dosage in order to maximize results.

5.7.1 Consistency with Literature Findings

There were an ample amount of research dedicated as references in this project to obtain clearer understanding on the efficacy of PSB to purify water and enhance the growth performance of red claw crayfish, *Cheraz quadricarinatus*.

5.7.2 Novel Insights and Variances

This project has provided original perspectives on the PSB and crayfish, in which supply new knowledge for future use.

5.8 Practical Implications and Recommendations

5.8.1 Application Strategies for Aquaculture Systems

Aquaculture systems use a variety of tactics to maximize output effectiveness, maintain sustainability, and reduce environmental impact. Careful site selection and management techniques to match facilities with environmental conditions are some of these tactics. The choice of species is important, taking into account environmental compatibility, ecological adaptability, and market demand. Cultured organisms depend

heavily on water quality management techniques, such as measuring dissolved oxygen and nutrient levels, to stay healthy and flourish.

Feeding procedures are designed to be effective and environmentally friendly, with an emphasis on maximizing feed composition and reducing waste. A focus on rotation techniques and stocking density helps avoid problems such as disease outbreaks, nutrient accumulation, and overcrowding. Recirculating Aquaculture Systems (RAS) is one of the technologies that is being used to maximize resource usage, improve biosecurity, and minimize water consumption.

5.8.2 Considerations for Sustainable Practices

Aquaculture sustainable practices take a comprehensive strategy to reduce environmental effect, guarantee economic viability, and give social responsibility top priority. One of the most important considerations is choosing the right location, where compatibility and environmental circumstances must be carefully considered. Market demand, ecological appropriateness, and the possibility of a smaller ecological footprint are taken into consideration while choosing a species. An essential function of water quality management is the observation and regulation of variables including temperature, dissolved oxygen content, and nutrient levels. The main goals of sustainable feeding methods are formulation optimization, waste reduction, and meeting the nutritional requirements of cultured species without causing harm to the environment. Proper stocking density and rotation practices are implemented to prevent issues like overcrowding, disease outbreaks, and excessive nutrient buildup.

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5.9 Limitations of the Study

5.9.1 Methodological Constraints

The inexperience with the spread plate approach made the cultivation and isolation of PSB extremely difficult. Cross contamination was one of the restrictions on the PSB plate spreading process. In addition, the equipment for monitoring water quality is also one of the limitations. There were some of the equipment that are not functional; the probe of turbidity on multiparameter was not functional and chemical for water quality parameter such as the API test kits had exceeded the expiry date. These constraints could be avoided if detailed selection of the equipment was practiced.

5.9.2 External Factors Influencing Results

One of the external factors that influence the result was the strategic site location for the project. The project was conducted in a laboratory, in which the rearing tank of the crayfish is in an enclosed area, hence less penetration of sunlight during the day. So, maximal effect can be observed if there is enough sunlight for the growth of PSB. In addition, the weather during PSB cultivation is one of the methodological problems. To reach the maturity stage, PSB cultivation needs to be exposed to continuous daylight during the day to promote microbial development. However, due of the intense rain, some attempts to cultivate this bacterium were unsuccessful.

5.10 Future Research Directions

5.10.1 Refinement of Photosynthetic Bacteria Application

Strategic site selection for the project is necessary in order to see better efficacy of PSB in the crayfish rearing tank. The site should be exposed to sunlight during the day

in the laboratory. In addition, a better handling during PSB cultivation is also crucial to avoid contamination.

5.10.2 Exploration of Additional Water Quality Parameters

Investigating new water quality measures necessitates a thorough evaluation that goes beyond the fundamental criteria that are frequently tracked. While basic indicators of water quality include pH, temperature, dissolved oxygen, and nutrient levels, other factors can give a more thorough knowledge of the aquatic ecosystem. A few other metrics related to water quality are Biochemical Oxygen Demand (BOD). The amount of oxygen used by bacteria during the breakdown of organic materials is measured by BOD. It offers information about how much organic pollution is present in water. Metals and trace elements come next. To determine possible contamination and toxicity issues, it is crucial to monitor the levels of trace elements and metals including lead, mercury, and copper. Water safety and possible fecal contamination can be inferred from the presence of microbiological markers, such as coliform bacteria, which can be assessed.

CHAPTER 6

CONCLUSION

A study on the efficacy of photosynthetic bacteria (PSB) towards the water quality in the rearing tank and the growth rate of red claw crayfish, *Cherax quadricarinatus* was conducted in the Post-mortem Laboratory University Putra Malaysia Bintulu Sarawak Campus which took place on 30th August 2023 until 31st October 2023. The study showed that the concentration and abundance of the photosynthetic bacteria (PSB) affects the water quality in the rearing tank of red claw crayfish, *Cherax quadricarinatus*. The water parameter that were recorded include total suspended solids (TSS), chemical oxygen demand (COD), ammonia, nitrite, nitrate, pH, temperature, turbidity, conductivity, total dissolved solids (TDS) and dissolved oxygen (DO). The average of weight and length of the crayfish were recorded, alongside with the survival rate and specific growth rate (SGR). The efficacy of photosynthetic bacteria from each treatment and growth performance were affected by the environmental conditions and the water quality in the rearing tanks.

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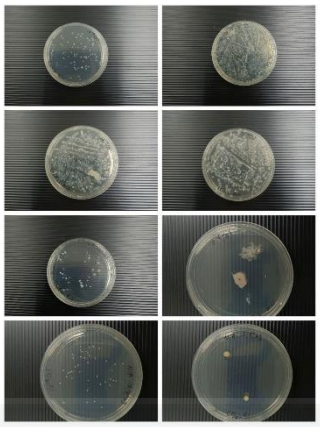
APPENDICES



Single colony of photosynthetic bacteria in different dilutions

Red claw crayfish, *Cherax quadricarinatus* sampling

Mature photosynthetic bacteria

Length measurement of red claw crayfish, *Cherax quadricarinatus*

Weight measurement of red claw crayfish, *Cherax quadricarinatus*

Umbrella fish trap set up for crayfish sampling

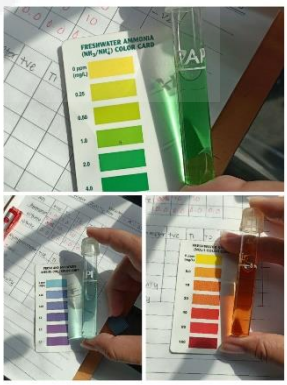
Red claw crayfish, *Cherax quadricarinatus* rearing tank condition before the addition of photosynthetic bacteria

Red claw crayfish, *Cherax quadricarinatus* rearing tank condition after the addition of photosynthetic bacteria

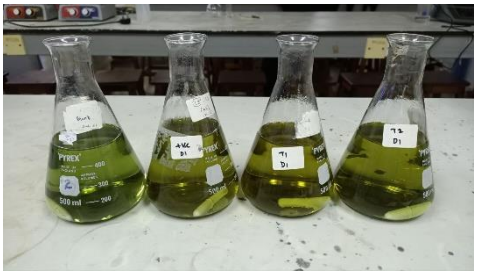
Photosynthetic bacteria pellet

Total suspended solids (TSS) analysis

Multiparameter reading in the water sample of crayfish rearing tank



API water test kit readings in the water sample of crayfish rearing tank



Chemical oxygen demand (COD) of the water sample in crayfish rearing tank