IMPROVEMENT OF FORMULATED FEEDS AND FEEDING MANAGEMENT FOR REDCLAW AQUACULTURE IN AUSTRALIA

Thesis submitted by

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ABSTRACT

The redclaw crayfish *Cherax quadricarinatus* has high potential for aquaculture in Australia. However, the farming of the species in the country has not fulfilled early high expectations after initial fast expansion in the 1980s and 1990s, and in recent years, there has been a decline in redclaw aquaculture production since 2006. Among various major obstacles identified, problems associated with feeds and feeding have been a major cause of concern for redclaw farmers for years. In particular, currently the commercial feeds available for the redclaw farming industry in Australia have poor water stability and are only available in one size; and the industry lacks an efficient feeding management standard. A series of laboratory experiments were hence conducted to tackle these problems.

To determine a feeding regime that ensures maximum efficacy, a total of 72 juvenile redclaw (18 per treatment) were cultured individually (caged) within a recirculating system and were fed at four different feeding intervals (every day (D1), every second day (D2), every third day (D3) and every fourth day (D4)) with the same average daily ration of the commercial redclaw feed (i.e. average 5% body weight per day for all treatments). The redclaw were cultured for 20 weeks and data collected to assess their performance. The survival of juvenile redclaw increased with reduced feeding intervals ranging from 77.8% in the D4 treatment to 88.9% for the D1 and D2 treatments, with an intermediate 83.3% survival recorded for the D3 treatment. No significant effect of the different feeding intervals on the growth performance and feed conversion ratio (FCR) was detected. Hence, feeding every day appears unnecessary and a longer feeding interval of up to once every four days for this size range might be recommended, particularly considering that in pond culture situation, there are naturally grown supplemental feeds available to redclaw.

In the attempt to improve water stability of the commercial redclaw feed, six different binders (agar, alginate, carboxymethyl cellulose, carrageenan, polyvinyl alcohol and starch) were added

at 3.0% dry weight, respectively to the crushed commercial redclaw diet and resultant new pellets compared for their water stability (i.e. physical form and dry matter lost (DML) after different durations of submergence in water), with the commercial redclaw diet as the control. Poor water stability of the commercial redclaw feed was confirmed as the feed totally disintegrated within 1 hour of immersion with a DML of 23.8%. All six binders significantly improved water stability but alginate was recommended as the overall best choice. The alginate was subsequently used as the binder incorporated at 5 concentrations (2.0%, 2.6%, 3.2%, 3.8%, and 4.4%) to evaluate the best inclusion level. The results showed that an inclusion level of 4.4% of alginate retained the best physical form of the pellet and produced the lowest DML overall of 16.2% after 24 hours immersion. The effect of pellet size (diameters: 1.0, 2.0, 3.0, 4.5, 5.0 and 7.0 mm) on water stability were also tested but appears to be a relatively minor factor.

The substantial reduction in wastage and the possibility of significantly improved water stability expected from the newly developed experimental diet (with alginate added at 4.4% as the binder) may lead to its less attractiveness to the redclaw and was tested by a feeding behavioural study on individual redclaw. To achieve this, behavioural experiments were designed to quantitatively assess and compare the feeding responses of redclaw to the introduction of the new pellets against the original commercial pellets, under identical feeding conditions.

The feeds used were both 4.5 mm in diameter and the results confirmed that the experimental diet remained water stable for a significantly longer time and as a result, had a significantly lower wastage level (5.0%) after 15 minutes of feeding compared to that of the commercial redclaw diet which crumbled quickly and with a significantly higher wastage (11.0%). It was further shown that in addition to its significantly improved water stability, the experimental feed was as attractive as the commercial diet to the redclaw as there was no significant difference in the mean

'time start feeding' as the redclaw responded to the introduction of both the experimental and commercial diets.

The final experiment examined the effects of pellet size on the feed wastage and feeding efficiency of three different sized redclaw using the experimental diet. The results showed clear evidence that using an inappropriate feed size led to significantly lower feeding efficacy and higher feed wastage and that the pellet size of 4.5 mm (the only size of commercial pellets available to redclaw farmers) were too big even for adult redclaw of individual weight of up to 50 g. Based on the results from this experiment, it was recommended that the most suitable pellet size for the juveniles (5 - 8 g), sub-adults (15 - 25 g) and adults (35 - 50 g) redclaw was 1.0, 2.0 and 3.0 mm, respectively.

This study has made several key findings for the improvement of feeding management and formulated feeds for redclaw crayfish farming. Feeding every day appears unnecessary and longer feeding intervals of up to once every four days are recommended for redclaw farmers to save on labour and other associated costs. The poor water stability of the currently available commercial redclaw feed in Australia can be substantially improved by incorporating 4.4% alginate of total diet dry weight as the binder. It is also recommended that instead of a single pellet size available at 4.5 mm only, the redclaw feed should be pelletised at 1.0 mm, 2.0 mm and 3.0 mm for juveniles, sub-adults and adult, respectively. These recommendations should enhance the productivity of the redclaw farming industry in Australia.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BW	Body weight
cm	Centimetre
CMC	Carboxymethyl cellulose
DML	Dry matter loss
DO	Dissolved oxygen
DW	Dry weight
FCR	Feed conversion ratio
g	Grams
h	Hour
ln	Natural log
L	Litre
L^{-1}	Per litre
mm	Millimetre
min	Minute
min^{-1}	Per minute
mL	Millilitre

NQCFA	North Queensland Crayfish Farmers Associations
n	Number of alive animals
Pers. Comm.	Personal Communication
SE	Standard error
SGR	Specific growth rate
t	Time
W_o	Initial pellet dry weight
PVA	Polyvinyl alcohol
Wa	Weight before moult
W_b	Weight after moult
W_i	Mean initial weight
W_t	Mean final weight
%WG	Percentage body weight gain
RIRDC	Rural Industries Research and Development Corporation

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The three freshwater crayfish families Parastacidae, Cambaridae and Astacidae are native to Australia, Europe and North America, respectively (Ackefors, 2000). Although there are around 100 species of freshwater crayfish belonging to the Parastacidae family, the majority of species are not commercially important due to their small size and slow growth rate (Whisson, 1999). Only three freshwater crayfish species in the *Cherax* genus, i.e. the redclaw *Cherax quadricarinatus*, the marron *Cherax tenuimanus* and the yabby *Cherax destructor* are commercially important and currently are being cultured in Australia (Horwitz, 1990; Lawrence and Jones, 2002). Redclaw (*C. quadricarinatus)* is native to Northern Australia, marron (*C. tenuimanus)* is native to South-West Australia and the yabby (*C. destructor)* is common to central and South-Eastern Australia (Figure 1.1) (Semple et al., 1990). Of these three species, redclaw has been identified as having the highest potential for aquaculture in tropical Australia (Jones, 1990; Treadwell et al., 1992). However, interest in the aquaculture and aquarium trade of redclaw over past decades have resulted in widespread translocations of the species, both within Australia (Figure 1.1, i.e. parts of Western Australia, New South Wales) and to regions of the world, including Southeast Asia, Central and South America, the United States of America and South Africa (Jones, 1990; Masser and Rouse, 1997; De Moor, 2002; Muzinic et al., 2004; Saoud et al., 2013).

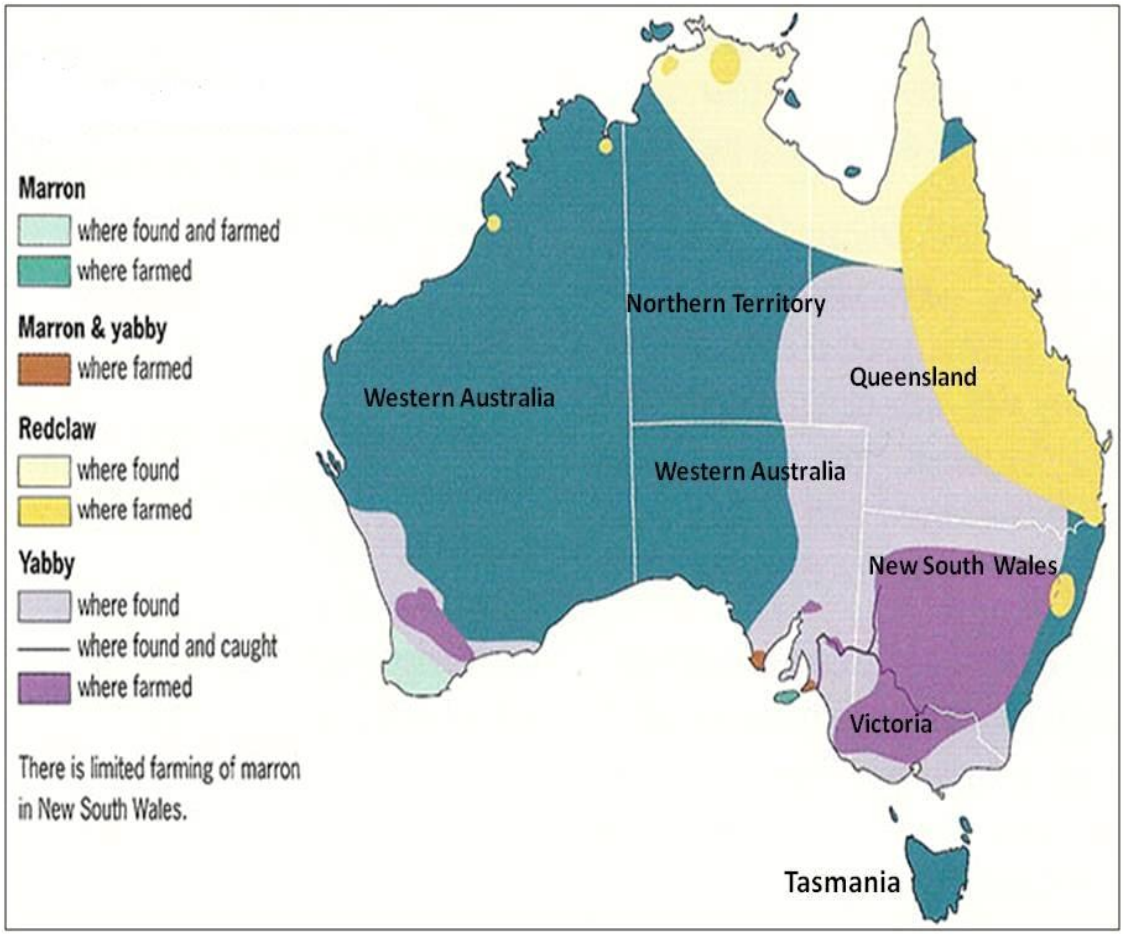


Figure 1.1 Natural and translocation distribution in Australia of the genus *Cherax* (Fisheries Research and Development Corporation, 2014).

1.2 Current status of redclaw aquaculture in Australia

Interest in redclaw aquaculture in Australia began in the mid-1980s (Jones and Ruscoe, 1996). The industry underwent a fast expanding initial phase with most farms being established throughout Queensland (Jones and Ruscoe, 1996). About 230 small farms were reportedly registered by 1990 (Jones, 1990), largely due to many biological, physical and commercial attributes that make the species a good candidate for aquaculture (Thompson et al., 2005). These include the following:

- It breeds easily, with no planktonic larval developmental stage.
- Has potential for selective breeding; many wild population strains.
- Tolerates high stocking densities.
- Requires low protein diet, not reliant on fishmeal.
- Lobster shape, flesh texture and flavour compatible to marine crustaceans.
- Market position as a high value crustacean.
- Meat recovery rate acceptable.
- Reaches commercial size after 9 months of grow-out (Food and Agriculture Organization (FAO), 2011 - 2013).

Unfortunately, despite all these positive attributes and optimistic forecasts, the redclaw farming industry in Australia has not fulfilled early high expectations and the industry has largely stagnated after initial fast expansion in the 1980s and 1990s. More recently, the redclaw aquaculture industry declined from its peak in 2006 and show a slow decline trend in both production and value (Figure 1.2) (Lobegeiger and Wingfield, 2006; 2009; 2010). For example, at the North Queensland Crayfish Farmers Associations' (NQCFA) Annual General Meeting in 2008, it was reported over 135 farms have no production at all and in addition that a couple of the larger redclaw farms were downscaling production while the properties were being offered for sale (Lobegeiger and Wingfield, 2009). Additionally, juvenile production decreased from $3.4 million in 2006 - 07 to $3.1 million in 2007 - 08 (Lobegeiger and Wingfield, 2009).

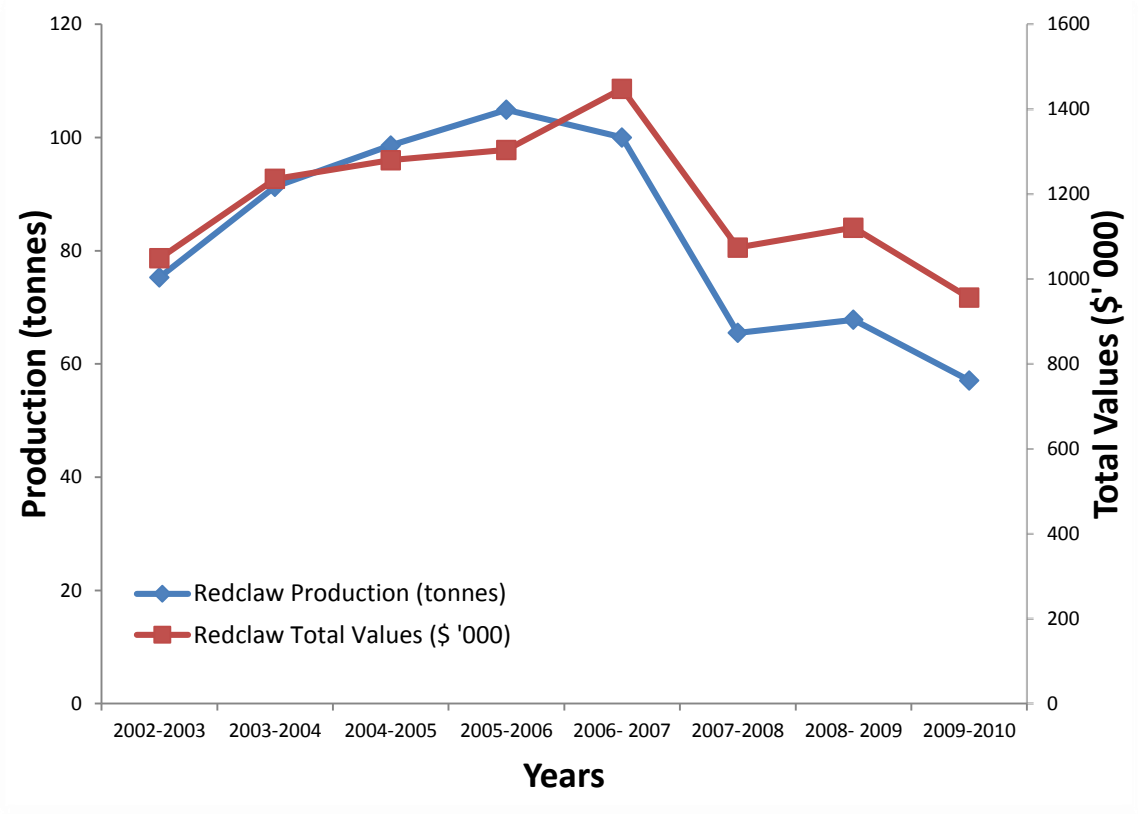


Figure 1.2 Redclaw aquaculture production and value trends in Queensland from 2002 - 03 to 2009 - 10 (based on Lobegeiger and Wingfield, 2006, 2009, 2010).

1.3 Impediments to redclaw aquaculture in Australia

Various problems have been identified to contribute to the declining production of the redclaw industry in Australia in recent years (Stevenson et al., 2013). In particular, an industry survey conducted in 2001 with the assistance of the Queensland Department of Primary Industries identified that a selective breeding program for faster growth, disease management, and improvement on feeds and feeding management, as the top priorities for the industry (Stevenson et al., 2013). The survey was used to formulate the Strategic Development Plan for the Australian Redclaw Industry. Since production of high quality redclaw juveniles has been a recurring issue and failures have often been attributed to their poor survival (Celada et al., 1989; Jones, 1990) and growth performance (Cortés-Jacinto et al., 2004), with funding support from the Rural Industries

Research and Development Corporation (RIRDC) of Australia, the redclaw selective breeding program was successfully implemented in 2009, which proved that it is possible to genetically improve redclaw to produce high quality and faster-growing crayfish (Stevenson et al., 2013). However, it was also pointed out that this advance cannot be capitalised upon without the availability of high quality, but low cost feeds specially designed for redclaw, as well as the establishment of a proper feeding management strategy for the industry (Stevenson et al., 2013).

The cost of feeds and feeding in modern aquaculture generally represent the greatest proportion of operational expense. In a high-intensive culture system, up to 70% of operating costs are attributable to feed (Sridhar, 1994). High-quality feeds enhance growth and minimise wastage. Therefore, to improve redclaw aquaculture production in Australia, the availability of a high quality redclaw formulated feed is obviously crucial (Mason, 1978; González et al., 2012). In addition, this needs to be incorporated with appropriate and adequate feeding management, since adopting a proper feeding management practice is also critical in enhancing production while reducing feed wastage and labour costs (Zelaya, 2005). Unfortunately, major problems with feeds and feeding have been present in the redclaw industry since its inception (Stevenson et al., 2013).

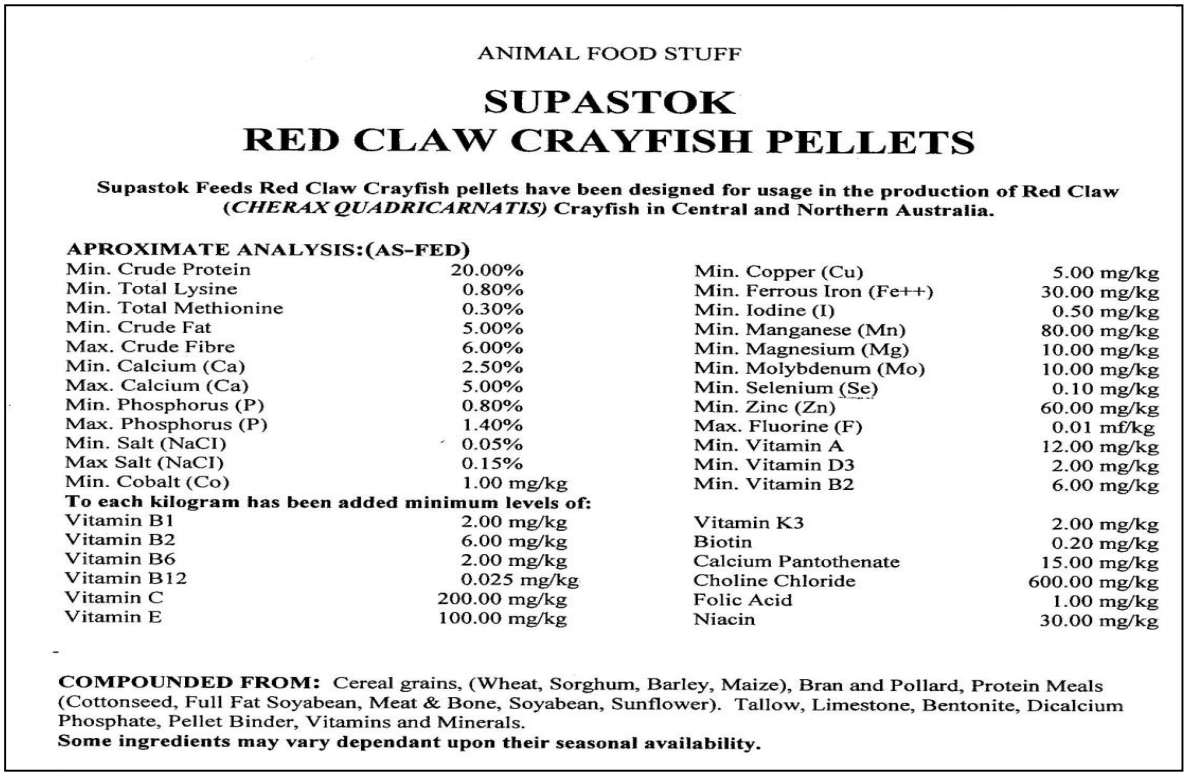
1.3.1 Formulation of feeds for redclaw

Understanding the function of the digestive enzymes secreted by redclaw, as well as ontogenetic changes in digestive enzyme activities (Xue et al., 1999; Figueiredo et al., 2001) is a necessary step to predict the ability of a species to utilize different dietary components of formulated feeds (Pavasovic et al., 2007). Due to the species omnivorous feeding habit, both animal and vegetable ingredients have been tested for the formulation of feeds for redclaw (Campana-Torres et al., 2006). Redclaw have been reported to possess the ability to modify their digestive enzyme secretions in response to a broad range of dietary ingredients including animal, single cell and

plant matter in their diet over time (Campana-Torres et al., 2006; Pavasovic et al., 2007; Saoud et al., 2012). Such information has been utilized in formulating cost-effective diets for redclaw (Figueiredo et al., 2001; Saoud et al., 2012).

On formulating a nutritionally balanced diet to meet redclaw requirements, past research has focused on the balance of major nutritional components, i.e. protein, carbohydrate and lipid (Cortés-Jacinto et al., 2004; Cortés-Jacinto et al., 2005; Pavasovic, 2008). Of these major nutritional components, protein is typically the most expensive component (Pavasovic, 2008). While fishmeal has been traditionally used as the major protein source for aquatic animals, asides from its high costs, the sustainability issue of fishmeal as the major protein source for aquaculture feed has also long been considered a major constraint (Watanabe, 2002). Hence, much nutritional research is underway to identify alternative dietary ingredients that are less expensive, more sustainable, palatable and nutritious to replace fishmeal (Muzinic et al., 2004; Thompson et al., 2005), which include a varieties of agricultural plant proteins, such as cereal grains, legumes and oilseeds (Allan et al., 2000).

Redclaw was found to have good digestibility for ingredients such as cereals (Lopez-Lopez et al., 2005). The ability of redclaw to digest ingredients containing high levels of starch means that the proportion of animal ingredients in their diets can be totally replaced to reduce feed costs, enabling better economic performance than other aquaculture species (Lopez-Lopez et al., 2005). This has led to the commercial redclaw feed available in Australia to contain a mixture of plant proteins and no fishmeal (Figure 1.3). Replacement of fishmeal by cereal grains and legumes in commercial redclaw feeds led to poorly bound pellets, often disintegrating and breaking up within minutes of being immersed in water (Figure 1.4). This has led to the major problem of low feeding efficiency, especially feeding behaviour and the physical processes of ingestion of redclaw (Ruscoe et al., 2005).



ANIMAL FOOD STUFF

SUPASTOK
RED CLAW CRAYFISH PELLETS

Supastok Feeds Red Claw Crayfish pellets have been designed for usage in the production of Red Claw (*CHERAX QUADRICARNATIS*) Crayfish in Central and Northern Australia.

APROXIMATE ANALYSIS:(AS-FED)

Min. Crude Protein	20.00%	Min. Copper (Cu)	5.00 mg/kg
Min. Total Lysine	0.80%	Min. Ferrous Iron (Fe++)	30.00 mg/kg
Min. Total Methionine	0.30%	Min. Iodine (I)	0.50 mg/kg
Min. Crude Fat	5.00%	Min. Manganese (Mn)	80.00 mg/kg
Max. Crude Fibre	6.00%	Min. Magnesium (Mg)	10.00 mg/kg
Min. Calcium (Ca)	2.50%	Min. Molybdenum (Mo)	10.00 mg/kg
Max. Calcium (Ca)	5.00%	Min. Selenium (Se)	0.10 mg/kg
Min. Phosphorus (P)	0.80%	Min. Zinc (Zn)	60.00 mg/kg
Max. Phosphorus (P)	1.40%	Max. Fluorine (F)	0.01 mf/kg
Min. Salt (NaCI)	0.05%	Min. Vitamin A	12.00 mg/kg
Max Salt (NaCI)	0.15%	Min. Vitamin D3	2.00 mg/kg
Min. Cobalt (Co)	1.00 mg/kg	Min. Vitamin B2	6.00 mg/kg
To each kilogram has been added minimum levels of:			
Vitamin B1	2.00 mg/kg	Vitamin K3	2.00 mg/kg
Vitamin B2	6.00 mg/kg	Biotin	0.20 mg/kg
Vitamin B6	2.00 mg/kg	Calcium Pantothenate	15.00 mg/kg
Vitamin B12	0.025 mg/kg	Choline Chloride	600.00 mg/kg
Vitamin C	200.00 mg/kg	Folic Acid	1.00 mg/kg
Vitamin E	100.00 mg/kg	Niacin	30.00 mg/kg

COMPOUNDED FROM: Cereal grains, (Wheat, Sorghum, Barley, Maize), Bran and Pollard, Protein Meals (Cottonseed, Full Fat Soyabean, Meat & Bone, Soyabean, Sunflower). Tallow, Limestone, Bentonite, Dicalcium Phosphate, Pellet Binder, Vitamins and Minerals.
Some ingredients may vary dependant upon their seasonal availability.

Figure 1.3 Ingredients of the redclaw formulated feed provided by the Ridley Agri Products Pty. Ltd.

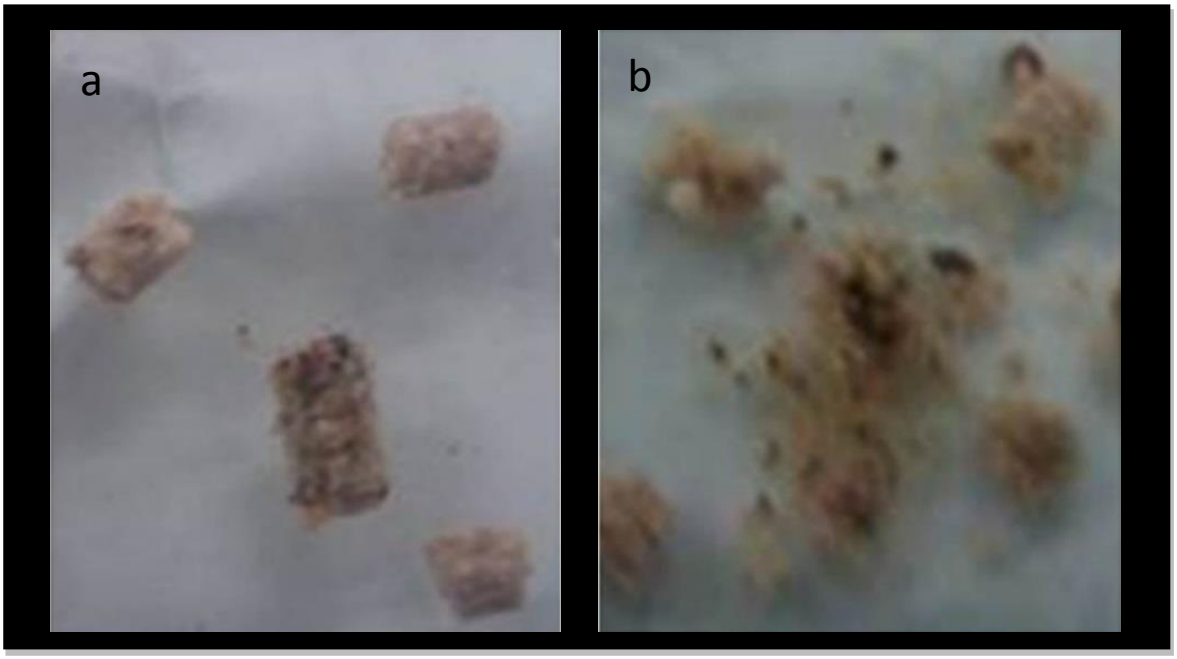


Figure 1.4 Commercial formulated pellet provided by the Ridley Agri Products Pty. Ltd a) pellet immediately after immersion in water and b) pellet disintegration 10 minutes after immersion in water.

1.3.1.1 Feeding behaviour of redclaw

Crustacean feeding behaviour differs substantially from that of fish, as crustaceans generally find food by chemoreception rather than visual cues. The fact that visual cues are not relied upon, poses a challenge when using formulated feed that lack feeding stimuli (D'Abramo, 2002). To ensure that formulated feed will be perceived by crustaceans as something suitable to eat, the food ideally should leach out a steady plume of attractants (Williams, 2007). Commercial formulated feeds for redclaw do not contain fishmeal; therefore, the leaching of chemo-attractants from other feed ingredients is crucial to preserve feed attractiveness. Research has shown that high inclusion of low-cost plant protein sources to replace fishmeal could result in reduced attraction, palatability or ingestion of feeds and hence lead to longer intervals between feed introduction and intake (Ahvenharju and Ruohonen, 2005). Longer intervals between feed introduction and intake lead to

feed disintegration and wastage. Formulated feed for redclaw may require a certain level of leaching out of chemical attractants to ensure feeding efficiency.

A number of chemical attractants were identified to stimulate feeding behaviour in crustaceans such as crayfish *Procambarus clarkii* (Corotto and O'Brien, 2002), prawns *Macrobrachium rosenbergii* (Harpaz et al., 1987; D'Abramo and Sheen, 1994) and shrimp *Penaeus wannamei* (Holland and Borski, 1993; Pittet et al., 1996). Leaching of amino acids found to be eliciting feeding behaviour for crustaceans considered to be carnivorous and carbohydrates, are the most stimulatory chemicals for eliciting feeding behaviour in crustaceans considered to be herbivorous or omnivorous (Kreider and Watts, 1998; Corotto and O'Brien, 2002). Behavioural analyses of feeding responses for the crayfish species *P. clarkii*, *Orconectes virilis* and *Orconectes rusticus* showed that carbohydrates produced stronger responses in food searching and substrate probing activity compared to amino acids (Tierney and Atema, 1988; Kreider and Watts, 1998). However, the initial feeding responses (i.e. orientation, approach and grasping) do not necessarily guarantee ingestion. It has been reported that once the feed reaches the mouth, oral receptors can determine ingestion or rejection of the feed (Kurmaly et al., 1990) and this final step in the feeding process is affected by food palatability (Harpaz, 1997).

When formulated feeds are exposed to water for prolonged periods, they often disintegrate and once disintegrated, they have been observed to be ignored by marron *C. tenuimanus*, another Australian freshwater crayfish (Jussila and Evans, 1998). Both economically and environmentally, disintegrated feed results in considerable financial loss (Ahvenharju and Ruohonen, 2005). Therefore, the feeding behaviour of crayfish places a high demand on the physical characteristics of formulated feeds, particularly their water stability (Sáez-Royuela et al., 2001).

1.3.1.2 Pellet water stability

Crayfish feeds with good water stability should minimise disintegration and excessive nutrient leaching prior to consumption by the animals (Ruscoe, 2005). Strategies for improving the water stability of formulated crayfish feeds have included both diet extrusion methods (Jussila and Evans, 1998) and incorporation of various binders in the formulation of the feeds (D'agaro and Lanari, 2004; Wolf, 2004; Ruscoe, 2005; Volpe et al., 2012). In the latter case, when a suitable binder is identified, an appropriate inclusion level should also be evaluated (Ruscoe, 2005). This is because diets containing too little binder may not be adequately water stable (Wolf, 2004). While alternatively, excessive high binder concentrations could result in a lack of nutritional value, due to the replacement of nutrients by binding agents which often have low nutritional values, and the addition of binders could also increases feed cost (Hashim and Saat, 1992; Durazo-Beltrán and Viana, 2001; Moond et al., 2011; Paolucci et al., 2011).

It has been reported that the addition of binders enhance the water stability of formulated diets that contain fishmeal as a major protein source for redclaw (Ruscoe, 2005) and other freshwater crayfish (Wolf, 2004; Volpe et al., 2008; 2012). However, formulated feeds with protein sources solely from low-priced, grain based protein, such as those commercial redclaw feeds used in Australia, have not been studied for crayfish.

It is believed that while alternative plant ingredients can be used to both replace fishmeal and reduce feed costs, they tend to create problems in terms of pellet integrity, as different feed ingredients have variable binding properties and particle size (Tumuluru, 2013), which can influence the binder choice (Dominy and Lim, 1991) and inclusion level (Durazo-Beltrán and Viana, 2001). For example, in their review of water stability of shrimp feeds, Lim and Cuzon (1994) concluded that ingredients that are hard to grind or have little to no binding properties, such as rice and oat hulls, are likely to result in pellets with poor water stability, because distribution of

each ingredient is not uniform enough to allow the binder to react properly (Meyers and Zein-Eldin, 1972). More than 42 per cent inclusion level of soybean material has also been reported to significantly decrease the water stability of pellets (Lim and Dominy, 1990). Durazo-Beltrán and Viana (2001) reported the difficulty in bonding fish silage to formulate pellets for abalone, probably because of the high content of hydrolysed protein. Therefore, when formulating crayfish feed, an appropriate binder needs to be identified when different feed ingredients are used, to ensure good water stability and minimise feed wastage.

The alternative of overly water stable pellets, could also be undesirable. For example, leaching of attractant molecules from tightly bound diets may be limited and may result in less attraction of the formulated feeds to the cultured animals (Partridge and Southgate, 1999). Additionally, the nutrients bound tightly may become more difficult to be digested by the animals (Balazs et al., 1973). Therefore, it is important that when formulating redclaw feeds, an appropriate balance is achieved on their water stability, acceptability and consumed efficiently at minimal wastage.

1.3.1.3 Pellet size

The size of pellets provided to crustaceans could also have a significant effect on the feed ingestion and wastage of feeds, as they break up into smaller fragments during the ingestion process and can become so small they can be no more utilised by the animals (Smith et al., 2009). Larger size of pellets may have the advantage of being more detectable by animals (Stradmeyer et al., 1988) and reducing animal energy expenditure on foraging. However, the pellet size that can be quickly detected by animals, might not be ingested readily if diameter of the pellet does not match with the mouthparts of the cultured crustaceans (Smith et al., 2009). Alternatively, if the pellet size is inadequate for the size of the cultured crustaceans, the feeding sequence (i.e. feed detection, acceptance and ingestion) may terminate at any stage. Consequently, to ensure a high feeding

success and efficiency, the pellet size needs to be appropriate to the feeding apparatus of the animals being fed.

It is well known that there is a direct relationship between fish mouth gap and pellet size; pellets of too large size for the mouth gape of a fish are often not effectively utilised and as fish grow in size the pellet size must also increase (Goldan et al., 1997). Although crustaceans generally do not swallow pellets like fish, they do use their mouthpart to chop pellets that are larger than their mouth before ingestion; such a process can generate considerable feed wastage. Juvenile and adult redclaw have been observed feeding only on large grain fragments when fed on steam pressed pellets of about 5 mm in diameter (Ruscoe et al., 2005). Fine granules were found propelled forward and away from the mouth by currents induced by the scaphognathites, as water is passed through the gill chamber of the redclaw (Ruscoe et al., 2005; Saoud et al., 2012). Similarly, Barker and Gibson (1977) describe the small particles in the vicinity of the mouth and feeding appendages of the European lobster, *Homarus gammarus*, appearing as a 'cloud of suspended matter being swept away from the oral region by the exhalent current of the branchial chamber' of the lobster. No attempt was made by the lobsters to eat small feed particles on the floor of the tank after they had eaten the larger pieces of the feed and had satisfied their appetite (Sheppard et al., 2002; Smith et al., 2009). Lobsters fed below satiation picked up and ingested small fragments (Smith et al., 2009). Actually, Hunt et al. (1992) reported that marine prawns ranging from 8 - 15 cm in total body length can ingest diet particles in the range of 8 - 20 μm in diameter. However, these fragments might require more energy and time to obtain the equivalent energy and nutritional benefits as larger ones, hence leading to reduced feeding efficiency and lower feed conversion ratios (D'Abramo, 2002; Smith et al., 2009). Additionally, due to leaching, fragmented feed would not have the nutrient composition as it was formulated (Smith et al., 2009). It is reported that feed wastage has been reduced from 50% to 19% when juvenile spiny lobster *Jasus edwardsii* were fed

ideal pellet sizes relating to its body size, as compared to those that are too large (Sheppard et al., 2002).

At present, commercial redclaw feeds in Australia are only available in one size (4.5 mm in diameter). This means that redclaw farmers have no option but to use such single size feeds for all developmental stages of redclaw. In general, the optimal situation is to increase feed size with the size of cultured animals, and in more established or developed aquaculture sectors, specifically formulated feeds are available in various sizes to suit different life stages of the animal. For example, barramundi pellets are available in eight sizes in Australia and similarly feeds for marine prawns come in a range of sizes and formulations. Sheppard et al. (2002) reported that for the spiny lobster *J. edwardsii,* the optimal pellet size for juveniles of 14 g was 3 × 3 mm and for larger individuals of 135 g, the size increased to 7 × 7 mm. Smith et al. (2009) also reported that the 1 mm diameter with 10 - 15 mm long pellets appear optimal for the tropical spiny lobster *Panulirus ornatus* of 2 g. While 3 mm diameter with 10 - 20 mm in length and 3 mm in diameter with 10 - 35 mm in length appear suitable for individuals of 50 - 60 g and over 100 g, respectively. Clearly, appropriate research has provided the basis for optimising food presentation which in turn has supported the growth of other aquaculture sectors. A similar approach is apparently needed for the redclaw industry.

1.3.2 Feeding management

Unlike terrestrial animals where feed consumption can be closely monitored and excess feeds retrieved, monitoring feed utilisation in aquatic environments is difficult and retrieving unutilised feeds problematic, particularly for benthic crustaceans. Therefore, good feeding practices and management are critically important in intensive aquaculture when using formulated feeds (D'Abramo, 2002). Poor feeding management can contribute significantly to the financial loss and

water quality degradation of an aquaculture farm (De Silva and Anderson, 1995). Frequent feeding is labour intensive, or requires installation of expensive automatic feeders (Sedgwick, 1979). Reducing the frequency of feeding may result in reduced growth rates and thus affects the economic viability of a farm (Cho and Bureau, 2002). Therefore, both under- and over-feeding can be detrimental to health and growth of the animal (Hernandez-Cortes et al., 1999), hence it is important to determine the best feeding interval of formulated feeds for redclaw aquaculture.

In more mature aquaculture sectors, such as Penaeid prawn aquaculture, it has been reported that excess feed was minimised by using a feeding chart as a guideline for feeding management (Carvalho and Nunes, 2006). Such a feeding chart was based on years of experience and research. Research on crayfish feeding management (Cortés-Jacinto et al., 2003) has not been conducted, in particular for redclaw. There are no standard feeding practices and management in Australia (John Stevenson; Pers. Comm.). The feeding interval is largely guesswork by the redclaw farmers and it varies from farmer to farmer, ranging from once per day to once per week (or even whenever a farmer has time) (John Stevenson; Pers. Comm.). Clearly, such variable practices arose from an absence of reliable information and research on best feeding practice. This lack of standardisation is a major concern since poor feeding management results in low feed efficiency and compromised productivity. Research needs to be conducting on feeding management to provide basis for the development of a proper feeding regime for the redclaw industry.

1.4 Aim and objectives of the study

The overall aim of the current study was to improve feeds and feeding management for redclaw aquaculture industry in Australia. The study is presented in six chapters. After the General Introduction chapter (**Chapter 1**), **Chapter 2** evaluates the different feeding intervals on the performance of juvenile redclaw using a commercial feed. The findings from this study should provide baseline information for improving feeding practices in Australia. **Chapter 3** reports the results of a series of three experiments evaluating the effects of various binders, binder concentrations and pellet sizes on the water stability of redclaw formulated feed. It identified the best binder as well as an optimal inclusion level of the binder that substantially improves the water stability of commercial redclaw formulated feeds. **Chapter 4** evaluates the acceptability and wastage of the commercial redclaw feed against the experimental feeds formulated based upon the best binder and binder concentrations identified in Chapter 3. **Chapter 5** examines ontogenetic changes in redclaw and the optimal pellet size for three different developmental stages of the redclaw. Through quantification and comparison of feeding efficacy and feed wastage when feeding three different size groups of redclaw with pellets of different sizes, the optimal size range of the pellets for each size group of redclaw was identified. The final General Discussion and Conclusion chapter (**Chapter 6**) highlights the main findings of the research and discusses their practical implications in a wider context.

CHAPTER 2: EVALUATING DIFFERENT FEEDING INTERVALS ON THE PERFORMANCE OF JUVENILE REDCLAW USING A COMMERCIAL FEED

2.1 Introduction

The benefits of using a formulated diet in modern aquaculture when compared to the use of trash fish or raw materials are enormous especially during the grow-out phase of the aquatic organisms. Such benefits include consistency, high nutritional value, ready availability, ease of storage, off the shelf convenience and reduced risk of bacterial contamination (Jones and Ruscoe, 1996; Rodriguez-Canto et al., 2002; Pavasovic, 2008). Despite the advantages of formulated feeds, feed still accounts for up to 70% of the total production costs in intensive aquaculture systems (Thompson et al., 2005). Therefore, good feeding management is crucial to minimise the overall production costs and consequently increase profitability.

Feeding management is an important factor in determining the profitability and success of an aquaculture farm and feeding frequency, or feeding interval, is one of the major considerations for feeding management. Conventional feeding strategies are adopted by many aquaculture farmers with a belief that more frequent feeding leads to faster growth and perhaps better food conversion rate in cultured animals. Indeed, it has been reported that splitting the same ration of formulated feeds into more feedings maximised feeding opportunities (Thomas et al., 2004), and also has the benefit of reducing nutrient leaching of formulated feeds (D'Abramo and Sheen, 1994; Velasco et al., 1999; Cho and Bureau, 2002; Smith et al., 2002). Hence, a less-than-ideal feeding frequency may result in reduced growth of cultured aquatic animals and therefore affect the productivity and profitability of aquaculture farming (Cho and Bureau, 2002). On the other hand, the high quality and consistency of formulated feed compared to natural feed could mean that less frequent feeding is sufficient to support good growth of aquaculture animals.

Devising a suitable feeding frequency may be directly linked to the feeding habits of cultured animals. Redclaw, like many other decapod crustaceans, is capable of consuming a large quantity of food and then refrain from feeding for a long period. The omnivorous feeding habit of the redclaw also means that under pond culture situations, less frequent feeding might be compensated by plenty supply of naturally grown foods available to redclaw in the ponds. Too frequent feeding could also disadvantage a farm, either by being labour intensive or require the installation of expensive automatic feeders (Sedgwick, 1979), thus increasing operational costs. The reduction of labour costs for farms is especially significant in developed countries such as Australia, where labour is very expensive. It is also particularly crucial for small farms, which is the case for most redclaw farms in Australia. Obviously, striking the right balance on feeding frequency is important as it can significantly affect its productivity and profitability.

Unfortunately to-date, optimal feeding management using formulated feeds for the culture of freshwater crayfish has not yet been well researched in intensive culture systems (Cortés-Jacinto et al., 2003; John Stevenson; Pers. Comm.). In particular, there is no established feeding management practice for Queensland's redclaw aquaculture industry. The feeding interval practiced in Queensland redclaw farms using the commercially formulated redclaw feed varies from one farm to another, and ranges from once per day to once per week or whenever a farmer has time (John Stevenson; Pers. Comm.). Clearly, research is urgently needed in this area for the Australian redclaw industry since both under- and overfeeding can be detrimental to the health and growth of the cultured animals. This study therefore examined the effect of four different feeding intervals, i.e. once per day, every second day, every third day and every fourth day, on the performance of juvenile redclaw over 140 days of continuous culture under controlled laboratory conditions. The range of feeding intervals adopted encompasses those most commonly used by Queensland redclaw farmers. The use of juveniles for the experiment was also based on the fact

that the main constraints in intensifying redclaw culture often include poor survival and growth of juveniles (Jones, 1990), with feed as a critical factor (González et al., 2012).

2.2 Methodology

2.2.1 Experimental animals

Redclaw juveniles were obtained from three Queensland redclaw farms, i.e. the "Captain Redclaw" farm located at Kelso, Townsville; the "AquaVerde Redclaw Hatchery and Farm" and another commercial farm both located at the Atherton Tablelands, North Queensland. The redclaw obtained from these farms were randomly mixed and acclimatised (temperature range 20 - 22°C) in two outdoor circular holding tanks of 2000 L capacity at the Marine and Aquaculture Research Facilities Unit (MARFU), James Cook University, Townsville, Queensland, Australia. The holding tanks were filled with de-chlorinated tap water and had numerous polyvinylchloride (PVC) pipes provided as shelter (De Bock and Greco, 2010). Aeration was provided by a blower through two air stones installed into each tank. A static culture system was adopted with 50% of the water in each tank exchanged every 4 days. The juveniles were fed to satiation as confirmed by observation of uneaten feed between 8 and 9 am daily in the morning daily with the commercially formulated redclaw diet produced by Ridley Agri products Pty. Ltd (20% crude protein, 5% crude fat and 6% crude fibre). Faeces and any leftover food were siphoned out every morning before feeding.

2.2.2 Culture system

The system used for the feeding interval experiment is illustrated in Figure 2.1. A cylindrical cage compartment was adopted to culture redclaw individually in order to minimise aggressive interactions, prevent breeding and avoid cannibalism of the experimental animals. A total of 72 cylindrical cage compartment culture units (18 cm high and 14.7 cm in diameter) made out of

high-strength polyethylene mesh (1 x 1 cm opening) were constructed (Figure 2.1 B) and six cages were kept in each of twelve 50 L rectangular tanks connected to a freshwater recirculating system (Figure 2.1 A). Each cage compartment was covered by a see-through plastic lid which was numbered for easy identification of each individual redclaw, and also allowed the accurate recording of moult interval and growth for all experimental animals (Figure 2.1 B). On top of each compartment lid, a heavy river stone was placed to prevent redclaw escaping from cages or tanks if the water level is high or equipment such as the airline tubing extends over the sides of a tank (Masser and Rouse, 1997).

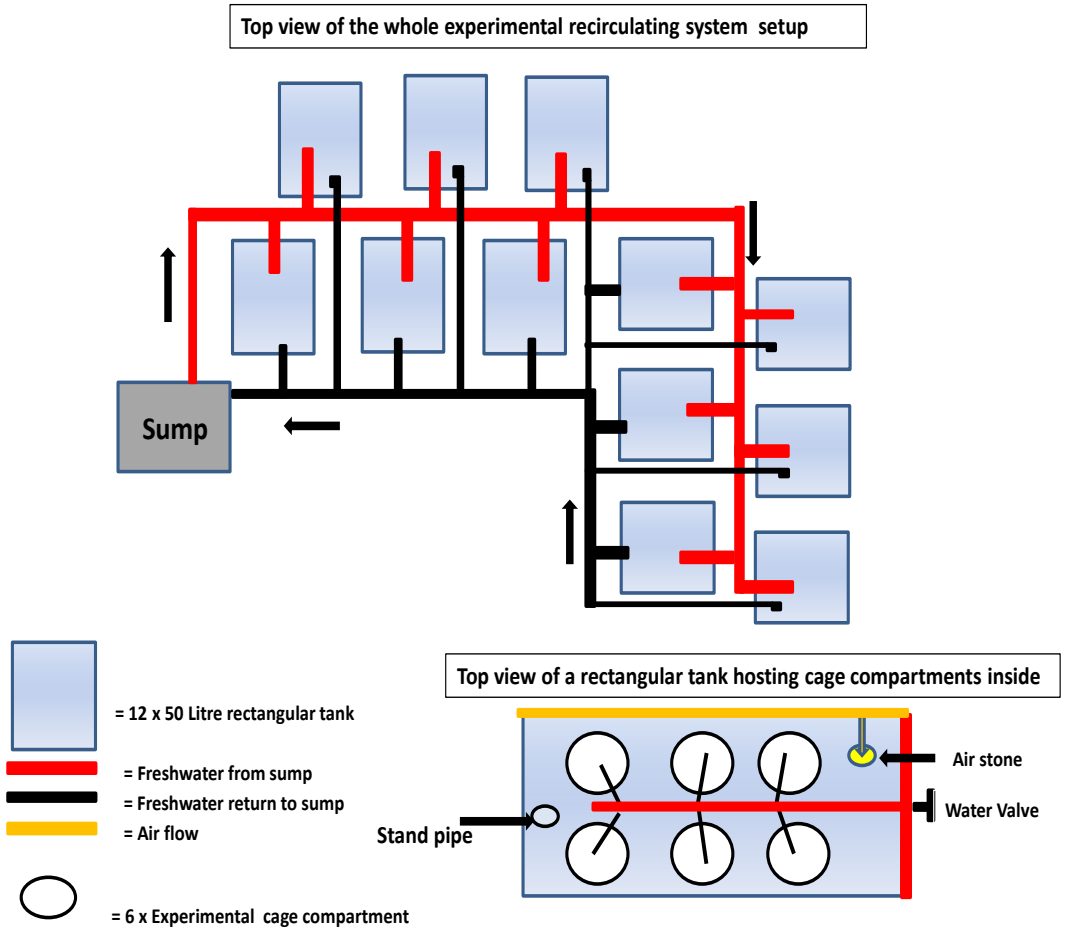
In each of the twelve 50 L rectangular tanks that hosted cage compartments, a water depth of 15 cm was maintained by a standpipe so that all cage compartments were approximately 3 cm above the water surface to deter escapes (Figure 2.1 A). The freshwater supply was pumped from a 3000 L sump tank and circulated into each cage compartment through a clear 5 mm tube that passed through a small hole in the cage lid and with an adjustable valve to control water flow (Figure 2.1 A and B). All 5 mm clear tubes supplied water to individual cages were connected to a 15 mm black inlet pipe extended through 2/3 of the length of the tank. A main water valve was fitted to the black inlet pipe to control the water flow (Figure 2.1 A). The flow rate was maintained from 3 to 4 L min^{-1} in each tank and water circulated freely through the perforated cage compartments before flowing back to the sump via the standpipe located at the opposite end of the inlet hose in each tank (Figure 2.1 A). Hence, consistent water quality was maintained for all culture units during the course of the study.

2.2.3 Experimental design and procedure

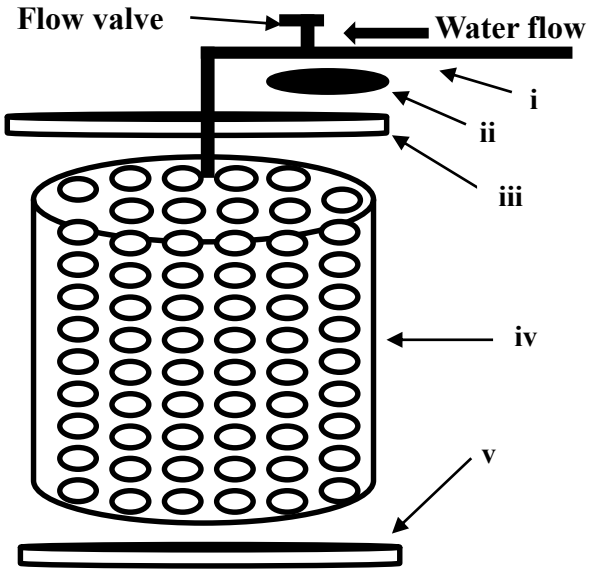
The feeding interval experimental design is outlined in Table 2.1. Four different feeding interval treatments were tested in which redclaw juveniles were fed once per day (D1), every second day

(D2), every third day (D3) and every fourth day (D4). A daily feeding ration equivalent to average 5% body weight (BW) of each experimental animal was maintained consistently across all treatments (Table 2.1). Feed rations were adjusted every fortnight, based on data obtained at each bi-weekly sampling time.

To start the experiment, a total of 72 juvenile redclaw (18 per treatment) with wet body weights ranging from 4 to 12 g were identified to be hard shell and selected from the outdoor holding tanks. They were stocked into individual cage compartments (see 2.2.2) and different sizes of redclaw were spread evenly throughout the four treatments so that a consistent mean initial wet body weight of 7.2 ± 0.3 g was achieved for each treatment. Each redclaw was kept in a cage culture unit which served as a replicate and the replicates of different treatments were distributed randomly among twelve 50 L tanks hosting the cage compartments.



A. View of experimental recirculating system used for this study.



B: Design of experimental cage compartment used to house individual juvenile redclaw for the feeding interval experiment. i: 5 mm clear pipe with adjustable valve to control water flow to each cage; ii: river stone to prevent escapes; iii: plastic dish serving as a lid; **iv**: cage compartment to permit water flow through; **v:** plastic dish serving a base. **NB** each lid has number for identification of each juvenile and a hole in the centre to allow clear pipe through to supply water.

Figure 2.1 Schematic diagram of freshwater recirculating system used for the feeding interval experiment.

After the juveniles were stocked in the cage compartments, they were allowed a one week acclimation period before the experiment started. During the acclimation period, all juveniles were fed to satiation as confirmed by observations of uneaten feed between 8 and 9 am daily on the commercial redclaw feed (Ridley Agri products Pty. Ltd: 20% crude protein, 5% crude fat and 6% crude fibre) to be used for the experiment. The commercial redclaw pellets had a size of 4.5 mm in diameter. Throughout the acclimation and subsequent experiment duration, a photoperiod of 12 h light: 12 h dark was maintained with a timer controlling 55 W fluorescent lights mounted in the ceiling of the laboratory.

Following acclimatisation, the experiment commenced with redclaw juveniles of different treatments fed the same diet on overall same ration but different feeding intervals as outlined in Table 2.1. The experiment lasted a total of 140 days and during the whole experimental duration, mortality and the presence of newly moulted individuals were checked and recorded daily before feeding, and any dead individuals and exuvia were thereafter removed from the cage compartments.

Table 2.1 Different feeding intervals (every day (D1), every second day (D2), every third day (D3) and every fourth day (D4)) for redclaw juveniles used to assess the effect of feeding intervals on performance response.

Treatment	Feeding Interval	Ration (% BW) at each feeding time
D1	every day (24 h)	5
D2	every second day (48 h)	10
D3	every third day (72 h)	15
D4	every fourth day (96 h)	20

Since the water recirculation rate (3 to 4 L min^{-1}) was not sufficient to flush all solid wastes out of the system, siphoning was carried out twice daily; the first round was to remove faeces 1 hour before feeding and the second round was to remove mounds formed by fine particulates from disintegrated feed 8 hours following feeding. To remove bacterial growth and scum formed on the tank surfaces and cage compartments housing the animals, every fortnight, following sampling of the redclaw for weighing, they were thoroughly scrubbed, rinsed, and new cleaned cage compartments used to replace the old ones. The used cage compartments were then soaked in clean water with added chlorine for disinfection. They were then left to dry and ready for reuse at the next cycle of tank cleaning.

Throughout the experiment, water temperature and dissolved oxygen (DO_2) were measured every day using a dissolved oxygen metre (Sper Scientific, model 850045). The water temperature was maintained between 24 - 27°C by a combined heater/chiller unit installed to the sump while DO_2 concentration was kept above 4 mg L^{-1} by aeration provided to each tank with an air stone. Levels of ammonia, nitrite, nitrate and pH were measured once per week using an API Aquarium Pharmaceuticals freshwater aquaculture test kit and maintained at <0.1 mg L^{-1} , 0 - 0.25 mg L^{-1}, 5 - 10 mg L^{-1} and 7.4 - 8, respectively. All these water quality parameters were within the acceptable ranges for redclaw (Masser and Rouse, 1997).

2.2.4 Data collection

Wet body weight (g), body length (mm) and carapace length (mm) of each experimental animal were measured at the beginning of the experiment and bi-weekly thereafter throughout the 140 days of the experimental duration. Prior to weighing, the juvenile redclaw were individually placed on a piece of absorbent towel to remove excess water and then weighed using a Mettler electronic balance to the nearest 0.001 g. The juveniles were then gently returned to their respective

compartments after weighing. Any juveniles that were found having just moulted within a day of weighing were weighed after 3 days of ecdysis since this duration is considered sufficient to allow them to enter the intermoult phase with stabilised body weights (Burton and Mitchell, 1987; Jones et al., 1996). Body length was measured from the beginning of the rostrum to the end of telson with a ruler, while carapace length was measured from the beginning of rostrum to the distal end of the carapace with a pair of Vernier Callipers (all the lengths were measured to the nearest 0.01 mm).

Growth performance of the redclaw was assessed by the increase in individual wet body weight (g), body length (mm) and carapace length (mm), as well as percentage weight gain (%WG) and specific growth rate (SGR, % day^{-1}) calculated based on weight and length data obtained using the following formula:

$$\text{Weight Gain (\%)} = \left(\frac{\text{Final Weight}}{\text{Initial Weight}}\right) \times 100$$

$$\text{Specific Growth Rate (SGR, \% day}^{-1}) = \left(\frac{(ln\,\text{Final Weight} - ln\,\text{Initial Weight})}{\text{Time}}\right) \times 100$$

Where *ln* is the natural logarithm, W_t is the mean final weight (g), W_i is the mean initial weight (g), and T is the duration of the experiment (days).

When a moult was identified, the moult interval and the number of moults were first recorded against the particular redclaw. However, the weighing of the crayfish was done 3 days after the moult event to allow the juvenile redclaw to enter the intermoult phase with a stabilised weight (Burton and Mitchell, 1987; Jones et al., 1996). The moult interval which is the duration of each moult interval for each juvenile during the 140 days experimental period was determined and the percentage weight increment after the moult (Wm, %) was then calculated based on following equation:

$$\text{Wm}\ (\%) = \left(\frac{\text{Wb} - \text{Wa}}{\text{Wb}}\right) \times 100$$

Where Wb is weight after a moult (g) and Wa is weight before a moult (g).

The overall feed conversion ratio (FCR) in each treatment over the experiment period was estimated based on the total amount of feed used over the 140 days duration as well as the increase in weight (i.e. final weight – initial weight) of the redclaw in the treatment:

$$\text{FCR} = \frac{\text{Dry feed offered}}{\left(\text{Final Weight}_{(g)} - \text{Initial Weight}_{(g)}\right)}$$

Overall survival of each treatment was calculated as the percentage of the initial number of redclaw surviving to the end of the rearing period:

$$\text{Survival}\ (\%) = \left(\frac{\text{final redclaw number}}{\text{initial redclaw number}}\right) \times 100$$

The dry weight (DW) and ash free dry weight (AFDW) of all surviving redclaw were measured at the end of the experiment. All redclaw were then euthanized by chilling in an ice water bath for approximately 8 hours. Any redclaw that moulted within 3 days of termination of the experiment were left for a full 3 days to allow hardening of their shell before they were sampled. All redclaw samples were then placed in an oven at 105°C for 24 hours until a constant dry weight was achieved. Thereafter, the dried samples were individually placed in a muffle furnace at 500°C for 8 hour to determine the AFDW of each sample.

2.2.5 Statistical analysis

Data are presented as means ± standard error (SE). A One way analysis of variance (ANOVA) was performed to detect any significant differences of measured parameters among the treatments with the significance level set at $P < 0.05$. Data were tested for normality and homogeneity prior to

the analysis, and where required, transformed (logarithmic and arcsine) to meet the requirements for performing the statistical analyses. All statistical analyses were performed using the statistical package SPSS (version 21).

2.3 Results

2.3.1 Survival rate and FCR

The overall survival rates of different treatments over the 140 days culture period increased with reduced feeding intervals ranging from 77.8% in the D4 treatment to 88.9% for the D1 and D2 treatments, with an intermediate 83.3% survival recorded for the D3 treatment. Unfortunately, statistical analysis was not possible due to the lack of replicates (Table 2.2). No significant difference was detected among different feeding intervals over the experiment duration for feed conversion ratio (FCR) ranging from 13.9 ± 0.1 to 14.1 ± 0.2 ($P > 0.05$; Table 2.2), although slight improvements in FCR with more frequent feeding were observed.

Table 2.2 Final survival and feed conversion ratio (FCR) (mean ± SE) of juvenile redclaw fed at four different feeding intervals: every day (D1), every second day (D2), every third day (D3) and every fourth day (D4), over a 140 day rearing period.

	Feeding interval treatments			
	D1	**D2**	**D3**	**D4**
FCR	13.9 ± 0.1	13.9 ± 0.1	14.1 ± 0.2	14.0 ± 0.1
Survival (%)	88.9%	88.9%	83.3%	77.8%

2.3.2 Moulting interval and moult increment

The various feeding intervals did not significantly affect weight increases at the first moult event ($P > 0.05$, Table 2.3). After the initiation of the experiment the moult interval of juvenile redclaw did not differ significantly for different feeding intervals but increased with lengthened feeding intervals ($P > 0.05$, Table 2.3). Juveniles which had moulted at the first moult event totalled 16 for the D1 and D2 treatments and 15 for the D3 and D4 treatments, however, by the end of the experiment, not all juveniles had progressed to their second moult. Those juveniles which had moulted twice over the 140 days experimental period totalled 11, 10, 9 and 7 for D1, D2, D3 and D4 treatments, respectively. The mean moulting interval of the second moult based on those individuals moulted for the second time did not differ significantly for all the treatments but shown to be longer as feeding intervals increases ($P > 0.05$, Table 2.3).

Table 2.3 Total moults, moulting intervals and percentage weight increments (Wm, %) (mean ± SE) of juvenile redclaw fed at four different feeding intervals: every day (D1), every second day (D2), every third day (D3) and every fourth day (D4), over a 140 days rearing period.

	Feeding interval treatments			
	D1	**D2**	**D3**	**D4**
First total moults	16.0	16.0	15.0	15.0
First moulting interval (days)	31.5 ± 1.5	31.9 ± 1.5	32.1 ± 1.5	32.3 ± 1.5
Second Wm (%)	28.7 ± 1.9	28.3 ± 1.9	27.1 ± 1.9	26.6 ± 2.0
Second total moults	11.0	10.0	9.0	7.0
Second moulting interval (days)	80.0 ± 1.8	80.5 ± 1.9	80.9 ± 2.0	81.3 ± 2.2
Second Wm (%)	18.2 ± 1.0	19.9 ± 1.0	20.4 ± 1.1	21.5 ± 1.2

2.3.3 Overall growth performance

The growth performance of the juveniles in terms of their final wet body weights, final dry weight (DW) and ash free dry weight (AFDW), final body length, final carapace length, specific growth rate (SGR) and percentage body weight gains (%WG) in response to the different feeding intervals, over the 140 days culture period are presented in Table 2.4. At the start of the experiment, the initial mean wet body weight, body length and carapace length of the juveniles for all the feeding interval treatments were uniform at 7.2 ± 0.3 g, 6.9 ± 0.9 mm and 3.2 ± 0.9 mm, respectively. Over the 140 days rearing period, final wet body weights, final body lengths and carapace lengths, were not significantly ($P > 0.05$; Table 2.4) different among the four feeding intervals although a slight improvement in these parameters was generally observed with shorter feeding intervals. Similar results were obtained for the SGR and %WG of the redclaw with no significant difference detected among all feeding interval treatments ($P > 0.05$) with a trend of slightly decreased SGR from 0.4 ± 0.0% day^{-1} for feeding daily (D1) to 0.3 ± 0.0% day^{-1} for feeding once every four days (D4) (Table 2.4). It was found that the DW increased with decreased feeding intervals (Table 2.4), but no significant differences ($P > 0.05$; Table 2.4) were detected between the treatments. There were also no significant differences ($P > 0.05$; Table 2.4) for juvenile AFDW among all treatments.

Table 2.4 Mean (± SE) of final wet body weight, dry weight (DW), ash free dry weight (AFDW), final body length and final carapace length, specific growth rate (SGR) and percentage body weight gain (%WG) of juvenile redclaw fed at four different feeding intervals: every day (D1), every second day (D2), every third day (D3) and every fourth day (D4), over a 140 days of rearing period.

	Feeding interval treatments			
	D1	**D2**	**D3**	**D4**
Final wet body weight (g)	12.7 ± 0.9	12.6 ± 0.9	12.5 ± 0.9	11.2 ± 0.9
DW	35.0 ± 0.4	34.6 ± 0.4	34.1 ± 0.5	33.8 ± 0.5
AFDW	33.5 ± 0.5	33.2 ± 0.5	32.7 ± 0.5	32.4 ± 0.5
Final body length (mm)	7.9 ± 0.2	7.9 ± 0.2	7.8 ± 0.2	7.8 ± 0.2
Final carapace length (mm)	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
SGR (% day^{-1})	0.4 ± 0.0	0.4 ± 0.0	0.4± 0.0	0.3 ± 0.0
%WG	75.5 ± 7.3	72.8 ± 7.3	72.0 ± 7.3	68.9 ± 7.6

2.4 Discussion

The results of this study showed that over a 140 days culture period the feeding intervals tested which ranged from feeding once every day to once every four days, had no significant effect on all parameters measured, including the growth performance and FCR of the juvenile redclaw held individually. These results are in agreement with a study of juvenile white-clawed crayfish *Austropotamobius pallipes* that changes in food supply frequency of once a day to twice daily did not improve the growth parameters over an 80 days culture period (Saez-Rayuela et al., 2001). In contrast, Ulikowski and Krzywosz (2006) and Mazlum et al. (2011) found that the same range of

feeding intervals as applied in the current study significantly affected the growth performance of early juveniles of narrow-clawed crayfish *Astacus leptodactylus.* In that study, early juveniles with initial mean body weight of 30.2 mg were used and cultured for 28 days. Mazlum et al. (2011) using slightly larger juveniles with an initial weight of 44.5 mg and cultured for 90 days found similar results. While many factors, including the species studied, the initial weight at stocking, the culture system used and the rearing period can significantly affect the outcomes of a feeding interval experiment, the differences between the current study compared to those by Ulikowski and Krzywosz (2006) and Mazlum et al. (2011) can most likely be attributed to substantial differences in the sizes of animals used for the experiment. It is well known that early juvenile crayfish grow substantially faster than late juveniles and have significantly higher metabolic rates (Lowery, 1988; Evans and Jussila, 1997). Additionally, their small body sizes limit their capacity to store ingested food, and it is likely that they need to be fed more regularly than large crayfish to achieve normal growth.

While statistical analyses were not possible due to the lack of replication, the survival rates slightly increase from 77% to 89% with reduced feeding interval. Survival rates of more than 80% are considered generally acceptable in crustacean studies (Cuzon and Guillaume, 1997). The high survival rates obtained over a prolonged 140 days period in the present study are likely attributable to the fact that juveniles were held in individual compartments that prevented cannibalism. Furthermore, water quality was also maintained generally near optima by the recirculation system as well as regular siphoning and cleaning of the culture units.

The SGR obtained in the current study were relatively low compared to results from other studies using compartmentalised individual rearing systems and similar initial sizes of juveniles for stocking. For example, Rodriguez-Canto et al. (2002) found that redclaw juveniles (average 6.9 g at stocking) reared for 150 days had a SGR at 0.96% day^{-1} based on wet body weight increase. In another study, juvenile redclaw which averaged 8.8 g at stocking and reared for 93 days exhibited

a SGR of 0.86% day^{-1}, also based on wet body weight increase (Barki et al., 2006). Both these studies fed the redclaw once daily and obtained much better SGR compared to the SGR obtained for the current study for the same feeding regime (once daily treatment). The poor growth performance of the redclaw in the current study probably could be attributable to poor water stability of the commercial redclaw feed used in the present study (see Chapter 3) as well as offering an unsuitable pellet size for the juveniles (see Chapter 5), which likely substantially reduced the utilization efficiency of the feed. In fact, when unstable feed was used to feed juvenile marron, *Cherax tenuimanus,* for 90 days, SGR values obtained were between 0.54 and 0.58% day^{-1} (Jussila and Evans, 1998), which are close to the results obtained in the current study.

Similarly, inadequate feed can depress the growth of crustaceans by lengthening the intermoult period and reducing the moult increment (Hartnoll, 1982). As a result, the intermoult period for the current study between successful moults was shown to be greater than 80 days and some of the juveniles for all the treatments did not moult twice over 140 days of rearing. Intermoult duration for the current study is longer than previously reported by Jussila (1997) when juveniles *Cherax tenuimanus* (initial weight of 11 g) were fed stable pellets and moulted twice compared to juveniles fed unstable pellets that only moulted once during 125 days of rearing. Average weight gain at first moult and second moult increase was shown to be less than 30% of the premoult weight for all treatments. The weight gain at moult is normally between 30% and 60% of the premoult weight for crayfish (Morrissy, 1984; Wolf, 2004) and these figures were higher than in the present study confirming the slower growth obtained in the current study. Mazlum et al. (2011) found that crayfish with minimal weight gains when cultured using poor quality feeds exhibited significantly improved weight gain when fed with high quality feed.

Similarly, FCRs from the present study are generally poorer than those reported for other crayfish (Manomaitis, 2001; Mazlum et al., 2011). Again, the poor quality of the feed used in the present study, particularly in terms of low water stability and inappropriate size, is likely to be the main

contributor to the outcome. For instance, it was revealed in our previous study that the commercial redclaw feed used for the current study had more than 23.8% of dry matter loss (DML) after immersed in water for 1 hour (see Chapter 3). In fact, Cuzon et al. (1994) concluded that feeds with a DML of over 10% after 1 hour immersion should be considered inadequate. In addition to the feed water stability issue, it was also observed that the size of pellets used appeared to be too large (4.5 mm diameter) for the redclaw juveniles and this resulted in longer feed fondling times and feed wastage (see Chapter 5). Sheppard et al. (2002) reported that by providing pellets of an ideal size to spiny lobster *J. edwardsii*, feed wastage can be reduced from 50% to 19%. Hence, feed wastage resulting from feed disintegration due to low water stability and also from redclaw fondling the inappropriately sized pellets are likely to be the two main contributors to the poor FCR values obtained in the present study. Clearly, development of formulated feeds with substantially improved water stability and providing redclaw with suitably-sized pellets to suit their ingestion process should help reduce the necessity for frequent feeding while simultaneously improving growth performance and FCR.

Meanwhile, Jusilla (1997) suggested that pellets which disintegrated quickly should not be administered at a rate of greater than 1% wet body weight per feeding because once the pellet has disintegrated, the fine particles which remain were observed to be largely ignored by the marron, *C. tenuimanus*, another Australian freshwater crayfish species closely related to redclaw. In the present study, the feeding ration at each feeding ranged from 5% to 20% body weight depending on the feeding interval treatments, which were higher than the 1% body weight suggested by Jusilla (1997) for low water stability feeds. However, no attempt was made to reduce the ration as that would lead to more frequent feeding and would not reflect what is being practised by Queensland redclaw farmers.

Besides the two main reasons of low water stability and unsuitable size of pellets that are likely to cause substantial feed wastage, the cage compartment culture system used for the current study

might also have contributed to feed wastage. It was observed that as the redclaw were restricted within the cage compartments, their ability to forage on feed particles that had spread outside the cage during their fondling of feed was restricted, and this could also lead to some feed wastage. However, the individual compartment culture system was necessary to prevent cannibalism, reduce aggressive social hierarchical interactions and minimise breeding that could have compounded the results.

In summary, it appears that within the range of feeding intervals tested, feeding frequency had no significant effect on the growth performance, FCR and survival of juvenile redclaw, hence feeding every day appears unnecessary and a longer feeding interval of up to feeding once every four days might be recommended to redclaw farmers save on labour costs. Such a recommendation is particularly valid when considering that in the present study, besides the formulated feed provided, there was no other food source available to the redclaw. However under actual pond culture situations, redclaw can utilise various natural feeds available in the ponds, and hence should be able to cope much better with longer feeding intervals. It is also worth noting that although the feed used in the current study was the same as that used by the Queensland redclaw farmers in pond culture, the results were obtained with redclaw cultured individually in laboratory compartmental units. Hence, whether less frequent feeding might increase the incidence of cannibalism in communal pond culture conditions is unknown. Furthermore, since redclaw is not a particularly cannibalistic species, with properly installed shelters and reasonable natural production existing in the pond, cannibalism might be able to be kept under control, however, further investigation is required to confirm this. Finally, it should also be noted that unlike what occurs under laboratory culture conditions, the uneaten feed that disintegrates in the pond water is probably not a total waste, as such feed might become the nutrients that drive natural pond productivity, which in turn could become additional feed for the redclaw.

CHAPTER 3: THE EFFECT OF BINDER TYPE, BINDER CONCENTRATION AND PELLET SIZE ON THE WATER STABILITY OF FEEDS FORMULATED FOR REDCLAW CRAYFISH

3.1 Introduction

Since the early 2000s, a mixture of fragmented grains have been used as a major protein source for the production of commercial redclaw crayfish feeds in Australia (Ruscoe et al., 2005). However, the resultant pellets have poor water stability, often disintegrating within minutes of being immersed in the water (see Chapter 1, Figure 1.4) when offered for feeding.

It is evident that while these alternative plant ingredients are used to replace fishmeal in an effort to reduce feed costs, they tend to create problems in terms of pellet integrity (Behnke, 1996). Diet ingredients have been reported to have different binding abilities and particle sizes (Tumuluru, 2013), that directly influence the binding capacity of the binders (Dominy and Lim, 1991) as well as their required inclusion level (Durazo-Beltrán and Viana, 2001). For example, in their review of water stability for shrimp diets, Lim and Cuzon (1994) concluded that ingredients which are hard to grind or have little or no binding properties, such as rice and oat hulls, are likely to result in pellets with poor water stability. More than a 42% inclusion level of soybean meal in diets has also been reported to significantly decrease the water stability of the resultant pellets (Lim and Cuzon, 1994).

Pellet quality with respect to its water stability is of considerable significance for crayfish, because they generally find food through chemoreception (Grasso and Basil, 2002; Ahvenharju and Ruohonen, 2005). After locating feed, crayfish process the feed by external mastication prior to ingestion (Sáez-Royuela et al., 2001). Due to this feeding behaviour, the duration that pellets remain stable in water is even more important (Meyers and Zein-Eldin, 1972).

High pellet water stability is defined as the retention of physical pellet integrity with minimal disintegration and nutrient leaching during immersion prior to consumption (Marchetti et al., 1999). Pellets that break up into small pieces and quickly leach nutrients (Obaldo and Tacon, 2001), particularly within the first 30 minutes of exposure to water (Genodepa et al., 2007), could lead to reduced water quality, poor animal growth, inefficient feed conversion, and low survival (Obaldo and Tacon, 2001).

A major method to increase pellet water stability of aquaculture feeds is to add certain binders, to hold the various feed ingredients together as reported by various researchers (D'agaro and Lanari, 2004; Ruscoe et al., 2005; Volpe et al., 2008; 2012). Of the different types of binders, carbohydrate binders were more frequently used for the formulation of crayfish feeds, following the study by Xue et al. (1999) which revealed that redclaw crayfish produce endogenous enzymes capable of digesting complex carbohydrates. The most frequently used carbohydrates binders include alginate, agar, carboxymethyl cellulose (CMC), starch and carrageenan (D'agaro and Lanari, 2004; Ruscoe et al., 2005; Volpe et al., 2008; 2012).

The concentration of a binder to be incorporated is another important consideration. Pellets containing binder concentrations that are too low may not be adequately water stable, resulting in the deterioration of water quality and the loss of valuable dietary nutrients (Wolf, 2004). On the other hand, overly high concentrations of binding agents can result in a lack of nutritional value to the animal due to the replacement of key nutrients by binding agents, which is also likely to increase the feed production costs (Hashim and Saat, 1992; Durazo-Beltrán and Viana, 2001; Moond et al., 2011; Paolucci et al., 2011). The optimal binder concentration is reportedly also affected by ingredient type and composition. For example, Durazo-Beltrán and Viana (2001) found that pellet stability increased as alginate proportion decreased to 0.5% for formulations containing

fishmeal, whereas in treatments with fish silage as the protein source, feed stability increased as the alginate proportion increased to 1.6%.

Additionally, pellet size is another factor which can possibly affect the stability of feeds in water, as generally larger pellets are considered to be more water stable (Obaldo and Tacon, 2001). The increase in surface area to volume ratio of smaller-sized pellets is considered to be attributed to its lower water stability (Obaldo and Tacon, 2001).

Therefore, the aims of this study were to evaluate whether the addition of various binders and at different concentrations might help to improve the water stability of the currently available commercial redclaw crayfish feed in Australia. This was achieved through a three-step trial. Firstly, six binders (alginate, agar, carrageenan, CMC, PVA and starch) were mixed at an identical concentration with crushed commercial redclaw crayfish pellets and subsequently re-pelletised as dry pellets; then the water stability of these pellets was compared to determine the best binder. Utilising a similar approach, the best binder identified was subsequently incorporated at a range of concentrations to determine the best inclusion level. Additionally, the potential effect of pellet size on diet water stability was also tested.

3.2 Methodology

3.2.1 Effect of different binders on pellet water stability

3.2.1.1 Feed formulation with six different binders

The commercial redclaw crayfish pellets (Ridley Agri Products Pty. Ltd) was used as the base diet in this study and first ground and then passed through a 710 μm sieve to break up any clumped particles. Thereafter, 200 g of sieved feed was weighed and mixed with each of the six binders, agar, alginate, CMC, carrageenan, PVA and starch (Sigma-Aldrich, Sydney), to make the pellets

for each binder treatment. Characteristics of the six binders tested are listed in Table 3.1. Each binder was weighed at 3% of the total diet dry weight and dissolved in 120 mL of 65°C distilled water, except for carrageenan which was dissolved in an extra 100 mL of distilled water (i.e. 220 mL). Each binder solution was then thoroughly mixed with ground commercial redclaw crayfish feed and kneaded until it formed soft dough. Subsequently, a small pelleter was used to pelletize the dough into spaghetti-like strands which were then placed on aluminium foil dishes and oven dried at 60^{o}C until a constant weight was achieved. Dried pellet strands were then broken manually to small sizes of approximately 5 mm, packaged in labelled plastic bags and stored at 10^{o}C to maintain the quality until used for the trial.

Table 3.1 List of the binders tested.

Binders	**Source**	**Nutritional value to redclaw crayfish**
Agar	Red seaweeds	Yes, carbohydrates
Carrageenan	Red seaweeds	Yes, carbohydrates
Alginate	Brown seaweeds	Yes, carbohydrates
Carboxymethyl (CMC)	Cellulose	Yes, carbohydrates
Polyvinyl alcohol (PVA)	Synthetic	No

Seven treatments were set up including six different binders and the commercial redclaw crayfish pellet as the control. All seven treatments were assessed for water stability firstly at 10 minutes, 30 minutes and then 1 hour. After this the control treatment was excluded due to the fact that it had totally disintegrated, however, the six experimental treatments were further tested for water stability at 2, 4, 8, 12 and up to 24 hours.

3.2.1.2 Assessment of pellet water stability: leaching and pellet physical form

The procedure for assessment of water stability of the various binder treatments was modified from D'agaro and Lanari (2004). Four replicates (n = 4) of identically 0.42 g pelleted feed from each binder and the control were immersed simultaneously at different times of immersion in 1 L glass beakers (labelled with respective treatment), filled with de-chlorinated freshwater and incubated in water baths at 27^{o}C (Figure 3.1). The water volume for each water bath was maintained by refilling with water of the same temperature to compensate for evaporation, and replicates of each treatment were randomly distributed in the water baths.

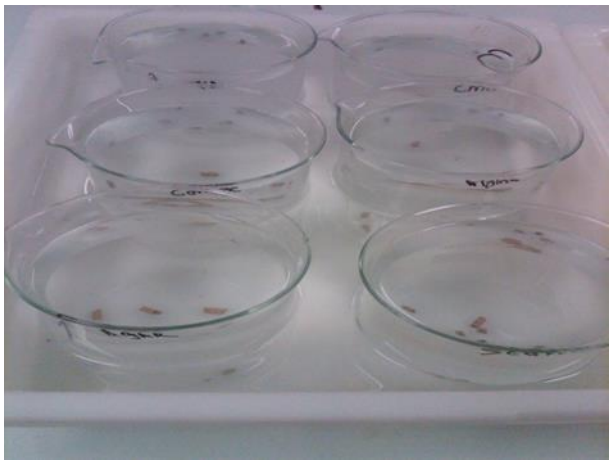


Figure 3.1 One litre glass beakers (labelled for treatment) filled with de-chlorinated freshwater and incubated in a water bath at 27^{o}C for assessing water stability of different binder treatments.

The leaching of material from the pellets, defined as percentage of dry matter loss (DML), and disintegration of the physical form of the experimental pellets was monitored at 10 and 30 minutes, and 1, 2, 4, 8, 12 and 24 hours. The pellets in each beaker were photographed at each monitoring interval with a digital camera (Samsung model ES 15), with 10.2 effective megapixels for high-definition pixel images.

The physical form of the pellets have a significant effect on leaching, e.g., the lager the intact pellet, the lesser the percentage dry matter lost. Hence, pellet physical form was classified into six

categories and considered to be still in pellet form if they were ‘stable’, ‘cracked’ or ‘divided’ (Figure 3.2). After classifying the pellet physical form, the solutions in the beakers was poured through a pre-weighed Whatman No. 1 filter paper (pre-dried at 60°C for 24 hours) to retain pellets or disintegrated particles, which were then oven dried at 60°C until a constant weight was achieved. DML of the experimental pellets was calculated as the difference between the initial and final weights at each sampling interval based on Cruz-Suarez et al. (2001) and Ruscoe et al. (2005). This was expressed as follows: $\mathrm{DML}\ (\%) = \left(\frac{\mathrm{Wo}-\mathrm{Wt}}{\mathrm{Wo}}\right) \times 100$

Where Wo is initial pellet dry weight (g), and Wt is dry weight at each sampling time t after immersion (g).

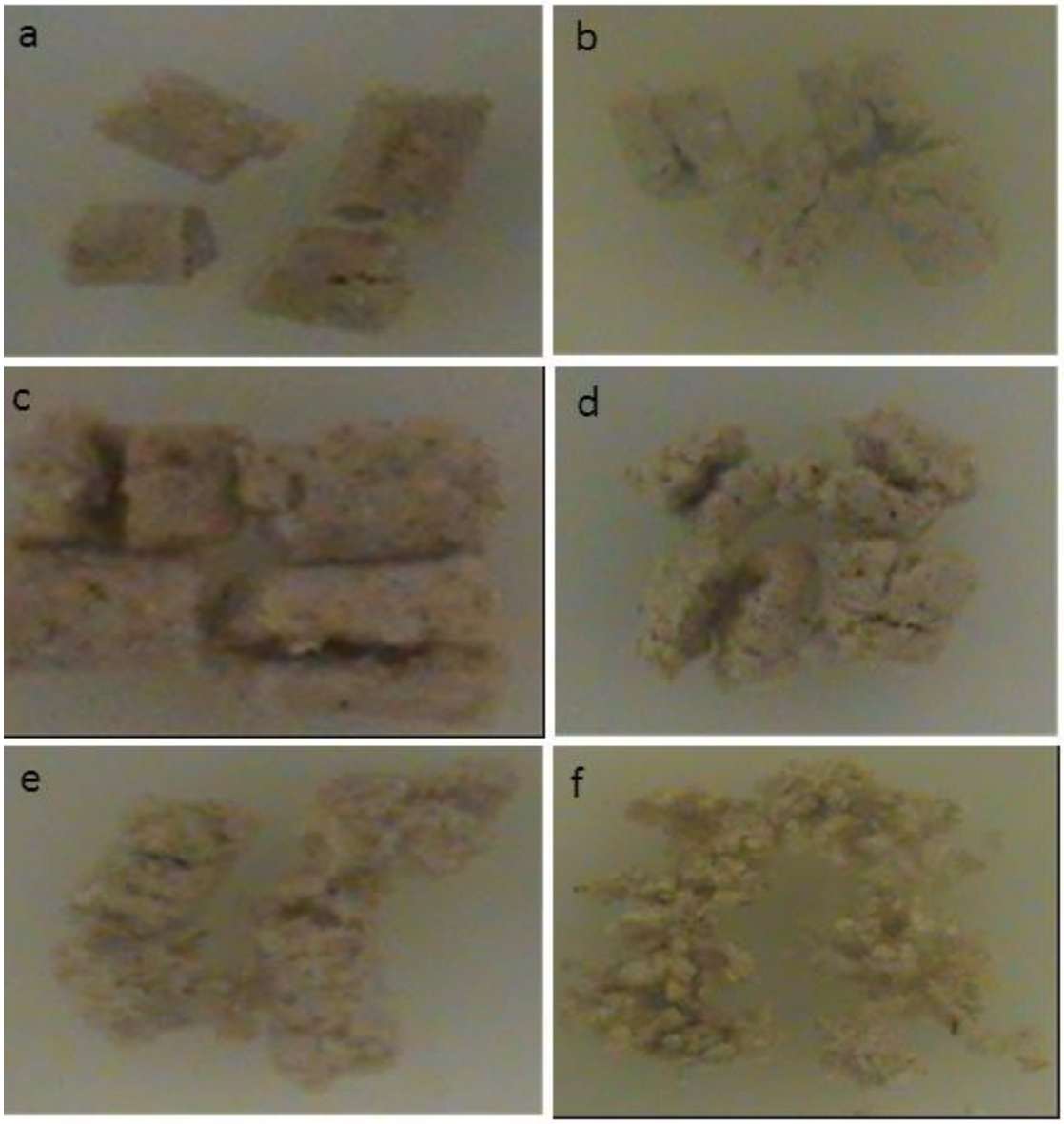


Figure 3.2 Classification of pellet physical form after immersion in water. a) Stable; b) Cracked; c) Partially divided; d) Totally divided e) Partially disintegrated; f) Totally disintegrated.

3.2.2 Effect of binder inclusion level on pellet water stability

Based on the results of the binder experiment (see 3.2.1), of the six binders tested, alginate was identified as the best binder treatment for optimal pellet water stability. Therefore, it was used for testing the best incorporation concentration for continued pellet water stability. Pellets bound with five alginate inclusion concentrations of 2.0%, 2.6%, 3.2%, 3.8% and 4.4% of the total diet dry weight were formulated and tested for their water stability. The procedure used for feed formulation (see 3.2.1.1) and assessment of water stability (see 3.2.1.2) of the resultant pellets were the same as for the binder experiment.

3.2.3 Effect of pellet size on pellet water stability

The general procedure for making pellets for this trial was the same as that described in section 3.2.1, with the exception that alginate (the best binder) was added at 4.4% (the optimal inclusion level), based on the best binder and binder concentration experiment, to improve the water stability of the resulting different pellets sizes. To make different sized pellets, seven dies with different sizes of 1.0, 2.0, 3.0, 4.0, 4.5, 5.0 and 7.0 mm were made and attached to a small pelleter, to pelletize the dough into spaghetti-like strands of these sizes (Figure 3.3). The resulting strands of each size were then dried at 60°C, and broken manually into pellets approximately 5.0 mm in length which were stored at 10°C until use. The procedure used for assessment of water stability of the pellets of different sizes was the same as for the binder experiment (see 3.2.1.2).

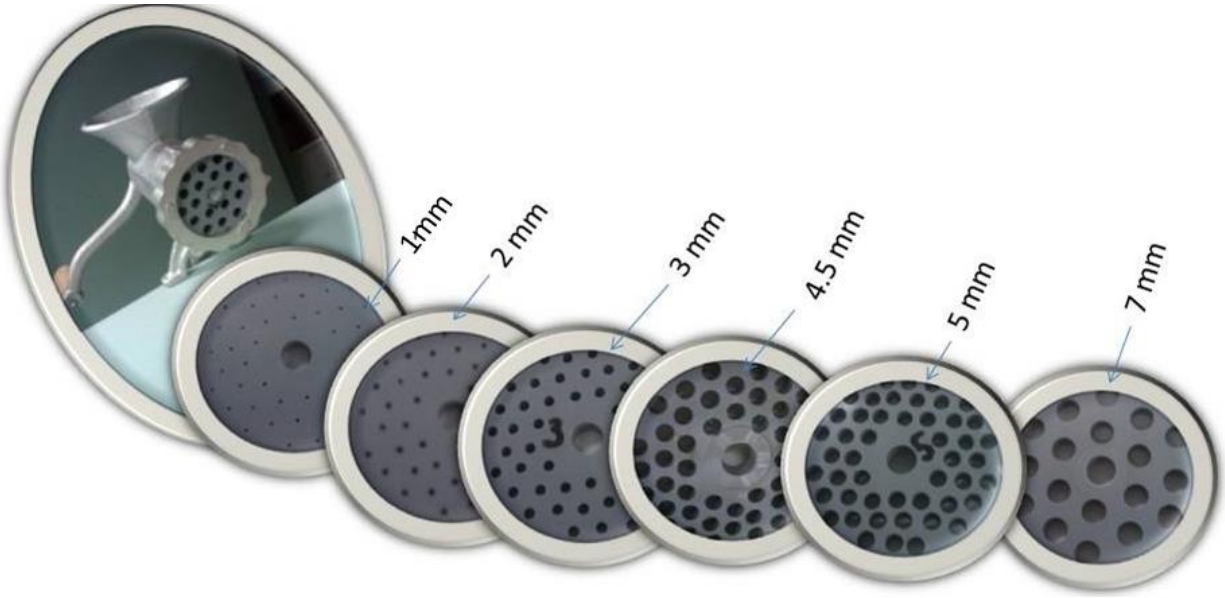


Figure 3.3 Seven different-sized dies of 1.0, 2.0, 3.0, 4.0, 4.5, 5.0 and 7.0 mm made for a pelleter for the production of strands with designated sizes.

3.2.4 Statistical analysis

All data are presented here as mean ± standard error (SE). Mean DML percentages were compared using a Factorial analysis of variance (ANOVA), followed by a Tukey's HSD test to separate the means at $P > 0.05$ among treatments to determine significant differences between them. For each analysis, the assumptions of the ANOVA were tested. All percentage data were arcsine transformed prior to analysis. All statistical analyses were performed using the statistical software package Statistica (version 10).

3.3 Results

3.3.1 The effect of binders on pellet water stability

3.3.1.1 Leaching and pellet physical form change during the first hour of immersion

As measured by percentage dry matter lost (DML%), leaching of the control pellets (i.e. original commercial redclaw crayfish pellets), was significantly higher, at 14.4 ± 1.0%, than in all binder treatments after 10 minutes of immersion. Of the six binder treatments, the CMC treatment had the highest leaching at 9.9 ± 0.7%, which was followed by starch, agar, PVA and carrageenan treatments, while alginate had the lowest leaching at only 5.6 ± 0.5%. Alginate had a significantly lower leaching level in comparison to other binders ($P < 0.05$), except for the carrageenan treatment where the differences were not significant. The trend remained largely the same after 30 minutes and 1 hour of immersion; where with increasing immersion time, the DML of each treatment increased steadily ($P < 0.05$, Table 3.2). At 1 hour of immersion, the control treatment lost almost one-quarter of its original dry weight (23.8 ± 0.7%), while the lowest leaching level of the best experimental treatment (i.e. the alginate) was less than half of this at 11.4 ± 0.5%. All other binders also showed significantly lower leaching when compared to the control (Table 3.2). Statistical analyses showed that the binder treatment, immersion time and their interaction all significantly affected DML (Factorial ANOVA: Treatment, $F_{6,63} = 94.091$, $P < 0.001$; Time, $F_{2,63} = 168.159$; $P < 0.001$; interaction, $F_{12,63} = 44199$, $P < 0.001$).

After 10 minutes of immersion, the physical form of all six experimental diets bound with different binders remained stable, but the control diet showed signs of cracking. The pellets of the control treatment were partially disintegrated within 30 minutes, and totally disintegrated within the first hour of immersion. All other pellets bound with experimental binders remained in a stable form after 30 minutes; however, by 1 hour of immersion, all but the pellets bound with alginate had cracked (Table 3.2).

Table 3.2 Percent dry matter lost (DML%) (mean ± SE) and pellet physical form change of formulated redclaw crayfish pellets bound with different binders and the control during 1 hour of immersion. [abc] Different superscripts within the same column indicate significant differences ($P < 0.05$).

	DML%			**Pellet physical form**		
Binder	**Immersion time**			**Immersion time**		
	10 min	**30 min**	**1 h**	**10 min**	**30 min**	**1 h**
Control	14.4 ± 1.0[c]	18.2 ± 1.2 [d]	23.8 ± 0.7 [c]	crack	partially disintegrated	totally disintegrated
Alginate	5.6 ± 0.5[a]	6.5 ± 0.81 [a]	11.4 ± 0.5 [ab]	stable	stable	stable
Agar	8.4 ± 0.6[b]	10.8 ± 0.5 [abc]	13.5 ± 0.4[ab]	stable	stable	cracked
Carrageenan	7.0 ± 0.9 [ab]	10.5 ± 0.3 [abc]	12.4 ± 0.6[a]	stable	stable	cracked
CMC	9.9 ± 0.7[b]	12.5 ± 0.7 [c]	15.9 ± 0.6 [b]	stable	stable	cracked
Starch	9.1 ± 0.5[b]	11.7 ± 0.3 [bc]	14.1 ± 0.4 [ab]	stable	stable	cracked
PVA	8.2 ± 0.4 [b]	10.7 ± 0.3 [abc]	13.9 ± 0.5[ab]	stable	stable	cracked

3.3.1.2 Leaching and pellet physical form change during 2 to 24 hours of immersion

Due to the fact that the commercial redclaw pellet control treatment had totally disintegrated within 1 hour of immersion, it was excluded from data collection after that time. During the period of 2 to 24 hours immersion, the DML differed significantly between binder treatments at each sampling interval, while the rate of DML change over time also varied among treatments (Factorial ANOVA: Treatment, $F_{5,90} = 73.15$, $P < 0.001$; Time, $F_{7,90} = 87.85$, $P < 0.001$; Table 3.3). A significant interaction was observed between binder treatments and immersion time (Factorial ANOVA: $F_{20,90} = 4.87$, $P < 0.001$). For example, after 2 hours of immersion, the PVA treatment had significantly higher DML (20.6 ± 0.6%) than that of the alginate (14.3 ± 0.4%) and agar treatments (15.7 ± 0.6%), but no significant difference was found when compared to the pellets bound with starch CMC or carrageenan ($P > 0.05$). After 8 hours of immersion, the PVA bound pellets showed significantly higher DML ($P < 0.05$; Table 3.3), than all other treatments and was the only binder treatment to lose more than 30 DML% after 12 hours of immersion (31.5 ± 1.1%). After 24 hours immersion, the pellets bound with alginate had the lowest DML (20.2 ± 1.2%), which was significantly lower than pellets bound with starch (25.1 ± 0.12%) and PVA (33.0 ± 1.8%) ($P < 0.05$) but was not significantly different from treatments bound with carrageenan (23.2 ± 1.4%), agar (23.4 ± 0.6%) and CMC (22.2 ± 0.4%) ($P > 0.05$). Leaching also appears to have more or less stabilised after 8 hours of immersion for the alginate, CMC and carrageenan treatments (Table 3.3).

Table 3.3 Percent dry matter lost **(**DML%) (mean ± SE) of formulated redclaw crayfish pellets bound with different binders after 2 to 24 hours immersion. [abc...l] Different superscripts within the same column indicate significant differences ($P < 0.05$).

	DML%				
	Immersion time				
Binder	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
Alginate	14.3 ± 0.4^{a}	16.2 ± 0.6^{c}	22.3 ± 1.0^{g}	21.3 ± 0.4^{h}	20.2 ± 1.2^{j}
Agar	15.7 ± 0.6^{a}	15.6 ± 1.2^{c}	19.7 ± 0.9^{fg}	21.7 ± 1.2^{h}	23.4 ± 0.6^{jk}
Carrageenan	16.9 ± 0.6^{ab}	17.1 ± 0.8^{cd}	21.1 ± 0.6^{fg}	20.3 ± 0.9^{h}	23.2 ± 1.4^{jk}
CMC	18.4 ± 0.5^{ab}	21.1 ± 0.4^{de}	22.2 ± 0.1^{g}	22.2 ± 0.1^{h}	22.2 ± 0.4^{jk}
Starch	16.1 ± 0.3^{ab}	16.4 ± 0.6^{c}	17.2 ± 0.5^{f}	23.4 ± 0.6^{h}	25.1 ± 0.8^{k}
PVA	20.6 ± 0.6^{b}	24.0 ± 0.7^{e}	27.6 ± 1.5^{h}	31.5 ± 1.1^{i}	33.0 ± 1.8^{l}

The pellet physical form also showed clear differences among treatments during the 2 to 24 hours immersion period, and these were apparently influenced by the source binders. At the end of a 2 hours immersion in water, pellets of all binder treatments were cracked except for PVA which had disintegrated and partially divided (Table 3.4). After 4 hours of exposure to water, pellets bound with carrageenan, CMC, starch and PVA were partially divided, but pellets bound with agar and alginate remained only cracked (Table 3.4). At 8 hours immersion, alginate, agar, carrageenan and CMC bound pellets remained only partially divided whereas pellets with PVA and starch had become totally divided. At 12 hours of immersion, pellets bound with binders sourced from seaweeds, i.e. alginate, agar and carrageenan, were only partially divided, whereas pellets bound with cellulose (CMC), corn (starch) and synthetic (PVA) types of binder were

totally divided. At 24 hours of immersion, the pellets bound with alginate retained the best physical form, and this was the only treatment showing partial division as its highest degree of disintegration within 24 hours of immersion. In fact, all pellets bound by carbohydrate-type binders maintained the general appearance of a pellet form, albeit in either a partially divided or totally divided state. Conversely, the pellets bound by PVA, the synthetic binder, were already partially disintegrated after 2 hours of immersion (Table 3.4).

Table 3.4 Pellet physical form change of formulated redclaw crayfish pellets bound with different binders after 2 to 24 hours of immersion.

Binder	Pellet physical form				
	2 h	**4 h**	**8 h**	**12 h**	**24 h**
Alginate	cracked	cracked	partially divided	partially divided	partially divided
Agar	cracked	cracked	partially divided	partially divided	totally divided
Carrageenan	cracked	partially divided	partially divided	partially divided	totally divided
CMC	cracked	partially divided	partially divided	totally divided	totally divided
Starch	cracked	partially divided	totally divided	totally divided	totally divided
PVA	partially divided	partially divided	totally divided	totally divided	partially disintegrated

3.3.2 The effect of binder inclusion level on pellet water stability

3.3.2.1 Leaching during 24 hours of immersion

The DML of diet treatments showed a general decrease with increasing alginate inclusion level and immersion time (Factorial ANOVA: Treatment, $F_{4,120} = 286.17$, $P < 0.001$; Time, $F_{7,120} = 1122.43$, $P < 0.001$; Table 3.5). A significant interaction was also observed between the binder concentrations and immersion times (Factorial ANOVA: $F_{28,120} = 9.98$, $P < 0.001$). For instance, after 10 minutes of immersion in water, the DML of pellets bound with alginate at a concentration of 3.8% and 4.4% were significantly lower than that of treatments with alginate inclusion levels of 2.0%, 2.6% and 3.2% ($P < 0.005$) (Table 3.5). After 30 minutes of immersion, the lowest DML corresponded to the treatment containing 4.4% alginate (5.8 ± 0.8%), which was significantly lower than the treatments containing alginate inclusion levels of 2.6% and 2.0% ($P < 0.005$), although not significantly different from the treatments containing alginate at 3.2% and 3.8%. Between 1 to 24 hours immersion times, all treatments containing 4.4% alginate had significantly lower DML (16.2 ± 0.2 %) than all other treatments tested ($P < 0.005$) (Table 3.5).

Table 3.5 Percent dry matter lost **(**DML%) (mean ± SE) of formulated redclaw crayfish pellets bound with alginate at different inclusion levels during 24 hours immersion. [abc...v]Different superscripts within a column indicate significant differences (P <0.05).

Alginate inclusion level (% total diet dry weight)	**DML%**							
	Immersion time							
	10 min	**30 min**	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
4.4	4.8 ± 0.2^{a}	5.8 ± 0.8^{c}	8.4 ± 0.1^{f}	8.0 ± 0.8^{i}	14.0 ± 0.5^{k}	15.8 ± 0.1^{m}	14.4 ± 0.7^{o}	16.2 ± 0.2^{s}
3.8	4.2 ± 0.2^{a}	7.2 ± 0.5^{cd}	11.3 ± 0.4^{g}	13.8 ± 0.2^{j}	17.6 ± 0.2^{l}	17.9 ± 0.4^{n}	16.8 ± 0.5^{p}	19.6 ± 0.1^{t}
3.2	6.8 ± 0.2^{b}	7.5 ± 0.2^{cd}	12.3 ± 0.1^{gh}	14.3 ± 0.0^{j}	17.0 ± 0.1^{l}	18.7 ± 0.1^{n}	19.7 ± 0.2^{q}	20.7 ± 0.1^{tu}
2.6	6.9 ± 0.2^{b}	9.1 ± 0.1^{de}	13.4 ± 0.3^{h}	14.7 ± 0.1^{j}	17.0 ± 0.2^{l}	19.0 ± 0.1^{n}	21.2 ± 0.0^{qr}	22.1 ± 0.1^{uv}
2.0	7.8 ± 0.8^{b}	10.4 ± 0.3^{e}	13.8 ± 0.2^{h}	15.0 ± 0.5^{j}	17.9 ± 0.2^{l}	18.9 ± 0.5^{n}	22.2 ± 0.3^{r}	23.9 ± 0.3^{v}

3.3.2.2 Pellet physical form changes during 24 hours of immersion

The pellets used in all treatments tested remained stable within the first 30 minutes of immersion. Between 1 and 24 hours immersion, the physical form of pellets generally showed better integration with increasing alginate concentration (Table 3.6). The treatments containing the lower alginate inclusion levels of 2.0%, 2.6% and 3.2% cracked after 1 hour of immersion, while the treatments with higher alginate concentrations of 3.8% and 4.4% cracked only at the end of 4 and 8 hours of immersion respectively (Table 3.6). Treatments bound with the lowest concentrations of alginate at 2.0% and 2.6% were partially divided at the end of 12 and 24 hours of immersion respectively, while those treatments containing higher alginate concentrations remained cracked but did not divide at the end of 24 hours of immersion (Table 3.6).

Table 3.6 Physical form of formulated pellets bound with alginate at different inclusion levels during 24 hours of immersion.

Alginate inclusion level	**Pellet physical form**							
(% total diet dry weight)	**Immersion time**							
	10 min	**30 min**	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
4.4	stable	stable	stable	stable	stable	cracked	cracked	cracked
3.8	stable	stable	stable	stable	cracked	cracked	cracked	cracked
3.2	stable	stable	cracked	cracked	cracked	cracked	cracked	cracked
2.6	stable	stable	cracked	cracked	cracked	cracked	cracked	partially divided
2.0	stable	stable	cracked	cracked	cracked	cracked	partially divided	partially divided

3.3.3 The effect of pellet size on water stability

3.3.3.1 Leaching during 24 hours of immersion

The DML of the diets tested decreased with increasing pellet sizes and immersion times (Factorial ANOVA: Treatment, $F_{6,168} = 64.43$, $P < 0.001$; Time, $F_{7,168} = 205.12$, $P < 0.001$). There were no indications of significant interactions between treatments and time (Factorial ANOVA: $F_{42,168} = 1.92$, $P > 0.05$). After 10 to 30 minutes of immersion, the DML remained the same for all pellet sizes tested ($P > 0.05$, Table 3.7). Between 1 to 2 hours of immersion, significant differences in DML were observed between pellet sizes of 7.0 mm vs. 4.0, 3.0, 2.0 and 1.0 mm ($P < 0.005$, Table 3.7). However, these differences were less apparent when compared with pellet sizes of 4.5 and 5.0 mm ($P > 0.05$). After 4 hours immersion, the DML of the 7.0 mm pellet size treatment differed significantly ($P < 0.005$) from the 1.0, 2.0 and 3.0 mm pellet sizes, but no significant difference ($P > 0.05$) was found in comparison to other treatments. Interestingly, pellet sizes of 1.0 mm (15.9 ± 1.6), 2.0 mm (15.6 ± 1.5) and 3.0 mm (15.3 ± 0.4) lost twice as much DML as 7.0 mm pellets did (7.8 ± 0.6) (Table 3.7). During the period of 8 to 24 hours of immersion, the DML differed significantly ($P < 0.005$) only between largest pellet size (7.0 mm) and smallest pellet sizes (1.0 and 2.0 mm), when compared to all other treatments and pellet sizes where the 1.0 mm (22.8 ± 1.9) and 2.0 mm (21.2 ± 0.6) sizes were the only treatments to lose more than 20 DML% after 24 hours of immersion (Table 3.7).

Table 3.7 Percent dry matter lost **(**DML%) (mean ± SE) of different pellet sizes bound with 4.4% alginate during 24 hours immersion.

[abc...q] Different superscripts within the same column indicate significant differences (P <0.05).

Pellet sizes (mm in diameter)	**DML%**							
	Immersion time							
	10 min	**30 min**	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
7.0	0.3 ± 0.0	2.0 ± 0.6	4.4 ± 0.6^{a}	6.3 ± 1.7^{c}	7.8 ± 0.6^{e}	9.0 ± 0.4^{h}	10.8 ± 0.6^{l}	13.0 ± 1.5^{o}
5.0	0.4 ± 0.1	2.2 ± 0.7	5.6 ± 0.8^{a}	8.1 ± 0.4^{c}	9.9 ± 0.5^{ef}	11.8 ± 0.6^{hi}	12.4 ± 1.1^{l}	13.1 ± 0.2^{o}
4.5	0.7 ± 0.1	3.5 ± 1.1	8.8 ± 0.6^{ab}	11.1 ± 0.8^{cd}	11.7 ± 0.4^{ef}	12.2 ± 1.6^{hi}	13.9 ± 1.0^{l}	14.3 ± 0.2^{op}
4.0	0.8 ± 0.0	5.6 ± 0.1	11.7 ± 0.4^{b}	12.6 ± 0.5^{d}	12.9 ± 0.4^{efg}	14.4 ± 1.3^{hik}	16.1 ± 0.9^{lm}	16.5 ± 0.4^{op}
3.0	0.9 ± 0.2	5.6 ± 0.6	12.7 ± 0.3^{b}	13.9 ± 1.5^{d}	15.3 ± 0.4^{fg}	15.7 ± 0.2^{ik}	16.4 ± 0.3^{lmp}	17.5 ± 0.3^{opq}
2.0	1.4 ± 0.5	5.8 ± 0.6	13.3 ± 1.6^{b}	14.7 ± 0.9^{d}	15.6 ± 1.5^{fg}	16.4 ± 0.7^{ik}	20.4 ± 1.1^{mn}	21.2 ± 0.6^{pq}
1.0	1.4 ± 1.4	6.6 ± 2.9	14.1 ± 1.7^{e}	15.3 ± 1.3^{d}	15.9 ± 1.6^{g}	18.8 ± 2.3^{k}	22.1 ± 1.8^{n}	22.8 ± 1.9^{q}

3.3.3.2 Pellet physical changes during 24 hours of immersion

All pellet sizes tested remained stable between 10 minutes and 1 hour of immersion. Between 2 and 24 hours of immersion, the physical form of the pellets generally showed better integration with increasing of pellet size (Table 3.8). Between 2 and 8 hours of immersion, the smallest pellet size remained partially divided, whereas other pellet sizes were still stable or only cracked. Pellet sizes of 5.0 mm and 7.0 mm maintained the best physical form, as they were cracked only at the end of 12 and 24 hours respectively, while other pellet sizes had cracked or become partially divided after an 8 hours of immersion (Table 3.8). After 24 hours of immersion, all treatments had retained their pellet form except for the 2.0 mm and 1.0 mm pellet sizes, which had become partially divided (Table 3.8).

Table 3.8 Physical form of different pellet sizes over 24 hours of immersion.

Pellet sizes (mm in diameter)	**Pellet physical form**							
	Immersion time							
	10 min	**30 min**	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
7.0	stable	stable	stable	stable	stable	stable	stable	cracked
5.0	stable	stable	stable	stable	stable	stable	cracked	cracked
4.5	stable	stable	stable	stable	stable	cracked	cracked	cracked
4.0	stable	stable	stable	stable	cracked	cracked	cracked	cracked
3.0	stable	stable	stable	cracked	cracked	cracked	cracked	cracked
2.0	stable	stable	stable	cracked	cracked	cracked	partially divided	partially divided
1.0	stable	stable	stable	partially divided	partially divided	partially divided	partially divided	partially divided

3.4 Discussion

Water stability is a critical factor in defining the quality of crayfish feeds (Volpe et al., 2012). The results of the present study clearly showed that both binder type and binder inclusion level had significant effects on the water stability of redclaw formulated feeds. The poor water stability of the currently available redclaw commercial feed in Australia can be substantially improved by incorporating alginate as the binder at an inclusion level of 4.4% of total diet dry weight. On the other hand, the effect of pellet size on water stability appears relatively minor.

The commercial redclaw feed tested as a control treatment confirmed its poor water stability as it disintegrated totally within 1 hour of immersion. Of the six binders tested, alginate, agar and carrageenan sourced from seaweed and CMC from cellulose sources, all performed better than the synthetic PVA and starch derived from corn. Alginate is the recommended overall best choice as a binder for retaining the best physical form of the pellet, and it also had the lowest DML overall after 24 hours immersion in water. These results are in agreement with those of D'agaro and Lanari (2004), which indicated that alginates are effective binders for binding pellets which contain grains as the major protein source. However, the results of more recent studies have reported that alginate is a poorer binder when compared to other carbohydrate binders (Volpe et al., 2008; 2012). The difference in results in comparison to this study are likely to be due to differences in the major protein sources used in the aforementioned studies, as fish meal and condensed fish soluble were used as the main protein sources (Volpe et al., 2008; 2012). It has been shown that when fish by- products are used as the main ingredients, alginate binders should be supplemented by a sequestering agent, such as sodium hexametaphosphate to achieve better water stability (Ruscoe et al., 2005). The alginate inclusion level was also shown to have significant effects on the water stability of the resultant redclaw pellets. Depending on the protein source used, alginates have been used at various concentrations for crayfish diets, ranging from 2.5% (Volpe et al., 2008), 3.0% (D'agaro and Lanari, 2004; Ruscoe et al., 2005) up to 5.0% (Ruscoe et al., 2005; Volpe et al.,

2012). For the inclusion range of 2.0% to 4.4% used in the current study, the highest inclusion level of 4.4% yielded the best results. Although higher alginate inclusion levels may further improve pellet water stability, overly-high levels of binder inclusion may lead to reduced dietary nutritional value, as increased binder inclusion levels are possible only at the cost of a reduction in other main diet ingredients, as well as incurring higher feed manufacturing costs. The effects of pellet size on water stability were shown to be relatively minor, as significant differences were found only between the largest pellet size (7.0 mm) and the smallest pellet sizes (1.0 and 2.0 mm) after 24 hours of immersion in water. A similar finding on the lower water stability of small pellets has been reported, and was attributed to the lower surface area to volume ratio of smaller pellets compared to larger ones (Obaldo and Tacon, 2001). In summary, this study showed that all six different binders tested could substantially improve the water stability of the current commercial redclaw pellet. Among the six binders, alginate incorporated at 4.4% of the total diet dry weight produced the best results and is thus recommended for adoption by the redclaw aquaculture industry to improve the quality of their products. The study further established that the effects of pellet size on the water stability of the improved commercial feed were relatively minor.

CHAPTER 4: FEEDING RESPONSE AND FEED WASTAGE OF REDCLAW FEEDING ON THE EXPERIMENTAL DIET VS. THE COMMERCIAL DIET

4.1 Introduction

Formulation of feeds for freshwater crayfish must also take into consideration their feeding behaviour (Wolf, 2004; Ruscoe et al., 2005; Volpe et al., 2012). Ideally, formulated feeds developed for crayfish should be nutritionally balanced, reasonably water stable, made from cheap and sustainable ingredients, and able to produce a strong feeding response as well as be easily handled for ingestion by the crayfish with minimal wastage (Hubbard et al., 1986; Jussila and Evans, 1998; Kreider and Watts, 1998; Tolomei et al., 2003). However, in practice, achieving all these simultaneously is not always easy since some of these criteria may mutually exclusive.

For example, a mixture of fragmented grains (e.g. wheat, sorghum, barley and maize) has been used as the major protein sources to totally replace expensive and unsustainable fishmeal for the production of commercial redclaw crayfish feeds in Australia. However, the resultant commercial redclaw pellets have poor water stability, often disintegrating within minutes of being immersed in water, leading to low feed efficiency and high wastage. Strategies for improving the water stability of formulated feeds have included both diet extrusion methods (Jussila and Evans, 1998) and incorporation of various binders (D'agaro and Lanari, 2004; Wolf, 2004; Ruscoe, 2005; Volpe et al., 2012). The addition of binders has been reported to enhance the water stability of formulated diets, but tightly bound pellets could also lower attractiveness and palatability for the target species (Partridge and Southgate, 1999; O'Mahoney et al., 2011). This is particularly true when considering that crayfish are known generally to find food by chemoreception rather than visual cues (D'Abramo and Sheen, 1994). The fact that visual cues are not relied on for foraging by crayfish poses a major challenge for any formulated feeds that may lack chemically-induced feeding stimuli release a few minutes after their introduction (D'Abramo, 2002).

Obviously, formulated diets may be water stable and nutritionally complete, but remain undesirable if they are not attractive to the animals. In fact, the formulation of a nutritionally balanced feed will be of little value if the crayfish cannot effectively locate and willingly consume the feed. In the studies reported in Chapter 3, alginate was identified as the best binder to improve the water stability of the commercial redclaw pellets and this binder optimal incorporation concentration was also defined. The addition of alginate at the ideal concentration has led to dramatically improved water stability with lower nutrient leaching of the pellets as compared to the commercial pellets with same ingredients except alginate as the binder (Chapter 3). However, it is not clear if this might lead to the pellets being less well accepted by redclaw. Therefore, it is necessary to evaluate if the newly developed experimental feed with the addition of alginate is still perceived by redclaw as being attractive and will incite a feeding response quickly upon introduction. To achieve this, behavioural experiments were designed to quantitatively assess and compare the feeding responses of redclaw to the introduction of the new pellets against the original commercial pellets, under identical feeding conditions.

So far, only a few studies have examined the feeding responses of redclaw or other freshwater crayfish species on formulated feeds (Volpe et al., 2008; Nguyen, 2012). From previous experiments, redclaw were observed to use their mouthparts to crush and macerate the commercial pellets, leading to high feed wastage (Nguyen, 2012). However, there are no studies to date quantifying the feed wastage when feeding redclaw with formulated feeds. Ruscoe et al (2005) has commended that quantifying the actual amount ingested by freshwater crayfish is one of the greatest difficulties in nutritional research with the species. While that is probably true in the case of pond culture, estimating feed wastage is possible under controlled aquarium condition, and this should provide a useful indication on feed quality. Obviously, feed wastage is another important indicator of the suitability and quality of feeds that are formulated for redclaw, therefore, the aim of the study presented in this chapter was to compare and evaluate the attractiveness and wastage

of the experimental diet with alginate added as the best binder at optimal concentration against the current commercial redclaw pellet.

4.2 Methodology

4.2.1 Experimental animals

Redclaw used for the experiment were obtained from the same farms as mentioned in Chapter 2 (see 2.2.1) except that for this experiment, the weights of the redclaw used ranged between 35 and 50 g. The same procedure of acclimation as described in Chapter 2 (see 2.2.1) was also adopted. Redclaw were fed between 8 and 9 am daily with raw soybeans purchased from a local shop to satiation as confirmed by observation of uneaten feed for a week prior to the experiment to avoid any potential influence of prior feeding experience on commercial pellets that may bias the results.

4.2.2 General experimental procedure

A total of 36 healthy redclaw with intact feeding appendages of both sexes were randomly selected from the outdoor holding tanks for the feeding experiment. The redclaw were individually marked by gluing a numbered Dymo tape to their carapace for identification so that none of them used a second time. Before being individually assigned into an experimental unit for the experiment, each of the redclaw was identified to be hard shell and washed with freshwater to remove any fine food particles that may have adhered to their body.

Each experimental unit consisted of a 20 L glass aquarium (51 × 27 × 25 cm) filled with de- chlorinated water to approximately 80% of its capacity and was gently aerated by an air stone (Figure 4.1). Between neighbouring aquaria, a black plastic panel was inserted, covering the full length of the aquarium side wall to totally block the view of the experimental redclaw to the neighbouring aquaria. Black sheets were also provided on the bottom of aquaria as a non-slip

surface. A submerged heater with thermo-control was placed in each tank to keep water temperature at approximately 27^{o}C.

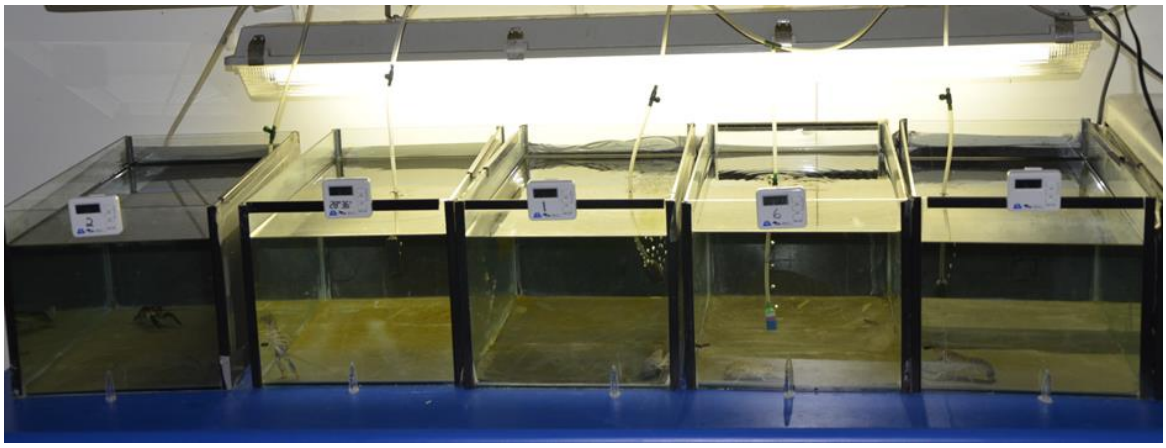


Figure 4.1 Illustration of the experimental units used for redclaw feeding behaviour experiment. Note a stopwatch was attached on the front of each aquarium to record the time of feeding response.

4.2.3 Feeding behaviour observation

Prior to the experiment, the redclaw used for the experiment were introduced individually to one of the aquaria and starved for 72 hours. This procedure allowed the redclaw to acclimatise to the experimental environment, while also preventing faeces being produced during the subsequent experimental duration. Shortly before the introduction of the feed to the aquarium to start the feeding experiment, each glass aquarium was siphoned thoroughly to remove any faecal material and air delivery through the air stone was restricted to minimise water turbulence. The submerged heaters which were used to maintain water temperature were also removed to avoid obstructing the movement of the animals during feeding. After all these were done, either the experimental (incorporating 4.4% alginate) or the commercial redclaw pellets (Ridley Agri Products Pty Ltd,

Australia) were introduced into the bottom of the tank via a PVC pipe that guided the pellets to a distance of approximately 10 cm from the redclaw in the aquarium. Both diets used for the test were 4.5 mm in diameter and identical in weight of 60 mg (equivalent to the average weight of a single commercial redclaw pellet).

Behavioural responses of the redclaw to the introduction of the experimental or the commercial diet were observed personally as well as recorded digitally via a video camera recorder (Sony Handycam DCR-SX65) for later replay and confirmation. A white fluorescent light was attached to the top side of the aquarium for clear video recording. Stop watches that were mounted to the aquaria were activated from the time the feed was introduced to record: a) 'Time starting feeding': defined as the time required from pellet introduction to the time when the experimental redclaw started to hold on to the pellet; and b) 'Time spent feeding' or 'Food handling time': defined as the interval between when the redclaw started grabbing the pellets to the time the feeding behaviour ceased.

The feeding observation was replicated 18 times for each of the diets tested using different redclaw. The observation duration was 15 minutes for all redclaw tested. Occasionally, more than 10 min elapsed following pellet introduction of experimental diet and commercial diet, an experimental redclaw still did not react to the introduced pellets. When such situation occurred, the experiment was terminated with a new acclimated redclaw used to repeat the test. Those redclaw did not respond to the diet induction in 10 minutes were excluded for the data analysis. The number of excluded redclaw were similar among treatments.

4.2.4 Estimation of feed wastage

Once feeding behaviour observation was completed for each redclaw, the animal was removed from the experimental aquarium, and the water inside was subsequently drained and filtered

through 1 μm glass fibre filter paper (Pall Corporation, Australia). The filter paper with feed material collected on it was subsequently oven dried at 60°C until a constant weight was achieved and weighed to the nearest 0.0001 g using a Sartorius electronic balance. The dry weight of the unconsumed feed was estimated by deducting the total weight of filter paper post-experiment with the pre-weighted original weight prior to the experiment. The feed wastage was expressed as the dried unconsumed feed as a percentage of the initial 60 mg pellet introduced.

4.2.5 Statistical Analysis

Student's t-tests were used to compare the differences of 'Time starting feeding', 'Time spent feeding' and the feed wastage of the two diets tested and significance level was set at $P < 0.05$. All statistics were carried out using the SPSS statistics package (version 2.1).

4.3 Results

4.3.1 General feeding behaviour

Redclaw generally exhibited a sequential initial feeding response for the diet introduced: firstly an increased antennule flicking, which is followed by the first two pairs of walking legs extended to probe the surface of the aquarium bottom and moved towards the location where the diet was introduced. Before reaching the pellet, the first pair of walking legs was observed to often point toward the mouth, showing preparation for food consumption, or alternatively a taste response. When the redclaw located the pellet, they most often used the walking legs or the third pairs of maxillipeds to pick up and bring the pellet to their mouthparts although occasionally claws were used. Once the pellet was at the mouthpart region, the first, second and third pairs of maxillipeds were used to hold the pellet to the mouthpart for ingestion. Due to the fact that the 4.5 mm pellet used in this experiment appeared to be too large to be ingested as whole by the redclaw, the pellet

was always observed being firstly crushed or masticated to small precise by the mandibles. The mandibles crushed the pellet into small pieces and pushed some of them inside the mouth using the maxillae, while others dropped to the floor of the tank. Even while a redclaw was masticating its meal, its walking legs often continued to probe for the next piece of the crushed pellet and upon locating it, held onto it with the small chelae on the ends of walking legs and waited to be put it into the mouth after those in the mouth had been consumed.

4.3.2 Feeding on the commercial redclaw diet

The typical process of a redclaw feeding on the commercial diet is illustrated in Figure 4.2. Since the commercial pellet normally started to crumble and disintegrate quickly after introduction into the water (Figure 4.2 a), the redclaw were often observed to flatten themselves against the floor of the aquaria and use only the third maxillipeds to scoop the fragments of the disintegrated pellet into their mouthparts. The first pair of their walking legs was subsequently positioned below the third maxillipeds with the claws next to mouthparts to prevent the loss of further crumbled small food particles (Figure 4.2 b). In the cases where the pellet had disintegrated or for crushed food particles dropped onto the aquarium floor, redclaw were observed to feed only on large fragments (Figure 4.2 c). They did not attempt to feed on the smaller particles that were scattered on the aquarium floor, and feeding activity usually ceased after larger particles were consumed, leading to high feed wastage (Figure 4.2 d).

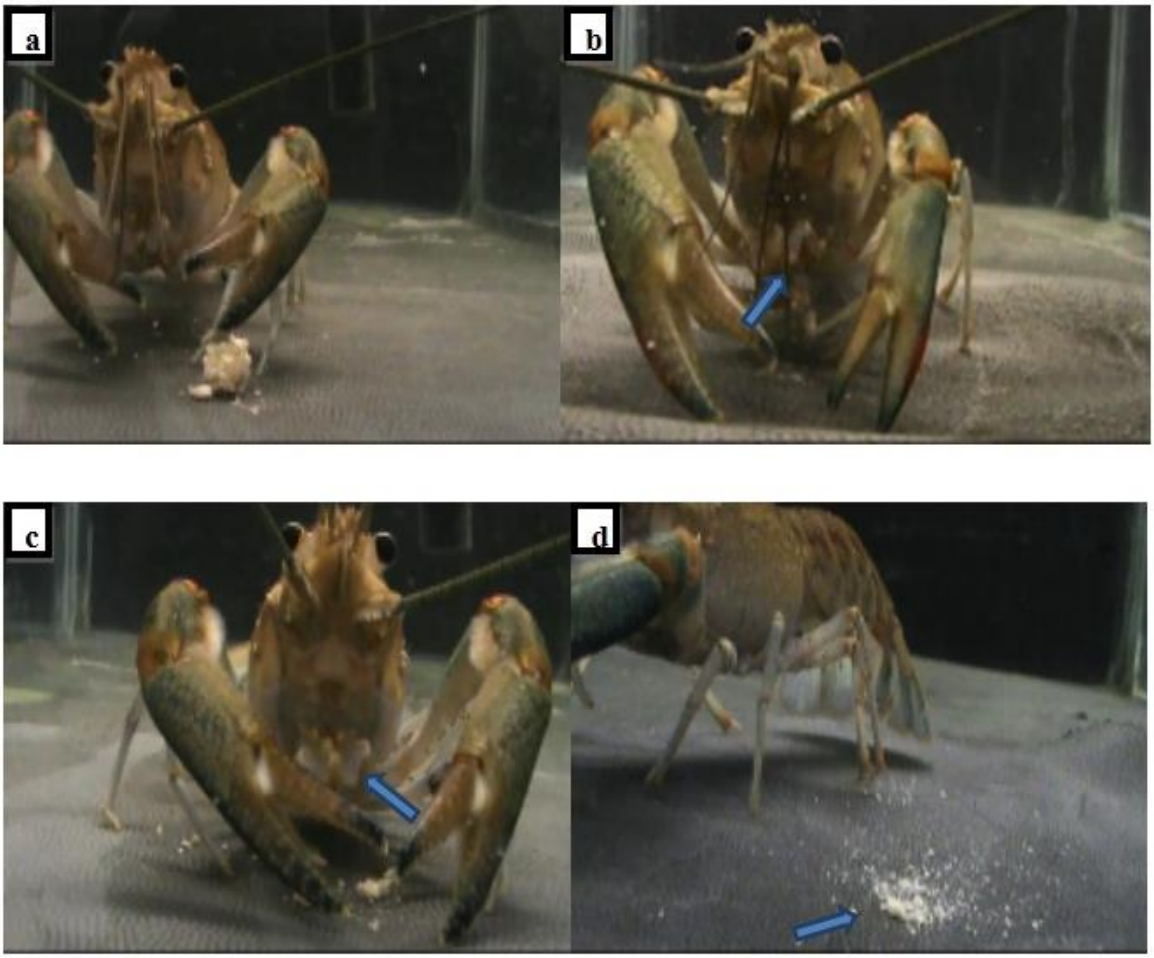


Figure 4.2 Snapshots of video record on the typical sequential of feeding response of redclaw on the commercial redclaw pellet. a) Approaching crumbling pellet; b) Mouthpart surrounded by small food particles (arrow); c) Pellet disintegrated into small pieces by either mouthpart crashing or due to low water stability, only large fragments (arrow) were picked up and fed upon; and d) Dusts of pellet scattered on the floor (arrow) and the redclaw showed no attempt to consume them.

4.3.3 Feeding on the experimental diet with improved water stability

The typical process of a redclaw feeding on the experimental diet with improved water stability is illustrated in Figure 4.3. The experimental pellet largely remained intact after introduction into the aquarium (Figure 4.3 a), as a result, in the majority of cases, redclaw were observed to use their first pair of walking legs to manipulate the pellet to their mouthparts. However, as the size of the pellet (4.5 mm in diameter) appeared to be too big for the redclaw to swallow as whole and the pellet was often stuck onto the mouthpart like a "candy bar". The redclaw were subsequently observed to attempt to crush the pellet, which was often unsuccessful at the first attempt. However, in the subsequent crushing attempts, with all three pairs of maxillipeds manipulating the pellet in a circular fashion to reposition the pellet (Figure 4.3 b), the pellet was eventually broke into smaller pieces and consumed by the redclaw. Unlike the commercial redclaw pellet, the fragmented experimental pellet generally did not further disintegrate into dust, making it possible for the redclaw to consume the most of the broken down pellet with the help of mandibles, the third maxillipeds and the first two pairs of walking legs (Figure 4.3 c), resulting in low feed wastage (Figure 4.3 d).

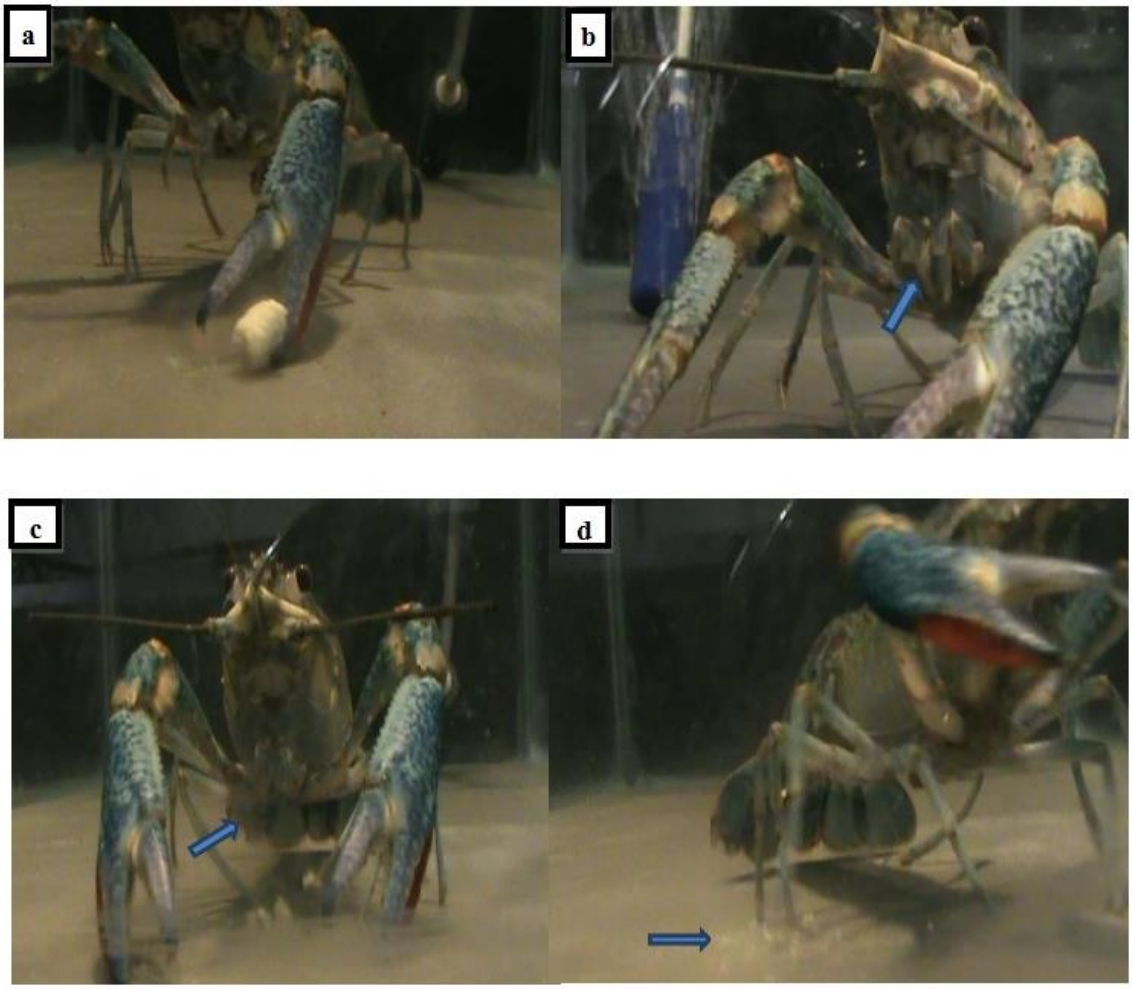


Figure 4.3 Snapshots of video record on the typical sequential of feeding response of redclaw on the experimental diet. a) Picking up largely intact pellet; b) Fondling pellet (arrow) using mouthparts; c) The pellet broken down into smaller pieces (arrow) which did not further disintegrate. While ingesting one pieces of pellet in the mouthpart, the redclaw simultaneously held on to another pieces of broken down pellet by the small chelae on the end of the walking legs; and d) Fewer dusts left (arrow).

4.3.4 Time starting feeding, time spent feeding and feed wastage of redclaw feeding on the experimental diet vs. the commercial diet

When feeding responses of redclaw to the experimental and the commercial diets were compared, no significant difference in the mean 'Time start feeding' (i.e. the time from the pellet introduction to the time when the redclaw started to hold on to the pellet with its appendages) was detected between the two diets (experimental diet: 1.92 ± 0.17 minutes vs. the commercial diet: 1.89 ± 0.24 minutes) ($P > 0.05$). However, 'Time spent feeding' or 'food handling time', was significantly shorter for the commercial redclaw diet (5.3 ± 0.1 minutes) as compared to the experimental diet (6.3 ± 0.2 minutes) ($P < 0.05$) (Table 4.1). On the other hand, 'Feed wastage', expressed as the unconsumed feed as the percentage of the initially introduced pellet weight, was significantly higher for the commercial redclaw diet (11.0 ± 0.0%) than that of the experimental diet (5.0 ± 0.0%) ($P < 0.05$) (Table 4.1).

Table 4.1 Time starting feeding, time spent feeding and feed wastage of redclaw feeding on the commercial redclaw pellet and the experimental diet. Data are presented as mean ± S.E.; data of a same row with different superscript letters are significantly different ($P < 0.05$).

	Diet type	
	Commercial redclaw diet	**Experimental diet**
Time start feeding (min)	1.89 ± 0.24	1.92 ± 0.17
Time spent feeding (min)	5.3 ± 0.1[a]	6.3 ± 0.2[b]
Feed wastage (%)	11.0 ± 0.0%[b]	5.0 ± 0.0%[a]

4.4 Discussion

The sequence of initial feeding responses of redclaw crayfish to either the experimental or commercial redclaw diet was very similar, the positive initial feeding responses, such as increased antennular flicking and active probing with the first two pairs of walking legs observed soon after the introduction of either diet, indicate that the experimental diet with significantly improved water stability could rival the commercial redclaw diet in its attractiveness to the redclaw. This is further confirmed by the fact that no significant difference was detected between the 'Time starting feeding' for two diets.

However, the positive initial feeding responses do not necessarily lead to ingestion in crustaceans. For example, in the giant freshwater prawn *Macrobrachium rosenbergii*, it has been observed that although identical initial feeding responses were shown for both plain and bitter pellets, subsequent maxilliped handling behaviour during ingestion was clearly different. It was concluded that the sets of chemoreceptors guiding the initial response of feeding were likely to be different from those used for ingestion (Steiner and Harpaz, 1987). In fact, once the feed reaches the mouthparts of decapod crustaceans, oral chemoreceptors are likely to determine ingestion or rejection of a feed, and this final step in the feeding process of crustaceans is affected by food palatability (Harpaz, 1987; Kurmaly et al., 1990). Both diets tested in the current study appeared to be perceived by the redclaw as palatable since once both diets reached the mouthparts of the redclaw, they were ingested with no rejection or spitting out observed. This is not surprising as the experimental diet was prepared by re-pelleting the commercial redclaw diet with the same basal ingredients except that alginate was added to bind the ingredients more tightly. The binder alginate is a carbohydrate and has also been reported to elicit a strong feeding response in crayfish (Tierney and Atema, 1988; Kreider and Watts, 1998).

Although both diets appeared to be palatable, redclaw could not swallow the whole of both diets with a diameter of 4.5 mm. In comparison, they spent longer time fondling/repositioning the experimental diet with their mouthparts and feeding appendages attempting to break it down into smaller pieces than they did with the commercial diet. This behaviour largely reflected the substantially enhanced water stability of the experimental pellets as they were observed to remain largely intact before handling by the redclaw. Conversely, the poor water stability of the commercial redclaw pellet caused it to crumble soon after being introduced into the aquaria. Once such crumbled pieces of the commercial pellet reached the mouthparts, less fondling was observed as those feed fragments were already soggy and relatively easily ingested by the redclaw. Although the redclaw spent significantly longer time in handling the experimental diet, it is worth noting that mean difference in handling time was only about 1 minute between the two diets, hence unlikely to have any significant impacts on redclaw performance.

While both diets tested in the current study resulted in feed wastage because the redclaw did not attempt to feed on feed dust scattered on the bottoms of the aquaria resulted from either feed disintegration or mastication of the feeds by the redclaw due to their unsuitable size. Similar observations were reported by Ruscoe (2005) and Nguyen (2012) on redclaw of different sizes as well as in the hairy marron, *C. tenuimanus*, another Australian freshwater crayfish, which was observed to ignore feed when it became disintegrated (Jussila and Evans, 1998). Similarly, the feeding behaviour was reported in juvenile spiny lobster, *J. edwardsii,* where the attractiveness of the diet declined with increasing immersion time and no attempt was made by the juvenile lobsters to re-handle the small food materials dropped onto the floor of the tank (Sheppard et al., 2002; Tolomei et al., 2003). In the present study, the redclaw were most likely under starvation since they had been starved for 72 hours prior to the experiment and only being fed a pellet of 60 mg in weight, hence, it suggests that even under such starving situation, the redclaw would not feed on particles that are too small for them to consume.

In summary, the present study showed that understanding the feeding behaviour of a targeted aquaculture species and the corresponding physical and biochemical characteristics of formulated feeds developed for the species are critical for the feed formulation to enhance the culture productivity. The experimental diet developed in Chapter 3 aimed at improving poor water stability of the current commercial redclaw feed has achieved the goal, and with the significantly improved waters stability and low leaching rate, the experimental feed can still rival the commercial redclaw feed in its attractiveness and acceptability to the redclaw. With significantly improved water stability, the experimental diet remained intact for a significantly longer time in water and, as the result, had a significantly lower wastage level (5.0%) when compared to that of the commercial redclaw diet which crumbled quickly (11.0%). It is expected that under a pond culture situation with water movement, large bottom space and interactions among redclaw, the difference in feed wastage of the two diets is likely to be amplified than under the present controlled aquarium condition.

Finally, it is worth noting that even for the experimental pellet with high water stability when the pellet size was too big for the mouthpart of the redclaw to process, the pellet needed to be broken down into smaller pieces first before being ingested as was observed in the present study with the pellet size of 4.5 mm in diameter. This handling process resulted in an average wastage level of about 5.0% for the experimental feed. It has been reported that in juvenile spiny lobster, *J. edwardsii*, up to 50% of formulated feed pellets were wasted through inefficient handling of pellets of unsuitable sizes; however, with changes in pellet dimension to match the feeding ability of the lobsters, the wastage were reduced by as much as 19% (Sheppard et al., 2002). Obviously, for redclaw, it is also likely that the feed wastage could be further minimised by using pellet size that match the handling abilities of the mouthpart of the redclaw to be fed and this needs to be addressed by further experiments.

CHAPTER 5: EFFECT OF PELLET SIZE ON FEEDING EFFICACY AND FEED WASTAGE OF THREE DIFFERENT SIZE GROUPS OF REDCLAW

5.1 Introduction

Efforts to maximise feed utilisation should consider the feeding behaviour of cultured species at different developmental stages. The ingestion of feed in crustaceans involves a sequence of events of feed detection, acceptance and ingestion, and the pellet size plays an important role in feeding efficacy and feed wastage of crustaceans (Smith et al., 2009). While larger size feed particles may have the advantage of being more detectable by the crustaceans, if the pellet size is too large, the feeding sequence may terminate at any stage leading to feed wastage. Alternatively, while large crustaceans may consume small-sized feed particles, more energy and time are required to capture an equivalent weight of smaller feed particles, resulting in reduced feeding efficacy and lower feed conversion ratios (D'Abramo, 2002). Furthermore, as demonstrated in Chapter 4 (see Figure 4.2 d and 4.3 d), too small feed particles may also be ignored by large crustaceans. Both situations translate into low productivity and financial loss for the aquaculture farmers. Therefore, an effective mean to ensure a high feeding efficacy and low feed wastage is to tailor the size of the diet to ontogenetic changes in the feeding capabilities of the cultured animals (Sheppard et al., 2002).

Studies on crayfish feeding behavior under laboratory conditions (Loya-Javellana et al., 1993; Cronin et al, 2002; Meakin et al., 2008) showed developmental changes in feeding appendages, which was also reflected in selective consumption of feed items. The feed items that are easily handled are normally ingested more readily. Unfortunately, at the present, commercial redclaw feeds in Australia are only available in one pellet size (4.5 mm in diameter). This means that redclaw farmers have no option but to use this single sized feed for all developmental stages of redclaw. As demonstrated in Chapter 4, such sized pellet appeared to be too large even for the

adult redclaw with a size of 35 to 50 g. Given this, the aim of this study was to identify the optimal size of pellets for different developmental stages of redclaw to benefit redclaw famers through improved productivity.

5.2 Methodology

5.2.1 Experimental animals

Experimental animals were obtained from the same farms as in Chapter 2 (see 2.2.1) except that for this experiment, the weight of redclaw used for the experiment ranged from 5 to 50 g. The acclimatisation procedure was the same as described in Chapter 2 (see 2.2.1) while the feeding condition prior to the experiment was the same as described in Chapter 4 (see 4.2.2).

Healthy animals with intact appendages were sorted into three size groups: juveniles (5 - 8 g), sub- adults (10 - 15 g) and adults (35 - 50 g). In order to avoid animals being used more than once for the test, sub-adults and adult animals were marked by gluing a numbered Dymo tape to their carapace while juveniles were not labelled due to their vulnerability to handling stress. Instead, a set of 3 spare tanks were used to separate juveniles that had been used from those that hadn't.

5.2.2 Preparation of pellet of various sizes

Pellets of different size with varying diameters were manufactured with an identical pellet length of 5 mm. The details of the procedure are described in Chapter 3 (see 3.2.3).

5.2.3 Experimental design and experimental procedure

Redclaw were transferred individually from their communal holding tank and randomly assigned into a 20 L glass aquarium for the experiment. Based on its size category, each redclaw was offered pellet with one of the three different sizes to be tested (Table 5.1). That is, 1.0, 3.0 and 4.5 mm for juveniles (5 - 8 g); 2.0, 3.0 and 4.5 mm for sub-adults (10 - 15 g) and 3.0, 4.5 and 7.0 mm for adults (35 - 50 g), noting that the commercial pellet size of 4.5 mm was included in all size categories for testing as the control.

The glass aquaria used for the experiment were the same as showed in Figure 4.1 (Chapter 4). Prior to the experiment, all redclaw were starved for 72 hours. The experimental procedure prior to the experiment was the same as what was described in Chapter 4 (see 4.2.3). The experiment commenced with an identical 60 mg pellets (equivalent to the average weight of a single commercial redclaw pellet) of each size being introduced to the bottom of the aquarium via a PVC pipe that guided them to a distance of approximately 10 cm from the redclaw in the aquarium. Subsequently the feeding behaviour was observed and recorded both through personal observation as well as digitally video camera recording (Sony Handycam DCR-SX65) for later replay and confirmation. Stop watches mounted to the aquarium walls were activated from the time the feed was introduced to record: a) 'Time starting feeding', defined as the time required from pellet introduction to the time when the redclaw started to hold on to the pellet with its appendages, and b) 'Time spent feeding' or 'Food handling time', defined as the interval between when the redclaw started to grab the pellet introduced to the time the feeding behaviour ceased.

Each pellet size was replicated 18 times using different redclaw of a same size category. The experiment duration was 15 minutes for all redclaw tested. Occasionally, more than 10 min elapsed following pellet introduction, an experimental redclaw still did not react to the introduced pellets. When such situation occurred, the experiment was terminated with a new acclimated redclaw of a

same size category used to repeat the test. Those redclaw did not respond to the diet induction in 10 minutes were excluded for the data analysis. The number of excluded redclaw were similar among treatments. After recording each redclaw for 15 minutes, the water was discarded and the aquarium washed and re-filled with fresh water for the next experiment.

Table 5.1 Pellet sizes tested on redclaw of three size categories.

Developmental stage	**Body weight**	**Pellet sizes tested**
Juveniles	5 - 8 g	1.0, 3.0 and 4.5 mm
Sub-adults	15 - 25 g	2.0, 3.0 and 4.5 mm
Adults	35 - 50 g	3.0, 4.5 and 7.0 mm

5.2.4 Estimation of feed wastage

The feed wastage measurements were carried out using identical procedures as described in section 5.2.3 except that the measurement was replicated for the first 12 redclaw for each treatment.

5.2.5 Statistical analysis

Data are expressed as mean ± standard error (SE). A One-way ANOVA was performed to detect any significant differences between the 'Time starting feeding', 'Time spent feeding' and feed wastage among treatments. This was followed by a Tukey's HSD test to separate the means with a significance level of $P < 0.05$. All statistical analyses were performed using the statistical software Statistica (version 2.1).

5.3 Results

5.3.1 General feeding behaviour

The different pellet sizes tested significantly affected feed detection, handling time and feed wastage by the different developmental stages of redclaw. In general, each size group of redclaw exhibited the same sequential initial feeding response for each pellet size introduced while juveniles responded with more rapid feeding movements which was reflected by generally shorter times recorded for the 'Time starting feeding' (Tables 5.2, 5.3 and 5.4). It was also noted that when the redclaw had located the pellets, in the majority of cases the adults used their claws while juveniles and sub-adults more often used their walking legs to pick up and bring the pellets to their mouthparts. Generally, the bigger the pellet size, the quicker they were detected for all size groups of redclaw (Tables 5.2, 5.3 and 5.4). Once the pellet was near the mouthpart region, all size category of redclaw used their first, second and third pairs of maxillipeds to ensure that the pellet was placed in the mouthpart for fondling/repositioning prior to ingestion. The appropriate sized pellets tested for each size group generally had a significantly reduced time required for manipulation as they were often ingestion as whole without the need for further fondling/repositioning. On the other hand, the big sized pellets that were quickly detected by redclaw were not ingested readily as they were too large for the mouth of the redclaw, leading to substantially longer handling time as well as higher feed wastage (Tables 5.2, 5.3 and 5.4). No attempt was observed for all size group of redclaw tested to feed on the small dust-like feed particles that ended up scattered on the experimental aquarium floor.

5.3.2 Juvenile redclaw fed pellets with different sizes

'Time starting feeding' by the juveniles was significantly shorter for the biggest pellet size tested (4.5 mm) as compared to that of smaller sizes of 3.0 and 1.0 mm ($P < 0.005$) while no significant difference was detected between 3.0 and 1.0 mm pellets (Table 5.2). On the 'Time spent feeding, it was observed that the small pellet size of 1.0 mm was grasped with only one chela on the ends of first pair of walking legs and rapidly carried to the mouth and ingested as whole with a time spent on feeding only 1.1 ± 0.1 min. When pellet size increased to 3.0 mm, juveniles more often used their first pair of walking legs to bring pellet closer to their mouthparts and as the 3.0 mm pellet size appeared still too big for the mouth size of the juveniles to ingest as whole, juveniles were subsequently observed to attempt to crush the pellets and spent more than twice the time handling the pellets (2.7 ± 0.2 min) (Table 5.2). The pellet size of 4.5 mm was clearly way too large for the juveniles as they were observed to flatten themselves against the floor of the aquaria, with the first two pairs of walking legs functioning together to position a pellet to be scooped by third maxillipeds to begin the feeding sequence. The third maxillipeds were normally used to ensure that the 4.5 mm pellet was placed into the mouthpart for fondling/repositioning prior to ingestion, and it took more than 8 times longer for the juveniles to handling the pellet (9.7 ± 0.3 min) than that of 1.0 mm pellet ($P < 0.05$) (Table 5.2). The bigger pellet size of 4.5 mm was chopped up into small pieces by the juveniles using their feeding appendages, resulting in dust of feed dissipating into the water column, which led to 14 times higher feed wastage when compared to that of the 1.0 mm pellet size and twice more wastage compared to the 3.0 mm pellet ($P < 0.001$) (Table 5.2). Based on the results showed in Table 5.2, the pellet size of 1.0 mm diameter is recommended as the suitable size for juvenile redclaw of 5 - 8 g as it required the shortest handling time with the lowest feed wastage.

Table 5.2 Time starting feeding, Time spent feeding and Feed wastage of the juvenile redclaw fed on 3 different size pellet. Data are presented as mean ± S.E.; data with different superscript letters within a same row are significantly different (P <0.05).

Redclaw size category (g)	**Pellet size (mm in diameter)**	**Time starting feeding (min)**	**Time spent feeding (min)**	**Feed wastage (%)**
Juveniles (5 - 8 g)	1.0	3.1 ± 0.2^{a}	1.1 ± 0.08^{c}	1.0 ± 0.0^{f}
	3.0	3.1 ± 0.2^{a}	2.7 ± 0.18^{d}	7.0 ± 0.0^{g}
	4.5	1.8 ± 0.1^{b}.	9.7 ± 0.3^{e}	14.0 ± 0.0^{h}

5.3.3 Sub-adult redclaw fed pellets with different sizes

In the case of sub-adults of 15 - 25 g, it took significantly shorter time of 2.9 ± 0.4 min to start feeding on the largest pellet size tested (4.5 mm) and as pellet size decreased to 3.0 mm and 2.0 mm, the 'Time starting feeding' increased to 3.1 ± 0.6 and 3.7 ± 0.6 min respectively (P <0.05) (Table 5.3). No significant difference (P >0.05) was detected for 'Time starting feeding' between pellet size of 3.0 mm and 2.0 mm. A pellet size of 2.0 mm was observed to be grasped by sub-adults with only one chela on the ends of the first pair of walking legs and it took the shortest handling time (0.6 ± 0.7 min) to be ingested as whole by the redclaw. While sub-adults might use only one chela on the ends of their first pair of walking legs to grasp and manipulate the 3.0 mm pellets to their mouthparts, they were most often observed to use their first pairs of walking legs as well to help. The average time of the sub-adults spent on handling of larger 3.0 mm pellets was 3 times longer than that of the 2.0 mm pellets (P <0.05) (Table 5.3). On the other hand, sub-adult redclaw appeared still had difficulty in handling the 4.5 mm pellets, often required the first pair of walking legs functioning together to lift the pellet to the mouthparts, and on average, took about

9 times and almost 3 times longer time in handling the food as compared to that of 2.0 mm and 3.0 pellets, respectively (P <0.001) (Table 5.3). Meanwhile, feed wastage (8.0 ± 0.0%) resulted from feeding on the biggest pellet of 4.5 mm was significantly higher than that of 3.0 mm pellet (6.0 ± 0.0%) and 4 times higher than that of 2.0 mm pellet (2.0 ± 0.0%) (P <0.001) (Table 5.3).

Table 5.3 Time starting feeding, Time spent feeding and Feed wastage of the sub-adult redclaw fed on 3 different size pellet. Data are presented as mean ± S.E.; data with different superscript letters within a same row are significantly different (P <0.05).

Redclaw size category (g)	**Pellet size (mm in diameter)**	**Time start feeding (min)**	**Time spent feeding (min)**	**Feed wastage (%)**
Sub-adult	2.0	3.7 ± 0.6^{a}	0.6 ± 0.7^{c}	2.0 ± 0.0^{f}
(15 - 25 g)	3.0	3.1 ± 0.6^{a}	1.8 ± 0.1^{d}	6.0 ± 0.0^{g}
	4.5	2.9 ± 0.4^{b}	5.0 ± 0.4^{e}	8.0 ± 0.0^{h}

5.3.4 Adult redclaw fed pellets with different sizes

For the adult redclaw of 35 - 50 g, similarly the biggest pellet size (7.0 mm) tested was detected significantly quicker with a shorter 'Time starting feeding' at 2.1 ± 0.3 min at compared to that of the size of 4.5 mm and 3.0 mm, which took 3.4 ± 0.3 and 3.9 ± 0.3 min, respectively (P <0.05) while no significant difference was detected between the 4.5 and 3.3 mm pellets (P >0.05) (Table 5.4). It was observed that the adults most often used only one chela on the first pair of walking legs to bring the 3.0 mm pellet to the mouthparts to be ingested as whole. On the other hand, a pellet of 4.5 mm was most often grasped by the adults with first pair of walking legs and after which, the pellet was fondled by the mouthpart. The adults were also observed to use their claws to pick up the biggest sized pellets of 7.0 mm and transfer them to the mouthpart while the

third maxillipeds ensured that the pellet was hold at the mouthparts for fondling/repositioning and crushing by the mouthparts into small pieces prior to ingestion. Average feed handling time by the adults for the pellet size of 3.0 mm was the shortest at 0.7 ± 0.1 min but increased significantly to 2.0 ± 0.2 min and 3.8 ± 0.6 min as the pellet size increased to 4.5 mm and 7.0 mm, respectively ($P < 0.001$) (Table 5.4). Meanwhile, feed wastage was the lowest at 2.0 ± 0.0% from the 3.0 mm pellets and increased significantly ($P < 0.001$) to 5.0 ± 0.0% and 9.0 ± 0.0%, respectively for the bigger pellets of 4.5 mm and 7.0 mm) (Table 5.4).

Table 5.4 Time starting feeding, Time spent feeding and Feed wastage of the adult redclaw fed on 3 different size pellet. Data are presented as mean ± S.E.; data with different superscript letters within a same row are significantly different ($P < 0.05$).

Redclaw size category (g)	**Pellet size (mm in diameter)**	**Time start feeding (min)**	**Time spent feeding (min)**	**Feed wastage (%)**
Adult	3.0	3.9 ± 0.3^{a}	0.7 ± 0.1^{c}	2.0 ± 0.0^{f}
(35- 50 g)	4.5	3.4 ± 0.3^{a}	2.0 ± 0.2^{d}	5.0 ± 0.0^{g}
	7.0	2.1 ± 0.3^{b}	3.8 ± 0.6^{e}	9.0 ± 0.0^{h}

5.4 Discussion

The results of the present study reveal that the pellet sizes significantly affected feed detection, feed handling time and feed wastage and different size category of redclaw preferred different sizes of pellets. With high attractiveness of the experimental feed to redclaw as shown in Chapter 4, all pellet sizes tested in this study were positively accepted although the detectability of pellets was generally quicker in the juveniles as compared to the sub-adults and adults when offered the same size of 4.5 mm pellets. This is similar to the feeding response of juvenile yabby *Cherax*

destructor of less than 15 g, whose feeding mode was adapted to prey on zooplankton and involves rapid searching for food items as compared to larger sized yabby (Meakin et al., 2008). Meanwhile, bigger sized pellets generally were detected quicker for all size category of redclaw tested and 'Time starting feeding' increased with smaller pellets. Such feeding response is in agreement with other studies that found in crustaceans, food particle size affected feed detection and feed selectivity (Nunes and Parson, 1998). Loya-Javellana et al. (1993) also observed that decayed plant material was detected quicker by redclaw compared to zooplankton. However, the data on feed detection from the present study should be treated with caution as they came from laboratory experiments in which strong light as well as shallow and clear water was used, which allowed to maximum utilization of visual cues by the redclaw. Under the pond culture situation, however water often are murky and substantially deeper, visual cue may not play a significant role in feed detection by redclaw.

More importantly, it has been reported that food capture success ultimately related to the chela diameter in crustaceans (Hindley and Alexander, 1978; Nunes and Parson, 1998; Meakin et al., 2008; Smith et al., 2009). The bigger pellets that were quickly detected by redclaw in the current study was actually took substantially longer to be ingested by the redclaw with substantially higher feed wastage due to their sizes were too big to be ingested by the redclaw as whole and the process of breaking up of the pellets with the mouthparts by the redclaw led to longer handling time and substantial higher feed wastage. These findings are in agreement with Smith et al. (2009) who found that a particle size that was quickly detected by crustaceans might not be ingested rapidly if the diameter of the feed does not match the feeding appendages of the cultured crustaceans. Although crustaceans can feed on pellets that are too large for their mouth by shedding them down to smaller pieces prior to ingestion, the process is clearly time and energy consuming and inevitably leads to more food wastage because a proportion of fragmented feed was observed not being re-captured and ingested. On the other hand, although the smaller sized pellets generally

took slightly longer (1 to 2 min on average) to elicit a response, the appropriate sizes allowed them to be ingested as whole without further fondling, which substantially reduced the time and energy required for manipulation while reduce the feed wastage.

The results of the current study showed that feed wastage could range from hardly any (1.0% to 2.0%) when fed the respective optimal sized pellets to different size categories of redclaw (i.e. 1.0 mm for the juveniles, 2.0 mm for the sub-adults and 3.0 mm for the adults) to up to 8% to 14% when the pellet size were way too large. It was also shown that optimal pellet size increased with the size of redclaw as 1.0 mm was found to be the best size for the juveniles of 5 - 8 g while 2.0 mm was most suitable for the sub-adults of 15 - 25 g and 3.0 mm most appropriate for the adults of 35 - 50 g. Similar results have been reported by Sheppard et al (2002) for the spiny lobster *Jasus edwardsii* as it was found that when fed juveniles of 14 g with pellets of 3 × 3 mm, feed wastage reduced to 19% from 50% when fed those pellets that were too large. However, for larger *J. edwardsii* of 135 g, pellet size increased to 7 × 7 mm with a similar result. It was hence concluded that less nutrient loss and feed wastage can be achieved if cultured *J. edwardsii* were fed ideal sizes of pellets relating to their body size (Sheppard et al, 2002).

In summary, this study showed that pellet size can significantly affect feeding efficacy and wastage and for different size categories of redclaw, the optimal pellet size is different.

At 4.5 mm in diameter, the only size of commercial redclaw pellets currently available to redclaw farmers in Australia is too large even for the adult redclaw up to 50 g. It is proposed that similar to other more mature aquaculture sectors, such as saltwater prawns and barramundi for which commercial feeds are available in multiple sizes to suit different life stages, commercial pellets manufactured for the redclaw industry should also come in with different sizes and it is recommended that for the juveniles of 5 - 8 g, the sub-adults between 15 - 25 g and the adult redclaw of 35 - 50 g, the most suitable pellet size is 1.0, 2.0 and 3.0 mm in diameter, respectively.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

At the outset of this study, despite all the positive attributes of the target organism and optimistic forecasts, the expansion of aquaculture for this species has been hindered by poor growth and survival during early stages. Various problems have been identified such as poor growth and survival that contribute to the declining production of the redclaw industry in Australia in recent years, and this has mainly been attributed to major problems relating to feeds and feeding (Stevenson et al., 2013). Unfortunately to date, optimal feeding management for crayfish has not yet been achieved when using only one (pellet) size of formulated feed that has poor water stability in intensive culture systems and in particular for redclaw (Cortés-Jacinto, 2003; John Stevenson; Pers. Comm.).

The feeding practice for the Queensland redclaw aquaculture industry varies from once per day to once per week depending on the farmer's operational criteria and experience using the only available commercially manufactured diet. As a result, based on the experiment described in Chapter 2 it was necessary for this study to examine the range of feeding intervals (once every day, every second day, every third day and every fourth day) that Queensland redclaw famers are practicing to allow practical conclusions on the growth performance of juvenile redclaw to be made. However, within the range of feeding intervals tested in the current study, growth parameters, FCR and survival of juvenile redclaw held individually were not affected, but slightly improved with decreased feeding intervals. Growth performance of juvenile redclaw and FCR, except for the survival rate of juveniles, were not satisfactory compared to other studies over a period of 140 days. Inadequacies of feed availability and relatively poor food utilization values might be attributable to lengthening the intermoult period and reducing the moult increment, leading into slow growth of juveniles rather than the feeding intervals tested. These were confirmed by poor water stability of the commercial redclaw feed used in the present study which totally disintegrated within 1 hour of immersion with a dry matter loss (DML%) of 23.8%

(Chapter 3 results). However, the individual cage compartment culture system used for the current study restricted the animals within cage compartments, limiting their continuous foraging on feed particles that spread outside the cage during their longer feed fondling time, because the pellet size of 4.5 mm (only available in Australia) used is definitely unsuitable for juvenile redclaw (Chapter 5 results). Survival rate (~ 80%) was satisfactory, but the long term survival (>140 days) of juveniles fed such a poorly water stable diet with no other food source available to the redclaw is unknown.

Although the poorly water stable diet at only one size used in the current study is commonly used by the Queensland redclaw industry in farm pond culture with no standard feeding management practice, uneaten feed resulting from disintegration and external mastication during the ingestion process could have allowed for nutrients to drive natural pond productivity. This may allow for redclaw to use natural food resources in the pond until a sufficient number of satiation meals are taken when the availability of feed is not sufficient. However, once a poorly water stable diet has disintegrated in an intensive culture system, marron *C. tenuimanus*, another Australian freshwater crayfish have been observed to preferentially feed on intact pellets rather than pieces of fragmented feed (Jussila and Evans, 1998). Both economically and environmentally disintegrated feed results in considerable financial loss (Ahvenharju and Ruohonen, 2005). Therefore, if this commercial redclaw diet is to be used in intensive culture systems where there is no natural feed available for juvenile redclaw, further study aimed to improve water stability and ideal pellet size of the currently available commercial redclaw feeds in Australia is essential to improve growth and feed utilisation efficiencies.

A step forward to improve formulated redclaw feed firstly was to identify the best binder and secondly the optimal binder concentration, to formulate a pellet size that is more water stable. These aspects were addressed below and in more detail in Chapter 3.

Crayfish feeds with good water stability should minimise disintegration and excessive nutrient leaching prior to consumption, because crayfish generally find food through chemoreception rather than visual cues, and as result the interval between offering a feed and its ingestion can often exceed the time that the pellet will remain stable (D'Abramo and Sheen, 1994; Sáez-Royuela et al., 2001). After locating feed, the diet must remain intact as they capture feed particles by using the major chelipeds and walking legs in a grasping fashion, for transfer to the mouth where external mastication prior to ingestion occurs with some of the food being lost (Sáez-Royuela et al., 2001; Grasso and Basil, 2002; Ahvenharju and Ruohonen, 2005; Ruscoe, 2005). However, in an effort to reduce feed costs since the early 2000s, replacement of fishmeal by cereal grains and legumes in commercial redclaw feeds led to poorly bound pellets (Ruscoe et al., 2005). The resultant pellets of the commercial redclaw diet examined for the current study totally disintegrated within the first hour of immersion compared to the improved water stability diet bound with six binder types (alginate, agar, carrageenan, CMC, PVA and starch). As a result, our findings highlighted the successful development of a formulated diet for redclaw with the addition of binders to enhance water stability. However, of the six binder types tested for the current study to improve diet water stability, alginate could substantially improve the water stability of the current commercial redclaw pellet because it gave best pellet physical form combined with the overall lowest DML over 24 hour immersion.

Although the use of binding agents has advantages as mentioned above, excessively high concentrations of binder may cause increase feed costs, a reduction in diet digestibility and loss of valuable dietary nutrients (Meyer et al., 1972; Patridge and Southgate, 1999). Alternatively, too little binder may not be adequately water stable, resulting in deterioration of water quality (Meyer et al., 1972; Patridge and Southgate, 1999). For the current study, alginate was tested at a range of concentrations (2.0%, 2.6%. 3.2%, 3.8 % and 4.4%) and the highest inclusion level of 4.4%

alginate is recommended for best results in relation to physical form and overall lowest DML after 24 hour immersion.

After identifying alginate as the best binder type at an optimal concentration of 4.4% total dry weight it was then incorporated for the current study to formulate the range of pellet sizes (1.0, 2.0, 3.0, 4.5, 5.0 and 7.0 mm diameter) to be tested for water stability. Water stability was found to have a direct correlation to pellet size (Obaldo and Tacon, 2001). A lower water stability of smaller pellet sizes has been reported because the surface area to volume ratio is increased as compared to larger pellets (Obaldo and Tacon, 2001). The current findings showed that pellet size had a minor effect on water stability due to using the same best binder type at optimal concentration, which had already provided good results for water stability from the previous experiment.

Improved formulated feed may be water stable and offers minimal leaching, but at the expense of the loss of qualities of attraction, palatability or digestion (Patridge and Southgate, 1999; D'Abramo, 2002). This is particularly true when considering that crayfish feeding behaviour differs substantially from that of fish, as crustaceans generally find food by chemoreception rather than visual cues (D'Abramo and Sheen, 1994). The fact that visual cues are not relied on for foraging by crayfish poses a major challenge for any formulated feeds that may lack chemically-induced feeding stimuli released a few minutes after introduction to the culture ponds (D'Abramo, 2002). For example, it was not clear for the current study if using either a commercial redclaw feed that does not contain fishmeal or a more tightly bound experimental diet prepared by re-pelleting the commercial redclaw with alginate added as a binder might lead to the pellets being better accepted by redclaw. Research has shown that the leaching of attractant molecules from feed may be limited if a high inclusion of low-cost plant protein sources replace fishmeal, or if a diet is tightly bound (Partridge and Southgate, 1999; Ahvenharju and Ruohonen, 2005;

O'Mahoney et al., 2011). To ensure that formulated feed will be perceived by crustaceans as something suitable to eat, the food ideally should leach out a steady plume of attractants, or otherwise, if the diet is rejected then death from starvation is inevitable (Williams, 2007). Therefore, to warrant that the experimental diet developed in Chapter 3 aimed at improving poor water stability of the current commercial redclaw feed has achieved great success, behavioural experiments in Chapter 4 were designed to quantify redclaw feeding responses to the new pellets with alginate added as the binder, against the original commercial redclaw diet.

The findings showed that the experimental diet rivalled the commercial redclaw feed in its attractiveness and acceptability to the redclaw. This is not surprising as the experimental diet was prepared by re-pelleting the commercial redclaw diet with the same basal ingredients, except that alginate was added to bind the ingredients more tightly (Chapter 3). A likely reason for this is that the binder alginate is a carbohydrate (Table 3.1), and has been reported to elicit a strong feeding response in crayfish (Tierney and Atema, 1988; Kreider and Watts, 1998). Observations on the commercial redclaw diet showed it usually started to crumble or disintegrate soon after introduction into the water, and redclaw were observed to only feed on the larger fragments of the crumbled pellet or cease feeding after it had totally disintegrated. The same feeding behaviour has been reported when the hairy marron, *C. tenuimanus*, another Australian freshwater crayfish species, was observed to ignore feed when it became disintegrated (Jussila and Evans, 1998). In contrast, the experimental diet remained intact and redclaw were subsequently observed to attempt to crush the pellet into smaller pieces which resulted in smaller dust-like feed particles scattering on the aquarium floor. However, small feed particles have been observed to be picked up and ingested by spiny lobster, *Panulirus ornatus,* when fed was below satiation levels (Smith et al., 2009). It was also expected that the redclaw used in the current experiment were under satiation feeding levels since they had been starved for 72 hours prior to the experiment and were only fed a pellet of 0.06 g for either the experimental or commercial redclaw diet treatments, hence, this

suggests a feeding behaviour difference between redclaw and *P. ornatus*. As a result, both diets tested resulted in feed wastage by redclaw refusing to feed on disintegrated feed in the commercial redclaw diet treatment and feed dust scattered on the glass aquarium for the experimental diet. Therefore, it was important for the current study also to quantify the feed wastage levels for both diets tested.

Obviously, feed wastage is another important indicator for the suitability and quality of feeds that are formulated for redclaw. However, there are no studies to date quantifying feed wastage when feeding redclaw with formulated feeds. Ruscoe et al (2005) has recommended that quantifying the actual amount ingested by freshwater crayfish is one of the greatest difficulties in nutritional research with the species. While that is probably true for pond culture, it is possible to estimate feed wastage under controlled aquarium conditions, which should provide a useful indication of feed quality. Therefore, to quantify feed wastage in the current study, each animal was removed from its experimental glass aquarium once feeding behaviour observations were completed and all the water in the tank subsequently drained and filtered to collect unconsumed feed material.

With significantly improved water stability, the experimental diet remained intact for a significantly longer time in water and as a result, had a significantly lower wastage level (5.0%) when compared to the commercial redclaw diet which crumbled quickly (11.0%). However, considering that crayfish masticate feed externally before ingestion, feed wastage for the experimental diet can be minimised further by using pellet sizes that match the handling abilities of the mouthparts of the redclaw and this was addressed as described below and in more detail in Chapter 5.

The feed handling efficiency of crustaceans represents a key issue for the development of formulated diets in aquaculture. Up to 50% of formulated feed pellets were wasted through inefficient handling of unsuitable pellet sizes fed to juvenile spiny lobster, *Jasus edwardsii*;

however, with changes in pellet dimension to match the feeding ability of the lobsters, this wastage was reduced by as much as 19% (Sheppard et al., 2002). Unfortunately, Queensland commercial feed producers manufacture only one size (4.5 mm diameter) of pellets for all developmental stage of redclaw. Therefore, Chapter 5 aimed to determine the optimal pellet size for different developmental stages of redclaw.

Our results highlighted that the bigger pellet size tested had the advantage of being detected quickly by all different size groups of redclaw examined, but not ingested efficiently as the diameter of the pellet did not match with the mouths of all sizes of redclaw observed. This indicates that the effort of shredding a larger pellet size may be energetically costly and results in significantly long time spent feeding and increased feed wastage. The pellet size of 4.5 mm, which is the only available size of commercial pellets was observed to be unsuitable for all developmental stages of redclaw tested up to a size of 50 g due to prolonged feed handling times and high levels of feed wastage. Based on the results of the current study, the optimal feed size increases with animal size hence the optimal size of pellets for juvenile redclaw of 5 - 8 g is 1.0 mm, for sub-adults of 15 - 25 g this increases to between 2.0 to 3.0 mm, and for 35 - 50 g adults a pellet size of 3.0 mm is the best. However, since the current study highlights that redclaw took longer to respond on the small pellet size, in the pond culture system animals may find difficulties in detecting small pellets due to other factors (ie., the presence of sediment, natural feed, feed dispersal, inter-animal behavioural relationships) and may be compensated by the higher numbers of small pellet per area which will increase the probability of food encounters (Nunes and Parson, 1998).

In conclusion, the major findings of this study were:

1. A cost effective feeding management practice demonstrated in Chapter 2 whereby feeding every day appears unnecessary and a longer feeding interval of up to feeding once every four days might be recommended to redclaw farmers to save on labour costs.

2. Significantly improved water stability of commercial redclaw pellets achieved in Chapter 3 when commercial redclaw pellets were first ground and incorporated with alginate at 4.4% of total diet dry weight.

3. In Chapter 4, improved water stability of the experimental diet rivalled the commercial redclaw feed in its attractiveness and acceptability with lower wastage levels.

4. Optimal pellet size observed to increase with size group of redclaw in Chapter 5 hence, optimal sizes of pellets for juvenile redclaw of 5 - 8 g is 1.0 mm, for sub-adults of 15 - 25 g this increases to 2.0 and for 35 - 50 g adults a pellet size of 3.0 mm is the best.

The findings of this study have provided baseline information leading to improvements of feed and feeding practices for Australian redclaw. However, since these experiments were conducted in the laboratory and in individually compartmentalised controlled conditions, with clear water (i.e. no natural pond productivity present), it is recommended that the improved feed and feeding management strategies outlined be evaluated in ponds as well, where natural productivity may contribute significantly to nutrition of the redclaw.

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