Development Of An Insect-Based Encapsulated Feed For Shrimp Aquaculture

Brooke Samantha Kyle

Submitted in fulfilment of the requirements for the degree of
Master of Science (Research)

School of Science
Faculty of Health and Environmental Science
Auckland University of Technology
2022

Acknowledgements

I could not have undertaken this journey without my wonderful supervisor, Andrea Alfaro. Andrea's immense knowledge, wisdom and supervisory assistance are unmatched. Andrea's support throughout this process has been unwavering, she has supported me, encouraged me and given me motivation in times I truly needed it.

Secondly, I would like to express my deepest gratitude to Sara Masoomi Dezfooli for her incredible support as my co-supervisor. Sara has gone above and beyond what is thought of as a co-supervisor. Sara has read every single piece of writing, given me lessons in chemistry, taught me the chemical proximate analysis necessary for this thesis and amongst everything, has been so kind, supportive and understanding throughout this entire process. I couldn't have gotten to where I am today without her.

A very special thanks to the ABRG group in its entirety. Ming, Shaneel and Awanis for their assistance with my feeding trial, Ming for showing up whenever I needed him even on weekends, Natalia for her support, the statistics lessons and advice, Leonie and Tim for their knowledge and advice and the rest of ABRG for checking in on me and for their support. I would also like to extend my sincere thanks to Adrian Owens for his support as laboratory technician, his chemistry knowledge, his laughter and his support throughout this process as well as Tim Layt for his trust, support in me and for allowing me to teach others the chemistry I had learnt throughout this process.

I would be remiss in not mentioning the support of my partner Brandon throughout this time. He has supported me an unbelievable amount throughout this process, he helped me catch the shrimp for the feeding trial, assisted me with grammar and mathematical equations and has been my emotional support throughout this process. I couldn't have done this without his love and support. Lastly, I would like to mention my parents. My mum for her love and listening ear throughout this process and my dad for his continued support throughout my six years of study, for his kind words, his support, his advice and for giving me his passion and love for the ocean. I wouldn't be the woman I am today without him.

Abstract

The aim of this thesis was to create an innovative shrimp aquafeed with encapsulation technology and varying concentrations of mealworm meal to improve the sustainability of current shrimp aquaculture production. This feed was developed and tested on a non-commercial shrimp species (*Palaemon affinis*) as a proxy for application of shrimp aquaculture. Laboratory analyses and feeding trials were conducted to compare the encapsulated feed with a commercial feed for *P. affinis* growth parameters, feed sustainability and effect on water quality parameters. The results indicate that the encapsulated feed had significantly less matrix erosion over 48 hours in seawater than the commercial feed. Additionally, an eight-week feeding trial was conducted to assess the effectiveness of the encapsulated mealworm diets on shrimp. Four diets were used in the feeding trial: a commercial diet (CF) with predominantly fishmeal as the protein source and three encapsulated mealworm diets with varying concentrations of fishmeal: mealworm ratios (F70, F50 and F30). The final weight and length displayed significant differences in shrimp fed higher concentrations of mealworm meal (F50, F30). However, it was discovered that there was a correlation between the weight gain of shrimp and the lipid concentration in the diets suggesting that the weight gain was due to the increased lipids in mealworms rather than the diets themselves. Uniquely, shrimp fed F50 were found to excrete excess lipids in their diets, resulting in no significant difference in bodily lipids in comparison to the lower concentration of mealworm diets. This suggests that shrimp fed slightly elevated levels of lipids than required may be able to discard the excess lipids in their faecal matter. Additionally, feed utilisation, feed ingested, palatability and survival all indicated no significant differences between commercial and encapsulated diets. Overall, the results indicate that an encapsulated mealworm diet can be utilised to enhance water quality parameters in shrimp aquaculture and indicate that alternative, sustainable insect-based protein sources such as mealworms have the potential to replace fishmeal in varying concentrations.

Table of Contents

List of Figures

List of Tables

List of Equations

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: 

Date: 30/10/2022

Chapter 1: Introduction and Literature Review

1.1 Shrimp Aquaculture

1.1.1 The Global Food Challenge and Aquaculture

Annual global consumption of seafood more than doubled between 1960 and 2022 (Guillen et al., 2019; IBISWorld, 2022). Fisheries stocks are unable to meet the demand for seafood globally, and fishers have exploited more than half of the worlds fish stocks trying to meet demands (Jacquet & Pauly, 2007). This has resulted in undeniable damage to fish species globally, destroying and altering ecosystems in the process (Jacquet & Pauly, 2007). Kidane and Braekkan, 2021 reported that global demand for seafood has been higher than the global supply (Kidane & Brækkan, 2021). Aquaculture has been able to alleviate some of the pressure on wild fish stocks, supplying more than half of all seafood for human consumption. Aquaculture is therefore vital for filling the seafood demand gap (Gephart et al., 2020).

1.1.2 Shrimp Aquaculture

The shrimp aquaculture industry holds importance on a global economic scale. The culture of shrimp supports economies around the world and has one of the highest annual growth rates seen in all aquaculture production (Arthur et al., 2002). In 2017, shrimp aquaculture supplied 4.92% of the worlds total production and supplied 13.71% of the total economic value. This equated to shrimp having the 8th highest production in aquaculture and the 2nd highest economic value worldwide (Junning et al., 2019). In 2019, shrimp aquaculture rose to approximately 8% of the total global production and approximately 27% of the global economic income within the aquaculture industry (OCED, 2022). These statistics show that global shrimp aquaculture is a growing industry with an economically valuable trade (Leung & Engle, 2008).

1.1.3 Culture Methods

Within shrimp aquaculture, there are three main farming classifications: extensive, semi-intensive, and intensive farming (Anderson, 2002). Having three farming classifications with differing requirements make shrimp farming a viable option across the globe. Extensive farming involves a stocking density of less than 25,000 post larval shrimp per hectare. This system is common in countries such as Vietnam, Guyana, Bangladesh and Indonesia

(Anderson, 2002; Honculada, 1998). Extensive practices are used when there is a lack of economic resources and stocking density is kept to a minimum to remain managable for farmers. These systems are run predominantly on natural resources, with limited to no excess substituted feeding (Anderson, 2002; Rosenberry, 1991). Semi-intensive farming is when the stocking density is between 100,000-300,000 post larval shrimp per hectare. This is a system which is more affordable than intensive culture, while still large enough to meet international demand. These systems use mechanical pumps for water exchange and use formulated feeds for prime growth performance. Semi-intensive culture systems are used in Ecuador, Honduras, Mexico, Australia and New Zealand (Anderson, 2002; Honculada, 1998). Intensive farming involves more than 300,000 post larval shrimp per hectare. This is attributable to the considerable control that can be had over abiotic and biotic conditions inside an intensive aquaculture system. However, there are greater costs associated with this system (Rosenberry, 1991; Sakami et al., 2008). Extensive, Semi-intensive and intensive farming practices all offer viable options for commercial shrimp production and contribute to economic importance globally.

1.1.4 Production and Economic Value

Commercial shrimp production has shown linear growth since the 1970's, economically increasing at a rate of 10% per annum (Figure 1; OECD, 2021). This linear growth is likely attributable to increasing knowledge on culturing techniques, technology and disease prevention and control (OECD, 2021; Rosenberry, 2001). For example, in 1975 global production was approximately 22,400 tonnes and grewn to 118,900,667 tonnes in 2020, valuing over 35 billion USD (Figure 2; OECD, 2021). Shrimp aquaculture equates to more than 55% of the shrimp for consumption, indicating that aquaculture is the primary source of shrimp globally (Biao & Kaijin, 2007; Chamberlain, 2010; New, 2002; OECD, 2021; Rosenberry, 2001). The culture of shrimp species is one of the fastest growing aquaculture markets in the world, exhibiting an increase in production, economic return and surpassing the percentage of shrimp from the fishing industry every year (OECD, 2021). This increase demonstrates that the demand for shrimp aquaculture continues to grow and therefore so will the industry to support this demand.

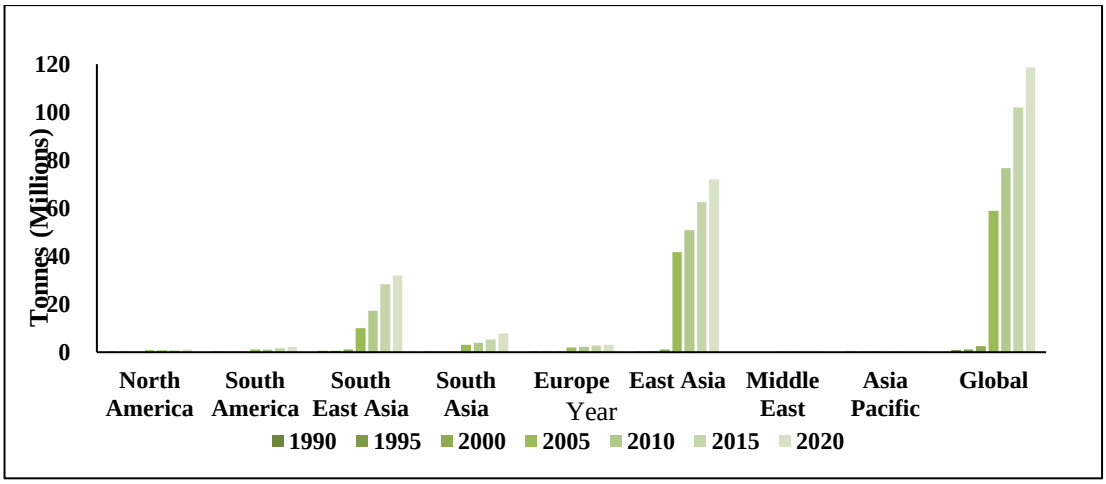

Figure 1: Shrimp Aquaculture production per area between the years 1990-2020 expressed as tonnes (FAO, 2009; OECD, 2021).

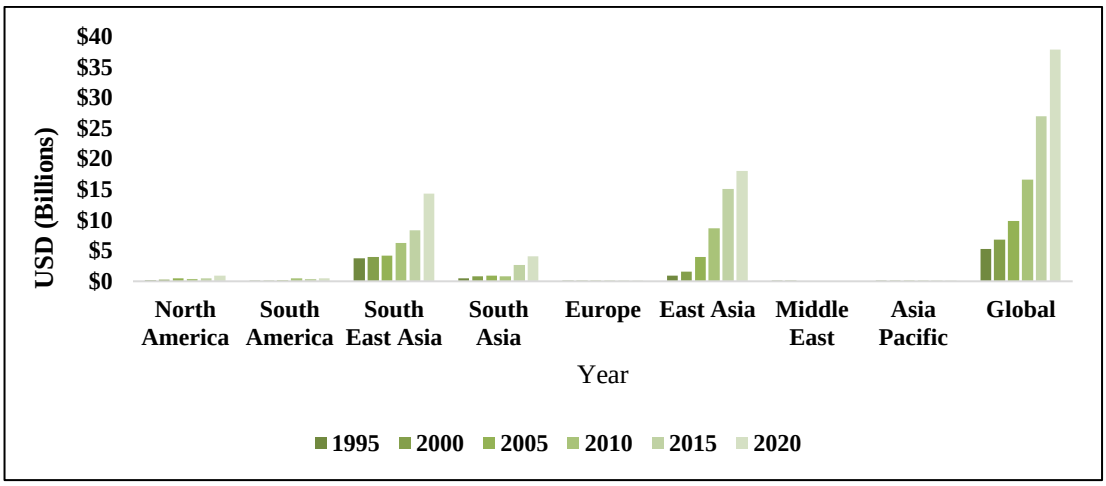

Figure 2: Shrimp aquaculture economic value per area between the years 1990-2020 expressed in USD (FAO, 2009; OECD, 2021).

1.1.5 Commercialised Species

The culture of shrimp for commercial sale consists of over 400 species from 22 different families (FAO, 2019). Although over 400 species are reared for commercial purposes, four species provide a substantial proportion of the global supply (Josupeit, 2004; OECD.Stat, 2020), which include *Litopenaeus vannamei* (synonym *Penaeus vannamei*), *Penaeus monodon*, *Fenneropenaeus chinensis* (synonym *Penaeus chinensis*) and one freshwater species, *Macrobrachium rosenbergii* (Josupeit, 2004; OECD.Stat, 2020). *L. vannamei*, *P. monodon* and *P. chinensis* contributed to 38% of saltwater shrimp culture in 2019 (OECD, 2020). *M. rosenbergii* is the most cultured freshwater species of shrimp contributing 89% of freshwater shrimp culture in 2019 (OECD, 2020). The four most cultivated species for commercial production are produced at such high rates due to the easy rearing capabilities, high rates of reproduction and survival, lower susceptibility to disease as well as being

adaptable to temperature, salinity, oxygen and stocking density changes (Holthuis, 1980; Karplus et al., 2000; New, 2002). For example, Panama began cultivating two different shrimp species in the 1970's, *P. stylirostri* and *L. vannamei*. It was discovered that in comparison, *P. stylirostri* was highly aggressive in nature, less tolerable to salinity and temperature changes and exhibited a much slower growth rate (Funge-Smith & Briggs, 2003; Rosenberry, 2001). A secondary consideration for producers is species export value. The four top producing species have a higher export value than most other species, therefore these species contribute most to producers due to their favourable characteristics and high export values (Khemundu & Banerjee, 2019).

1.1.6 Palaemonidae Family

Palaemonidae is a family of shrimp from the order Decapoda (WoRMS, 2021). The family contains more than 1200 species from over 100 genera (FAO, 1983). It is recorded that the family originated in the late Jurassic to early Cretaceous period and originated in freshwater, later inhibiting brackish and saltwater environments (Pereira, 1991). Palaemonidae can be found in freshwater, brackish and saltwater environments throughout the tropics and subtropics and can be found in estuaries, rivers, rockpools, and coral reefs. Palaemonidae has been found to feed mostly on small invertebrates and detritus (Day, 2001; Holthuis, 1952). The family Palaemonidae incorporates the species, *Palaemon affinis*. From this family, species from the *Palaemon* and *Macrobrachium* genus are used in commercial productions (FAO, 1983).

1.1.7 *Palaemon affinis*

Palaemon affinis is a species of shrimp from the genus Decapoda and the family Palaemonidae, which also encompasses the well-known commercially produced freshwater shrimp *Macrobrachium rosenbergii* (Miller et al., 2005). *M. rosenbergii* could not be sourced for this study and therefore the closest related species that could be sourced in New Zealand for use for this study was *P. affinis*. *P. affinis* is endemic to New Zealand and can be found inhabiting a range of habitats from intertidal rockpools to estuaries around New Zealand. Their tolerance to a variety of salinities is extensive, *P. affinis* can survive in as low as 0.5 ppt and survive well in 35-38 ppt. Temperature tolerance is also high, *P. affinis* live in areas where the temperature is as low as 6° and have been shown to survive in temperatures upwards of 24° (Kirkapatrick & Jones, 1985). Published findings on *P. affinis* are scarce and therefore information on the maximum length, weight and size of the species is undetermined.

Preliminary studies have shown that *P. affinis* can grow as large as 90mm and there may be a correlation between growth and available space although this would need to be further studied for official confirmation. Day, 2001 found during field studies the maximum TBL of *P. affinis* examined was 54mm (Day, 2001). During this research, the maximum TBL of *P. affinis* in the study was 36mm and the maximum weight was 0.39g.

The life stage of the animals as well as sex was undetermined due to their small size. The sex of crustaceans is usually determined using external morphology although when the animal is small externally non-sexable males are very morphologically close to that of females and may be incorrectly considered females. Research has not been conducted on the minimum size that individuals can be sexed and therefore to avoid incorrect data, sex and life stage were not determined (Day, 2001).

1.1.8 Worldwide Aquaculture for Palaemonidae

Palaemonidae is an important family in the aquaculture of shrimp, specifically due to its freshwater genus *Macrobrachium*. *Macrobrachium* is a globally recognised genus with many species from the genus being cultivated for commercial purposes. Specifically, *Macrobrachium rosenbergii* is the most widely cultivated freshwater species in the world totalling nearly 90% of freshwater shrimp production, the other 10% of freshwater species used in commercial shrimp culture also belong to the family Palaemonidae (OECD, 2020). In 2019, Palaemonidae species contributed more than 460,000 tonnes to commercial shrimp production and over 2 billion USD which equated to approximately 8% of total shrimp economic revenue (OECD, 2022). Palaemonidae is a widely and highly cultivated family contributing to shrimp aquaculture on a global production and economic scale.

1.1.9 New Zealand Palaemonidae Aquaculture

In New Zealand, only one farm has been developed for commercially producing shrimp. Huka Prawn Park, located in Taupo of the North Island in New Zealand have built a facility for culturing *Macrobrachium rosenbergii*. Developed in 1987, Huka Prawn Park uses semi-intensive farming techniques and geothermal heat waste from a geothermal power station close by for shrimp culture. The farm has a total of nineteen ponds and can hold approximately 150,000 individual *M. rosenbergii*. The species was chosen as it is the most commercially produced freshwater species with the highest reproductive output. *M. rosenbergii* produces a large number of eggs, grows to a commercial saleable size quickly and females can spawn up to five times per year, giving accessibility to year-round production (Huka-Prawn-Park).

Currently Huka Prawn Park imports their commercial shrimp feed from Australia, created by Ridleys. The prominent ingredient in the feed is fishmeal, which is the main ingredient for the majority of commercially formulated shrimp feeds globally (Gasco et al., 2018). The feed is imported from Australia because there is a lack of specifically formulated feed with local ingredients for farming the species in New Zealand.

1.1.10 Factors Affecting Production

Nutrition is a predominant factor which has a profound effect on shrimp production globally. Nutrition can influence abiotic factors, such as water quality parameters as well as biotic factors such as disease control and prevention, with consequences for production outputs and the economic value of stock (Hasan, 2001). Specific nutritional profiles can also be used to improve immune responses and overall health, such as the use of chitin (insect source) in the feed to boost the immune system (Motte et al., 2019). Unlike fishmeal, insect meal contains chitin which can be as high as 13% in mealworm larvae (Motte et al., 2019; Song et al., 2018). Indeed, previous studies have that nearly half of the factors that contribute to the diseases experienced by Tiger Shrimp, for example, are directly or indirectly related to the nutritional adequacy of diets (Boonyaratpalin, 1996). More recently, reviews conclude that feed additives and nutrition have been shown to minimise the use of antibiotics in shrimp culture and reduce disease while promoting growth and stimulating immune response (Dawood et al., 2018; Emerenciano et al., 2022).

1.2 Nutritional Requirements

1.2.1 Core Nutritional Requirements of Commercial Shrimp

Shrimp require protein for muscular growth, maintenance and essential amino acids (Das et al., 1996). Carbohydrates as a source of energy, while lipids represent a source of essential fatty acids, metabolic energy and as an alternative source of protein for growth, as well as vitamins and minerals, which are essential for survival (Boonyaratpalin, 1996; Davis, 2005; Mukhopadhyay et al., 2013). Nutritional requirements are a key component and consideration for commercial shrimp production. When these requirements are unfulfilled the individual will not grow to a commercial grade size, weight and length, which can be detrimental in the commercial sector (Craig et al., 2017).

1.2.2 Protein

Protein is a core nutritional requirement which aids in the maintenance, growth and supply of essential amino acids, which are crucial to the individual's function and survival (Cowey & Forester, 1971; Craig et al., 2017; Davis, 2005; Fox et al., 2006). A minimum dietary protein level of approximately 30% is required for all species of shrimp (Davis, 2005). Lower levels of protein can lead to a reduction in growth and weight, since the animal then starts to withdraw protein from tissues to maintain vital physiological functions (Lee & Lee, 2018). However, if the protein percentage in the dietary feed is too high, the feed costs will increase and the excess protein can lead to excess of nitrogen in the water as well as poor water quality (Lee & Lee, 2018). Andrews et al. (1972) and Davis 2005 found that protein levels between 14-25% caused an increased mortality rate and decreased growth rate for several species of penaeid shrimp (Andrews et al., 1972; Davis, 2005; Nesara & Paturi, 2018). Protein is therefore an essential nutritional requirement and a vital aspect of commercial feed nutrition.

1.2.3 Carbohydrates

Carbohydrates are an important nutrient group in shrimp feeds as they are a main source of energy, and the cheapest form of energy to include into a supplementary feed (Davis, 2005). Carbohydrates are also important for the formulation of supplementary feeds as they assist in the binding of the feed, which makes pellets more durable and stable in water (Davis, 2005). A minimum dietary carbohydrate level of 25% is required for most species of shrimp. Lower percentages can interrupt growth and energy performance (Davis, 2005). Indeed, a study on juvenile *P. monodon* shrimp showed that growth and survival decreased with a carbohydrate percentage of less than 32% (Chuntapa et al., 1999). Carbohydrates are an essential form of energy for shrimp in commercial production, but they are also essential to the stability of the feed itself, making it another important consideration in a commercial supplementary feed.

1.2.4 Lipids

Lipid in shrimp feeds are a source of essential fatty acids, which maintain the integrity of cellular membranes and are important for many physiological processes (Mukhopadhyay et al., 2013). Lipids contain approximately twice the energy of carbohydrates and proteins, but the cost of ingredients high in lipids are more expensive than that of carbohydrates. Therefore lipids are included in shrimp feeds for essential fatty acids purposes only rather than used as a main source of energy (Davis, 2005). The minimum dietary lipid percentage is approximately 2% if the lipid percentage is any lower than 2% it has the potential to affect the growth and

the survival of the animal. This is because lipids are a part of cell membranes, they help with the absorption of vitamins A,D,E and K as well as help to regulate metabolic processes, hormones, moulting and growth (Davis, 2005; Nesara & Paturi, 2018). Sheen and Chen in 1992 reported that a diet containing lipids less than 8% for juvenile *P. monodon* reported in lower growth rates than 8 and 10% respectively (Boonyaratpalin, 1996; Sheen & Chen, 1992). Therefore, lipids have an important function in maintaining physiological processes and supplying energy for growth and survival.

1.2.5 Vitamin and Mineral Nutritional Requirements

In addition to protein, carbohydrates and lipids, vitamin C is known to aid in hydroxyproline and collagen formation in shrimp. A lack of vitamin C may lead to black spots, clear muscle tissue, a soft exoskeleton, weak connective tissues, damaged gill covers, slow growth, incomplete moulting's and high mortality rates (Boonyaratpalin, 1996). Minerals are also important for shrimp health, since they are involved in the structural components that make hard tissues, such as the shrimp exoskeleton and some soft tissue as well. Minerals, such as calcium, phosphorus and potassium aid in the function of osmoregulation, acid base balance and the production on membrane potentials (Boonyaratpalin, 1996; Davis, 2005). Nutritional requirements extend past the main three nutritional components. Therefore, a supplementary feed should include a mixture of suitable protein, carbohydrate, lipid and energy levels as well as an adequate addition of essential vitamins and minerals.

1.3 Current Commercial Feeds and The Need for Change

1.3.1 Commercial Feed

Current commercial feeds on the market are designed to provide shrimp with optimal nutrition such as adequate protein, carbohydrates, lipids, energy, vitamins and minerals for the best growth performance possible. There are many commercial shrimp feeds on the market that cater to all life stages of shrimp from companies such as Ridleys, Indica, Reed Mariculture, Cargill, CP Prima and BioMar. Although, there is limited information about feed ingredients and formulations creating difficultly in providing an in-depth review of the feeds. However, all complete commercial shrimp feeds currently contain fishmeal. Fishmeal is the most commonly used protein source for commercial shrimp feeds due to its high protein level, suitable amino acid profile, high digestibility, lack of anti-nutritional factors which have an effect on the nutrition uptake, availability and high palatability (Jackson, 2009). Fishmeal is made by cooking, drying and grinding complete fish or fish by-products to obtain a fish

powder called fishmeal (Saleh et al., 2022). However, fishmeal is an unsustainable ingredient and alternative plant and insect protein sources will likely become more prominent in future commercial feeds.

1.3.2 Sustainability

Sustainability has become a concept mentioned profusely around commercial aquaculture feeds within the last decade. The definition of sustainability is complex, as there are many considerations involved, such as the timeline of sustainability, human impacts, human wellbeing and environmental impacts and rejuvenation (Johnson et al., 2007). However, Johnston et al. (2007) described sustainability as relating to a method of harvesting or acquiring a resource so that the resource is not depleted or permanently damaged (Johnston et al., 2007). In relation to the sustainability of commercial shrimp feeds, specifically fishmeal, the main detrimental factors are the high environmental impacts of overexploitation of fish stocks, species and juvenile fish, overfishing, habitat degradation and by-catch. Indeed, Barlow (2003) found that approximately one-third of wild caught fish contributes to fishmeal production (Barlow, 2003). Myo et al., 2018 found that bottom trawling (Figure 3) provides up to 70% of the fish used for fishmeal in Myanmar, Thailand, Malaysia and Yangon (Myo et al., 2018). Bottom trawling is a known cause of habitat degradation, by-catch and overfishing which affects coral reefs, seagrass, sponge gardens, rock gardens, seahorses, cetaceans, reptiles, molluscs and juvenile fish (Stiles et al., 2010). Additionally, fishmeal is becoming a high cost ingredient, as the demand increases. Currently, fishmeal is the most expensive protein commodity in aquaculture feeds (El-Sayed & Abdel-Fattah, 2019). The use of fishmeal in aquafeed results in over exploitation of fish stocks (Hulefeld et al., 2017). Therefore, many alternative ingredients such as soybean meal, chickpea flour, black soldier fly larvae and mealworm meal are being researched and developed to provide more sustainable feeds for shrimp aquaculture.

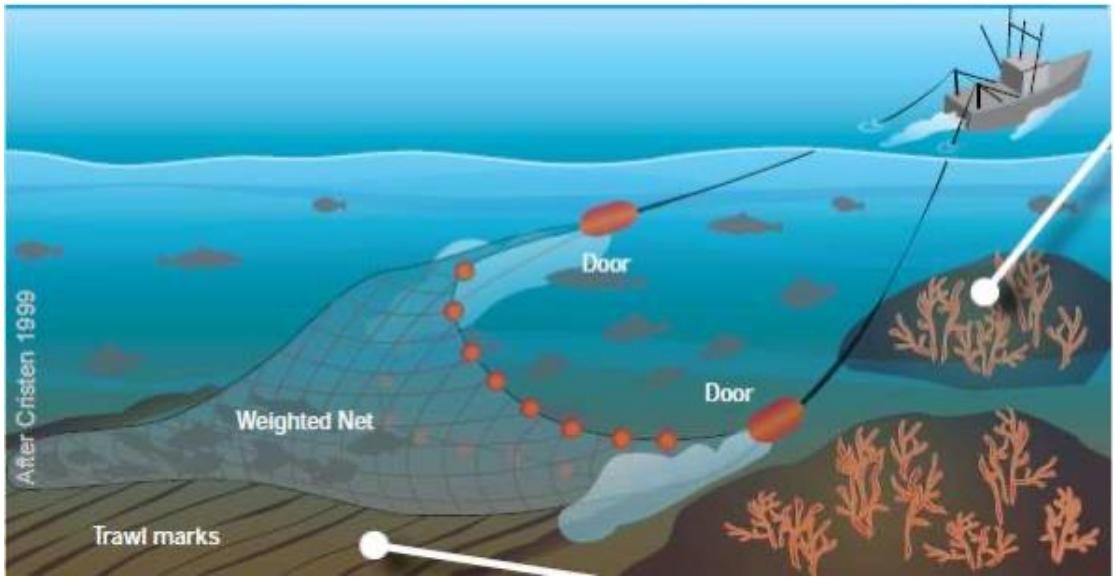

Figure 3: A diagram of bottom trawling (Stiles et al., 2010).

1.3.3 Mealworm Meal

Mealworm production requires less land area, water and energy than fish aquaculture, making them a sustainable alternative to fishmeal (Panini et al., 2017). Production of mealworms emits fewer greenhouse gases and ammonia than other protein alternatives such as fishmeal, soybean meal and poultry meal (Motte et al., 2019; Panini et al., 2017). Mealworms can be fed different types of organic waste for example expired feeds, food waste and organic plant matter (Ko et al., 2020; Ruschioni et al., 2020). Mealworms contain high amounts of protein, lipids and energy with a low ash content. Mealworms are also rich in essential amino acids, mono- and poly-unsaturated fatty acids, minerals and vitamins (Mastoraki et al., 2019; Motte et al., 2019). These properties are all highly suitable to support shrimp and their nutritional needs (Motte et al., 2019).

Shrimp have responded well to mealworm meal in previous studies and have the appropriate parameters for shrimp nutrition. Additionally, mealworms contain chitosan which has the potential to improve defence mechanisims and stress resistance in shrimp (Brol et al., 2021). Panini et al., 2017a found that *L. vannamei* were not affected by replacing fishmeal with mealworm meal from 25-100% in the shrimp diet, although there was a lack of methionine and an increase in lipid content in the feed. The addition of gelatine, alginate and chitosan in the feed could improve these parameters making mealworm more suitable for a replacement diet in shrimp aquaculture which can be achieved through encapsulation. Additionally, Choi et al., 2018 found that a 50% mealworm replacement resulted in higher live weight gain, higher specific growth rate, better feed conversion ratio and higher levels of immune markers. Moreover, Motte et al., 2019 found that insect meal has positive growth performance on

shrimp as well as the ability to improve immunity and disease resistance due to immune-enhancing compounds. Therefore, mealworm was chosen for this study due to the nutritional benefits, disease-resistant properties and the ability to reduce the environmental impact of a commercial aqua-feed (Mastoraki et al., 2019; Motte et al., 2019; Panini et al., 2017; Selaledi & Mabelebele, 2021).

1.3.4 Feed Waste

Shrimp farming produces high concentrations of nutrient waste due to the leaching and matrix erosion of commercially formulated feeds (Burford & Williams, 2001). Within commercial aquaculture, 30% of feed is not consumed and approximately 25% is excreted by the animal (Muqsith et al., 2019). Nutrient leaching can also occur when the solubility and moisture content of the feed is too high (Amran et al., 2021). However, leaching can also take place due to the unique feeding behaviours expressed by adult decapods, such as shrimp. Shrimp, are slow feeders, residing at the bottom of tank systems and are highly aggressive when handling feed. This combination sets challenges such as stability, for feed developers when formulating a high quality feed (Amran et al., 2021). Nutrient leaching of pelleted feeds can increase the amount of nitrogen and phosphorous within the system causing eutrophication (Fei, 2004). Elevated nutrient conditions can cause health problems by supressing the immune system and can increase mortality rates (Yang et al., 2018). Shrimp aquaculture is known for issues with disease as well as shrimp lacking adaptive immunity (Gao et al., 2016). Shrimp are developing diseases that are immune to antibiotics and are forming antibiotic resistance in bacteria (Seethalakshmi et al., 2021). When nutrients are high and community composition changes, pathogenic bacteria become dominant in the system, resulting in the proliferation of diseases. Yang et al., 2018 suggested that shrimp disease control should focus less on antibiotics and instead on finding a more sustainable system, which can be achieved by minimising nutrient pollution. Therefore, nutrient leaching of commercially formulated feeds can cause detrimental water quality parameters and lead to compromised immune systems in shrimp, causing a range of infections and diseases. One of the solutions to this is to change commercial feeds formulations in ways that reduce waste and increase stability. Encapsulation of entire commercialised feeds could be the solution to feed waste, leaching and water quality deterioration and the addition of immunity enhancing ingredients such as chitosan could improve shrimp immunity leading to less disease and higher survival rates.

1.3.5 Encapsulation

Encapsulation is the chemical and physical process of compartmentalising and isolating an ingredient, bioactive, amino acid or other nutrient and providing an internal environment that protects the nutrient inside from the external environment (Assesfa & Abunna, 2018). Additionally, encapsulation can comprise various chosen materials that can be used for palatability, solubility, pH balancing, added nutrition, health benefits and more (Assefa & Abunna, 2018; Dezfooli et al., 2019). The use of encapsulation has been applied for many decades. In the 1980s a study on supplementation was conducted by microencapsulating live feed to provide marine larvae with more sustainable, leach-free diets (Jones et al., 1984). However, encapsulation has developed significantly in terms of variability and useability. Some of the most recent studies conducted on encapsulation include supplementary microencapsulation systems for nutrient delivery, supplementary encapsulation of amino acids, encapsulation of supplemented live feeds, encapsulation of enzymes to assist in the digestibility of plant-based feeds and encapsulation of probiotics for shrimp using alginate and chitosan (Adilah et al., 2022; Ishthiaq et al., 2021; Mahotra et al., 2022; Uniyom et al., 2022). The most recent studies have been aimed toward bioactives, probiotics, supplementary nutrients, the health of aquatic species and reducing matrix erosion (Adilah et al., 2022; Dezfooli et al., 2019; Ishthiaq et al., 2021; Jaroensaensuai et al., 2022; Mahotra, 2022; Mahotra et al., 2022; Pulgara et al., 2021; Uniyom et al., 2022). However, research gaps still exist, especially with a complete encapsulated feed replacement that incorporates all of an animal's nutritional requirements rather than encapsulated additives alongside a pelleted feed.

The current body of research on encapsulation includes encapsulation for supplementation, bioactives, probiotic use and increased water stability. Encapsulation for supplementation and increased water stability however follows the same methodology which is problematic. The methodology is to incorporate microencapsulated beads into a powdered commercial feed which is then pelleted. This methodology can be seen in recent research on encapsulation with supplementary methionine, curcumin and egg yolk for added ascorbic acid (Bhoopathy et al., 2021; Jaroensaensuai et al., 2022; Mahotra, 2022). The issue with this methodology is that it enhances the exposure of the pelleted feed to seawater, a gap in the knowledge that is yet to be identified in other literature. Complete encapsulation of the supplement and the powdered feed has the potential to solve this issue which is a method that can be seen in the encapsulation of probiotics to prevent leaching and erosion by Masoomi Dezfooli et al., 2022, the entire probiotic and all of the necessary properties are encapsulated in a gelatine and alginate bead for the best protection against matrix erosion (Dezfooli et al., 2022). This method displayed by Masoomi Dezfooli et al., 2022 could be used as an encapsulation method and formula to

encapsulate an entire feed with supplements or probiotics added. To further improve all the current research, bioactives, probiotics, supplements and feed could be encapsulated together with gelatine and alginate coating. This would prevent leaching, matrix erosion and water quality deterioration which leads to increased absorption of all the targeted additives.

1.4 Thesis Aims

Based on an extensive literature review, there is a major research gap on finding more sustainable shrimp feeds that contain alternative sources of protein as substitutes for fishmeal. Such feeds should have consistent low matrices of erosion to minimise waste due to leaching, and to minimise environmental impacts due to increased nutrient levels in the water. To address this research gap, this thesis aims to investigate the use of insects as a source of protein in formulated and encapsulated feeds for shrimp cultivation. It is envisaged that the results of this thesis will contribute to more sustainable solutions for the shrimp aquaculture industry and could assist in the prevention of disease outbreaks, increased stock survival and overall health of shrimp.

The research objectives of this thesis are:

Aim One: To create a shrimp feed with desirable parameters (protein, carbohydrate, lipid and energy content) similar to current commercial feeds and to compare partial replacements of fishmeal with mealworm meal as a more sustainable ingredient.

Aim Two: To reduce the matrix of erosion of the feed by using innovative encapsulation technology and improving water quality parameters that lead to disease outbreaks, wastage, and nutrient leaching.

Aim Three: To trial the formulated and encapsulated feed on glass shrimp *Palaemon affinis* to assess survival, growth and nutritional benefits.

1.5 Significance

Fishmeal is a finite resource that is becoming more unsustainable every year with significant species declines, overfishing, habitat destruction and global change (Hall, 2011). Fishmeal is currently the most common ingredient in aquafeeds for its nutritional profile however, commercial feed made with fishmeal is unsustainable, costly, water-soluble, damaging to water quality and unsuitable for preventing disease. An aquafeed that solves these issues is a known necessity for the future of shrimp aquaculture globally. An alternative protein source such as mealworm meal combined with innovative encapsulation technology could be the

solution to all outlined issues. Mealworm meal is sustainable, requires less land area, fewer greenhouse emissions and is nutritionally adequate for shrimp requirements. Mealworms have proven to be successful in varying concentrations in other studies globally, however, on their own they do not provide a complete solution to the issues that shrimp aquaculture experiences. Encapsulation coats a powdered feed in a gel-like bead using innovative technology and ingredients to improve water solubility, leaching, matrix erosion, sustainability and disease prevention. Encapsulation has been successful in many studies to encapsulate probiotics and supplements, but it has never been used to encapsulate and create a feed replacement. This study used mealworm meal as a protein alternative and an encapsulation formula to create an innovative feed replacement. The encapsulation formulation includes gelatine and alginate for palatability and to prevent leaching, a powdered feed containing mealworm meal to assist in meeting shrimp nutritional requirements and sustainability, calcium carbonate and chitosan for increased health benefits and disease prevention. Mealworm meal and encapsulation together could solve the problems currently seen in shrimp aquaculture. There is potential for this feed to be trialled at New Zealand's shrimp aquaculture facility Huka Prawn park and exported to other facilities around the world which will contribute to sustainable solutions for the shrimp aquaculture industry and could assist in the prevention of disease outbreaks, increased stock survival and overall health of shrimp globally.

Chapter 2: Research Design and Methods

2.1 Experimental Diets

For the feeding trial, three encapsulated experimental diets were created in the chemistry lab at Auckland University of Technology and one commercial feed was produced by Ridleys™. The three encapsulated diets contained: fishmeal, mealworm, corn starch, corn flour and grape pomace (Table 3). The diets contained three different ratios of fishmeal to mealworm meal. Diet one (F70) contained 70% fishmeal and 30% mealworm meal for the protein component. Diet two (F50) contained 50% fishmeal and 50% mealworm meal and diet three contained 30% fishmeal and 70% mealworm meal. Each of the diets was created to have similar protein and energy although the carbohydrate and lipid content did vary due to mealworms having a higher lipid content than fishmeal. According Davis 2005 and Nesara & Paturi, 2018, it is important to maintain the protein level among the diets so that accurate comparisons can be made on growth and survival. Thus, the composition of the trial diets was created to have similar protein levels to reduce any bias between diets (Table 5; ((Davis, 2005; Nesara & Paturi, 2018)). Each of the experimental diets was encapsulated with innovative technology (Figure 4; ((Dezfooli et al., 2022)), to assist shrimp with the intake of feed with less nutrient waste. The commercial diet was unencapsulated to maintain the true nature of the diet so that it could be accurately compared to our experimental diets.

Table 1: Composition of ingredients within the four diets that were used in this experiment. Included are the ingredients necessary and the amount present in 100g of feed.

Diets	**Label**	**Fishmeal (g)**	**Meal Worms (g)**	**Corn Starch** (g)	**Corn Meal (**g)	**Grape** Pomace (g)	Total (g)
1	Commercial (UE)	N/A	N/A	N/A	N/A	N/A	N/A
2	F70 (E)	40.0g	20.5g	11.5g	26.0g	2.0g	100g
3	F50 (E)	28.0g	36.0g	10.0g	24.0g	2.0g	100g
4	F30 (E)	15.5g	52.0g	10.5g	20.0g	2.0g	100g

Key: (E) Encapsulated, (UE) Un-Encapsulated

Table 2: The protein percentage of fishmeal and mealworm and the ratio of fishmeal to mealworm within the diets.

Diets	**Label**	Fishmeal **(%)**	Mealworm **(%)**	Ratio of FM to MW
1	Commercial (UE)	100%	0%	0%
2	F70 (E)	68.94%	27.05%	2.55%
3	F50 (E)	48.50%	47.74%	0.98%
4	F30 (E)	27.10%	69.61%	2.57%

Table 3: Pre-calculated composition of protein, carbohydrate, lipid and energy that were used in the four diets for this experiment and the percentage of their presence in each diet based on the relevant literature.

Diets	**Label**	**Protein (%)**	**Carbohydrates (%)**	**Lipid (%)**	**Energy (kcal/kg)**
1	Commercial (UE)	~46.78%	~34.40%	~12.52%	UNKNOWN
2	F70 (E)	39.86%	37.98%	10.64%	4071.085 kcal/kg
3	F50 (E)	39.66%	35.77%	13.76%	4255.34 kcal/kg
4	F30 (E)	39.29%	33.59%	16.82%	4429.585 kcal/kg

2.1.1 Diet Formulation

Mealworm, grape pomace and corn starch required a drying or cooking process prior to formulation. The mealworms were oven dried in an oven in the Food Science Lab at Auckland University of Technology at 104°C for seven hours. Once the mealworms were dried, they were ground up in a Nutribullet blender until it turned into fine powder which was placed in a container in the fridge at 4°C. Selaledi & Mabelebele (2021) found that the effect of freezing and oven drying mealworms resulted in no significant difference in the nutrient, protein, and

amino acid content of the mealworm powder. Therefore, for convenience in this study, the mealworms were oven dried.

Grape pomace needed to be dried before formulation since it had a high moist content to start with. This ingredient was oven dried at 104°C for twelve hours. Once the grape pomace was dried, it was ground up with a Nutribullet into course powder and placed into a container in the fridge.

Corn starch was gelatinised by slowly adding water to the corn starch on top of the stove according to manufacturer's instructions and placed onto an oven tray in an oven at 104°C for three hours. The corn starch was removed from the oven and broken up into smaller pieces and placed back into the oven for an hour. The corn starch was then removed from the oven and ground up in a Nutribullet blender and placed back in the oven for an additional hour. Then, the ingredient was taken out and ground up again to create a very fine powder. Corn starch can sometimes be left uncooked in diets used in the aquaculture industry (Fu, 2005; X. Y. Wu et al., 2007). However, Aaqillah-Amr et al. (2021) suggested that gelatinisation of starch helps to improve feed digestibility in decapods.

Once all the ingredients were in a powder form and placed into appropriate containers, they were weighted out using a kitchen scale and placed into containers for each diet and mixed. A total of 200 grams of each diet were created. A homogenous sample (100g) was taken from each diet for protein, carbohydrates, lipid and energy analyses, and to ensure that the protein was similar across all diets.

2.1.2 Encapsulation of Diets

2.1.2.1 Bead Preparation

For the preparation of the encapsulated beads, a protocol was created by Sara Masoomi. Sara designed this protocol to encapsulate probiotics however, the protocol was taken and altered by encapsulating feed instead while maintaining the fundamental preparation protocol (Dezfooli et al., 2022). This protocol included a combination of biopolymers to develop double-layered capsules for encapsulating shrimp feed (Figure 4). The physicochemical properties of the developed capsules were characterised to minimise matrix erosion in seawater and maximise the palatability of the encapsulated feed.

The double-layer encapsulated beads were prepared in two stages, the first layer created the core of the bead which contained the chosen feed (F70, F50, F30), Alginate 2% w/v, Gelatine 4% w/v, dH_2O and calcium carbonate. To create the first layer, the ingredients were placed into a 500ml beaker on a stirrer at 280rpm to create a homogenous mixture and set to 60°C.

The second layer or “outer layer/shell” of the bead was created using Chitosan 0.4% w/s in 1% v/v lactic acid and Calcium Chloride 1.5M. The chitosan solution (layer 2) was poured into two 500ml beakers and placed onto two stirrers at 150rpm. The alginate, dried feed ingredients (F70, F50, F30) and gelatine solution (layer 1) was first created and turned into a thick gel-like liquid, once this solution reached 60°C it was removed from the stirrer and left until it reached 30°C. Once layer 1 had cooled, it was added slowly dropwise into the chitosan solution (layer 2). The reaction of layer 1 meeting layer 2 created small encapsulated beads. The chitosan layer coats the gel-like liquid of the gelatine layer and turns it into an encapsulated bead (figure 4).

Once the encapsulated feed (beads) had been created, they were left to sit in the chitosan solution for 40 minutes. The beads were then removed by pouring them into a standard kitchen sieve and rinsing with dH_2O. Once they were rinsed and gently patted dry with a paper towel, they were dried slowly at 70°C for 8 hours for storage until they were used. Drying was not found to disrupt any of the ingredients, nutrients or polymers and was therefore chosen as the best and most practical method for this study.

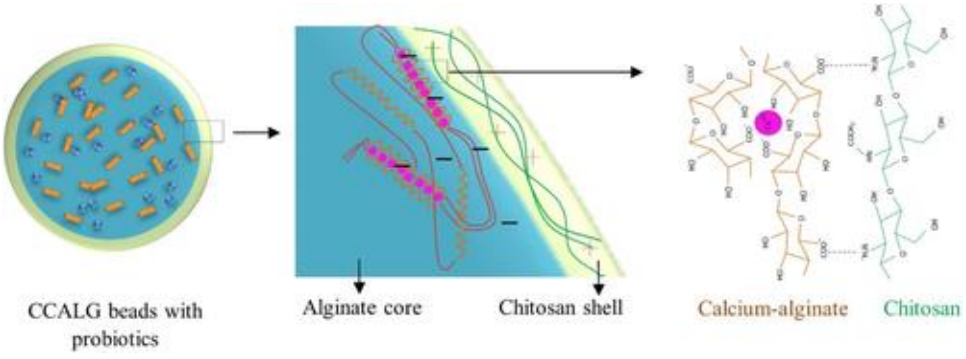

Figure 4: The diagram shows Calcium alginate Chitosan coated beads with probiotics and the formation of chitosan-coated alginate polyelectrolyte complex (Dezfooli et al., 2022)

2.1.3 Encapsulation and Characterisation

While analysing the efficiency, formulation and characterisation of the encapsulated beads, ground-down commercial feed was used as the feed component inside of the beads. The commercial feed was used inside of layer 1 initially to test different variations of alginate to gelatine. This was to ensure that the ratios of layer 1 to layer 2 would initiate the correct reaction of the solutions to create encapsulated beads. Some formulations of layer 1 created

tubes rather than spherical beads and tweaking of the solutions needed to be made to ensure the encapsulation formula was suitable before creating the three mealworm meal encapsulated diets (F70, F50 and F30).

The determination of encapsulation yield, sinking rate, palatability, water absorption, and matrix erosion were performed on encapsulated beads containing commercial feed rather than the three mealworm-based encapsulated diets. This was done to enable a more accurate comparison of the encapsulation aspect alone, while excluding the influence of other feed ingredients. Furthermore, insufficient time was available to conduct these tests on the three mealworm encapsulated beads.

The calculation of efficiency and characterisation of the encapsulated beads was performed using the below equations in Microsoft Excel.

2.1.3.1 Encapsulation Yield

To calculate the yield of the encapsulation process, Equation 1 was used. The mass of the dried beads obtained is the encapsulated feed once it has been oven dried and the mass of dried polymeric ingredient mixture is all dried ingredients that went into making that encapsulated feed in solution one including alginate, gelatine, calcium carbonate and the original dried feed ingredients.

$$\textit{Encapsulation Yield (\%)} = \frac{M1}{M2} X100 \quad (1)$$

Equation 1: Encapsulation Yield (%) Where M1 is the mass of dried obtained beads (g) and M2 is the mass of dried polymeric-ingredient mixture (g).

2.1.3.2 Sinking Rate

The sinking time of beads (n = 10) was measured by placing individual beads in a 50ml glass measuring cylinder with a 19.5cm height and a 2.6cm diameter that was filled 17cm high with standard seawater at room temperature (Dezfooli et al., 2022). The height of the seawater in the cylinder was 17cm. The time that an individual bead sunk from the surface of the seawater

at 17cm to the bottom of the cylinder was recorded as its sinking time. The measurements were taken for 10 individual beads.

2.1.3.3 Palatability

Palatability was conducted by comparing the amount of commercial shrimp feed (CF) consumed compared to the amount of encapsulated commercial shrimp feed (ECF) consumed (n = 15 each) (Dezfooli et al., 2022). Two identical 16-L tanks, light blue in colour with a 294mm height, 325mm width and 334mm length filled with 13L of seawater at 16° were used for this test. The test was done in the AUT aquaculture lab with the same conditions as the experiment which consisted of natural daylight lamp settings of 12H day 12H night cycles. Shrimp were chosen at random to conduct this test. Shrimp were starved for 24 hours prior to the palatability test. 15 CF and 15 ECF beads and pellets were fed to 30 shrimp. The tank was checked for any leftover CF/ECF after 1 hour and the amount of uneaten CF/ECF beads or feed pellets remaining was counted. Palatability testing was replicated three times at 10am on alternating days to give 24 hours of starvation in-between.

2.1.3.4 Water Absorption

To determine the water absorption of the encapsulated beads, wet and dried beads were submerged in standard seawater at six different time points and their swollen weights were analysed (Afzali & Boateng, 2022). Dried beads (0.5g) were separated into three replicate 100ml beakers with a 5cm diameter and 7.2cm height with 30ml seawater at room temperature. The beads were then weighted at different time points to calculate the water absorption rate of the swollen beads at and among times: 1hr, 2.5 hours, 4 hours, 6 hours, 24 hours and 48 hours. To weigh the beads at the varying time points, the seawater was gently poured into an empty beaker leaving the beads at the bottom of the current beaker, they were then gently removed and placed briefly onto a paper towel to remove any residual water and then placed into a plastic weighing dish and weighed. The water was removed and replaced between each weighing time. The percentage of water absorption was determined using Equation (2).

$$\textit{Water Absorption (\%)} = \frac{(W1 - W0)}{W0} X100 \quad (2)$$

Equation 2: Water Absorption (%) Where W1 is the weight of swollen beads after time submerged in seawater and W0 is the initial weight of the beads before submergence in seawater.

2.1.3.5 Matrix Erosion

To analyse the stability of the encapsulated feed in seawater, beads were analysed after incubation in seawater after 24 and 48 hours (Dezfooli et al., 2022). Dried encapsulated beads (0.5g) were weighed and placed into three replicate 100ml beakers, containing 30ml of seawater at room temperature. This was compared to the current commercial feed. After 24 and 48 hours the feeds were removed from the beakers, gently patted dry with paper towels, placed into plastic weighing dishes and dried at 70°C for 7 hours. The feed was then removed from the oven and weighed. The dried weight of the feeds was measured and the percentage of matrix erosion in the feed was determined using Equation (3).

$$\textit{Matrix Erosion (\%)} = \frac{(W0 - W1)}{W0} X100 \quad (3)$$

Equation 3: Matrix Erosion (%) Where W1 is the dried weight of the feed after incubation in seawater and W0 is the initial dry weight of the beads before submergence in seawater.

2.2 Feeding Trial

2.2.1 Collection of Experimental Animals

The collection of *Palaemon affinis* took place at the Shore Road Reserve Estuary located in Remurea, Auckland, New Zealand (-36.8644129442, 174.79192355) (Figure 5). The method of collection followed protocols from the fish department team leader Kim Evans at Kelly Tarltons Auckland aquarium as the safest and most effective method of collecting *P. affinis* (Kelly Tarltons, 2021). During a preliminary collection, 60 individual *P. affinis* were collected in under 30 minutes. Once the required number of approximately 180 *P. affinis* were collected, they were transported in buckets containing brackish estuarine water to the Auckland University of Technology.

Figure 5: The collection point of the experimental animals: Shore Road Reserve Estuary located in Remurea, Auckland, New Zealand.

2.2.2 Culture Water and Maintenance Conditions

The feeding experiment took place in the aquaculture laboratory, Auckland University of Technology, Auckland. Sixteen replicate tanks were set up prior to the collection of *P. affinis.* The 16-L tanks were light blue in colour with a 294mm height, 325mm width and 334mm length. Each tank was filled with 13L of standard salt water. An airline was provided to each tank for an adequate oxygen supply. The temperature of each system was maintained between 14 and 16°C for the duration of the experiment. The light intensity and photoperiod were 12H Light (6am – 6pm) and 12H dark cycle (6pm – 6am) to mimic conditions in the shrimp natural habitat (Lim et al., 1997; Wang et al., 2020). Water exchanges and tank cleaning occurred three times weekly to reduce stress from water quality parameters. Water parameters were tested periodically throughout the experiment, this included a standard salt water quality testing kit from API Marine for ammonia, nitrites, nitrates, and pH.

2.2.3 Feeding Trial

Sixteen tanks were used for the feeding trial with ten shrimp in each tank. Shrimp were fed one of four treatments diets: CF, F50, F70 and F30 four replicates, 10 shrimp per replicate and diet treatment and 160 shrimp across the experiment. Before the trial commenced, shrimp were acclimatised by placing them into a large clear glass tank with salt water slowly added over several hours. Once shrimp were acclimatised, wet weight and total body length measurements (TBL) were recorded and shrimp were placed into their replicate tank system (Day, 2001). Length measurements were analysed with ImageJ, a measuring software where total body length measurements of the shrimp were taken in cm increments (Plichta et al., 2021). Shrimp

were fed a feeding ratio that equated to 10% of their body weight once daily. Any uneaten food was removed at the time of the next feeding (Ettefaghdoost et al., 2018).

2.3 Data Collection

2.3.1 Uneaten Food & Faecal Matter Collection

To collect the uneaten feed and faecal matter a siphon was used with two sieves, one 150μm sieve for collecting the uneaten food and one 80μm sieve for collecting the finer faecal matter particles (Figure 6). Once collected, the uneaten food and faecal matter for each tank were placed into a corresponding labelled petri-dish and placed into an oven at 35°C until the next collection date. Once the uneaten feed and faecal matter were dried, they were removed from the oven and weighed. The dried faecal matter for each tank was transferred to another labelled petri-dish and kept dry in a cool place until proximate analysis for the protein, carbohydrate and lipid content could be performed. The faecal matter of shrimp is encased in a peritrophic membrane which enables the faecal matter to stay intact and further prevents nutrient leaching which was helpful upon the proximate analysis (Figure 7 ((Panini et al., 2017)).

Figure 6: Collection of the uneaten food and faecal matter with a siphon, two sieves, one 150um sieve and one 80um sieve and a bucket to collet any unwanted seawater.

Figure 7: Faecal matter of shrimp encased in a peritrophic membrane under a microscope at 10X measuring 644.94 µm.

2.3.2 Growth Performance

To analyse the growth parameters of the shrimp, wet weight, length measurements (TBL) and moults were used. At the beginning and at the end of the experiment, wet weight and length measurements were taken whereas moults were recorded during the entirety of the experiment.

2.3.2.1 Weight Measurements

To weigh the shrimp, individuals were scooped out of their tank system using an open/close method tea strainer to reduce the risk of appendages getting stuck or tangled in a net. Wet weight was taken by placing the animal into a bucket with 150mls of seawater on a tared precision laboratory balance with a 0.001g accuracy scale and the measurement was recorded. To ensure this method worked for this specific animal, dry weights were taken from 15 shrimp and compared with the wet weighing method. Since the results were the same for each individual shrimp between the two methods, the wet weight was used for this study to reduce the stress on the animals and avoid jumping.

2.3.2.2 Length Measurements using ImageJ

Length measurements were recorded by placing a 15cm ruler on the bottom of the tank, ensuring that all shrimp were situated on the bottom of the tank and a photo was taken using an iPhone 13 camera. The images were then uploaded to ImageJ, a measuring software and total body length (TBL) measurements of the shrimp were taken in cm increments (Plichta et al., 2021). The data were then transferred to excel for further analysis.

2.3.2.3 Moults & Survival

Moults were recorded during each data collection day. Before the collection of uneaten food and faecal matter, the tanks were examined for any moults. The moults were recorded when a half or a full moult was found in a tank. If two half moults were recorded in the same week, this would be changed to a full moult in the data sheet.

2.3.2.4 Analysing Growth Performance

Growth parameters were analysed by total moults, specific growth rate (SGR), mean weight gain (%), mean TBL growth (%) and Survival (%) which were calculated with the following equations:

Specific growth rate (SGR) was performed on the average weight and length of shrimp in each tank. Initial weight was adjusted to account for the number of shrimp post/mortalities before calculating weight change by using Equation 4.1. SGR was performed in excel using Equation (4.1).

$$\textit{Adjusted Initial Weight} = \frac{\textit{Initial Weight Change}}{\textit{Initial Number of Shrimp}} \textit{ X Final Number of Shrimp} \quad (4.1)$$

$$\textit{SGR (\% per day)} = 100 \text{ X } (W2 - W1) / T \quad (4.2)$$

Equation 4.2: Specific Growth Rate (SGR) Where W2 is the final average weight/length of the shrimp in each tank, W1 is the initial average weight/length of the shrimp in each tank and T is the experiment time in days (Du & Niu, 2003; Fóes et al., 2016).

Weight Gain was determined in excel using Equation (4.1 followed by 5)

$$Weight\ Gain\ (\%) = \frac{W2 - W1}{W1} X100 \quad (5)$$

Equation 5: Weight Gain (G) Where W2 is the final average weight of the shrimp in each tank and W1 is the initial average weight of the shrimp in each tank (Sudaryono et al., 1995).

Total Body length (TBL) growth was determined in excel using Equation (6).

$$TBL\ Growth\ (\%) = \frac{L2 - L1}{L1} X100 \quad (6)$$

Equation 6: TBL Growth (%) Where L2 is the final average TBL of the shrimp in each tank and L1 is the initial average length of shrimp in each tank (Sudaryono et al., 1995).

2.3.3 Proximate Composition

2.3.3.1 Diets, Faecal matter and Whole-Body Sample Preparation

Analysis of the four diets used for the growth trial were analysed for protein, carbohydrate and lipid content. To do this, the three encapsulated diets containing mealworm were analysed before the encapsulation process and the commercial feed was ground down from its pelleted form into a fine powder to be analysed.

The faecal matter of shrimp from each diet treatment was analysed for protein, carbohydrate and lipid content. The dried, ground faecal matter from each was then used for further analysis (Hawkins et al., 1989)

The whole-body of the shrimp from the replicate tanks for each of the diet treatments were starved for 24 hours, analysed for final weight and TBL and then freeze-dried for further analysis (Liu et al., 2012). The shrimp from each of the tanks were placed into 50ml centrifuge tubes with the tank label written on the side and placed into the freezer at -18°C. Three days after the shrimp had been frozen, they were removed from the freezer and the caps of the centrifuge tube were removed. Holes were put into the top of the tube caps to prepare the tubes for the freeze dryer. Once the holes were drilled into the caps, the tubes were put into glass jars and placed into the freeze dryer. Shrimp were left to freeze dry for 72 hours (Lim & Dominy, 1990). Once shrimp were dry, the shrimp from each tank were placed into a mortar and pestle and ground down into a powder to use for analysis.

2.3.3.2 Protein, Carbohydrate and Lipid Proximate Analysis

2.3.3.2.1 Protein Analysis

The protein content of the diets, faecal matter and whole-body shrimp was determined using a modified Bradford colorimetric protein assay. The Bradford assay is based on the ability of the dye to bind to amino acid residues in protein molecules (AOAC, 2019; Becker et al., 1996). The assay was modified by Seyedehsara Masoomi Dezfooli at Auckland University of Technology to enable the assessment of protein in diets, faecal matter and whole body shrimp. The modification involved specific dilution tables based on the assumed protein level and the type of matter analysed. The protocol required 10mg of dried sample and recommended three replicates for each analysis. The multi-well plate format was used, with bovine serum albumin (BSA) powder as the standard and Bradford Reagent as the reagent. The protocol involved filling the multi-well plate according to the specified procedure, followed by spectrophotometric analysis using a spectrometer machine. The resulting data was then analysed in Microsoft Excel, with a standard curve created using the absorbance values of the BSA standards and the reagent. The absorbance values of the samples were recorded and adjusted for dilution factors, and the percentage of protein in each sample was calculated based on the standard curve.

2.3.3.2.2 Carbohydrate Analysis

To determine the carbohydrate content in diets, faecal matter, and whole-body shrimp, a modified Anthrone principle was employed. The Anthrone method is a colorimetric assay that relies on the reaction between a dye and the total sugars in a sample, similar to the protein assay (Sapkota, 2020). The modified method developed by Seyedehsara Masoomi

Dezfooli and Brooke Samantha Kyle at Auckland University of Technology included dilution tables for each sample type, based on the expected carbohydrate levels. Each sample required 30 mg of material, and triplicate analyses were recommended. The protocol involved using a multi-well plate, glucose as a standard, and "Anthrone Reagent" as the dye reagent. After filling the multi-well plate according to the protocol, the plate was analyzed using spectrophotometry, and the results were recorded and analyzed using Excel. A standard curve was generated using glucose standards, and the absorbance values of the samples were adjusted for dilution factors to calculate the percentage of carbohydrates in each sample. The modified Anthrone method was precise, accurate, and allowed for reproducible results.

2.3.3.2.3 Lipid Analysis

A method was developed by Seyedehsara Masoomi Dezfooli at Auckland University of Technology to analyze the lipid content in diets, faecal matter, and whole-body of shrimp. This method is based on the Bligh and Dyer method from 1959, which involves the extraction of lipids from a cell suspension using chloroform and methanol, as described by Kumar et al. in 2015. The updated protocol is used to determine the total lipid content in a sample. To perform the analysis, 30mg of sample is required, and three replicates are recommended for each sample. The sample is mixed with chloroform, methanol, and dH20, and the resulting mixture is separated into two layers. The chloroform layer is then transferred to another centrifuge tube and dried using nitrogen gas until only the lipid remains. The weight of the centrifuge tube is measured before and after the extraction process, and the initial weight is subtracted from the final weight to calculate the total lipid content. The resulting data is then transferred into Excel for further analysis using Equation (7).

$$Lipid\ (\%) = \frac{W1 - W2}{W2} X100 \quad (7)$$

Equation 7: Lipid (%) Where W1 is the initial weight of the centrifuge tube and W2 is the second weight of the centrifuge tube with the lipid inside.

2.3.4 Energy

Energy was calculated in excel using the Atwater system where specific caloric values are assigned to each macronutrient: protein: 4 kcal.g, carbohydrate: 4 kcal/g and lipid:

9 kcal/g. This was used to calculate the total energy value of the four diet treatments (Merrill & Watt, 1955; Southgate & Joint, 1981)

2.3.5 Feed Utilisation

Feed given to each tank during the feeding trial was calculated at 10% of tank body weight per day and uneaten feed was collected to calculate feed utilisation. This was done by analysing Feed Ingested (g), the total feed ingested over the feeding trial. This equation was modified slightly to suit the data of this study from two Feed Ingested equations previously outlined by Azhar et al., 2021 and Wu et al., 2020. Feed Conversion Ratio (FCR) which is calculated to see what the conversion is of feed consumed to weight gain. The lower the ratio, the more effective that feed is at providing weight gain and Protein Efficiency Ratio (PER) which is calculated to find the conversion of protein consumed to weight gain. The higher the ratio, the more effective the protein in that feed is at providing weight gain. Additionally, when analysing the data matrix erosion of each diet treatment was added to Feed Ingested for accuracy, as shown in Equation (8).

$$Feed\ Ingested\ (G) = \quad FG - (ME \times FG) - FU \tag{8}$$

Equation 8: Feed Ingested (G) Where FG is the feed given, ME is the matrix erosion rate of the feed and FU is the feed uneaten (Azhar et al., 2021; X. Wu et al., 2020).

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Feed\ Ingested\ (g)}{Gain\ in\ shrimp\ weight\ (g)} \tag{9}$$

Equation 9: Feed Conversion Ratio (FCR) Where feed ingested is calculated as the total feed ingested by shrimp during the feeding trial and gain in shrimp weight is the total weight gained by shrimp per tank (Binalshikh-Abubkr & Hanafiah, 2022; Fóes et al., 2016).

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{Gain\ in\ shrimp\ wet\ weight\ (g)}{} \tag{10}$$

Protein intake (g)

Equation 10: Protein Efficiency Ratio (PER). Where gain in shrimp weight is the total weight gained by shrimp per tank during the feeding trial and protein intake is the protein (g) in the total amount of food ingested by shrimp per tank during the feeding trial (Binalshikh-Abubkr & Hanafiah, 2022).

To calculate the survival of shrimp within each of the diet treatments the below equation was used to analyse the survival percentage shown in Equation (11).

$$Survival\ (\%) = FS / IS \ \ X100 \tag{11}$$

Equation 11: Survival (%) Where FS is the final number of shrimp per diet treatment and IS is the initial number of shrimp per tank (Sudaryono et al., 1995).

2.3.6 Statistical Analysis

To ensure that all data sets were normally distributed, the Shapiro Wilk test was employed. Once it was confirmed that the data sets were indeed normal, the diet treatments were compared to one another as well as to growth, proximate composition, and feed utilization using a one-way analysis of variance ($p < 0.05$). In cases where the ANOVA showed significant differences, a Tukey test was performed at a 95% confidence interval ($p < 0.05$) to determine which group means had a significant difference (Binalshikh-Abubkr & Hanafiah, 2022). Diet treatments were categorized as independent variables while growth (weight/length), proximate composition (protein, carbohydrates, lipids), and feed utilization (uneaten feed) were categorized as dependent variables. For proximate analysis, statistical analysis was performed using Microsoft Excel.

Additionally, RStudio was used for linear model regressions to see if there was a relationship between certain variables. The weight and length data contained some mortalities creating more complexity with the inability to compare individual animals, therefore averages of weight (g) and length (cm) per replicate tank were compared to other variables in RStudio.

Chapter 3: Results

3.1 Encapsulation And Characterisation

3.1.1 Encapsulation Yield

The dried weight of the encapsulated beads generated was 50g and the mass of the dried polymeric feed mixture was 62.5g indicating an encapsulation yield of 80%.

3.1.2 Sinking Time

The average±SD sinking rate of dried encapsulated beads was 3.24±0.46 seconds .

Table 4: Sinking rate of dried encapsulated beads

Encapsulated Bead	Sinking (Seconds)	Average	Standard Deviation
1	3.29	3.24	0.46
2	3.53		
3	3.76		
4	3.23		
5	3.54		
6	3.54		
7	3.18		
8	2.89		
9	2.96		
10	2.24		

3.1.3 Palatability

A one-way ANOVA indicated that there was no statistically significant difference in palatability between the commercial and encapsulated feeds ($F_{(1, 4)} = 1$, $p = 0.37$). Observations of the shrimp indicated that the animals approached the encapsulated and commercial feed with similar frequency, with no obvious preference.

Table 5: Palatability test on *P. affinis* using 30 commercial feed pallets and 30 encapsulated beads. The numbers below are individual pallets/beads that were eaten out of the total number given.

Palatability	Commercial Feed	Encapsulated Beads
1	15	15
2	15	15
3	15	13

3.1.4 Water Absorption

Results indicate that the encapsulated dried beads containing commercial feed absorb water and reach approximately 95% absorption at the 24-hour mark. After 48 hours, the beads start to experience water loss as indicated by a 92.26% reading at 48 hours (Figure 8).

A one-way ANOVA indicated a statistically significant difference between time and the amount of water uptake. The time periods that signified significant differences when further analysed using Tukey's HSD test of multiple comparisons are outlined in the graph below (Figure 8).

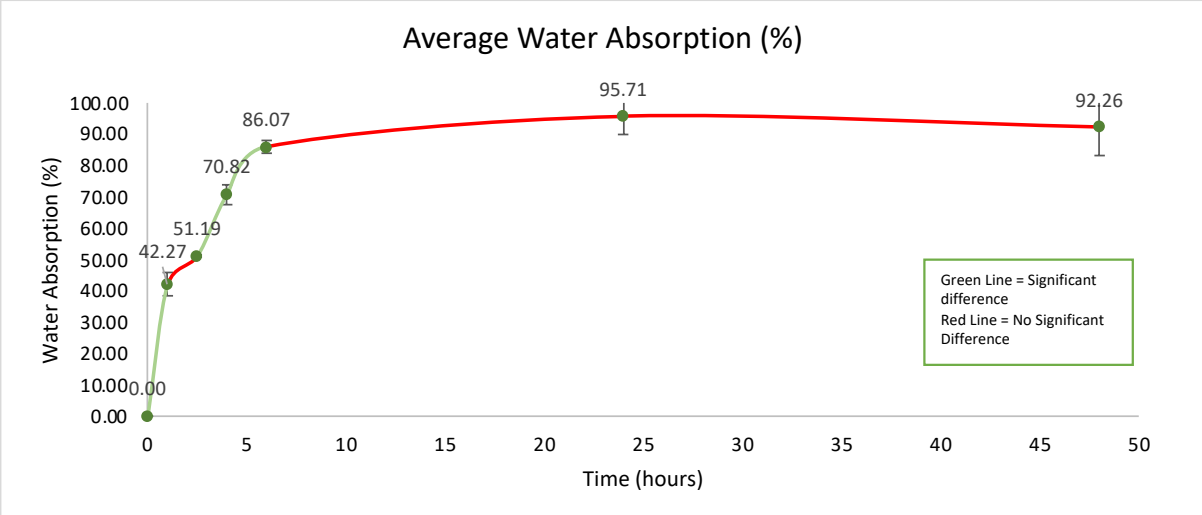

Figure 8: The average water absorption (%) of dried encapsulated beads across different time points from 0 to 48 hours after submergence.

3.1.5 Matrix Erosion

The results from the one-way ANOVA indicate that there is a statically significant difference between the matrix erosion rates of the diets ($F(3, 8) = [56.94]$, $p = 9.678E^{-06}$). A Tukey Test

revealed that the matrix erosion rate was significantly different between the commercial feed at 24 hours and the encapsulated feed at 24 hours (p = [0.000048], 95% C.I = [4.245, 11.259]). However, there was no significant difference between the commercial feed at 48 hours and the encapsulated beads containing commercial feed at 48 hours (p = [0.5134]). These results are displayed in figure 9.

To further examine the matrix erosion of the feed and the impact each feed has on water quality parameters, seawater was tested from tanks containing the encapsulated feed and the commercial feed after 48 hours. Seawater was examined for ammonia, nitrate, nitrite and pH. Nitrite levels for the tank containing the commercial feed pallets and the encapsulated feed pallets were under 0.25ppm, nitrate was under 5.0ppm and pH was not in the high range colour pallet displayed by the API saltwater master test kit card. However, ammonia levels were significantly higher in the seawater from the tank containing the commercial feed showing an ammonia reading between 0.50 and 1.0ppm in comparison to the ammonia level in the seawater from the tank containing the encapsulated feed between 0 and 0.25ppm (Figure 10).

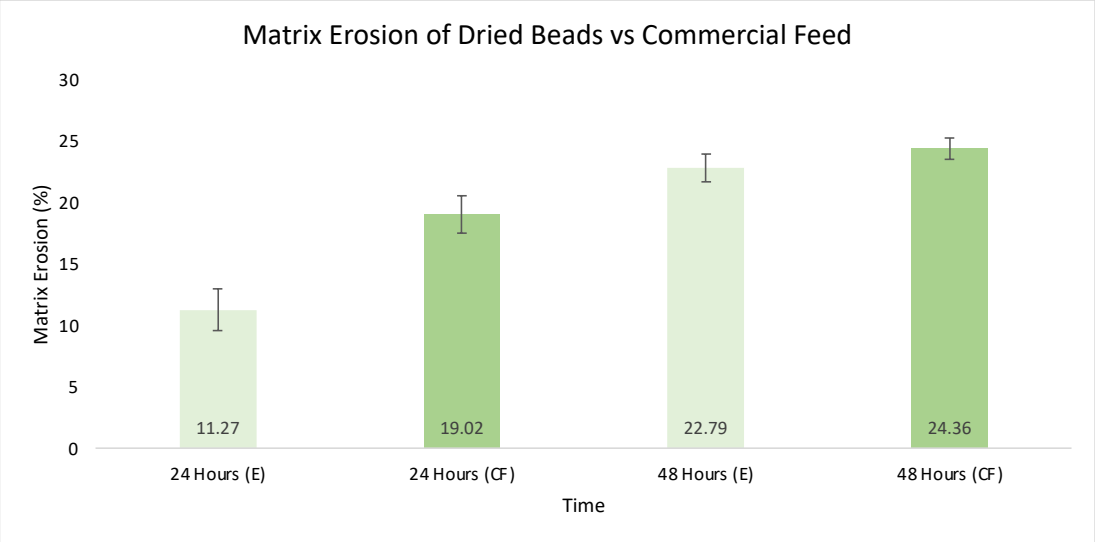

Figure 9: The average matrix erosion rate (%) of dried encapsulated beads compared with commercial shrimp feed at times: 24 hours and 48 hours with error bars representing standard deviation.

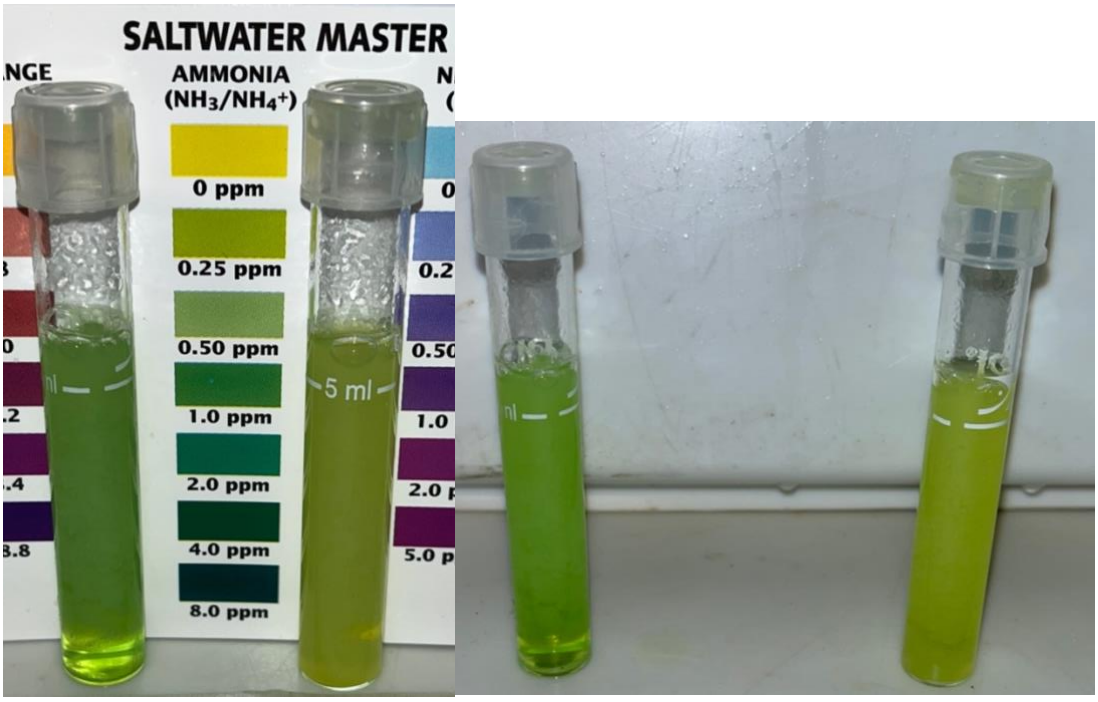

Figure 10: The ammonia level according to the 'SALTWATER MASTER TEST KIT' by API Marine on the seawater taken from 'CF' Commercial Feed seawater and the encapsulated diet. CF/Commercial feed is on the left in both pictures and the encapsulated feed is on the right.

3.2 Growth Performance

3.2.1. Weight Gain and Specific Growth Rate

The shrimp in the commercial diet treatment and F70 treatment had a mean weight gain of 13.33% across the feeding trial, shrimp from the F50 diet treatment had a mean weight gain of 23.08% and shrimp from the F30 diet treatment had a mean weight gain of 33.33% (Table 6). A one-way ANOVA exhibited no significant difference between the mean weight gain of shrimp per tank $F(3, 12) = [3.053]$, $p = 0.07$). Additionally, a one-way ANOVA exhibited no significant difference between SGR % and the diet treatments $F(3, 12) = [2.475]$, $p = 0.11$).

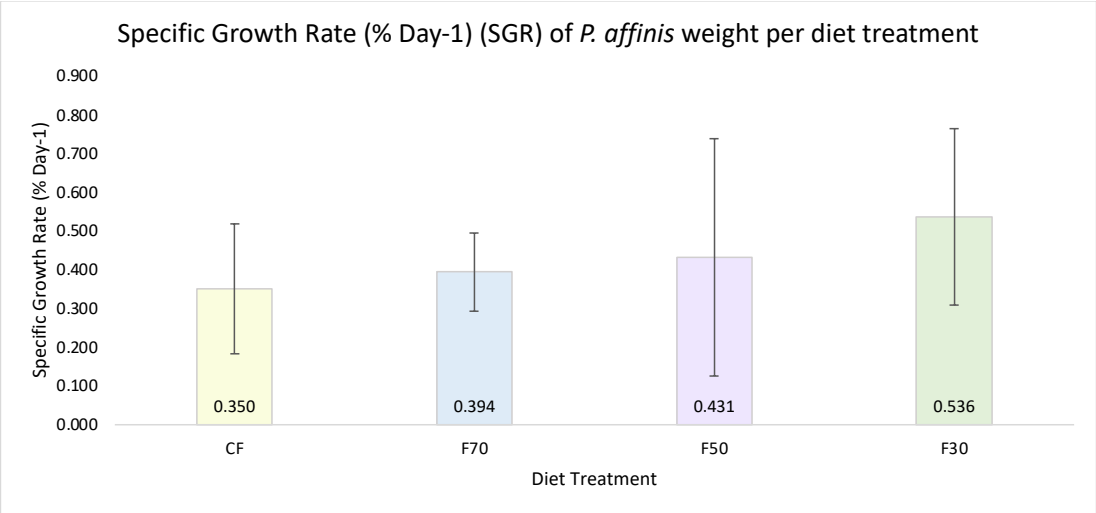

Figure 11: The Specific Growth Rate of *P. affinis* weight (g) on average per day per diet treatment for the duration of the feeding trial.

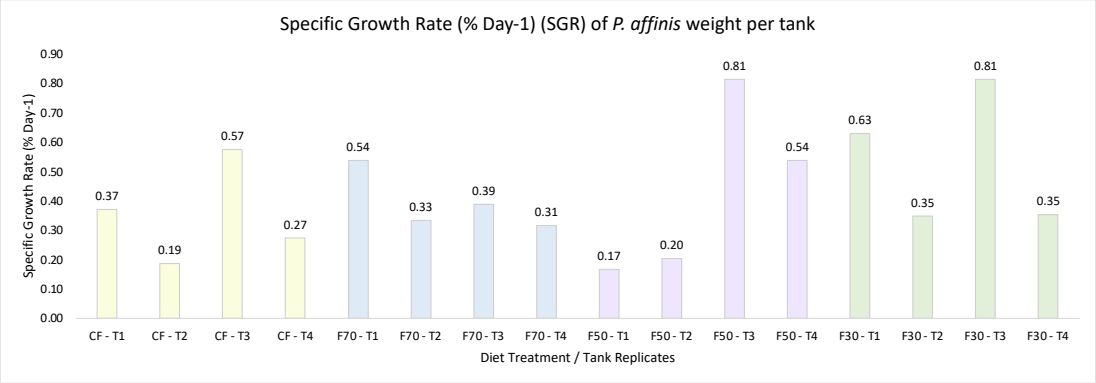

Figure 12: The Specific Growth Rate of *P. affinis* weight (g) on average per day per replicate tank for each diet treatment for the duration of the feeding trial.

R-studio was used to create linear models of the relationship of weight change (equation 4.1). In R-Studio, weight change was compared to the following parameters: diet treatment, amount of food eaten, protein, carbohydrate and lipid content (in diets and whole-body shrimp), tank replication and total moults. There was no significant relationship between the diets and weight gain.

The only significant relationship found was between lipids in the diets and bodies of shrimp with weight change . Lipid content in the diets significantly predicted weight change in shrimp ($\beta = 2.098^{E-04} \pm 9.719^{E-05}$, $t_{14} = 2.159$, $p < 0.04$). Similarly, lipid content in the whole body of shrimp from the different diet treatments significantly predicted weight change in shrimp ($\beta = 0.00003 \pm 0.0001$, $t_{14} = 2.288$, $p < 0.03$).

Table 6: Growth performance of *Palaemon affinis* based on initial and final weight (g).

Average Weight Gain Performance	CF	F70	F50	F30
Initial Weight (g)	0.15 ± 0.05	0.15 ± 0.04	0.13 ± 0.06	0.12 ± 0.05
Final Weight (g)	0.17 ± 0.06	0.17 ± 0.05	0.16 ± 0.07	0.16 ± 0.05
Weight Change (g)	0.02 ± 0.011	0.02 ± 0.005	0.03 ± 0.018	0.04 ± 0.007
Mean Weight Gain (%)	13.33	13.33	23.08	33.33
Specific Growth Rate (%)	0.0003	0.0313	0.0469	0.0625

3.2.2 TBL Growth and Specific Growth Rate

The commercial diet had the lowest TBL growth rate at 5.81% overall, the best TBL growth rate was F50 at 13.03% (Table 7). A one-way ANOVA found that there was no statistically significant difference between TBL growth and diet treatment F(3, 12) = [1.233], p = 0.34). R-Studio was used to create linear models of the relationship of TBL growth. TBL growth was compared to the following parameters: diet treatment, amount of food eaten, protein, carbohydrate and lipid content (in diets and whole-body shrimp), tank replication and total moults. There were no significantly significant relationships between any of the variables tested and TBL growth.

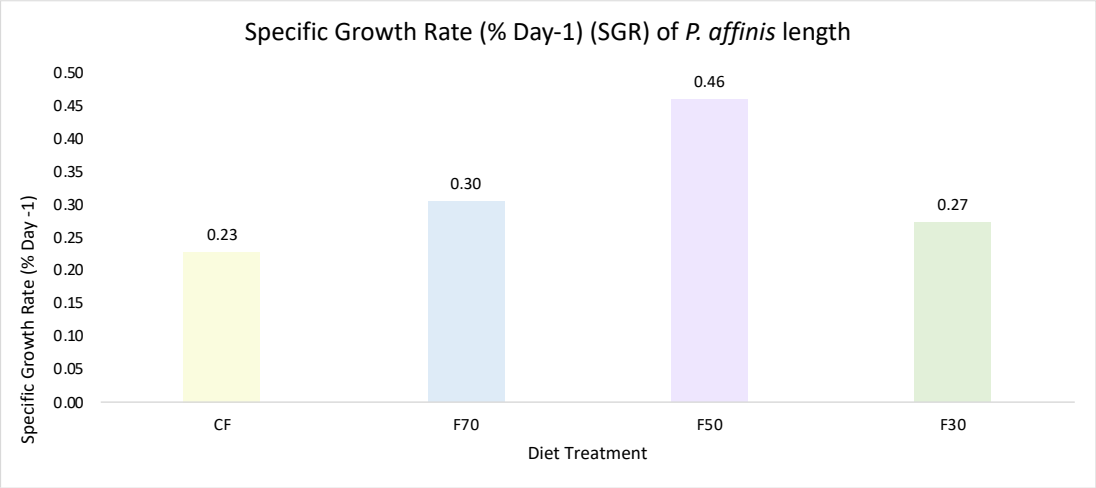

Figure 13: The Specific Growth Rate of *P. affinis* length (cm) on average per day per diet treatment for the duration of the feeding trial.

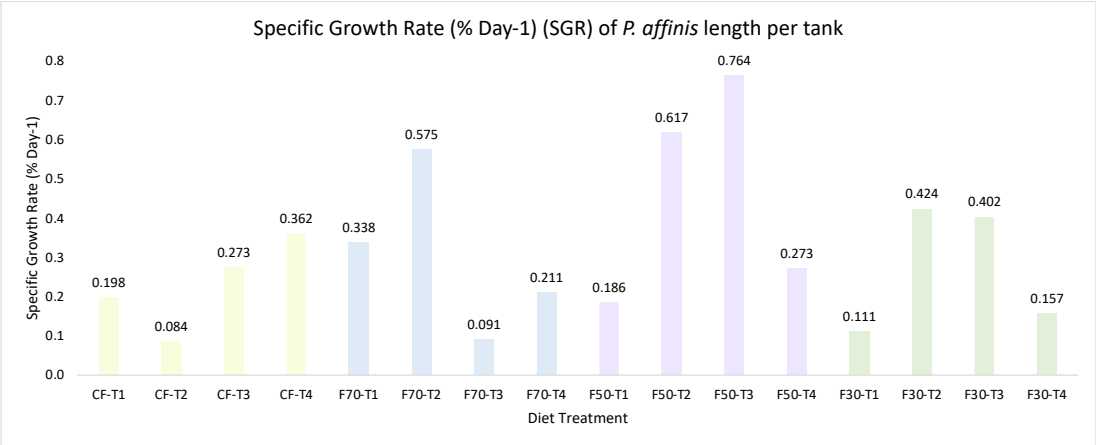

Figure 14: The Specific Growth Rate of *P. affinis length* (cm) on average per day per replicate tank for each diet treatment for the duration of the feeding trial.

3.2.2.1 Number of Moults

The number of moults per tank and diet treatment were analysed in R-Studio with a linear regression model and were found not to correlate with the length of shrimp from the respective diet treatments ($\beta = 0.0025 \pm 0.012$, $t_{14} = 0.226$, $p < 0.82$) or any other variables.

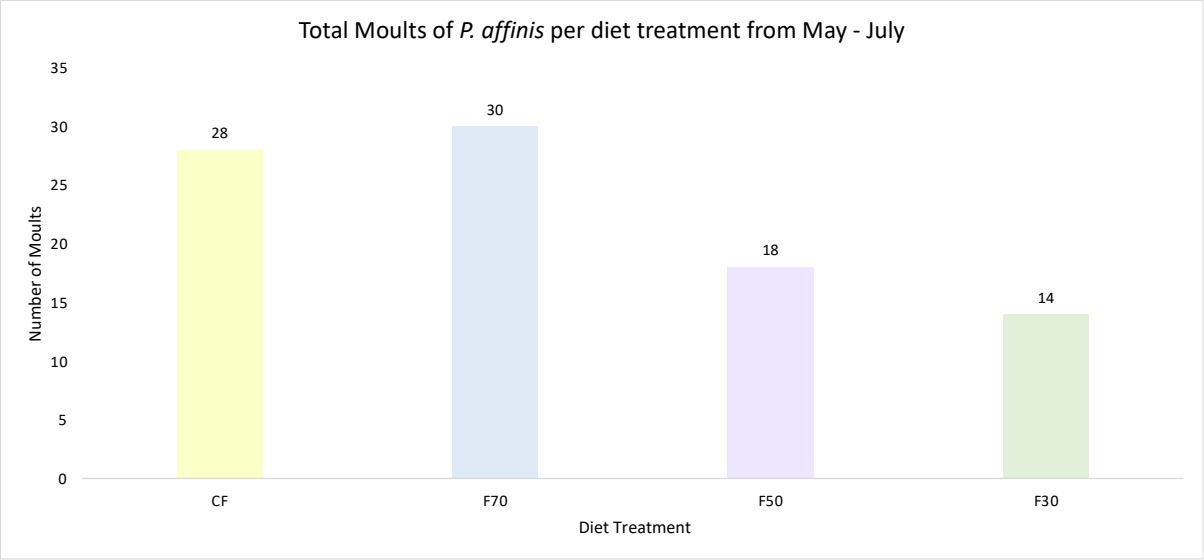

Figure 15: The total number of moults from *P. affinis* per tank per diet for the duration of the feeding trial.

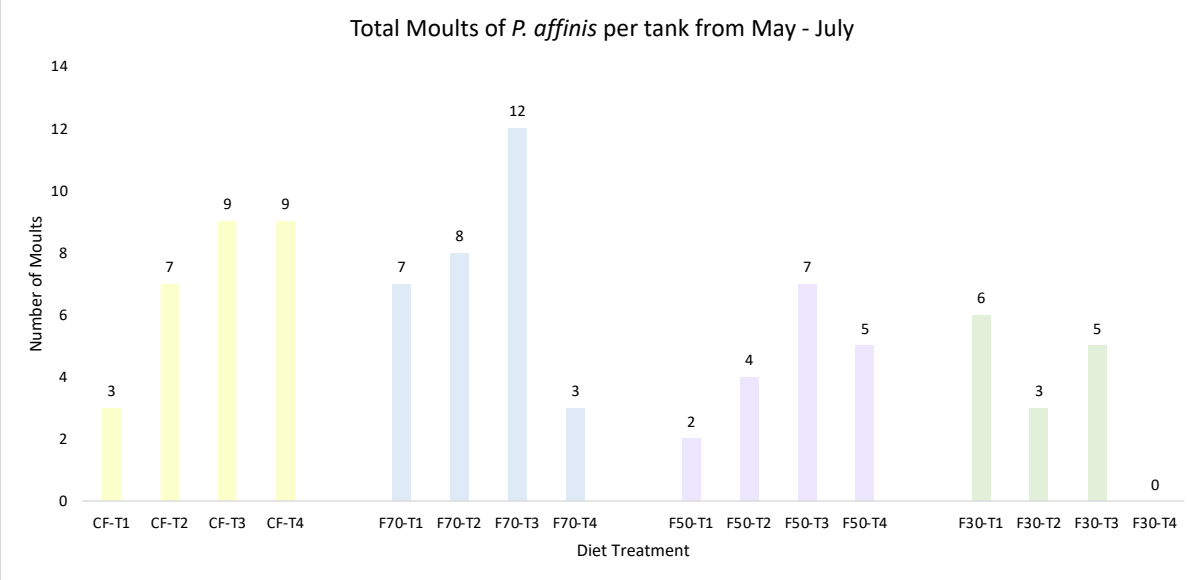

Figure 16: The total number of moults from *P. affinis* per replicate tank for each diet treatment for the duration of the feeding trial.

Table 7: Length performance of *Palaemon affinis* based on initial and final length (cm).

Average Growth (TBL) Performance	CF	F70	F50	F30
Initial Length (cm)	2.512 ± 0.29	2.526 ± 0.24	2.257 ± 0.42	2.316 ± 0.32
Final Length (cm)	2.658 ± 0.30	2.721 ± 0.28	2.551 ± 0.36	2.491 ± 0.31
Length Change (cm)	0.15 ± 0.08	0.19 ± 0.13	0.29 ± 0.18	0.18 ± 0.10
Mean TBL Growth (%)	5.81 ± 2.92	7.72 ± 5.68	13.03 ± 8.82	7.56 ± 4.85
Specific Growth Rate (%)	0.23 ± 0.12	0.30 ± 0.21	0.46 ± 0.28	0.27 ± 0.16
Number of Moults	28	30	18	14

3.3 Proximate Composition

3.3.1 Diet Composition

The results of the chemical analysis determined that the average protein percentage of the commercial feed was 46.78%, F70 was 39.22%, F50 was 40.96% and F30 was 41.90% (Table

8). The results from the one-way ANOVA indicated that there was no statistically significant difference between protein in the diets ($F(3, 4) = [6.084]$, $p = 0.06$).

Proximate analysis determined that the commercial diet displayed a lower carbohydrate content than the encapsulated diets (Table 8). Furthermore, results from the one-way ANOVA indicated that there was a statistically significant difference between the carbohydrate content within the diets ($F(3, 7) = [17.929]$, $p = 0.001$). A Tukey Test found that the mean value of carbohydrates was significantly different between the commercial feed and F70 ($p = [0.001]$, 95% C.I = [7.253, 22.297]), the commercial feed and F50 ($p = [0.005]$, 95% C.I = [3.698, 17.154] and the commercial feed and F30 ($p = [0.003]$, 95% C.I = [4.772, 18.228]). However, there was no statistically significant difference between any of the encapsulated diets. Indicating that there was a statistically significant difference between the carbohydrate content of the commercial feed in comparison to the three encapsulated diets created.

The lipid content of the commercial feed was found to have a similar concentration to F70 and F50 however, the lipid content in F30 was much higher at 18.57% (Table 8). A one-way ANOVA comparing the lipid in the diets indicated a statistically significant difference ($F(3, 8) = [13.368]$, $p = 0.001$). A Tukey Test found that the lipid content was significantly different between the commercial feed and F30 ($p = [0.007]$, 95% C.I = [1.888, 10.197]), F70 and F30 ($p = [0.002]$, 95% C.I = [3.242, 11.551]) as well as F50 and F30 ($p = [0.005]$, 95% C.I = [2.232, 10.541]). Indicating a significant difference between F30 and all the other diets.

The energy levels across the diets vary with CF at 4377.00, F70 at 4136.80, F50 at 4527.80 and F30 at 5183.30 (Table 8).

Table 8: Proximate composition of the commercial diet and three encapsulated diets

Diets	CF	F70	F50	F30
Protein (%)	46.78 ± 2.86	39.22 ± 0.43	40.96 ± 2.28	41.90 ± 0.46
Carbohydrate (%)	34.40 ± 0.93	40.62 ± 2.90	44.83 ± 2.47	45.90 ± 3.24
Lipid (%)	12.52 ± 0.80	10.48 ± 0.06	12.18 ± 1.58	18.57 ± 2.36
Energy (Kcal/kg)	4377.00	4136.80	4527.80	5183.30
Lipid:Carbohydrate Ratio	25:70	1:4	4:15	2:5

3.3.2 Faecal Matter Composition

The average protein in the faecal matter of shrimp from the diet treatments was below 3% (Table 9). A one-way ANOVA comparing the protein in the faecal matter of shrimp between the difference diets indicated that there was a statistically significant difference between the protein in the faecal matter of shrimp between the different diets ($F(3, 44) = [6.278]$, $p = 0.001$). A Tukey Test found that the protein was significantly different between F70 diet and F50 diet ($p = [0.00048]$, 95% C.I = [0.533, 2.249]). However, there was no statistically significant difference in the protein between any of the other diet treatments.

A one-way ANOVA revealed that there was a statistically significant difference between the carbohydrate content within the faecal matter of shrimp between the diet treatments analysed ($F(2, 15) = [131.597]$, $p = 3.0767^{E-10}$). A Tukey Test found that the value of carbohydrates was significantly different between all analysed groups. CF and F70 ($p = [6.7978^{E-06}]$, 95% C.I = [3.107, 6.501]), CF and F30 ($p = [1.8889^{E-10}]$, 95% C.I = [8.888, 12.282]) and F70 and F30 ($p = [6.9618^{E-07}]$, 95% C.I = [4.084, 7.478]).

Upon comparison of the lipids in the faecal matter of shrimp there was a visible differentiation between the diet treatments (Figure 17.1, Table 9). A one-way AVOVA found that there was a statistical difference between the lipid content in faecal matter from different diet treatments ($F(3, 38) = [54.754]$, $p = 7.2625^{E-14}$). Tukeys HSD Test for multiple comparisons concluded that the value of lipids was significantly different between all analysed groups except for between CF and F70 ($p = [0.280]$, 95% C.I = [-7324, 3.800]).

The percentage of lipids across the four diets within the whole body, faecal matter and diets of shrimp were graphed to visualise the trend in lipids across the diet treatments (Figure 17.1). The lipid content in the faecal matter of shrimp has a noticeable increasing lipid content across the diet treatments in comparison to the whole-body and dietary lipids (Figure 17.1) The faecal matter of shrimp was found to best fit a trendline of exponentially increase ($R^2 = 0.9742$) across the diet treatments (Figure 17.3).

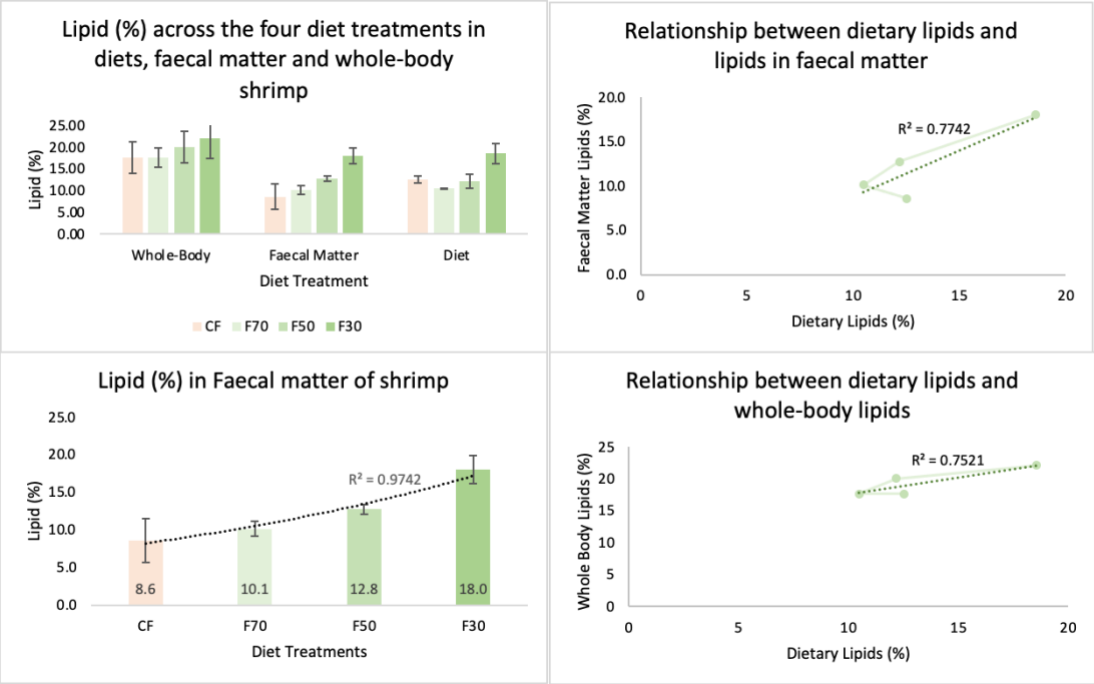

Figure 17.1: The average percentage of lipids across the four diet treatments within the diets, faecal matter and whole-body of shrimp.

Figure 17.2: The relationship between dietary lipids and faecal matter lipids fitted to a linear trendline

Figure 17.3: The average percentage of lipids across the four diet treatments in faecal matter of shrimp fitted to an exponential trendline

Figure 17.4: The relationship between dietary lipids and whole-body lipids fitted to a linear trendline.

Table 9: Proximate composition of faecal matter collected from *Palaemon affinis*

Faecal Matter	CF	F70	F50	F30
Protein (%)	2.61 ± 0.69	1.84 ± 0.49	3.17 ± 1.13	2.55 ± 0.69
Carbohydrate (%)	10.51 ± 0.40	15.31 ± 0.46	UNAVAILABLE	21.09 ± 1.86
Lipid (%)	8.60 ± 2.89	9.90 ± 1.00	12.77 ± 0.66	18.04 ± 1.81

3.3.3 Whole-Body Composition

The protein per milligram in whole-body shrimp was found to have a statistically significant difference shown by the results of a one-way ANOVA F(3, 28) = [6.744], p = 0.0014). A Tukey Test found that there was a statistically significant difference between the protein percentage of CF and F50 (p = [0.0074], 95% C.I = [0.152, 1.165]) additionally with CF and F30 (p = [0.0021], 95% C.I = [0.152, 1.165]).

The carbohydrates in whole-body shrimp were shown to have a significant statistical difference when analysing the results of a one-way ANOVA (F(3,19) = [3.764], p = 0.02). A Tukey Test found that there was a statistically significant difference between the carbohydrate content in the commercial feed and F30 (p = [0.021], 95% C.I = [0.041, 0.586]).

The lipids in whole-body shrimp had a significant difference indicated by one-way ANOVA (F(3,44) = [4.194], p = 0.011). A Tukey Test found that there was a significant difference between the lipid percentage between the commercial feed and F30 (p = [0.023], 95% C.I = [0.482, 8.492]) as well as between F70 and F30 (p = [0.022], 95% C.I = [0.494, 8.504]).

Table 10: Proximate composition of whole body *Palaemon affinis*

Whole-Body	CF	F70	F50	F30
Protein (%)	33.66 ± 2.92	27.81 ± 2.51	27.39 ± 0.40	26.52 ± 4.59
Carbohydrate (%)	0.50 ± 0.19	0.72 ± 0.15	0.65 ± 0.18	0.82 ± 0.07
Lipid (%)	17.65 ± 3.68	18.46 ± 2.19	20.37 ± 3.66	22.13 ± 4.80

3.4 Feed Utilisation

3.4.1 Feed Ingested

Shrimp in the commercial diet ingested 33.29% of the feed given on average, F70 ingested 32.54%, F50 consumed the most at 38.08% and F30 consumed the least at 31.68% (Figure 18). A one-way ANOVA concluded that there was no statistically significant difference between the total feed ingested per diet treatment (F(3,12) = [1.50], p = 0.30).

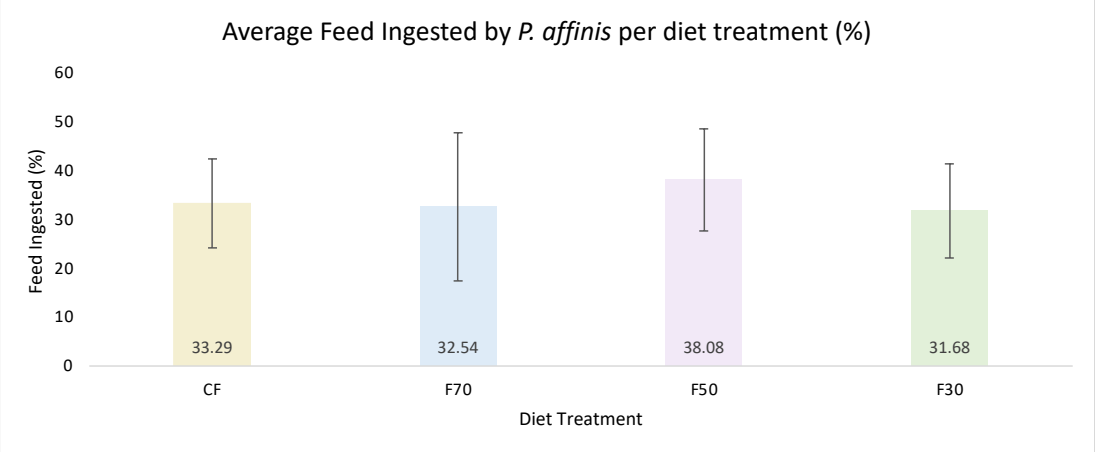

Figure 18: The calculated average feed ingested by *P. affinis* across the four diet treatments during the duration of the feeding trial and displayed as a percentage (%).

3.4.2 Feed Conversion Ratio

The results of the FCR were not within the ranges of other studies conducted and examined on shrimp FCR's which range between 1.20 – 2.99 (Motte et al., 2019; Mukhopadhyay et al., 2013; Panini et al., 2017). The feed conversion ratio of the commercial feed was the highest, with the least food converted to weight gain at 14.42, F70 was 13.28, F50 was 11.04 and F30 was the lowest at 7.49 with the most food converted to weight gain (Figure 19, Table 11). However, a one-way ANOVA discovered there was no statistically significant difference between the FCR values ($F(3,12) = [0.89]$, $p = 0.47$).

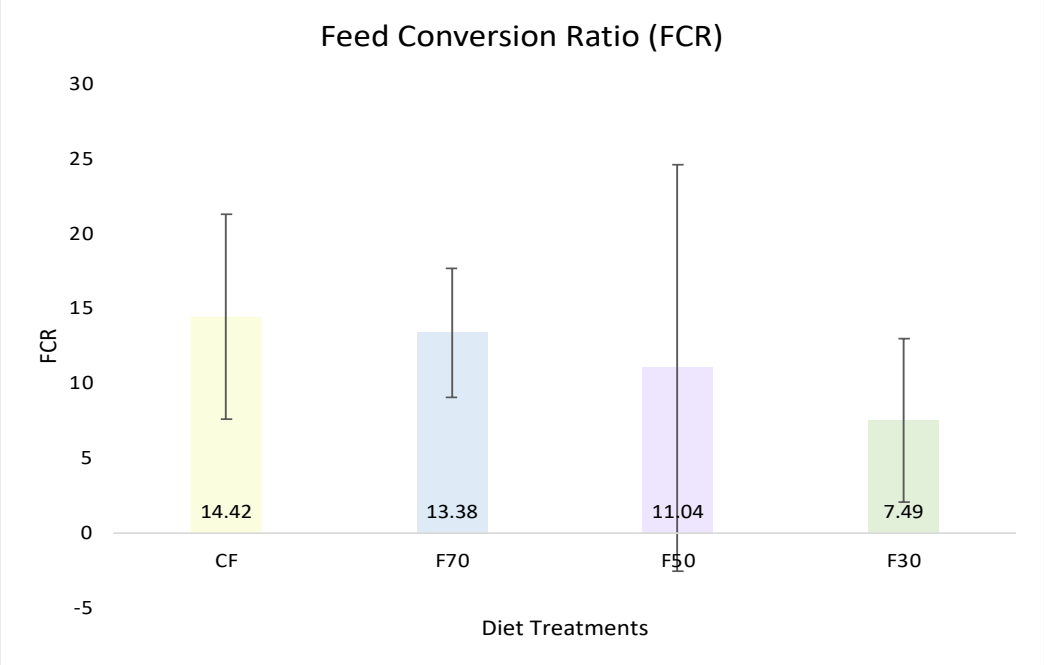

Figure 19: The feed conversion ratio (FCR) of *P. affinis* across the four diet treatments during the feeding trial with error bars representing standard deviation.

3.4.3 Protein Efficiency Ratio (PER)

The PER of CF was shown to be the lowest at 0.15, F70 at 0.19, F50 at 0.22 and F30 at 0.32 (Figure 20, Table 11). Overall a one-way ANOVA discovered no statistically significant difference between the PER values ($F(3,12) = [1.66]$, $p = 0.29$.

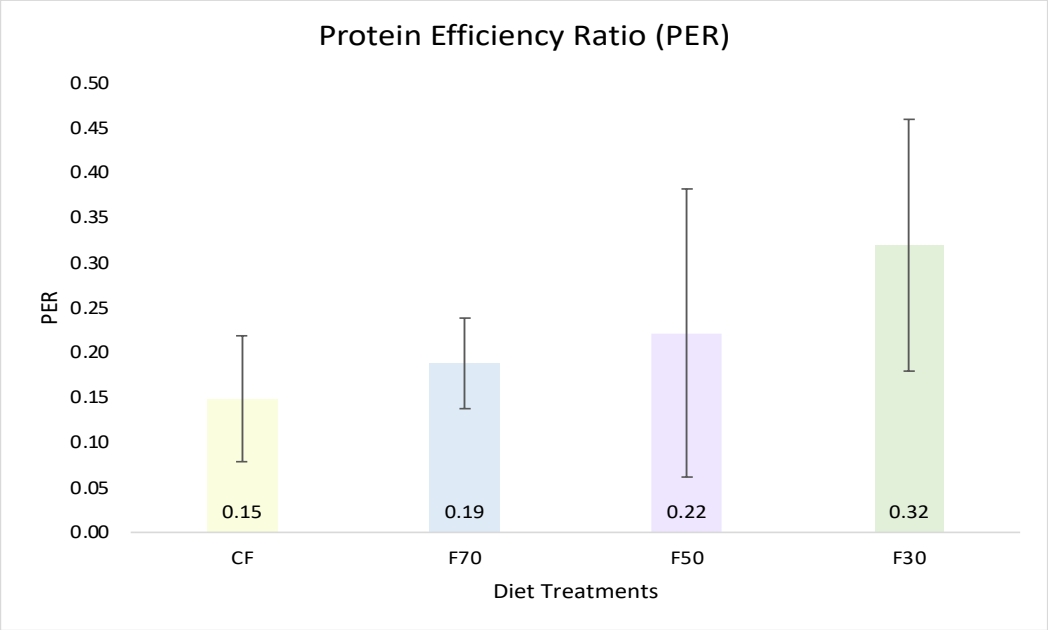

Figure 20: The protein efficiency ratio (PER) of *P. affinis* across the four diet treatments during the feeding trial with error bars representing standard deviation.

3.4.4 Survival

F70 was found to have the highest survival rate of 100% and F30 had the lowest at 80% survival (Figure 21, Table 11). A one-way ANOVA concluded that there was no significant difference in the survival between shrimp from different diet treatments (F(3,12) = [2.103], p = 0.153).

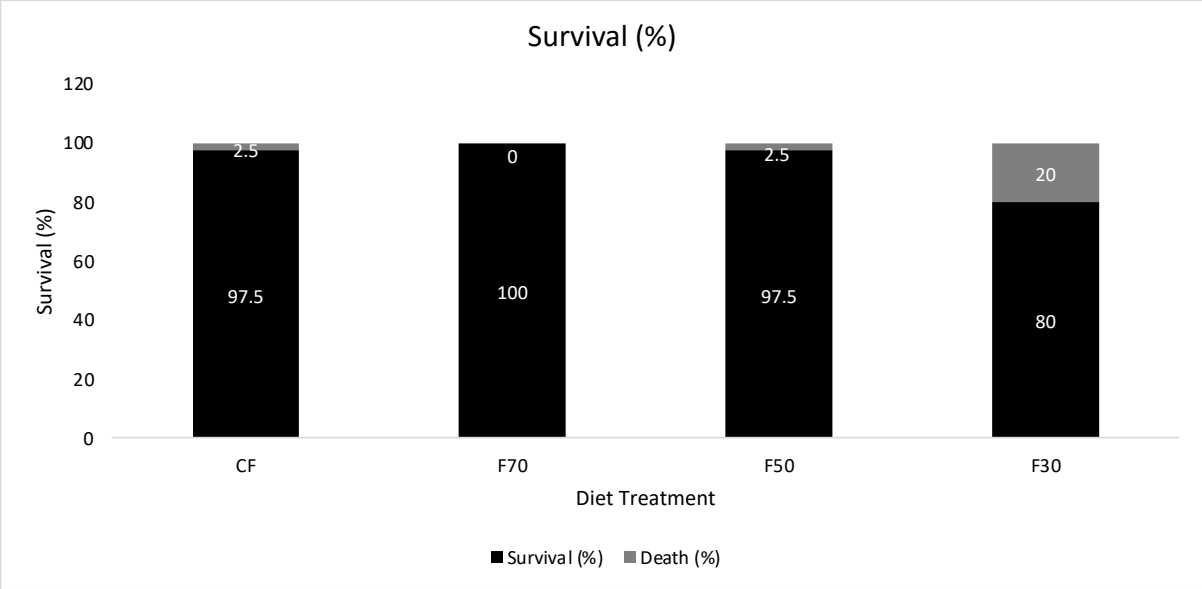

Figure 21: The total survival and death of *P. affinis* per diet treatment during the feeding trial (%).

Table 11: Feed utilisation of *P. affinis*

Feed Utilisation	CF	F70	F50	F30
Feed Ingested (g)	2.70 ± 0.16	2.81 ± 0.51	2.54 ± 0.39	2.17 ± 0.68
Feed Conversion Ratio (FCR)	14.42 ± 6.83	13.38 ± 4.31	11.04 ± 13.60	7.49 ± 5.47
Protein Efficiency Ratio (PER)	0.15 ± 0.07	0.19 ± 0.05	0.22 ± 0.16	0.32 ± 0.14
Survival (%)	97.5	100	97.5	80

Chapter 4: Discussion

4.1 Encapsulation And Characterisation

4.1.1 Encapsulation Yield

The dried ingredients used in the formulation mixture equated to 62.5g of alginate, gelatine, calcium carbonate and dried feed. The dried weight of beads generated was 50g indicating a yield of 80%.

4.1.2 Water Absorption

The results graphed in figure 8 indicates that the amount of time encapsulated beads are in water affects the water absorption of beads, suggesting that the encapsulated beads can be submerged in seawater for up to 48 hours before any noticeable effects of water absorption starts to appear.

4.13 Matrix Erosion

It was found that the encapsulated feed had more stability in seawater and less erosion than the commercial feed between 0 and 24 hours. However, the encapsulated feed had a similar stability and erosion rate between 24 and 48 hours when compared with the commercial feed. This indicates that the encapsulated diet performs better, with less erosion, increased stability and potentially influences better water quality parameters than the current commercial feed within 0 to 24 hours, which could be beneficial on a commercial basis (Figure 9). The results from the ammonia test on the seawater taken from a tank containing commercial feed and a tank containing encapsulated feed further suggest that the matrix erosion rate of encapsulated beads is lower, with lesser effect on water quality parameters than the current commercial feed (Figure 10).

4.2 Growth Performance

4.2.1. Mean Weight Gain and Specific Growth Rate

R-Studios linear regression model found that there was a relationship between the lipid content of the diets and the lipid content in the bodies of shrimp and weight gain. In future, defatted

mealworms could be used to ensure weight is not affected by the additional lipids found in mealworms. Furthermore, the lack of significant difference in the mean weight gain and SGR % is likely due to high between-tank variation (Figure 12). This indicates that statistically, there is no significant difference between the weight change of shrimp between diet treatments, however, mean growth rate and SGR were highest in shrimp from the F30 diet overall.

Overall, it can be presumed that if the additional lipids were not present in the diets that the weight change of shrimp from the different diet treatments would perform similarly. Indicating that with the reduction of lipids, weight gain would be comparable to the current commercial feed and appropriate for commercial use.

4.2.2. TBL Gain and Specific Growth Rate

The lack of significant relationships between length gain and the analysed variables (diet treatments, protein, carbohydrate and lipid content) could be due to high between-tank variation creating a lack of statistical relationships and differences with other variables (Figure 14).

There was no positive or negative significant relationship between length and the diet treatments. This signifies that the encapsulated diets have the same effect on the tbl growth of shrimp as the commercial diet and could be appropriate for commercial use.

4.2.3 Moulting

Moulting is usually an indicator of growth in shrimp (Panakorn, 2018). However, moulting did not have a significant relationship with length change. Healthy moulting occurs when the current exoskeleton can no longer hold the muscle gain although moult frequency is not always an indicator of such growth. If shrimp were stressed this could induce moulting which would not lead to growth (Panakorn, 2018). Stress can be brought on by many things including water changes (Lemos & Weissman, 2021). Water changes were frequent during the duration of the feeding trial and could have potentially increased the stress in some shrimp and therefore created a lack of a statistically significant relationship between length change and moulting. Another reason behind the lack of significance could be because shrimp were only tended to every 48 hours, shrimp may have moulted during that time and the moults may have been eaten. Moulting fluid found in old exoskeletons contains agents such as amino acids and enzymes that are strong feeding stimulants and attract shrimp to feed on them (Lemos &

Weissman, 2021). Therefore, shrimp may have eaten the moults in their respective tanks before they were counted creating a lack of relationship between moulting and length.

In summary, the growth performance of shrimp was not statistically significant between the diet treatments which suggests that shrimp have a similar growth response to the encapsulated mealworm-based diets at all replacement levels to the commercial feed. Panini et al., 2017 found that the growth parameters evaluated exhibited satisfactory results with all variations of mealworm inclusion in their study and displayed no negative effects on any growth parameters (Panini et al., 2017). Furthermore, Motte 2019 found that growth performance was better in diets that included 25 – 75% defatted mealworm inclusion than fishmeal (Motte, 2019). This signifies that an encapsulated diet with mealworm meal should give similar growth results as the current commercial feed for commercial shrimp.

4.3 Proximate Composition

4.3.1 Diet Composition

4.3.1.1 Protein

The commercial feed had the highest percentage of protein in the diets, this is likely due to this feed being a grow-out feed for shrimp with a higher protein content to encourage the fast growth of commercial prawns. The requirement of protein in a diet for *M. rosenbergii* is between 35-40% but during larvae development, higher protein and lipids are required (Nesara & Paturi, 2018).

4.3.1.2 Carbohydrates

Chemical analysis revealed that the carbohydrate content of the three encapsulated diets had higher carbohydrate contents than anticipated, the targeted amount of carbohydrates was between 33-35% (Table 3). The increase in carbohydrate content could be due to the addition of mealworm as the total targeted carbohydrate ingredient inclusion was similar across the three diets (Table 3). Therefore, the addition of mealworms may have impacted the carbohydrate content more than originally anticipated.

4.3.1.3 Lipids

The high lipid content in the diets was due to the addition of mealworm meal. Mealworms are high in lipids, therefore, when adding further mealworm meal to the diet, the lipid content simultaneously increases (Panini et al., 2017; Son et al., 2020).

4.3.1.4 Energy

The diets in this study contain an energy level between 4136 Kcal/kg and 5183 Kcal/kg and a range of Lipid: Carbohydrate ratios with F70 displaying a ratio of 1:4 (Table 8). D'Abramo (2011) suggested that protein sparing is maximised when the dietary lipid to carbohydrate ratio is 1:4. The energy levels were targeted between 4000 – 4429 Kcal/kj although when proximate analysis was completed those levels were higher than anticipated. Although it did not seem to have any adverse effects on shrimp growth or survival. In future, the proximate analysis of each ingredient should be completed to accurately assess the energy level within the suggested optimal ranges to ensure that shrimps nutritional requirements are being met at optimal levels.

4.3.2 Faecal Matter Composition

4.3.2.1 Protein

The average protein in the faecal matter was below 3% indicating that the shrimp utilised majority of the protein in the diets they were given (Table 9). However, the Tukey Test performed indicated no significant difference in the protein between any of the diet treatments except for F70 and F50. It is unclear why there was a significant difference between the protein level in the faecal matter of shrimp between diets F70 and F50.

4.3.2.2 Carbohydrates

Statistical analysis revealed that there was a significant difference between the carbohydrate content within the faecal matter of shrimp between all analysed groups. Therefore, it is likely the inclusion of mealworms is adding additional carbohydrates to the diets and causing the rise seen in their feed and discarded in the faecal matter. The discarding of carbohydrates at these percentages could indicate that they were eating more carbohydrates than was necessary for energy requirements. Little research has been conducted on *P. affinis* due to their small size and that they are not a commercially produced species which could mean their nutritional requirements slightly differ from the requirements of *M. rosenbergii* on which the feeds were based, resulting in the potential for more macronutrient waste. Another possibility is the inclusion of corn starch and meal of which the carbohydrate content might be higher than that of the commercial diet.

4.3.2.3 Lipids

The percentage of lipids excreted by shrimp fitting an exponential trend could suggest that shrimp only retain lipids necessary for energy and maintenance and excrete the lipid content that is not required (Figure 17.3). This is further explored by figure 17.2 and figure 17.4 displaying a weak relationship between the lipid content of diets, faecal matter and whole-body. This could suggest that the encapsulated diets higher in mealworm and lipid content, could still be suitable for shrimp due to the potential ability to excrete lipids that are not required by the animal as the exponential trend of lipid excretion cannot be well-explained by other factors. However, to explore this possibility further increased replication, more data points and a wider range of samples would need to be included to make a confirmed conclusion of this possibility.

There are some limitations that require mentioning on the faecal matter collected from shrimp. Initially, when conducting the carbohydrate proximate analysis using the Anthrone method, there were some difficulties in finding the necessary dilution level for the faecal matter. Therefore, this required some pre-analysis which led to depleting F50 faecal matter for proximate analysis. Furthermore, during the feeding trial there were some mortalities of shrimp in the diet treatments, therefore some of the tanks had less variation of faecal matter than others due to there being fewer animals to collect faecal matter from, notably in the F30 treatment. In future, more tanks should be set up during the feeding trial and faecal matter should be collected from those tanks to trial any dilution difficulties.

4.3.3 Whole-Body Composition

4.3.3.1 Protein

The significant difference in proximate composition of proteins in whole-body shrimp could be due to: digestibility, the processing of the mealworms, lack of essential amino acids for the building of proteins or the lipid content. There are scientific articles which suggest Chitin, a compound in mealworms, could have the potential to reduce the digestibility of proteins by reducing transit time in the gut. Additionally, chitin may also have the potential to affect the digestion of amino acids (Akiyama et al., 1989; Clark et al., 1993; Panini et al., 2017), similarly with Chitosan (Shi-Yen & Yi-Ping, 1998). However, Motte et al. 2019 suggests that nutrient digestibility is due to the absence of processing meal and the absence of amino acid supplementation rather than the presence of chitin (Motte et al., 2019). Additionally, Panini et al. 2017 state that the amount of lipid in the diet and fatty acids can influence digestibility

(Panini et al., 2017). Therefore, the significant difference between the commercial diet and the F50 and F30 diets may be due to an issue with protein digestibility for one or more of the above reasons. To remedy this, amino acid supplementation could be a suitable option as well as defatted mealworm meal.

4.3.3.2 Carbohydrates

The significant difference between the carbohydrate content in shrimp from the commercial diet and F30 may be due to shrimp in the F30 diet absorbing more carbohydrates from the diet given. As shown in table 10 shrimp seem to be able to discard excess carbohydrates from their diets in their faecal matter. Although, this may only be to an extent of what they are given and therefore F30 is significantly higher than F30 as seen in the diets.

4.3.3.3 Lipids

Results of the lipid analysis of whole-body shrimp indicate that shrimp fed a mealworm diet between 30-50% replacement, may be able to discard the excess lipids from the mealworm inclusion. This is due to the significant difference in lipids between most diet treatments faecal matter. However, F30 with a 70% replacement, still has significantly different lipid percentages in the whole bodies of shrimp suggesting the lipid content may be too high to discard for this percentage of replacement. Panini et al., in 2017 found a similar trend in the effects of dietary lipids on whole bodies of shrimp. They discovered that shrimp fed a fishmeal replacement of above 25% mealworm inclusion showed an increase in body lipid content stating that this is due to the lipid deposition in the hepatopancreas and the muscle tissue of shrimp (Panini et al., 2017). In future, a defatted mealworm could be beneficial in decreasing the lipid content with high replacement levels.

4.4 Feed Utilisation

4.4.1 Feed Ingested

There was no significant difference between the feed ingested and the diet treatments. This indicates that there is likely no difference in palatability between the different diet treatments, confirming the palatability test (Table 5). Shrimp feed to fulfil an energy requirement, if there is no statistically significant difference in the amount of feed consumed by shrimp per diet treatment this could be an indication that shrimp are able to fulfil that energy requirement by eating a similar amount of the encapsulated mealworm diets, similarly to the amount of feed to fulfil energy requirements with the commercial feed.

4.4.2 Feed Conversion Ratio

The results of the FCR were not what is typically seen in other similar studies conducted on shrimp species. This is likely due to *P. affinis* being a non-commercial species, with a small standard size and the small growth expressed by *P. affinis* in comparison to a commercial species with a much larger size and growth (D'Abramo & Sheen, 2012). The results of weight gain showing a significant relationship to lipids in the higher mealworm diets is likely the reason for F30 having the most feed ingested to weight gain ratio (FCR). Motte et al. 2019 found that FCR was best in diets containing 25-100% inclusion of defatted mealworm in comparison to a fishmeal-based diet (Motte et al., 2019). Therefore, it is likely that the FCR of mealworm diets would be similar to the current commercial feed if the lipid content was decreased.

4.4.3 Protein Efficiency Ratio (PER)

The results are not within the ranges of PER from previous studies between 1.40 – 2.40. D' Abramo, 2011 found that protein efficiency ratios for crustaceans likely differ from species-species (D'Abramo, 2011; Motte et al., 2019; Mukhopadhyay et al., 2013). This could be one reason for the difference, or it could be that the lipid concentrations are higher in the higher inclusion of mealworm diets. Therefore, it is likely that the PER would more-closely resemble the commercial feeds PER if the lipid levels were reduced.

4.4.4 Survival

There was found to be no significant difference in the survival rate of shrimp from different diet treatments however, CF had a 97% survival rate and F30 had an 80% survival rate which does show there were more deaths in F30. It was found not to be due to the type of feed as there was no relationship between the type of need and the number of shrimp post-feeding trial ($\beta = -3.697 \pm 0.1956$, $t_{14} = -1.89$, $p < 0.08$). During the feeding trial, some contamination of certain tanks occurred due to a build-up of algae. The tanks that were affected by contamination were analysed to see if there was a relationship between contamination and the number of shrimp post-feeding trial. The results from the linear model performed in RStudio signified that the contamination of tanks significantly predicted the number of shrimp post-feeding trial ($\beta = -2.00000 \pm 0.172$, $t_{14} = -11.65$, $p < 2^{E-16}$). This indicates that contamination is likely the reason behind the deaths of shrimp. Another possible reason for the difference could have been cannibalism. Only a certain number of shrimp bodies were found after death, therefore some deaths could have been related to cannibalism. Shrimp are known to have

cannibalistic behaviour, especially when they are around other shrimp that are moulting (Lemos & Weissman, 2021).

4.5 Limitations

There were some limitations in this study that require mentioning. Not being able to conduct the feeding trial with the four diets and examine growth, proximate composition, feed utilisation and survival on a commercialised shrimp species was a limitation. The closest relative to the commercial shrimp species in New Zealand is *P. affinis* as it is in the same family as *M. rosenbergii*. However, it would have been ideal to test these diets on a commercial species. Furthermore, *P. affinis* is a comparatively small shrimp species which meant that there was less faecal matter and less whole-body shrimp at the end of the study than if it was conducted with a larger species. This limited the amount of analysis that could be achieved such as protein, lipid and carbohydrate proximate analysis and prevented amino acid and fatty acid analysis as the samples were limited. In future, it would be beneficial to examine the listed effects of the diets on a larger commercialised shrimp species such as *M. rosenbergii* to see how the diets affect a commercial species in comparison to *P. affinis.*

Chapter 5: Conclusions

5.1 Thesis Aims and Research Questions

Overall, the encapsulated mealworm diets were comparable to the current commercial diet and provided similar growth, survival and feed utilisation.

Additionally, it was discovered that an encapsulated diet significantly improved the erosion rate, water quality parameters and sustainability of the feed and would likely aid in the reduction of disease in shrimp aquaculture due to increased desirable water parameters. All three encapsulated diets would make suitable replacements in shrimp aquaculture and with minor adjustments, the encapsulated diets could provide improved nutritional, growth and survival parameters in comparison to the current commercial feed.

5.2 Research Reflection, Approach and Limitations

The encapsulated diets were proven to have increased stability, sustainability and less degradation in seawater than the current commercial feed and provided the same palatability. The growth and feed utilisation displayed statistically similar results between shrimp fed the encapsulated diets and the current commercial feed. The diets were found to be statistically similar aside from the lipids and carbohydrates in some diets however these exhibited no effect on shrimp growth, survival, health and nutritional parameters. Unexpectedly, *P. affinis* were found to potentially discard high levels of lipids and carbohydrates in the higher concentrated diets which proved to be successful in lowering the bodily lipids of shrimp fed diet F50. This suggests that shrimp fed a diet containing higher than the desired lipids could potentially be discarded to a degree in shrimp faecal matter. The survival and moults of shrimp results likely arose due to contamination, lack of separation and the potential consumption of moults or other shrimp.

5.3 Future Recommendations

A recommendation would be to have a commercialised species to conduct a feeding trial on with the same encapsulated diets created. The diets were designed to be nutritionally compatible with *Macrobrachium rosenbergii*, the current commercial shrimp species in New

Zealand. Being able to test the diet created on this species would solidify the results of the diets on growth, survival, FCR, SGR and PER due to the nutritional parameters being catered to this species and the species displaying certain size and growth comparable to other studies. Additionally, it would be beneficial to be able to identify individual shrimp giving a better indication of growth results. This could be done by splitting individual shrimp up inside tanks. Clear plastic tanks could be used with plastic dividers to separate individual shrimp, this would lead to the ability to label individual shrimp, their initial weights, and length and create the ability to identify any individual moults. Having shrimp separated would also prevent any cannibalism and likely give a truer interpretation of survival. However, this would need to be researched, examined, and trialled to ensure that there is no compromise on shrimp behaviours. Furthermore, the diets themselves would benefit from an amino acid premix to further improve the PER of the diets and ensure no amino acids are lacking, as mealworms are low in the amino acid methionine which could interfere with protein building in shrimp muscle. Lastly, defatted mealworms would make this diet lower in lipid content which would give a truer representation of shrimp weight gain.

5.4 Contribution to the Current Body of Knowledge

Other studies have been conducted on the inclusion of mealworm as an alternative protein source for the prawn aquaculture industry, but never in an encapsulated bead with multiple properties to boost shrimp immunity, aid in palatability, reduce matrix erosion and improve sustainability in its entirety. Encapsulation has also been used before in aquaculture, but never to improve the sustainability of a shrimp aquafeed. Therefore, both mealworms and encapsulation in this study are entirely unique and innovative concepts. The results found that an encapsulated mealworm diet was successful and indicate that an encapsulated mealworm diet could be a way into the future for shrimp aquaculture, to lower the inclusive of fishmeal and overall improve the sustainability of shrimp aquafeed globally which is a current battle within the aquaculture sector.

Bibliography

Adilah, R. N., Chiu, S. T., Hu, S. Y., Ballantyne, R., Happy, N., Cheng, A. C., & Liu, C. H. (2022). Improvement in the probiotic efficacy of Bacillus subtilis E20-stimulates growth and health status of white shrimp, *Litopenaeus vannamei* via encapsulation in alginate and coated with chitosan. *Fish & shellfish immunology*, *125*, 74-83.

Afzali, M., & Boateng, J. (2022). Composite fish collagen-hyaluronate based lyophilized scaffolds modified with sodium alginate for potential treatment of chronic wounds. *Polymers*, *14*(8), 1550.

Akiyama, D. M., Coelho, S. R., Lawrence, A. L., & Robinson, E. H. (1989). Apparent digestibility of feedstuffs by the marine shrimp *Penaeus vannamei* boone. *日本水産学会誌*, *55*(1), 91-98.

Amran, A. M., Ariffin, H., Mohamad, A. N., Rahim, A. I. A., Muyassar, A. H., Noordin, N. M., & Mhd, I. (2021). Use of pelleted diets in commercially farmed decapods during juvenile stages: A review. *Animals*, *11*(6), 1761.

Anderson, J. L. (2002). Aquaculture and the Future: Why Fisheries Economists Should Care. *Marine Resource Economics*, *17*, 133-151.

Andrews, J. W., Sick, L. V., & Baptist, G. J. (1972). The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture*, *1*, 341-347.

AOAC. (2019). *Official Methods of AnalysisTM, 21st Edition*: AOAC International.

Arthur, J. R., Michael, J. P., Subasinghe, R. P., Reantaso, M. B., & Macrae, I. H. (2002). *Social and Economic Impact and Mangement of Shrimp Disease among small-scale farmers in Thailand and Viet Nam*. Retrieved from Rome:

Assefa, A., & Abunna, F. (2018). Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary medicine international*, *2018*.

Azhar, H., Siddiqui, H., & Hussain, M. (2021). Effect of varying dietary lipid and protein levels on growth performance, feed utilization, and body composition of black tiger shrimp (Penaeus monodon) reared in a biofloc system. *Aquaculture International*, *29*(1), 287-299.

Barlow, S. M. (2003). Encyclopedia of food sciences and nutrition. In: Academic.

Becker, J. M., Caldwell, G. A., & Zachgo, E. A. (1996). *Biotechnology: a laboratory course*: Academic Press.

Bhoopathy, S., Inbakandan, D., Rajendran, T., Chandrasekaran, K., Kasilingam, R., & Gopal, D. (2021). Curcumin loaded chitosan nanoparticles fortify shrimp feed pellets with enhanced antioxidant activity. *Materials Science and Engineering: C*, *120*, 111737.

Biao, X., & Kaijin, Y. (2007). Shrimp farming in China: Operating characteristics environmental impact and perspectives. *Ocean & Coastal Mangement*, *50*(7), 538-550.

Binalshikh-Abubkr, T., & Hanafiah, M. M. (2022). Effect of supplementation of dried bioflocs produced by freeze-drying and oven-drying methods on water quality, Growth performance and proximate composition of Red Hybrid Tilapia. *Journal of Marine Science and Engineering*, *10*(1), 61.

Boonyaratpalin, M. (1996). *Nutritional Requirements of Commercially Important Shrimps in the tropics*. Retrieved from Jatujak, Bangkok 10900, Thailand:

Brol, J., Müller, L., Prates, E. C. A., de Farias, B. S., Pedrosa, V. F., de Almeida Pinto, L. A.,. Ventura-Lima, J. (2021). Dietary chitosan supplementation in *Litopenaeus vannamei* reared in a biofloc system: effect on antioxidant status facing saline stress. *Aquaculture, 544*, 737034.

Burford, M. A., & Williams, K. C. (2001). The fate of nitrogenous waste from shrimp feeding. *Aquaculture, 198*(1-2), 79-93.

Chamberlain, G. (2010). History of Shrimp Farming. *The Shrimp Book*, 1-34.

Choi, I.-H., Kim, J.-M., Kim, N.-J., Kim, J.-D., Park, C., Park, J.-H., & Chung, T.-h. (2018). Replacing fish meal by mealworm (*Tenebrio molitor*) on the growth performance and immunologic responses of white shrimp (*Litopenaeus vannamei*). *Acta Scientiarum. Animal Sciences, 40*.

Chuntapa, B., Piyatiratitivorakul, S., Nitithamyong, C., Viyakarn, V., & Menasveta, P. (1999). Optimal lipid: carbohydrate and protein: energy ratios in semi-purified diets for juvenile black tiger shrimp *Penaeus monodon* Fabricius. *Aquaculture Research, 30*(11-12), 825-830.

Clark, D., Lawrence, A. L., & Swakon, D. (1993). Apparent chitin digestibility in penaeid shrimp. *Aquaculture, 109*(1), 51-57.

Cowey, C. B., & Forester, J. R. M. (1971). The essential amino-acid requirements of the prawn *Palaemon serratus.* The growth of prawns on diets containing proteins of different amino-acid compositions. *Journal of Marine Biology, 10*, 77-81.

Craig, S. R., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding.

D'Abramo, L. R. (2011). Nutritional Requirements of the Freshwater Prawn *Macrobrachium rosenbergii*: Comparisons with Species of Penaeid Shrimp. *Reviews in Fisheries Science, 6*(1-2), 153-163.

D'Abramo, L. R., & Sheen, S. (2012). Nutritional requirements, feed formulation, and feeding practices for intensive culture of the freshwater prawn *Macrobrachium rosenbergii*. *Reviews in Fisheries Science, 2*(1), 1-21.

Das, N. N., Saad, C. R., Ang, K. J., Law, A. T., & Harmin, S. A. (1996). Diet formulation for *Macrobrachium rosenbergii* (de Man) broodstock based on essential amino acid profile of its eggs. *Aquaculture Research, 27*(7), 543-555.

Davis, A. (2005). *Nutrient requirements of penaeid shrimp*. Retrieved from

Dawood, M. A., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture, 10*(4), 950-974.

Day, C. A. (2001). *An Introduction to the Ecology and Behaviour of the Prawn, Palaemon affinis Milne-Edwards, 1837 .(Crustacea: Decapoda: Natantia).* (Masters of Science in Zoology Masters). University of Canterbury, Canterbury, New Zealand.

Dezfooli, S. M., Bonnot, C., Gutierrez-Maddox, N., Alfaro, A., & Seyfoddin, A. (2022). Chitosan coated alginate beads as probiotic delivery system for New Zealand black footed abalone (Haliotis iris). *Journal of Applied Polymer Science*, e52626.

Dezfooli, S. M., Gutierrez-Maddox, N., Alfaro, A., & Seyfoddin, A. (2019). Encapsulation for delivering bioactives in aquaculture. *Reviews in Aquaculture, 11*(3), 631-660.

Du, L., & Niu, C. J. (2003). Effects of dietary substitution of soya bean meal for fish meal on consumption, growth, and metabolism of juvenile giant freshwater prawn, Macrobrachium rosenbergii. *Aquaculture Nutrition, 9*(2), 139-143.

El-Sayed, & Abdel-Fattah. (2019). *Tilapia culture*: Academic Press.

Emerenciano, M. G., Rombenso, A. N., Vieira, F. d. N., Martins, M. A., Coman, G. J., Truong, H. H., . . . Simon, C. J. (2022). Intensification of penaeid shrimp culture: an applied review of advances in production systems, nutrition and breeding. *Animals, 12*(3), 236.

Ettefaghdoost, M., Noveirian, H. A., & Falahatkar, B. (2018). Growth performance, feed efficiency and whole-body chemical composition of the oriental river prawn, Macrobrachium nipponense, fed different dietary protein and lipid levels. *Iranian Journal of Fisheries Sciences, 17*(3), 585-602.

FAO. (1983). *PALAEMONIDAE: Palaemonid Shrimp*. Retrieved from

FAO. (2009). *Fish as feed inputs for aquaculture, practices, sustainablity and implications*. Retrieved from Rome, Italy:

FAO. (2019). *Fisheries and Aquaculture*. Retrieved from: https://www.fao.org/fishery/en/species?page=1&q=prawns&advanced=&filter=family%3DPALAEMONIDAE#search

Fei, X. (2004). *Solving the coastal eutrophication problem by large scale seaweed cultivation.* Paper presented at the Asian Pacific Phycology in the 21st Century: Prospects and Challenges: Proceeding of The Second Asian Pacific Phycological Forum, held in Hong Kong, China, 21–25 June 1999.

Fóes, G., Krummenauer, D., Lara, G., Poersch, L., & Wasielesky Jr, W. (2016). Long term storage and the compensatory growth of white shrimp Litopenaeus vannamei in aquaculture ponds. *Latin American Journal of Aquatic Research, 44*(3), 588-594.

Fox, J. M., Davis, A. D., Wilson, M., & Lawrence, A. L. (2006). Current status of amino acid requirement research with marine penaeid shrimp. *Avances en Nutrición Acuícola*.

Fu, S. J. (2005). The growth performance of southern catfish fed diets with raw, precooked cornstarch and glucose at two levels. *Aquaculture Nutrition, 11*(4), 257-261.

Funge-Smith, S., & Briggs, M. (2003). The introduction of Penaeus vannamei and P. stylirostris into the Asia-Pacific region. *International Mechanisms for the Control and Responsible Use of Alien Species in Aquatic Ecosystems, Jinghong, Xishuangbanna, People's Republic of China*, 26-29.

Gao, X., Zhang, X., Lin, L., Yao, D., Sun, J., Du, X., . . . Zhang, Y. (2016). Passive immune-protection of Litopenaeus vannamei against Vibrio harveyi and Vibrio parahaemolyticus infections with anti-vibrio egg yolk (IgY)-encapsulated feed. *International Journal of Molecular Sciences, 17*(5), 723.

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., & Caruso, G. (2018). Fishmeal alternative protein sources for aquaculture feeds. In *Feeds for the aquaculture sector* (pp. 1-28): Springer.

Gephart, J. A., Golden, C. D., Asche, F., Belton, B., Brugere, C., Froehlich, H. E., . . . Jones, R. C. (2020). Scenarios for global aquaculture and its role in human nutrition. *Reviews in Fisheries Science & Aquaculture, 29*(1), 122-138.

Guillen, J., Natale, F., Carvalho, N., Casey, J., Hofherr, J., Druon, J. N., . . . Martinsohn, J. T. (2019). Global seafood consumption footprint. *Ambio, 48*(2), 111-122.

Hall, G. M. (2011). Fishmeal production and sustainability. *Fish processing–Sustainability and new opportunity. Blackwell Publishing Ltd., UK*, 207-235.

Hasan, M. R. (2001). *Nutrition and feeding for sustainable aquaculture development in the third millennium.* Paper presented at the Aquaculture in the third millennium. Technical proceedings of the conference on aquaculture in the third millennium.

Hawkins, A., Widdows, J., & Bayne, B. (1989). The relevance of whole-body protein metabolism to measured costs of maintenance and growth in Mytilus edulis. *Physiological Zoology, 62*(3), 745-763.

Holthuis, L. B. (1952). *A general revision of the Palaemonidae (crustacea Decapod Natantia) of the Americas. II. The subfamily Palaemonidae*.

Holthuis, L. B. (1980). *Shrimp and Prawns of the World* (Vol. 1). Leiden, The Netherlands: Food and Agriculture Organisation of the United Nations (FAO).

Honculada, P. J. (1998). *Tropical Shrimp Farming and its Sustainability*. Retrieved from Tigbauan, Philippines:

Huka-Prawn-Park. History.

IBISWorld. (2022). *Seafood Consumption*. Retrieved from: https://www.ibisworld.com/nz/bed/seafood-consumption/24/

Ishthiaq, I. B., Ahmed, J., & Ramalingam, K. (2021). Probiotics in Brackish Water Fish Farming: A Special Focus on Encapsulated Probiotics.

Jackson, A. (2009). The continuing demand for sustainable fishmeal and fish oil in aquaculture diets. *International Aquafeed, 12*(5), 32-36.

Jacquet, J. L., & Pauly, D. (2007). The rise of seafood awareness campaigns in an era of collapsing fisheries. *Marine Policy, 31*(3), 308-313.

Jaroensaensuai, J., Wongsasulak, S., Yoovidhya, T., Devahastin, S., & Rungrassamee, W. (2022). Improvement of Moist Heat Resistance of Ascorbic Acid through Encapsulation in Egg Yolk–Chitosan Composite: Application for Production of Highly Nutritious Shrimp Feed Pellets. *Animals, 12*(18), 2384.

Johnston, P., Everard, M., Santillo, D., & Robèrt, K. H. (2007). Reclaiming the definition of sustainability. *Environmental science and pollution research international, 14*(1), 60-66.

Jones, D., Holland, D. L., & Jabborie, S. (1984). Current status of microencapsulated diets for aquaculture. *Microencapsulation and Artificial Cells*, 275-288.

Junning, C., Zhou, X., Xue, Y., Lucente, D., & Lagana, C. (2019). Top 10 species groups in global aquaculture 2017. *Rome: Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations*.

Karplus, I., Malecha, S., & Sagi, A. (2000). *The Biology and Management of Size Variation*. Retrieved from

Khemundu, G. R., & Banerjee, K. (2019). Culture of Macrobrachium rosenbergii using indigenous floral extract in the hilly terrains of Koraput district of Odisha, India. *Heliyon, 5*(10), e02312.

Kidane, D. G., & Brækkan, E. H. (2021). Global seafood demand growth differences across regions, income levels, and time. *Marine Resource Economics, 36*(3), 289-305.

Kirkapatrick, K., & Jones, M. B. (1985). Salninity Tolerance and Osmoregulation of a Prawn Palaemon affinis Milne Edwards (Caridea:Palaemonidae). *Journal of Marine Biology, 93*, 61-70.

Ko, H., Kim, Y., & Kim, J. (2020). The produced mealworm meal through organic wastes as a sustainable protein source for weanling pigs. *Journal of animal science and technology, 62*(3), 365.

Lee, C., & Lee, K. (2018). Dietary protein requirement of Pacific white shrimp Litopenaeus vannamei in three different growth stages. *Fisheries and Aquatic Sciences, 21*(1), 1-6.

Lemos, D., & Weissman, D. (2021). Moulting in the grow-out of farmed shrimp: a review. *Reviews in Aquaculture, 13*(1), 5-17.

Leung, P., & Engle, C. R. (2008). *Shrimp culture: economics, market, and trade*: John Wiley & Sons.

Lim, C., Ako, H., Brown, C., & Hahn, K. (1997). Growth response and fatty acid composition of juvenile Penaeus vannamei fed different sources of dietary lipid. *Aquaculture, 151*(1-4), 143-153.

Lim, C., & Dominy, W. (1990). Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (Penaeus vannamei). *Aquaculture, 87*(1), 53-63.

Liu, X., Ye, J., Wang, K., Kong, J.-H., Yang, W., & Zhou, L. (2012). Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, Litopenaeus vannamei. *Aquaculture Research, 43*(5), 745-755.

Mahotra, M. (2022). Encapsulation systems for nutrients delivery in aquaculture.

Mahotra, M., Yu, H., Xu, Q., Liew, W. C., Kharel, S., Tan, L. S. E., & Loo, S. C. J. (2022). Solid Lipid Microparticles as leaching free, slow-release encapsulation system for Methionine in aquaculture. *Aquaculture, 557*, 738342.

Mastoraki, M., Vlahos, N., Patsea, E., Chatzifotis, S., Mente, E., & Antonopoulou, E. (2019). The effect of insect meal as a feed ingredient on survival, growth, and metabolic and antioxidant response of juvenile prawn Palaemon adspersus (Rathke, 1837). *Aquaculture Research*, 1-12.

Merrill, A. L., & Watt, B. K. (1955). *Energy value of foods: basis and derivation*: Human Nutrition Research Branch, Agricultural Research Service, US ….

Miller, A., Murphy, N., Burridge, C., & Austin, C. (2005). Complete mitochondrial DNA sequences of the decapod crustaceans Pseudocarcinus gigas (Menippidae) and Macrobrachium rosenbergii (Palaemonidae). *Marine Biotechnology, 7*(4), 339-349.

Motte, C., Rios, A., Lefebvre, T., Do, H., Henry, M., & Jintasataporn, O. (2019). Replacing Fish Meal with Defatted Insect Meal (Yellow Mealworm Tenebrio molitor) Improves the Growth and Immunity of Pacific White Shrimp (Litopenaeus vannamei). *Journal of Animals, 9*(258).

Mukhopadhyay, P. K., Rangacharyulu, P. V., Mitra, G., & Jana, B. B. (2013). Applied Nutrition in Freshwater Prawn, Macrobrachium rosenbergii, Culture. *Journal of Applied Aquaculture, 13*(3-4), 317-340.

Muqsith, A., Harahab, N., Mahmudi, M., & Fadjar, M. (2019). *The estimation of loading feed nutrient waste from vannamei shrimp aquaculture pond and carrying capacity of coastal area in Banyuputih sub-district Situbondo Regency.* Paper presented at the AIP Conference Proceedings.

Myo, A., Darbanandana, T., Boutson, A., & Kaewnern, M. (2018). Status of Trash Fish from Bottom Trawl Fishery and Utilization in Myeik Township, Tanintharyi Region, Myanmar. *Journal of Fisheries and Environment, 42*(3), 1-12.

Nesara, K. M., & Paturi, A. P. (2018). Nutritional requirement of fresh water prawn and shrimps: A review. *Journal of Entomology and Zoology Studies, 6*(4), 1526-1532.

New, M. (2002). *Farming Freshwater Prawns: A Manual for the Culture of the Giant River Prawn (Macrobrachium rosenbergii)*. Marlow, United Kingdom: Food and Agriculture Organisation of the United Nations (FAO).

OECD. (2021). *Aquaculture Production*. Retrieved from: https://www.oecd-ilibrary.org/agriculture-and-food/data/fisheries/production-from-aquaculture_data-00225-en

OECD. (2022). *Fisheries: Production from aquaculture*. Retrieved from: https://www.oecd-ilibrary.org/agriculture-and-food/data/fisheries/production-from-aquaculture_data-00225-en

Panakorn, S. (2018). Managing shrimp moulting. *Aquacult Asia Pac, 14*, 18-21.

Panini, R. L., Eduardo, L., Freitas, L., Guimarães, A. M., Rios, C., Silva, M. F. O. d., . . . Amboni, R. D. M. C. (2017). Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. *Journal of Aquaculture, 473*, 115-120.

Panini, R. L., Pinto, S. S., Nóbrega, R. O., Vieira, F. N., Fracalossi, D. M., Samuels, R. I., . . . Amboni, R. D. M. C. (2017). Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp Litopenaeus vannamei. *Food Research International, 102*, 445-450.

Pereira, S. (1991). The cladistics, taxonomy, biogeography, and evolutionary history of the shrimp family Palaemonidae (Crustacea: Decapoda: Caridea).

Plichta, Z., Kobak, J., Maciaszek, R., & Kakareko, T. (2021). All Shades of Shrimp: Preferences of Colour Morphs of a Freshwater Shrimp Neocaridina davidi (Decapoda, Atyidae) for Substrata of Different Colouration. *Animals, 11*(4), 1071.

Pulgara, R., Mandakovica, D., Salgadoa, P., Venegasb, L., Ortiza, D.,˜a-Neirae, A. P., & Wacykb, J. (2021). Micro-encapsulated grape pomace extract (MGPE) as a feed additive improves growth performance, antioxidant capacity, and shifts the gut microbiome of rainbow trout. *Journal of Aquaculture, 544*.

Rosenberry, B. (1991). World Shrimp Farming 1991. *Aquaculture Digest*.

Rosenberry, B. (2001). World Shrimp Farming 2001. *Aquaculture Digest*.

Ruschioni, S., Loreto, N., Foligni, R., Mannozzi, C., Raffaelli, N., Zamporlini, F., . . . Osimani, A. (2020). Addition of olive pomace to feeding substrate affects growth performance and nutritional value of mealworm (Tenebrio molitor L.) larvae. *Foods, 9*(3), 317.

Sakami, T., Fujioka, Y., & Shimoda, T. (2008). Comparison of microbial community structures in intensive and extensive shrimp culture ponds and a mangrove area in Thailand. *Fisheries Science, 74*(4), 889-898.

Saleh, N. E., Wassef, E. A., & Abdel-Mohsen, H. H. (2022). Sustainable Fish and Seafood Production and Processing. In *Sustainable Fish Production and Processing* (pp. 259-291): Elsevier.

Seethalakshmi, Rajeev, Kiran, & Selvin. (2021). Shrimp disease management for sustainable aquaculture: innovations from nanotechnology and biotechnology. *Aquaculture International, 29*(4), 1591-1620.

Selaledi, L., & Mabelebele, M. (2021). The Influence of Drying Methods on the Chemical Composition and Body Color of Yellow Mealworm (Tenebrio molitor L.). *Insects, 12*(333).

Sheen, S. S., & Chen, S. J. (1992). Optimum dietary lipid requirement level of the tiger prawns, Penaeus monodon. *Aquaculture, 92*, 203.

Shi-Yen, S., & Yi-Ping, Y. (1998). Chitin but not chitosan supplementation enhances growth of grass shrimp, Penaeus monodon. *The Journal of nutrition, 128*(5), 908-912.

Son, Y.-J., Choi, S. Y., Hwang, I.-K., Nho, C. W., & Kim, S. H. (2020). Could defatted mealworm (Tenebrio molitor) and mealworm oil be used as food ingredients? *Foods, 9*(1), 40.

Song, Y., Kim, M., Moon, C., Seo, D., Han, Y. S., Jo, Y. H., . . . Kim, Y. W. (2018). Extraction of chitin and chitosan from larval exuvium and whole body of edible mealworm, Tenebrio molitor. *Entomological Research, 48*(3), 227-233.

Southgate, D., & Joint, F. (1981). The relationship between food composition and available energy.

Sudaryono, A., Hoxey, M. J., Kailis, G., & Evans, L. H. (1995). Investigation of alternative protein sources in practical diets for juvenile shrimp, Penaeus monodon. *Aquaculture, 134*(3-4), 313-323.

Uniyom, N., Chumkam, S., Triwutanon, S., & Jintasataporn, O. (2022). Development of Encapsulation and Coating for Protease on Shrimp Feed. *Int. J. of Aquatic Science, 13*(1), 368-378.

Wang, Y., Zhang, C., Li, Y., Zhang, H., Wang, J., & Feng, F. (2020). Effects of dietary glycerol monolaurate on the growth performance, digestive enzymes, body composition and non-specific immune response of white shrimp (Litopenaeus vannamei). *Aquaculture Reports, 18*, 100535.

Wu, X., Li, F., Z, S., & Huang, J. H. (2020). Effects of dietary protein and lipid levels on growth performance, feed utilization, and body composition of juvenile Pacific white shrimp Litopenaeus vannamei (Boone, 1931). . *Aquaculture Research, 51*(6), 2369-2379.

Wu, X. Y., Liu, Y. J., Tian, L. X., Mai, K. S., Yang, H. J., & Liang, G. Y. (2007). Effects of raw corn starch levels on growth, feed utilization, plasma chemical indices and enzyme activities in juvenile yellowfin seabream Sparus latus Houttuyn. *Aquaculture Research, 38*(12), 1330-1338.

Yang, W., Zheng, C., Zheng, Z., Wei, Y., Lu, K., & Zhu, J. (2018). Nutrient enrichment during shrimp cultivation alters bacterioplankton assemblies and destroys community stability. *Ecotoxicology and environmental safety, 156*, 366-374.