Australian Government

Australian Centre for International Agricultural Research

Final report

project

Assessing the potential for low-cost formulated diets for mud crab aquaculture in Australia, Indonesia and Vietnam

project number	FIS/2000/065
date published	July 2010

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final report number	FR 2010-13
ISBN	978 1 921615 99 3
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

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1 Acknowledgments

The authors would like to thank David Mann, Marko Pavasovic, Tom Asakawa and Beverley Kelly at BIARC for their technical assistance. Astaxanthin was kindly provided by Dr Linda Browning of DSM Nutritional Products (Australia). Our gratitude is also extended to Dr Richard Smullen of Ridley Aqua-Feed (Australia) for supplying fishmeal and Mr John Harsant of Radford Park Aquafeeds (Australia) for supplying soybean, canola and cotton seed meals.

The authors would also like to thank Dr Kevin Williams and Dr David Smith (CSIRO) for their expertise in the design of formulated mud crab diets.

2 Executive summary

Mud crabs of the genus *Scylla* are a valuable source of nutrition and provide income for many coastal communities in the Asia-Pacific region. Currently, mud crab farming is well established throughout Southeast (SE) Asia with most mud crab farmers using trash fish, bivalve meats or animal by-products as feeds. This traditional feeding practice is unsustainable and the development of low-cost, formulated diets for grow-out is viewed by ACIAR as a priority research issue.

The current ACIAR project sought to build on earlier progress by aquaculture research teams in Australia (Bribie Island Aquaculture Research Centre; BIARC), Indonesia (Gondol Research Institute for Mariculture: GRIM) and Vietnam (Research Institute for Aquaculture No. 3; RIA3) Vietnam. The major aim was to determine if artificial diets formulated for local *Scylla* species could provide alternatives to traditional, but unsustainable, grow out feeding practises. Key outputs of the project are summarised under the headings of the three main objectives;

Objective 1: To evaluate the potential of formulated feeds to replace traditional diets used in mud crab aquaculture.

Outcome: ***Artificial, fishmeal-based pellet feeds displayed the potential to replace trash fish for mud crab grow out.***
An improved dietary formulation based on a lobster diet (Smith *et al*, 2005) was developed in collaboration with nutrition experts Dr Kevin Williams and Dr David Smith (CSIRO Marine Research, Cleveland, Australia). Feeding trials were conducted at BIARC using this diet and resulted in crab growth rates that were approximately 90% of those obtained using a fresh diet (fish, squid and mussel). Two pilot studies were also conducted at RIA3 (Vietnam) in earth ponds to assess the feasibility of using formulated diets for farm scale production systems. The results of these pilot studies were encouraging. Specifically, growth rates for crabs fed experimental diets in earth ponds were;

- substantially higher than those obtained in a laboratory-based culture system
- equivalent to those exhibited by crabs fed trash fish.

Finding such as these demonstrate the potential of formulated pellet diets to supplement or replace traditional mud crabs diets. These outcomes also help to justify the need to produce larger batches (tonnes) of experimental feeds for more extensive feeding trials in production systems typically used for mud crab aquaculture in SE Asia (ie. earth ponds).

Objective 2: To determine critical nutritional requirements and evaluate key feed ingredients

Outcome: ***Low cost, locally available feed ingredients were identified that were readily digested by mud crabs.***
A consistent finding of this study was the high capacity of *Scylla* species to digested feed meals derived from various animal and plant-based products. Specifically, digestibility coefficients were obtained for test ingredients that were equivalent (or superior) to those obtained for fishmeal, which is traditionally the major source of protein in formulated aquaculture diets. For example, high digestibility coefficients were obtained for prawn head meal in studies conducted in Vietnam and Indonesia using *S.paramamosian.* Likewise, sub-adult *S.serrata* demonstrated the capacity to readily digest poultry meal in studies conducted in Australia. An unexpected outcome of the current project was the high apparent digestibility of many plant-based ingredients. For example, digestibility coefficients were obtained for soybean meal that were significantly higher than the corresponding values for locally available fishmeal. Finding such as these strongly suggest that mud crabs have the potential to access nutrients from a variety of common

feed ingredients. Based on such data, we recommend that high protein (>30%) feed ingredients which can be readily digested by *Scylla* species (such as prawn head meal, poultry meal and soybean meal) be further investigated for their potential to replace fishmeal in formulated mud crab diets.

Outcome: ***Data on the crude protein and lipid requirements of local mud crab species was obtained.***
In laboratory-based culture systems, juvenile crabs demonstrated best growth performance when fed artificial diets containing at least 43% crude protein (36% digestible protein) and between 9% to 15% lipid. It is important to note that this is significantly less protein and more lipid, respectively, than contained in many artificial prawn diets that have been used for mud crab aquaculture. Such findings are encouraging and suggest it may be possible to;

- reduce diet cost by reducing the amount of expensive, marine animal-based protein (ie. fishmeal) required for mud crab diets, and,
- increase the energy density of mud crab diets by incorporating higher levels of lipid.

Despite these promising results, growth rates in laboratory-based culture systems were significantly less than those reported for mud crabs reared in commercial production facilities (ie. earth ponds). As a consequence, a pilot study was conducted at RIA3 to assess the potential of experimental diets to support semi-intensive mud crab aquaculture in earth ponds. An important outcome of this pilot study was that growth for all diet treatments was substantially higher than that obtained for corresponding treatment groups in laboratory-based investigations. Moreover, the growth performance of juvenile *S.paramamosian* fed a formulated diet (43% crude protein and 15% lipid) was equivalent to that obtained using trash fish.

Objective 3. To formulate and evaluate improved mud crab diets.

Outcome: ***Several animal and plant-based ingredients showed the potential to replace fishmeal in formulated mud crab diets.***
Krill meal, poultry meal, corn gluten meal and soybean meal all demonstrated the capacity to replace 20% or 40% of fishmeal without significantly reducing growth performance in laboratory-based studies. Interestingly, crab growth in laboratory facilities was significantly less than that expected for semi-intensive production systems. To assess the potential of candidate ingredients to replace fishmeal in farm scale production systems, a pilot study was conducted in earth ponds at RIA3 to assess the potential of soybean meal to replace fishmeal in formulated mud crab diets. Results were highly encouraging. Specifically, a formulated diet containing 40% soybean meal supported growth which was equivalent to that obtained using trash. We therefore recommend that diet formulations incorporating low cost alternatives to fishmeal (as developed in the current project) be further tested in more extensive farm-based trials to assess their suitability for adoption by the mud crab aquaculture industry in SE Asia.

3 Background

Mud crabs in the genus *Scylla* (Figure 1) are a valuable source of nutrition and provide income for many coastal communities in the Asia-Pacific region. In recent years, however, many mud crab fisheries have experienced over fishing that has threatened capacity to meet future demand from local and export markets. As a consequence, there has been significant investment directed towards developing profitable and sustainable mud crab aquaculture industries, particularly in SE Asia.

Initially, the limited supply of wild caught crab seed was identified as a major factor restricting industry growth. To address this issue, ACIAR supported several collaborative projects for laboratory (FIS/1992/017) and commercial (FIS/1999/076) scale crablet production in Indonesia, Vietnam, the Philippines and Australia. Techniques were developed to facilitate large scale hatchery production of crab seed. The uptake of improved hatchery technologies has been particularly successful in Vietnam where now there are over 100 private hatcheries supplying seed to local mud crab farmers (Thach. *pers. com*.)

Fig. 1. An individual *Scylla serrata* adult (a) and *S.serrata* harvested from earth ponds at BIARC (b).

With the issue of hatchery seed supply largely resolved, attention has shifted towards other factors that have impeded expansion of mud crab aquaculture. In 2003, ACIAR conducted a scoping study that identified cannibalism and a lack of appropriate supplemental feeds as major factors limiting industry growth in the Asia / Pacific region (Fielder and Allan, 2004). In particular, the use of low value trash fish or expensive artificial prawn diets for grow out was considered unsustainable. As a consequence, the development of low-cost grow out diets based on local ingredients was identified by ACIAR as a key strategy to increase feed resource security for the expanding mud crab aquaculture industry.

Although artificial diets have been used for crab aquaculture in the past (Mann and Paterson, 2004), most have been designed for *Penaeid* species and as such contain high levels of relatively expensive marine animal-based feed ingredients, such as fishmeal. There is a general consensus that the global supply and cost of high quality fishmeal for artificial feeds is insufficient to meet the demands of a rapidly expanding aquaculture sector. To address this issue, ACIAR is funding a number of research programmes in the Indo-Pacific region aimed at replacing fishmeal in aquafeeds with cost-effective alternatives (eg. FIS/2006/141).

Identifying alternatives to fishmeal for aquafeeds requires information on the capacity of candidate species to digest and assimilate nutrients from potential feed ingredients. In recent years, evidence has emerged that the mud crab digestive system has the capacity to digest many relatively cheap plant-based feed ingredients. For example, enzymes required to digest plant-based carbohydrates such as amylase and cellulase have been detected in the mud crab digestive system (Pavasovic *et al*, 2004). Moreover, high digestibility coefficients have been obtained using various flours and plant-based feed meals incorporated into mud crab diets (Catacutan *et al*, 2003; Tuan *et al*, 2006). Inclusion of relatively cheap ingredients, such as plant-based meals, into artificial diets provides an opportunity to significantly reduce feed costs for *Scylla* species.

The current project sought to build on earlier progress by ACIAR-funded mud crab aquaculture research teams in Australia (BIARC), Indonesia (GRIM) and Vietnam (RIA3) Vietnam. Specifically, experiments were conducted to determine if artificial, formulated diets could provide alternatives to traditional feeding practises for grow out (eg. trash fish). This requires diets that are;

- Cheaper than the diets currently used to support commercial mud crab culture in Australia and SE Asia.
- Able to provide equivalent or superior performance to currently available crustacean feeds.
- Less likely to impact on water quality than diets currently used for mud crab aquaculture.
- Based on readily available ingredients that minimise feed resource risks.

The current research project initially combined studies on *in vivo* digestibility and gross nutrient requirements in *Scylla* species. Information obtained was then used to; 1) develop and trial artificial diets formulated to meet the specific nutritional requirements of local *Scylla* species in Australia, Indonesia and Vietnam and, 2) minimise fishmeal content in diets through the use of locally available, low-cost alternatives.

4 Objectives

1. To evaluate the potential of formulated feeds to replace traditional diets used in mud crab aquaculture.

Growth trials were conducted in partner countries to validate the capacity of experimental pellet feeds to support mud crab grow out. Initially, growth trials were conducted in laboratory-based cellular systems to prevent cannibalism. Juvenile crabs were fed experimental or reference diets, such as trash fish or artificial prawn feeds. Subsequently, small scale growth trials were conducted in earth ponds at RIA3 to assess the feasibility of using experimental diets for larger scale, farm-based feeding trials.

2. To determine critical nutritional requirements and evaluate key ingredients

This study was conducted in two parts. In the first part, the digestibility of selected animal and plant-based ingredients in formulated mud crab diets was assessed at each partner organisation. Ingredient selection was based primarily on local availability and price.

In the second part of this study, investigations were conducted to determine the crude protein and energy requirements of local mud crab species (*S.serrata* or *S.paramamosain*) during grow out.

3. To formulate and evaluate improved diets.

Experimental diets containing locally available, low cost ingredients were tested for their ability to promote growth of mud crabs in laboratory and pond-based culture environments. A key aim of this study was to adjust diet formulation to obtain acceptable growth performance while minimising fishmeal content.

5 Methodology

5.1 General Approach

In the current project, basic research was conducted into the nutritional requirements of mud crabs during grow out. There was, however, a capacity building component included in the project that involved training crustacean nutrition scientists and a postgraduate research students from RIA3 and GRIM in methodologies of crustacean nutrition research (8.2).

Research for the current project was conducted at three locations;

- BIARC, Bribie Island, Australia
- GRIM, Bali, Indonesia
- RIA3, Nha Trang, Vietnam

Candidate species used for experiments were the local *Scylla* species, *S.serrata* (Australia) or *S.paramamosain* (Indonesia and Vietnam).

Three main studies were conducted during the current project;

1. **Determination of the apparent digestibility of selected feed ingredients in formulated mud crab diets (5.4).**

2. **Evaluation of critical dietary nutrient requirements for mud crabs during grow out (5.5)**. As part of these studies, comparisons were made between the performance of experimental diets and diets traditionally used for mud crab grow out (eg. trash fish or prawn pellets).

3. **Assessment of the potential of digestible, low cost feed ingredients to replace fishmeal in formulated grow out diets for mud crabs (5.6)**. Initially growth trials were conducted using laboratory-based culture systems. The findings of these laboratory-based studies were then used as a basis for pilot studies to test the feasibility of using artificial diets for semi-intensive pond-based crab aquaculture.

5.2 The Research Team

Project team members were drawn from four institutions. The principle investigator was Associate Professor Peter Mather who with Dr Alex Anderson and Dr Neil Richardson (all at QUT) coordinated project activities.

BIARC (Australia)
Onsite supervision of experiments was performed by Dr Brian Paterson with assistance from Mr David Mann and Ms Beverley Kelly.

GRIM (Indonesia)
Onsite supervision of experiments was performed by Mr Ketut Suwirya

RIA3 (Vietnam)
Onsite supervision of experiments was performed by Mr Nguyen Co Thach with assistance from Mr Phuong Ha Truong and Mr Le Vinh.

5.3 Experimental culture systems

All digestibility (and most growth) trials were conducted using laboratory-based culture systems that were supplied with partly recirculated flowing seawater. Crabs were held individually in plastic containers to prevent cannibalism and allow precise monitoring of feed input. An example of a culture system used for this study is shown in Figure 2.

Fig. 2. Individual crab containers (a) and recirculation system (b) at BIARC, typical of those used for mud crab digestibility and growth trials conducted in FIS/2000/065.

5.4 Methods for Study 1: *Determination of the apparent digestibility of selected feed ingredients in formulated mud crab diets.*

Many plant and animal-based feed ingredients have been reported to have potential as replacements for fishmeal in formulated aquafeeds (Tacon 1994). For example, Catacutan *et al*. (2003) and Tuan *et al*. (2006) reported high digestibility coefficients for a variety of animal and plant-based ingredients incorporated into formulated *S.serrata* diets. The objective of the current study was to extend upon the findings of Catacutan *et al*. (2003) and Tuan *et al*. (2006) and evaluate the potential of locally selected feed meals for inclusion in diets formulated for mud crab species cultured in Australia (*S.serrata*), Indonesia and Vietnam (*S.paramamosain*). The criteria used to select test feed ingredients was;

- Local availability
- Low cost (less than fishmeal)
- High crude protein content (preferably >30% by weight) so as to offer the potential to replace fishmeal as a major source of protein in formulated diets

5.4.1 Animals

All digestibility trials were conducted using laboratory-based recirculation culture systems similar to that shown in Figure 2. Water temperature was maintained within a range (27.5

+ 0.5°C). To obtain sufficient material for analysis, sub-adult crabs were selected that had an average body weight of 89g ± 18g (BIARC), 125g ± 7.2g (RIA3) or 120.5g ± 7.1g (GRIM). At the commencement of the study, crabs were assigned randomly into treatment groups and housed individually in plastic containers (19.5cm x 28cm x 22cm). Crabs were kept in these containers for one week to acclimate to the culture conditions prior to the start of the experiment and fed a control diet of a commercial prawn feed or trash fish as indicated. Crabs were fed experimental diets twice daily at a feeding rate of 4-5% body weight (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected. A daily record was kept of mortalities in each test group. Faecal material at the bottom of the tank was typically collected by syphoning into a plastic sieve and gently rinsed for one minute in distilled water before removing individually using forceps. To collect sufficient material for analysis, faecal material from three crabs in each treatment was pooled (n=4 / treatment). All samples were lyophilized and stored at -20°C until required for analysis.

5.4.2 Experimental diet design

Experimental diets were formulated by combining test ingredients with a reference diet in a 30%:70% ratio, on a dry weight basis with 0.5% Chromic oxide (Cr_2O_3) added as an inert indicator to allow the calculation of digestibility coefficients for dry matter (ADMD), crude protein (ACPD), crude lipid (ACLD), fibre (ACFD) or gross energy (AGED). The dough was then extruded through a 3 mm die to obtain pellets 5 to 10 mm in length. Pellets were cooked in a rice steamer for 10 min then dried at 45°C for 24 h. Pilot studies determined that there was no significant loss of detectable chromium (Cr) in feed pellets immersed in water at 26°C for 1h (data not shown). The specific composition of experimental diets formulated at each facility is detailed in Tables 1, 3 and 5 while the proximate nutrient content of test ingredients is shown in Tables 2, 4 and 6. Proximate composition of diets and faecal material were determined according to AOAC protocols (1984). The composition and proximate analysis of experimental diets and feed ingredients is detailed below (5.4.2.1 – 5.4.2.3).

5.4.2.1 Experimental diets for digestibility trial: BIARC (Australia)

Table 1. Composition (% dry weight) of the formulated diets prepared for the digestibility trial at BIARC.

Ingredient	Diet 1	2	3	4	5	6	7	8	9
Basal Diet (Turbo)	62.4	62.4	62.4	62.4	62.4	62.4	62.4	62.4	92.4
Fishmeal	30								
Meat meal		30							
Poultry meal			30						
Soybean meal				30					
Canola meal					30				
Lupin meal						30			
Cotton seed meal							30		
Yeast								30	
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Common ingredients[a]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6

[a] Common (g/100g): mineral and vitamin premix, (2); (kg^{-1} of total diet - 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1750 mg riboflavin; 1125 mg pyridoxine hydrochloride; 3750 mg cyanocobalamin; 25000 mg ascorbic acid; 500 000 mg colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin); astaxanthin (0.1); chromic oxide (Cr_2O_3) (0.5).

Table 2. Proximate nutrient composition (%) in dry matter of basal diet and test ingredients used in the digestibility trial at BIARC.

Ingredient	Dry Matter	Crude Protein (N x 6.25)	Crude Fat	Ash	Energy (Mj/kg)
Basal Diet; Turbo *P.monodon* feed	90.4	49.7	6.7	15.3	19.1
Fishmeal	91.7	75.5	8.7	17.2	15.9
Meat meal	97.7	59.6	13.4	20.4	16.9
Poultry meal	96.7	69.2	13.1	14.1	20.9
Soybean meal	88.3	53.2	1.9	7.2	20.6
Canola meal	90	44.1	3.8	7.4	21.5
Lupin meal	86.1	30.8	9.4	3.6	15.9
Cotton seed meal	89.2	48.4	2.4	7.3	19.3
Yeast	95.1	48.6	0.4	9.6	18.3

5.4.2.2 Experimental diets for digestibility trial: GRIM (Indonesia)

Table 3. Composition (% dry weight) of the formulated diets prepared for the digestibility trial at GRIM.

Ingredients	Reference diet (%)	Test diet (%)
Fishmeal	44.1	30.5
Prawn head meal	10.0	6.9
Squid liver meal	5.0	3.5
Defatted soybean meal	10.0	6.9
Casein	6.0	4.1
Wheat flour meal	5.5	3.8
Soybean oil	2.5	1.7
Fish oil	1.2	0.8
Lecithin	2.0	1.4
Mineral Mix	2.0	1.4
Vitamin Mix	2.5	1.7
Dextrin	6.2	4.3
CMC	2.0	2.0
Cr_2O_3	1.0	1.0
Test ingredients*	-	30.0
Total	100.0	100.0

* Individual test ingredients utilized for this trial are detailed in Table 4.

Table 4. Proximate nutrient composition (%) in dry matter of test ingredients used in the digestibility trial at GRIM.

Ingredients	Protein	Lipid	Fibre	NFE[1]	Ash
Fishmeal	64.36	7.61	1.85	6.80	19.38
Prawn head meal	34.69	4.47	5.61	21.81	33.42
Tiny prawn meal	59.97	4.23	4.80	5.85	25.15
Squid liver meal	46.60	17.76	2.30	25.73	7.61
Poultry feather meal	86.30	6.71	2.51	1.71	2.77
Corn gluten meal	60.89	15.22	3.36	19.24	1.29
Soybean meal (defatted)	54.13	3.31	3.62	32.02	6.92

[1] Nitrogen free extract = 100- (% crude protein+ % crude lipid + % crude fiber + % ash).

5.4.2.3 Experimental diets for digestibility trial: RIA 3 (Vietnam)

Table 5. Composition (% dry weight) of the formulated diets prepared for the digestibility trial at RIA3.

Ingredients (%)	Diets							
	RD	T1	T2	T3	T4	T5	T6	T7
Fishmeal	40.6	28.4	28.4	28.4	28.4	28.4	28.4	28.4
Krill meal	7.9	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Green mussel meal	5.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Defatted soybean meal	14.9	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Rice bran	4.0	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Wheat flour	10,9	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Fish oil	3.0	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Squid oil	5.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Cholestine	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Dicalci-P	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7
CMC	3.0	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Vi-Premix	3.0	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Oxide crom (Cr_2O_3)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fish meal	-	29.7	-	-	-	-	-	-
Small prawn meal	-	-	29.7	-	-	-	-	-
Prawn head meal	-	-	-	29.7	-	-	-	-
Soybean oil cake	-	-	-	-	29.7	-	-	-
Manioc flour	-	-	-	-	-	29.7	-	-
Rice bran	-	-	-	-	-	-	29.7	-
Wheat flour	-	-	-	-	-	-	-	29.7
Total	100	100	100	100	100	100	100	100

Table 6. Proximate nutrient composition (%) in dry matter of test ingredients used in the digestibility trial at RIA3.

Ingredients	Dry mater (%)	Crude lipid (%)	Crude protein (%)	Ash (%)
Fishmeal	93.2 ± 0.9	5.7 ± 0.0	62.1 ± 1.0	21.9 ± 0.0
Small prawn meal	88.5 ± 0.8	0.6 ± 0.3	34.6 ± 0.3	51.5 ± 0.7
Prawn head meal	91.4 ± 1.5	1.0 ± 1.5	31.0 ± 0.6	52.6 ± 0.2
Soybean meal	92.8 ± 0.7	3.2 ± 0.4	41.4 ± 0.5	6.7 ± 0.0
Manioc flour	94.0 ± 0.3	0.9 ± 0.8	10.3 ± 1.4	3.2 ± 0.1
Rice bran	95.6 ± 0.7	4.1 ± 1.1	8.8 ± 1.5	25.4 ± 0.5
Wheat flour	93.7 ± 0.3	1.0 ± 0.4	15.4 ± 1.2	1.5 ± 0.2

Crabs were fed experimental diets (n=12-18 crabs / treatment) twice daily at a feeding rate of 5% body weight (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected. A daily record was kept of mortalities in each test group. Faecal material at the bottom of the tank was collected by syphoning into a plastic sieve and removed individually using forceps. To collect sufficient material for analysis, faecal material from three crabs in each treatment was pooled (n=4 / treatment). All samples were lyophilized and stored at -20°C until required for analysis.

The indirect method of Furukawa and Tsukahara (1966) was used to calculate the digestibility coefficients of all diets tested. Briefly, 0.5g of feed or faecal material was added to 4.0mL of concentrated nitric acid (AnalaR grade, 16 M HNO_3) and incubated overnight at room temperature. Samples were then heated to 150°C for an additional 60 min. After cooling, samples were mixed with 5.0mL of concentrated perchloric acid (AnalaR grade, 70% $HClO_4$) then heated to 220°C for 30 min and 245°C for a further 30 min. After cooling, the absorbance of each sample was read at 346.5nm. For calibration purposes, the above protocol was repeated using known quantities of Cr_2O_3.

Coefficients for ADMD were determined using the formula:
ADMD = 100 – 100 (%Cr_2O_3 in feed / % Cr_2O_3 in faeces).
Coefficients for crude protein (ACPD) or gross energy (AGED) were determined using the formula:
APD = 100 – 100 [(%Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% protein or Mj/kg energy in faeces/ % protein or Mj/kg energy in feed)].

Apparent digestibility coefficients (ADC) of the test ingredients were calculated using the following equations described by Bureau, Harris and Cho (1999).
$ADC_I = ADC_T + ((1 – s) D_R / s D_I) (ADC_T – ADC_R))$; where: ADC_I = apparent digestibility coefficient of test ingredient; ADC_T = apparent digestibility coefficient of test diet; ADC_R = apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; s = proportion of test ingredient in test diet mash (i.e. 0.3 in this study); (1 – s) = proportion of reference diet mash in test diet mash (i.e. 0.7 in this study).

5.4.3 Statistical analyses

The significance of data were determined by one-way ANOVA (SPSS version 13.0) and post hoc comparison by Tukey's HSD. For all analysis the significance level of $p<0.05$ was used as standard.

5.5 Methods for Study 2: *Evaluation of critical dietary nutrient requirements for mud crabs during grow out.*

It has been shown in terrestrial animals that growth is characterised by a protein dependant phase where protein deposition is directly related to protein intake (Dunkin, 1990). Growth may also be characterised by an energy dependant phase where growth is not directly correlated with protein intake. Since the supply of protein is normally one of the greatest expenses incurred during aquafeed production, significant savings can be obtained by establishing a minimum level of protein required by diets to promote acceptable growth.

Previously, artificial diets used in mud crab aquaculture have been formulated for prawns which can require relatively high levels (~60%) of crude protein to promote acceptable growth. Evidence suggests, however, that good growth can be achieved in mud crab aquaculture without the need for the high levels of protein found in prawn diets. For example, ACIAR-funded John Allwright fellow Tuan Vu-Anh reported a protein requirement for mud crabs of approximately 50% at dietary energy level of 18.5 MJ/kg (PhD thesis). The objective of the current study was to determine requirements for dietary protein and lipid for local mud crab species cultured in Australia, Vietnam and Indonesia.

5.5.1 Animals for laboratory-based growth trials

Unless otherwise indicted, growth trials were conducted using laboratory-based recirculation culture systems with individual containers. Juvenile crabs were selected that had an average body weight of 0.65g $\pm$ 0.1g (BIARC), 0.65g $\pm$ 0.3g (RIA3) or 1.0g $\pm$ 0.25g (GRIM). At the commencement of the trial, crabs were assigned randomly into treatment groups (n=12-24 crabs / treatment).

5.5.2 Animals for pilot pond-based growth trial (RIA3 only)

At the completion of the laboratory–based growth trial at RIA3, a pilot study was conducted in earth ponds to examine the feasibility of using formulated pellet diets for semi-intensive mud crab aquaculture. Diet formulation was based on the outcomes of the laboratory-based growth trial. Two formulated diets were selected for this trial;

1. a diet containing 43% crude (36% digestible) protein / 10% lipid
2. a diet containing 43% crude (36% digestible) protein / 15% lipid

A control diet based on trash fish was also incorporated into the study.

Crablets were selected from the crab hatchery at RIA3 with an average weight of 1.00g ± 0.30g and average carapace width of 18.05mm ± 2.14mm. Crabs were then cultured for three months in earth ponds (2000m^2) at an initial density of 1 crab/2m^2. Diets were administered at 5% body weight per day. Two ponds (Figure. 3) were allocated to each treatment group (n=2).

The following environmental parameters for the pond water were also determined;

- Average of salinity (‰): 27 ± 1.25
- Average of pH: 7.87 ± 1.58
- Average temperature (^oC): 28.5 ± 2.10


Fig. 3. Earth ponds used for mud crab growth trials at RIA3.

5.5.3 Experimental diet design

At each partner institution experimental diets were prepared using local ingredients. Diets were formulated to contain a broad range of crude protein (26% - 55%) and crude lipid (2%-15%) levels. The specific composition of experimental diets formulated at each facility are detailed in Tables 7-10. At BIARC crabs were also fed a control commercial *Penaeid* diet (Turbo: CP Feeds, Thailand; see Table 2 for proximate analysis) while a control diet based on trash fish was included in the study conducted at RIA3.

Experimental diets were prepared by mixing dry dietary ingredients with oil and water until a crumbly dough consistency was achieved. The dough was then extruded through a 3 mm die to obtain pellets 5 to 10 mm in length. Pellets were cooked in a rice steamer for 10 min then dried at 45^{o}C for 24 h. Dried pellets were then crushed and sifted to obtain particles with a width from 0.7 mm to 1 mm (weeks 1 to 5 of growth trial) and 1 mm to 2 mm (weeks 6+ of growth trial). All diets were stored at 4^{o}C until required. Crabs were fed experimental pellet diets twice daily at a feeding rate of 5% body weight per day. Mud crabs were individually weighed once a week for 7-16 weeks. A daily record was kept of mortalities in each test group.

5.5.3.1 Experimental diets for study 2: BIARC (Australia)

Table 7. Composition (% dry weight) of experimental diets formulated to determine protein and lipid requirements of *S.serrata* at BIARC

Ingredients	Diet 1	2	3	4	5	6	7	8	9	10	11	12
Fishmeal[a]	23.2	23.3	23.3	25.6	36.7	36.8	23.8	49.1	50.3	21.1	56.2	55.3
Casein	-	-	-	8.8	-	-	20.8	0.9	-	33.6	6.1	6.75
Lipid[b]	0.3	5.3	10.35	-	4.1	9.1	-	3	7.9	-	2.3	7.35
Wheat Starch	58	52.4	47.5	45.4	39	34.2	33.5	25.7	20.8	22	12.6	8.1
Fullers earth	0.4	0.9	0.7	2.1	2.1	1.8	3.8	3.2	2.9	5.2	4.7	4.4
Common ingredients[c]	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Calculated												
Crude protein (%)	25	25	24.9	35	34.9	34.9	44.9	44.9	44.9	54.9	55.1	55
Crude lipid (%)	5	10	15	5	10	15	5	10	15	4.9	10	15
Ash (%)	8.4	8.9	8.7	10.6	12.2	11.9	12.1	15.4	15.2	13.3	18	17.6
Energy (Mj/kg)	17	17.8	18.7	17	17.8	18.7	17	17.8	18.7	17.1	17.8	18.7

[a] Supplied by Ridley Aqua feed, Australia. [b] Cod liver oil, [c] Common ingredients (g/100g): gluten, 5; Vitamin mineral premix, 3; $CaHPO_4$, 3; astaxanthin, 0.1; Minced dried squid, 5; Lecithin, 2.

5.5.3.2 Experimental diets for study 2: GRIM (Indonesia)

Table 8. Composition (% dry weight) of experimental diets used to determine crude protein requirements of *S.paramamosain* at GRIM.

Ingredients	Experimental diets					
	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50
Fishmeal	20.8	28.3	35.9	39.4	42.8	46
Prawn head meal	10	10	10	10	10	10
Squid liver meal	5	5	5	5	5	5
Defatted soybean meal	10	10	10	10	10	10
Casein	-	-	-	3	6	9
Wheat flour meal	5.5	5.5	5.5	5.5	5.5	5.5
Soybean oil	2.5	2.5	2.5	2.5	2.5	2.5
Fish oil	3.4	2.7	1.9	1.6	1.2	0.9
Lecithin	2	2	2	2	2	2
Mineral / vitamin mix	5.5	5.5	5.5	5.5	5.5	5.5
CMC binder	2	2	2	2	2	2
Dextrin	34.2	26.7	19.1	12.6	6.2	1.6
Nutrient composition (%)						
Crude protein	26	31.4	37.4	41.8	46.8	51.0
Crude lipid	11.5	12.7	11.2	12.0	11.2	11.2
Ash	11.3	10.9	11.2	12.4	12.5	12.7
Fiber	3.9	3.9	4.2	4.4	4.7	4.9

Table 9. Composition (% dry weight) of experimental diets used to determine crude lipid requirements of *S.paramamosain* at GRIM.

Ingredients	Test diets				
	CL -0	CL-3	CL-6	CL-9	CL-12
Fishmeal	29.0	29.0	29.0	29.0	29.0
Prawn head meal	2.0	2.0	2.0	2.0	2.0
Squid liver meal	3.0	3.0	3.0	3.0	3.0
Soybean meal	2.0	2.0	2.0	2.0	2.0
Casein	25.0	25.0	25.0	25.0	25.0
Wheat flour	2.5	2.5	2.5	2.5	2.5
Soybean oil	0.0	1.0	2.0	3.0	4.0
Fish oil	0.0	2.0	4.0	6.0	8.0
Lecithin	2.0	2.0	2.0	2.0	2.0
Vitamin Mineral mix	5.5	5.5	5.5	5.5	5.5
CMC binder	2.0	2.0	2.0	2.0	2.0
Dextrin	27.0	22.3	15.9	9.4	3.0
Cellulose	0.0	1.7	5.1	8.6	12.0
Calculated (%)					
Crude protein	48.2	48.1	48.0	48.4	48.0
Lipid[1]	1.82	4.9	7.8	10.7	13.5
Ash	13.6	12.0	12.5	12.6	12.2

[1]Prior to incorporation into formulated diets, lipid from all protein sources was extracted with chloroform: methanol (1:1).

5.5.3.3 Experimental diets for study 2: RIA3 (Vietnam)

Table 10. Composition (% dry weight) of experimental diets used to determine protein and lipid requirements of juvenile *S.paramamosain* at RIA3.

Ingredients (% of diet)	Diets 1 35/7	2 35/10	3 35/15	4 43/7	5 43/10	6 43/15	7 50/7	8 50/10	9 50/15
Fishmeal	26	25	26	38	40	41	54	55	56
Krill meal	8	8	8	8	8	8	8	8	8
Green mussel meal	5	5	5	5	5	5	5	5	5
Defatted soybean meal	13	15	15	15	15	15	12	11	6
Rice bran	5.5	10	10	6	4	4	5	5.5	5
Wheat flour	33	25.5	19.5	19.5	16.5	11	8.5	5	4.5
Fish oil	0.5	1	3.3	0.2	1	3	0	1	2.5
Squid oil	0.5	2.3	5	0.3	2.0	5	0.0	2	5
Cholestine	1	1	1	0.5	1	1	0.5	0.5	1
Dicalci-P	1	1	1	1	1	1	1	1	1
CMC binder	3	3	3	3	3	3	3	3	3
Vi-Premix	3	3	3	3	3	3	3	3	3
Total	100	100	100	100	100	100	100	100	100
Calculated (%)									
Crude protein	35.10	35.02	35.05	43.05	43.10	43.07	50.06	50.18	50.07
Digestible protein[a]	30.1	29.7	29.4	35.5	36	35.8	41.3	40.9	39.4
Crude lipid	7.12	10.01	15.01	7.10	10.0	14.95	7.1	10.11	15.05

[a] data calculated based on outcomes of digestibility study (Table 18)

5.5.4 Assessment of growth performance and feed utilisation

Biological parameters used for evaluating relative growth performances and nutrient utilization in this study were calculated as follows:

Average moulting interval (MI, day) = T/Mt
Intermoult period = Number of days between first and second moult
Survival rate (SR,%) = N_f x 100/N_i
Weight gain (WG,%) = (W_f-W_i) x 100/W_i
Specific growth rate (SGR% day^{-1}) = (ln W_f-lnW_i) x 100/T
Food conversion ratio (FCR) = D_f/WG

Where Mt is the number of each crab moult during culture period (T); N_i is the number of initial crabs; N_f is the number of final crabs; W_f is the final wet weight (g); W_i is the initial wet weight (g); T is the duration of trial in days; D_f is the dry feed intake (g). Dp is the dry protein intake (g).

Duration of growth trials conducted in laboratory-based culture systems;

- BIARC: 70 days
- GRIM: 56 days
- RIA3: 120 days

5.5.5 Statistical analyses

The effects of diets on growth performance were determined by one- way ANOVA (SPSS version 13.0) and post hoc comparison by Tukey's HSD. For all analyses the significance level of $p<0.05$ was used as standard. Regression analysis was used to test the weight gain response to protein and energy intake.

5.6 Methods for Study 3: *Assessment of the potential of digestible, low cost feed ingredients to replace fishmeal in formulated diets for mud crab grow out.*

Replacing fishmeal as a primary source of protein in aquafeeds is a global research priority for the aquaculture industry which is driven by declining fishmeal availability and a rapidly expanding aquaculture sector. To address this research priority, the following study investigated the potential of selected plant or animal-based ingredients to reduce fishmeal content in grow out diets formulated for juvenile mud crabs.

The specific formulation of reference diets was based on the outcomes of study 2 which established optimum protein and lipid levels in formulated mud crab diets at each partner institution. A series of experimental diets were also formulated where selected amounts of fishmeal were replaced by alternative feed meals including;

- poultry meal and soybean meal (BIARC)
- soybean meal and krill meal (RIA3)
- corn gluten meal and soybean meal (GRIM).

5.6.1 Animals for laboratory-based growth trials

Initially, growth trials were conducted using laboratory-based recirculation culture systems with individual containers. Juvenile crabs were selected that had an average body weight of 0.65g $\pm$ 0.1g (BIARC), 0.65g $\pm$ 0.3g (RIA3) or 1.0g $\pm$ 0.25g (GRIM). At the commencement of the trial, crabs were assigned randomly into treatment groups (n=12-24 crabs / treatment).

5.6.2 Animals for pond-based growth trials (RIA3 only)

At the completion of the laboratory–based growth trial at RIA3, a pilot study was conducted in earth ponds to examine the feasibility of using experimental diets for semi-intensive, pond-based mud crab aquaculture. Selection of diets was based on the outcomes of the laboratory-based growth trial (5.6.1) and included;

1. a diet containing 40% soybean meal (Diet 1, Table 14)
2. a diet containing 15% soybean meal (Diet 2, Table 14)

A control diet of trash fish was also selected for this trial.

Juvenile crabs were selected with an average weight of 1.1g $\pm$ 0.04g and a carapace width of 12.6mm $\pm$ 0.17mm. Crabs were then cultured for 90 days in earth ponds (65m^2) at an initial density of 1.3 crabs / m^2. Diets were administered at 5% body weight with three ponds allocated to each treatment group (n=3). It should be noted, that this growth trial was not intended to be a definitive study of the response of crabs to artificial diets in semi-intensive production systems. Instead, the key aim was to explore the feasibility of using experimental feeds and existing husbandry practises for larger scale, farm-based growth trials that will require significantly more resources than available for the current study.

5.6.3 Experimental diet design for study 3

At each partner institution a reference diet based on fishmeal was formulated. Diets were prepared by thoroughly mixing dry ingredients, followed by wet ingredients, until a crumbly dough consistency was achieved. Diet mixture was pressure pelleted using a meat grinder

with a 3mm die. Cooking and storage of diets was then performed as described previously (study 1). The specific composition of experimental diets formulated at each facility is detailed in Tables 11-14. It should also be noted that, where possible, diets were designed to permit estimation of digestible protein and energy content.

Crabs were fed experimental diets twice daily at a feeding rate of 5%-10% body weight per day. Mud crabs were individually weighed at the start of the feeding trial and once a week thereafter for 9-12 weeks. In the laboratory culture system a daily record was also kept of mortalities in each test group.

5.6.3.1 Experimental diets for study 3: BIARC (Australia)

Table 11. Composition (% dry weight) of the formulated diets (D) prepared for the fishmeal substitution trial at BIARC.

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Fishmeal	52.7	42.2	31.6	21.1	10.6	42.2	31.6	21.1	10.5	31.6	21.1	10.5
Poultry meal	-	11.5	23.1	34.5	46.1	-	-	-	-	11.5	17.3	23.1
Soybean Meal	-	-	-	-	-	14.4	28.8	43.2	57.6	14.4	21.6	28.8
Lipid	2.7	2.2	1.6	1.1	0.5	3.4	4	4.7	5.4	2.9	2.9	2.9
Wheat Starch	24.5	23.2	22.5	21.5	20.4	18.4	13	7	1	17.4	14	10.8
Fullers earth	2	2.8	3.1	3.6	4.3	3.5	4.5	5.9	7.3	4	5	5.8
Common ingredients[a]	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated												
Crude Protein (%)	47.5	47.6	47.6	47.6	47.6	47.2	46.8	46.5	46.4	47.2	47.0	46.8
Digestible protein (%)	41.1	41.1	41.1	41.1	41.1	41.1	41.1	41.1	41.1	41.1	41.1	41.1
GE (Mj/kg)[b]	17.8	17.8	17.8	17.9	17.8	17.8	17.9	17.8	17.8	17.8	17.8	17.8
DE (Mj/kg)[b]	16.4	16.1	15.9	15.6	15.3	16.3	16.2	16.4	16.0	16.0	15.8	15.7
Ash	14.8	15.4	15.5	15.8	16.4	15.5	15.6	16.2	16.9	15.8	16.3	16.5

[a] Common ingredients (g/100g): gluten, 5; Vitamin mineral premix, 3; $CaHPO_4$, 3; astaxanthin, 0.1; Minced dried squid, 5; Lecithin, 2.
[b] data obtained from results of study 1
All diets formulated to contain 10% lipid (calculated).
Energy values expressed as gross energy (GE) or digestible energy (DE)

5.6.3.2 Experimental diets for study 3: GRIM (Indonesia)

Table 12. Composition (% dry weight) of the formulated diets prepared for the fishmeal substitution trial at GRIM.

Ingredients	Diets				
	Fish meal	20% SBM[1]	40% SBM	20% CGP[2]	40% CGP
Fishmeal	52.7	42.2	31.6	42.2	31.6
Soybean meal	-	14.8	29.8	-	-
Corn gluten meal	-	-	-	16.0	31.0
Vitamin / mineral mix	4.5	4.5	4.5	4.5	4.5
Fish oil	2.7	2.7	2.7	2.7	2.7
Dextrin	24.5	24.5	24.5	24.5	24.5
Squid liver meal	5.0	5.0	5.0	5.0	5.0
Lecithin	2.0	2.0	2.0	2.0	2.0
Wheat flour meal	6.0	6.0	6.0	6.0	6.0
CMC binder	2.0	2.0	2.0	2.0	2.0
Fullers earth	0.6	1.0	0.0	0.0	0.0
Calculated(%)					
Crude protein	36.9	38.2	39.4	39.9	42.2
Digestible protein	31.7	33.5	35.4	33.5	34.8

5.6.3.3 Experimental diets for study 3: RIA3 (Vietnam)

Table 13. Composition (% dry weight) of the formulated diets prepared for the fishmeal substitution trial conducted in laboratory-based culture system at RIA3.

Ingredients	Diet 1 (fishmeal)	Diet 2 (20% SBM)	Diet 3 (40% SMB)	Diet 4 (20% KM)	Diet 5 (40% KM)
Fishmeal	55	48	41	46	38
Krill meal (KM)	0	0	0	12	23
Soybean meal (SBM)	0	12	23	0	0
Green mussel meal	5	5	5	5	5
Rice bran	4	4	4	4	4
Wheat flour	20	15	12	17	14
Fish oil	3	3	3	3	3
Squid oil	5	5	5	5	5
Cholestine	1	1	1	1	1
Dicalci-P	1	1	1	1	1
CM cellulose	3	3	3	3	3
Vitamin-mineral premix	3	3	3	3	3
Calculated[a] (%)					
Crude protein	39.9	39.8	39.5	40.4	41.1
Digestible protein	38.1	45.1	51.5	38.9	39.7

[a] Crude lipid adjusted to 13%

Table 14. Composition (% dry weight) of the formulated diets prepared for the fishmeal substitution trial conducted in earth ponds at RIA3

Ingredients	Diet 1 (%)	Diet 2 (%)
Fishmeal	28.4	41
Krill meal	5.5	8
Squid meal	4.1	5
Defatted soybean meal	40.1	15
Rice bran	0	4
Wheat flour	6.4	11
Fish oil	2.8	3
Squid oil	5	5
Cholestine	0.7	1
Dicalci - P	1	1
CMC binder	3	3
Vitamin / mineral premix	3	3
Total	100	100
Calculated (%)		
Crude protein	43.3	43.4
Digestible protein	36.3	35.8
Lipid	14.8	15.4

5.7 Methods for Extension Study: *Confirming optimum feeding rates and frequencies for mud crabs in laboratory-based culture systems*: BIARC (Australia)

In 2006, an ACAIR sponsored review of the outcomes of FIS/2000/065 suggested that lower than expected growth rates achieved during laboratory-based growth trials may have been due to suboptimal feeding rates or frequencies. As a consequence, a brief extension of the project was conducted exclusively at BIARC using *S.serrata.* The aim of this study was to identify issues that may have contributed to low growth rates when using formulated diets. Specifically, a laboratory-based growth trial was conducted with juvenile crabs exposed to the following treatments;

1. Four feeding rates (5%, 10% 20% dry feed weight / wet weight crab daily)
2. Two feeding frequencies (2x and 3x daily)
3. Three diets: i) an artificial fishmeal-based experimental pellet diet (KW), ii) a commercial *P. monodon* pellet diet [Turbo] or, iii) a natural diet based on a mixture of fish, molluscs and crustaceans

5.7.1. Culture System

Growth trials were conducted using a compartmentalised laboratory-based culture system supplied with flowing seawater. Individual containers were employed to prevent cannibalism. For all experimental treatments, crabs were supplied with recirculated, aerated seawater that was gravity fed through an electrically heated overhead tank. Water temperature was maintained within a range (27.5 $\pm$ 0.5^{o}C). Salinity and pH were maintained at 29.5 $\pm$ 0.8 $^{0}/_{00}$ and 8.15 $\pm$ 0.3, respectively.

5.7.2. Animals

Twelve single juvenile crabs (5th Crab instar or C5) per treatment. Progeny reared beforehand from a single wild sourced female and reared communally in a nursery pond until the correct size. Crabs were weighed when each moulted 3 times (+10 days) and 4 times (+ 10days), followed by a final weighing of all crabs at the termination of the trial.

5.7.3 Experimental Diets

The pelleted feeds, KW and Turbo, were partially crushed and sieved to provide particle size grades appropriate to different crab sizes according to the following table:-

Pellet size schedule

C6 to C8	1.5-2.0mm
C9 to C10	2.0-3.0mm
C11	2.5mm x 3-5mm

The composition of the KW diet is detailed in Table 15. The fresh flesh diets consisted of fish meat (*Mugil cephalus*), mussel (*Perna canaliculus*), squid tube meat (*Illex argentinus*) and prawn tail meat (*Penaeus merguensis*). These diets were cut into small squares ranging in size from approximately 3mm for the smaller crabs to 8mm for the largest instar. Feeding of the different diets was rotated on a daily basis such that on any day only one flesh diet type was provided.

Table 15. Composition (% dry weight) of the formulated diet (KW) prepared by Dr Kevin Williams (CSIRO) for the extension project conducted at BIARC.

Ingredient	(%)
Wheat flour	21
Fishmeal (65-67% CP)	53
Krill meal	15
Wheat gluten	6
Fish oil	1.5
Lecithin (Aqualipid 70)	2.1
Cholesterol	0.2
Carophyll Pink (8% Astaxanthin)	0.05
Stay-C 35	0.1
Vitamin premix	1.0
Total	100
Composition (as used)	
Dry matter (%)	91.6
Ash (%)	11.8
Crude protein (%)	50.2
Dig. Protein (%)	45.1
Gross Energy (kJ/g)	19.0
Dig. Energy (kJ/g)	16.7
Total lipid (%)	12.0

Dry feeds were broken to size, and delivered automatically using a pneumatically actuated automatic feeder. Four feeder magazines were aligned over each of the four rows of compartments in a tray. Each feeder magazine had holes in the underside of its case matching the spacing of cells in the array. A closely spaced series of tubes mounted through a slider in the magazines core made it possible to deliver several meals in succession into each cell by pulling the slider progressively over the ventral delivery holes during the course of the day using a pneumatic actuator.

When loading the magazine, the days ration for each crab was weighed and spooned into two or three tubes depending upon the number of meals designated for the crab. Fresh feeds were thawed finely chopped, weighed and fed to the crabs as required. Crabs in the fresh food treatment receiving 3 meals were fed an appropriate ration of pellets for the third meal because the fresh food did not suit the automatic feeder.

5.7.4 Feeding frequency

Two feeding frequency treatments were applied (2x and 3x per day). Feeding times (ie time of addition of a ration) were at 0800h and 1600h for the 2x treatment and 0800h, 1600h and 2400h for the 3x treatment. This design ensured the third feeding time fell in the middle of the extended nocturnal non-feeding period of the 2x feeding treatment. All pelleted feeds were dispensed automatically. At the required times a pneumatic actuator dropped a pre-weighed feed ration into each container. The flesh diets were dispensed manually. For practical reasons the ‘flesh diet fed 3x per day’ treatment received the KW (in-house standard formulated diet) at the 2400h feed.

5.7.5 Feeding quantity

There were three feeding rate treatments; 5, 10 and 20% BWt/day. The body weight estimate used for calculating feed ration for each crab was taken as the average weight for that crab's instar and was updated daily. Due to the hands-off approach to the husbandry of the animals each experimental crab could not be re-weighed following each moult. The total daily food ration was divided equally among the number of feeding times.

Feed quantities were calculated based on dry weight of feed to wet weight of crab. The ratio of wet weight to dry weight of each flesh diet was determined and this conversion factor used to calculate the dry weight equivalent of the wet, flesh diets fed.
No conversion factor was applied to the dry, pelleted diets.

5.7.6 Feed consumption estimates

On three days over a 3 week period the consumption rate of feed was estimated. Food remaining in each container 2 hours following the 0800h feed was collected by siphon and pooled for each diet treatment. Additionally feed that had been lost from the containers during the 2 hour period through the water exchange vents was collected at a single filtration point for each tray and the flesh component separated from the pellets. This retrieved waste food therefore contained feed from all treatments within each tray. The collected uneaten and waste feed was de-watered using filter paper, dried in a drying oven at 105°C for 16 hours and weighed to provide a dry weight. Accurate calculation of food consumed (dry wt IN – dry wt OUT) within each treatment was confounded by the loss of food from the containers however the data provides an estimate.

6 Achievements against activities and outputs/milestones

Objective 1: *To evaluate the potential of formulated feeds to replace traditional diets used in mud crab aquaculture.*

no.	Activity	outputs/ milestones	completion date	comments
1.1	Growth trial conducted at BIARC to compare artificial diets for *S.serrata* against a diet based on fresh marine animal flesh (A).	Growth responses of crabs to artificial diets were approximately 90% of those observed using a fresh diet.	Nov 07	Growth rates obtained for all treatments in laboratory-based culture system were significantly less than those which could be expected in farm production systems (earth ponds).
1.2	Growth trials conducted at GRIM and RIA3 to assess performance of experimental pellet diets for *S.paramamosian* (PC).	Growth of crabs in response to artificial diets in laboratory-based culture system was inferior to that obtained using a trash fish diet (RIA3). Pilot studies conducted in earth ponds (RIA3) demonstrated that growth of crabs obtained using artificial diets was equivalent to that achieved using trash fish.	Jan 06	Overall, artificial crab diets formulated at BIARC and GRIM produced higher levels of growth than were observed at RIA3 (using laboratory-based culture systems). Formulation of experimental diets initially used at RIA3 may be unsuitable for promoting optimum growth of crabs in laboratory-based culture systems. In pilot pond-based feeding trials conducted at RIA3, the best performing diet (43% CP / 15% CL) promoted growth which was equivalent to that obtained using trash fish.

PC = partner country, A = Australia

Objective 2: *To determine critical nutritional requirements and evaluate key ingredients*

no.	Activity	outputs/ milestones	completion date	comments
2.1	Assessment of nutrient digestibility of selected animal and plant-based ingredients in formulated mud crab diets (A and PC).	A range of animal, plant and single cell-based ingredients were identified which were readily digested by mud crabs.	Oct 05	For many ingredients tested, major nutrient digestibility coefficients were equivalent (or superior to) those obtained for locally available fishmeal. The mud crab digestive system appears well adapted to utilise nutrients from a variety of sources. Mud crabs demonstrated a high capacity to digest animal-based products such as prawn head meal and poultry meal. The high digestibility of many plant-based ingredients, such as soybean meal, provides a convincing rationale for considering inclusion of these ingredients in formulated mud crab diets.
2.2	Determination of basic nutrient requirements of mud crabs (A and PC).	Analysed responses of two mud crab species to different levels of dietary crude protein and lipid.	Mar 05	It was shown that artificial diets containing between 43% and 55% crude protein yielded the best growth responses. Diets formulated for mud crabs may not require the relatively high levels of protein that are contained in many commercial prawn diets. For both species of mud crab, diets containing relatively high levels of lipid (9% to 15%) yielded the best growth responses.

PC = partner country, A = Australia

Objective 3: *To formulate and evaluate improved diets.*

no.	Activity	outputs/ milestones	completion date	comments
3.1	Growth trials conducted in laboratory-based culture systems to assess the potential of selected feed meals for incorporation into mud crab diets (A and PC).	20% to 40% of fishmeal could be replaced by selected animal or plant-based feed ingredients without a significant reduction in growth performance.	Jan 06	In all partner countries, soybean meal demonstrated the capacity to partially replace fishmeal in grow out diets for juvenile mud crabs.
3.2	Pilot studies conducted in earth ponds at RIA3 to assess the feasibility of using soybean meal in diets for larger scale, farm-based feeding trials (PC).	In earth ponds, a diet containing 40% soybean meal promoted levels of growth which were equivalent to those obtained using trash fish.	Jan 06	The potential for using artificial diets with reduced fishmeal content for mud crab aquaculture should be further investigated in more extensive farm based feeding trials

PC = partner country, A = Australia

7 Key results and discussion

7.1 Determination of the apparent digestibility of selected feed ingredients in formulated mud crab diets.

7.1.1 Summary

The present study examined the capacity of mud crab species cultured in Australia (*S.serrata),* Vietnam and Indonesia *(S.paramamosian)* to digest a range of animal or plant-based meals commonly used for animal feed manufacture. Overall, sub-adult mud crabs demonstrated a high capacity to digest test ingredients. Apparent nutrient digestibility coefficients were generally high, ranging from 78% to over 90%. Moreover, with the exception of corn gluten and meat meal, most nutrient digestibility coefficients for test ingredients were equivalent, or superior to, those obtained for fishmeal. On the basis of these findings we suggest that there are readily available animal and plant-based ingredients in partner countries that warrant further attention for their potential to be routinely incorporated into artificial mud crab diets.

7.1.2 Results of digestibility trials conducted at partner institutions

7.1.3.1 Australia (*S.serrata*)

As shown in Table 16, the ADMD coefficients for most test ingredients were relatively high and not significantly different from that obtained for fishmeal, ranging from 79% to 88%. The ADMD value of meat meal, however, was significantly lower ($p<0.05$) than those obtained for all other test ingredients.

ACPD coefficients for all feed meals tested were relatively high, with values ranging from 86% to 97%. Interestingly, the ACPD value for yeast was significantly higher ($p<0.05$) than those obtained for all other test ingredients.

All plant-based feed ingredients demonstrated AGED values that were not significantly different ($p>0.05$) from fishmeal, ranging from 84% to 89%. The AGED coefficient for meat meal, however, was significantly less ($p<0.05$) than the values obtained for all other ingredients except cotton seed meal and poultry meal. By contrast, the highest value for AGED was obtained for yeast that was significantly higher ($p<0.05$) than those obtained for meat meal, cotton seed meal and poultry meal.

Survival rates for all treatments were high, ranging from 92% to 100% (data not shown).

Table 16. The apparent digestibility coefficients (%) of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) for yeast and selected animal feed meals utilised in formulated *S.serrata* diets at BIARC.

Ingredient	ADMD	ACPD	AGED
Basal Diet; Turbo prawn feed	83.2 + 0.5 ^{b}	90.4 + 0.5 ^{ab}	89.3 + 0.9 ^{cd}
Fishmeal	85.4 ± 1.7^{b}	88.3 ± 0.7^{ab}	87.8 ± 1.3^{cd}
Meat meal	67.0 ± 1.3^{a}	86.3 ± 0.9^{ab}	78.2 ± 0.8^{ab}
Poultry meal	78.9 ± 3.0^{b}	88.2 ± 2.1^{ab}	85.2 ± 1.9^{bc}
Soybean meal	80.4 ± 1.2^{b}	91.7 ± 0.5^{bc}	89.1 ± 0.9^{cd}
Canola meal	83.5 ± 4.7^{b}	87.6 ± 2.7^{ab}	87.5 ± 2.9^{cd}
Lupin meal	88.1 ± 1.6^{b}	89.1 ± 0.9^{ab}	89.9 ± 1.4^{cd}
Cotton seed meal	80.5 ± 0.8^{b}	86.8 ± 0.6^{ab}	83.9 ± 0.4^{abc}
Yeast	85.7 ± 3.2^{b}	96.8 ± 1.6^{c}	93.5 ± 1.7^{d}

Values are means ± standard error (n = 4; each replicate sample pooled from 3 crabs). Means in the same column with the same superscript are not significantly different (p>0.05) from one another

7.1.3.2 Indonesia (*S.paramamosian*)

As shown in Table 17, the ADMD coefficients for most feed meals tested were not significantly less than the value obtained for fishmeal (84.25%). The ADMD value of corn gluten meal (82.46%), however, was significantly less (p<0.05) than those obtained for all other test ingredients.

ACPD coefficients for all feed meals tested were relatively high with values ranging from 78% to 96%. Interestingly, the ACPD values for soy bean (96.05%) and poultry feather (95.14%) meals were significantly higher (p<0.05) than those for all other test ingredients. The only test ingredient to demonstrate a ACPD value significantly (p>0.05) less than fishmeal was corn gluten meal.

Table 17. The apparent digestibility coefficients (%) of dry matter (ADMD), crude protein (ACPD), crude lipid (ACLD) and gross energy (AGED) of selected feed ingredients utilised in formulated *S.paramamosain* diets at GRIM.

Diet	Test ingredient	ADMD (%)	ACPD (%)	ACLD (%)	AGED (%)
1	Local fish meal	84.25^{b}	86.12^{b}	57.62^{abc}	85.95^{b}
2	Prawn head meal	85.74^{d}	88.05^{bc}	71.25^{bc}	85.86^{b}
3	Tiny prawn meal	85.32^{c}	90.66^{c}	34.33^{a}	93.01^{bc}
4	Squid liver meal	87.15^{c}	90.02^{bc}	89.14^{c}	91.36^{bc}
5	Corn gluten	82.46^{a}	78.81^{a}	51.37^{ab}	71.13^{a}
6	Soy bean meal	89.20^{f}	96.05^{d}	43.25^{a}	98.48^{c}
7	Poultry feather meal	87.19^{e}	95.14^{d}	23.50^{a}	92.09^{bc}

Values in columns with the same superscript are not significantly different (p>0.05) from one another.

Lipid digestibility coefficients for tiny prawn (34.33%), soybean (43.25%) and poultry meal (23.5%) were significantly lower than the values for prawn head (71.25%) or squid liver meal (89.14%) ($p<0.05$). Nevertheless, none of the ingredients tested demonstrated lipid digestibility values that were significantly lower than that obtained for fishmeal (57.62%).

With the exception of corn gluten meal, all test ingredients demonstrated AGED coefficients that were equivalent to, or significantly higher ($p<0.05$) than that obtained for the local fishmeal (85.95%). Survival rates for all treatments were high, and ranged from 92% to 100% (data not shown).

7.1.3.3 Vietnam (*S.paramamosain*)

As shown in Table 18, the ADMD coefficients for most feed meals tested were not significantly different from the value obtained for fishmeal, ranging from 69% to 95%. The ADMD value of prawn head meal (69.4%), however, was significantly lower ($p<0.05$) than those obtained for soy bean (85.3%) or manioc (95.0%) meal.

ACPD coefficients for all feed meals tested were relatively high, with values ranging from 85% to 96%. In addition, ACPD values for all test ingredients were not significantly different to that obtained for the local fishmeal (86.1%).

Table 18. Digestibility of selected feed ingredients incorporated into formulated *S.paramamosain* diets at RIA3.

Ingredients	ADMD (%)	ACPD (%)	AGED (%)	ACFD (%)
Fish meal	78.5 ± 2.6^{bcd}	86.1 ± 2.5^{bc}	63.2 ± 5.1^{bc}	74.6 ± 4.1^{bc}
Small prawn meal	76.4 ± 3.0^{bcd}	86.3 ± 3.3^{bc}	27.1 ± 11.3^{a}	51.5 ± 8.0^{a}
Prawn head meal	69.4 ± 2.1^{ab}	84.9 ± 2.2^{b}	60.4 ± 3.4^{bcd}	88.4 ± 1.1^{cd}
Soybean meal	85.3 ± 1.5^{d}	95.9 ± 1.2^{c}	78.8 ± 1.0^{de}	83.1 ± 2.6^{bcd}
Manioc meal	95.0 ± 0.8^{d}	95.4 ± 1.6^{c}	84.7 ± 0.8^{e}	67.2 ± 5.4^{ab}
Rice bran	78.5 ± 0.7^{bcd}	94.2 ± 4.0^{c}	84.8 ± 0.6^{e}	88.2 ± 1.8^{cd}
Wheat flour	79.7 ± 1.5^{cd}	90.1 ± 3.4^{bc}	78.0 ± 1.3^{de}	74.2 ± 4.3^{bc}

Values in columns with the same superscript are not significantly different ($p>0.05$)

By contrast, there were dramatic differences in coefficients for gross energy digestibility with values ranging from 27.1% (small prawn meal) to 84.8% (rice bran). Of those ingredients tested, wheat flour, rice bran, manioc meal and soy bean meal demonstrated AGED values that were significantly higher ($p<0.05$) than that obtained for fishmeal (63.2%). Crude fibre digestibility of test ingredients incorporated into mud crab diets ranged from 51.5% (small prawn meal) to 88.4% (prawn head meal). Of these ingredients, only small prawn meal demonstrated an ACFD value that was significantly lower ($p<0.05$) than the local fishmeal (74.6%).

7.2 Evaluation of critical dietary nutrient requirements for mud crabs during grow out.

7.2.1 Summary

The aim of the present study was to determine basic nutrient requirements (protein and lipid) for the growth of juvenile mud crabs used in aquaculture in Australia (*S.serrata),* Vietnam and Indonesia *(S.paramamosian). S.serrata* juveniles demonstrated highest growth when fed diets containing between 45% and 55% crude protein and 15% crude lipid. Moreover, growth responses observed at BIARC for *S.serrata* fed best performing experimental diets were equivalent to those observed using a commercial prawn pellet diet.

Similar results were obtained in growth trials conducted in Indonesia and Vietnam using *S.paramamosian* juveniles. For example, at GRIM (Indonesia) highest growth was observed when juveniles were fed diets that contained between 45% and 50% crude protein and 9% and 15% lipid. These diets also produced highest body protein and lipid content.

Unfortunately, due to an oversight in the original design phase of this study it was not possible to express results obtained at BIARC or GRIM in terms of digestible protein or digestible energy. In particular, apparent nutrient digestibility coefficients were unavailable for casein which was a major component of diets formulated for this study. Fortunately, however, sufficient digestibility coefficient data was available at RIA3 (Vietnam) to permit expression of growth data in terms of digestible protein.

Another interesting outcome of the study conducted at RIA3 was that the culture environment had a significant impact on the response of *S.paramamosain* juveniles to experimental diets. Specifically, crabs fed best performing experimental diets in the laboratory-based culture system demonstrated significantly lower growth that those fed trash fish. By contrast, the growth response of juveniles to an experimental diet (43% CP / 15% CL) in earth ponds was equivalent to that obtained using a trash fish-based diet.

7.2.2 Results of growth trials conducted at partner institutions

7.2.1.1 Australia (*S.serrata*)

As shown in Table 19, growth responses were influenced by dietary crude protein and lipid content. Specifically, there were consistent increases in final weight and SGR values as the level of dietary protein was increased from 25% to 55%

Average feed conversion ratios (FCR) showed relatively little variation among treatments (ranging from 1.26 to 2.36), although the FCR values obtained using diet 3 (25% protein / 15% lipid) and the commercial prawn (Turbo) diet were significantly higher than that obtained for diet 1 (25% protein / 5% lipid).

Survival rates for all treatments were generally high, ranging from 83% to 100%.

Table 19. Mean values for final wet body weight, specific growth rate (SGR), feed conversion ratio (FCR) and survival for juvenile *S.serrata* fed formulated diets with different levels of digestible protein (CP) and crude lipid (CL) at BIARC. Culture period: 70 days.

Diet	Crude Protein / Crude Lipid (%)	Final weight (g)	SGR	FCR	Survival (%)
1	25/5	5.11 + 0.48^{a}	2.99 + 0.13^{a}	1.26 + 0.17^{a}	100
2	25/10	5.69 + 0.31^{ab}	3.09 + 0.06^{ab}	1.9 + 0.12^{ab}	92
3	25/15	5.73 + 0.42^{ab}	2.99 + 0.08^{a}	2.15 + 0.19^{b}	100
4	35/5	7.85 + 1.02^{abcd}	3.46 + 0.12^{abcd}	2.1 + 0.22^{ab}	100
5	35/10	6.41 + 0.45^{abc}	3.35 + 0.1^{abc}	1.99 + 0.13^{ab}	92
6	35/15	7.81 + 0.71^{abc}	3.44 + 0.11^{abc}	1.97 + 0.15^{ab}	100
7	45/5	8.53 + 0.71^{abcd}	3.54 + 0.09^{abcd}	1.88 + 0.16^{ab}	100
8	45/10	9.22 + 1.18^{bcd}	3.78 + 0.17^{cd}	1.85 + 0.24^{ab}	92
9	45/15	8.28 + 0.51^{abcd}	3.59 + 0.12^{bcd}	1.8 + 0.1^{ab}	92
10	55/5	10.87 + 1.27^{d}	3.99 + 0.14^{d}	1.74 + 0.19^{ab}	100
11	55/10	9.92 + 0.9^{cd}	3.9 + 0.16^{cd}	1.79 + 0.18^{ab}	83
12	55/15	10.69 + 1.27^{d}	3.83 + 0.16^{cd}	1.72 + 0.21^{ab}	92
13	Turbo	11.68 + 1.25^{d}	4.14±0.15^{d}	2.36 + 0.29^{b}	83

Data (Mean $\pm$ SE) within columns which have the same superscripts are not significantly different from one another ($p>0.05$). Initial wt = 0.65g $\pm$ 0.1. n=12

Subsequently, values for average weekly wet weights were subject to analysis of repeated measures using weight at t=0 as a covariate. The average initial weight of crabs at the commencement of the study did not differ significantly amongst treatments ($F=0.907$, $p= 0.535$). It was also shown that while the level of crude protein or lipid incorporated into diets had a significant effect on average weekly weight, ($p<0.01$) there was no evidence of any significant interaction occurring between these two dietary elements ($p>0.05$). As a consequence, the effect of dietary protein and lipid on crab weight was analysed independently and is shown in Figs. 4 and 5.

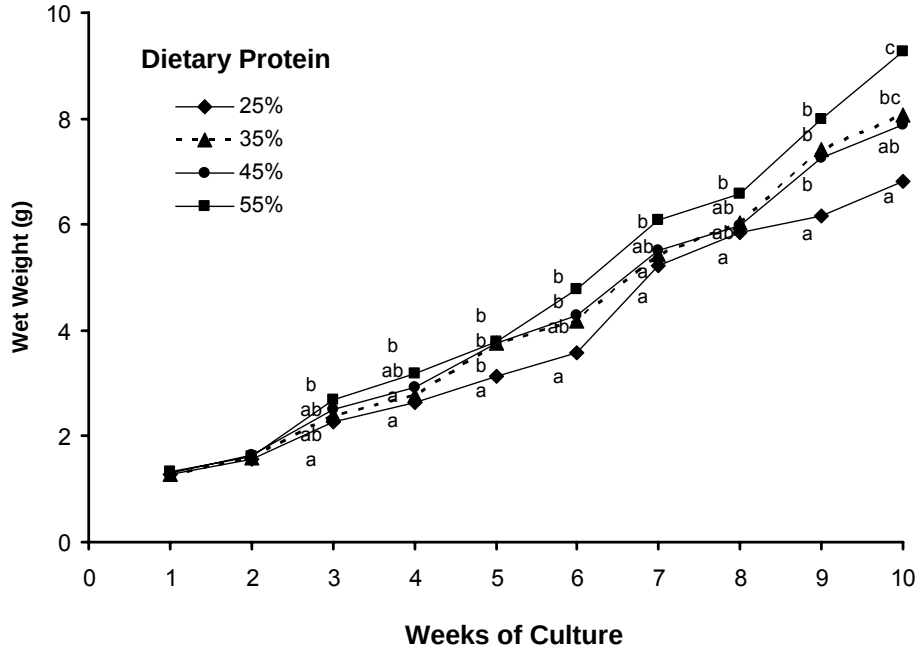

Fig. 4. The effect of dietary protein on average weekly weights of juvenile *S.serrata* at BIARC. Values are means (n=36 per group). Means at the same time point with the same letter script are not significantly different from one another ($p<0.05$).

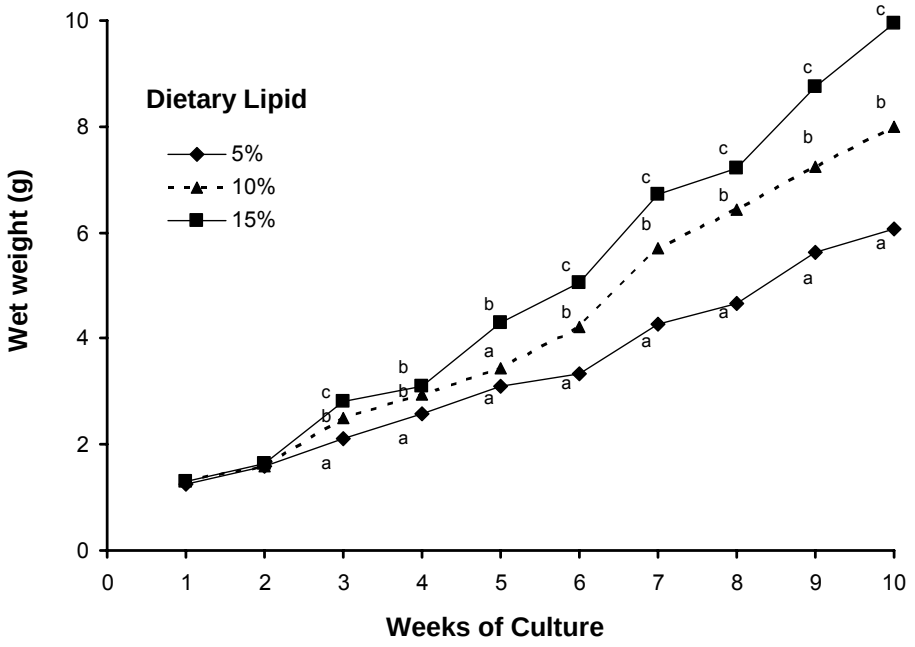

Fig. 5. The effect of dietary lipid on average weekly weights of juvenile *S.serrata* at BIARC. Values are means (n=48 per group). Means at the same time point with the same letter script are not significantly different from one another ($p<0.05$).

As demonstrated in Figure 4, significant differences were observed with the responses of crabs to different levels of dietary protein after three weeks of culture. Specifically, a general increase in average weekly weights was observed as the level of dietary protein was raised from 25% to 55%. For example, from week three onwards significantly higher ($p<0.05$) weight values were obtained for crabs fed diets containing 55% protein than were obtained for crabs fed diets containing only 25% crude protein. Nevertheless, increasing dietary protein from 45% to 55% did not yield any further increase in final weight.

As shown in Figure 5, there were significant differences ($p<0.05$) in the responses of crabs to different levels of dietary lipid. Specifically, progressively higher average weekly weights were obtained as the level of dietary lipid was increased from 5% to 15%. For example, from week five onwards the average weekly weights of crabs fed diets containing 15% lipid were significantly higher ($p<0.05$) than those obtained using diets containing either 5% or 10% lipid.

7.2.1.2 Indonesia (*S.paramamosain*)

Crude Protein Requirements

Dietary protein content had a significant ($p<0.05$) influence on final weight gain, carapace width (Table 20) and the protein and lipid content of juvenile mud crabs (Table 21). In particular, there was a progressive increase in final wet weight as the level of dietary protein was raised from 25% to 45%. Increasing dietary protein from 45% to 50%, however, did not yield any further increase in final weights of juvenile crabs. Broken line analysis of weight gain data indicated that the optimum dietary protein requirement for maximum growth of *S.paramamosain* juveniles under the reported experimental conditions was 47.6% (data not shown).

Survival rates for all treatments were generally high and ranged from 86.7% to 100%.

Table 20. Final mean weight, weight gain, carapace width and survival of *S.paramamosain* juveniles fed experimental diets containing different levels of crude protein at GRIM. Culture period: 56 days.

Experimental diets	Final weight (g)	Weight gain (%)	Carapace width (cm)	Survival (%)
CP-25	2.08 ± 0.30^{a}	510.7 ± 50.9^{a}	2.62 ± 0.06^{a}	93.3
CP-30	3.04 ± 0.18^{ab}	875.1 ± 84.2^{b}	2.89 ± 0.04^{b}	86.7
CP-35	4.32 ± 0.75^{bc}	1187.5 ± 106.0^{b}	2.98 ± 0.18^{bc}	100.0
CP-40	5.76 ± 0.82^{c}	1342.8 ± 105.4^{b}	3.19 ± 0.14^{c}	93.3
CP-45	7.05 ± 1.16^{d}	1942.6 ± 146.0^{c}	3.45 ± 0.07^{d}	93.3
CP-50	6.59 ± 1.22^{d}	1804.3 ± 184.1^{c}	3.45 ± 0.23^{d}	100

Initial weight 0.35 ± 0.08 g; initial carapace width 1.36 ± 0.09 cm. Values in the column with the same letter superscript are not significantly different ($p>0.05$). n = 18.

Subsequent analysis of body composition revealed that dietary protein levels also influenced crab body composition. In particular, as the level of dietary protein was raised to 45% there was a progressive increase in the level of crude protein in the crab body (Table 21). Likewise, it was shown that the protein content of formulated diets influenced the lipid content of the crab body. In particular, juveniles fed diets containing 30% or 35% crude protein accumulated significantly higher ($p<0.05$) levels of lipid than those fed diets containing 40% - 50% crude protein.

Table 21. Proximate composition of the whole body of *S.paramamosain* juveniles fed experimental diets containing different levels of crude protein at GRIM.

Experimental diet	Protein (%)	Lipid (%)	Fibre (%)	Ash (%)
CP-25	33.3 ± 0.2^{a}	5.8 ± 0.2^{ab}	7.5 ± 0.3	43.5 ± 0.0
CP-30	34.3 ± 1.1^{ab}	6.8 ± 0.4^{a}	7.3 ± 0.8	41.4 ± 2.1
CP-35	34.8 ± 0.6^{ab}	6.8 ± 0.9^{a}	7.4 ± 0.7	42.9 ± 1.8
CP-40	36.0 ± 1.3^{bc}	5.3 ± 0.3^{b}	7.4 ± 0.9	43.4 ± 0.7
CP-45	37.2 ± 1.6^{c}	5.4 ± 0.3^{b}	7.8 ± 0.6	42.3 ± 2.6
CP-50	36.9 ± 0.5^{c}	4.8 ± 0.3^{b}	7.7 ± 0.7	41.2 ± 2.2

Values in the column with the same letter superscript are not significantly different ($p>0.05$). n=18

Crude Lipid Requirements

A shown in Table 22, there was a progressive and significant ($p<0.05$) increase in final weight and weight gain as the level of dietary lipid was raised from 1.8% (CP-0) to 10.7% (CP-9). Final weight and weight gain values for crabs fed diets with 13.5% lipid (CP-12), however, were not significantly different from the values obtained using the CP-9 diet (10.7% lipid).

Table 22. Final weight and weight gain of *S.paramamosain* juveniles fed experimental diets containing different levels of lipid at GRIM. Culture period: 56 days.

Test diets	Final weight (g)	Weight gain (%)	Survival rate (%)
CL-0	1.30 ± 0.19^{a}	622.2 ± 80.1^{a}	88.9
CL-3	2.35 ± 0.10^{a}	1117.2 ± 51.0^{b}	83.3
CL-6	2.90 ± 0.21^{c}	1498.2 ± 116.3^{c}	100.0
CL-9	3.66 ± 0.38^{d}	1775.2 ± 107.0^{d}	88.9
Cl-12	3.59 ± 0.22^{d}	1821.4 ± 115.4^{d}	100.0

Initial weight 0.35 ± 0.08 g; initial carapace width 1.36 ± 0.09 cm. Values in the column with the same superscript are not significantly different ($p>0.05$). n= 18.

Table 23. Proximate composition of the whole body of *S.paramamosain* juveniles fed experimental diets containing different levels of lipid at GRIM.

Experimental diet	Protein (%)	Lipid (%)
CL-0	35.3 ± 0.8[a]	3.5 ± 0.3[a]
CL-3	35.0 ± 0.7[a]	3.8 ± 0.2[a]
CL-6	35.8 ± 0.5[a]	4.9 ± 0.3[b]
CL-9	35.2 ± 0.1 [a]	5.7 ± 0.1[c]
CL-12	35.5 ± 0.6[a]	5.8 ± 0.1[c]

Values in columns with the same letter superscript are not significantly different (p>0.05). n=18.

Subsequent analysis of body composition revealed that as dietary lipid content was raised to 10.7% there was a progressive increase in the amount of lipid detected in the crab body (Table 23). By contrast, the level of lipid in crab diets had no significant impact on the level of protein in the crab body.

7.2.1.3 Vietnam (*S.paramamosain*)

Laboratory-based growth trials

Table 24. Final weight and carapace width of *S.paramamosain* juveniles fed experimental diets at RIA3. Culture period: 120 days. Initial wt = 1.27g ± 0.1g

Diet CP%/CL%	Wet weight (g)	Carapace width (mm)	Survival Rate %
35/7	13.3 ± 1.13[ab]	42 ± 1.38[bc]	83
35/10	10.4 ± 1.03[ab]	37.8 ± 1.24[ab]	100
35/15	8.8 ± 0.96[a]	35.5 ± 1.25[a]	91.6
43/7	14.5 ± 1.39[ab]	42.1 ± 1.47[bc]	100
43/10	11.8 ± 1.14[ab]	39.9 ± 1.27[abc]	91.6
43/15	22.7 ± 1.5[c]	49.8 ± 1.42[d]	95.8
50/7	15.5 ± 0.94[b]	44.0 ± 0.89[cd]	100
50/10	15.6 ± 1.42[b]	43.4 ± 1.24[bc]	100
50/15	16.0 ± 1.27[bc]	43.8 ± 1.12[c]	100
Trash fish	32.9 ± 2.99[d]	56.5 ± 1.74[e]	91.6

Values in columns with the same letter script are not significantly different (p>0.05). n= 24.

As shown in Table 24, the highest wet weight and carapace width values were achieved using the diet containing 43% crude protein (36% digestible protein) and 15% lipid. Increasing crude protein level above 43%, however, did not significantly increase mud crab growth performance.

Another major finding of this study was that the increase in wet weight and carapace width exhibited by crabs fed trash fish was significantly higher ($p<0.05$) than that observed in response to any of the artificial diets.

Pond-based growth trials

A comparison of wet weight (Fig. 6) or carapace width (Fig. 7) values obtained after three months of culture revealed no significant difference between values obtained for crabs fed experimental diet based on 43% crude protein and 15% lipid and crabs fed trash fish. By contrast, wet weight and carapace width values obtained for crabs fed the experimental diet based on 43% crude protein and 10% lipid were significantly lower than all other treatment groups. No significant differences were observed when FCR values from all treatment groups were compared (Table 25).

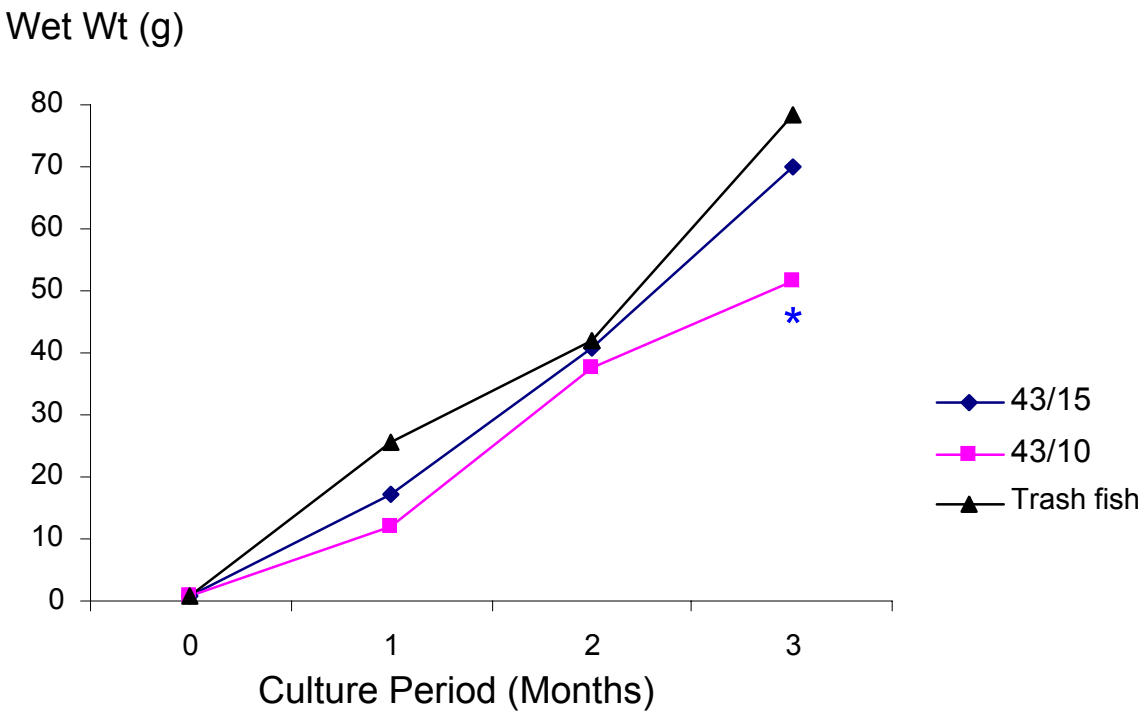

Fig. 6. Final wet weight of *S.paramamosain* juveniles fed experimental diets in pond-based trials at RIA3. An asterisk indicates the data is significantly different to that obtained for the trash fish-based diet ($p<0.05$). n = 2 ponds / treatment

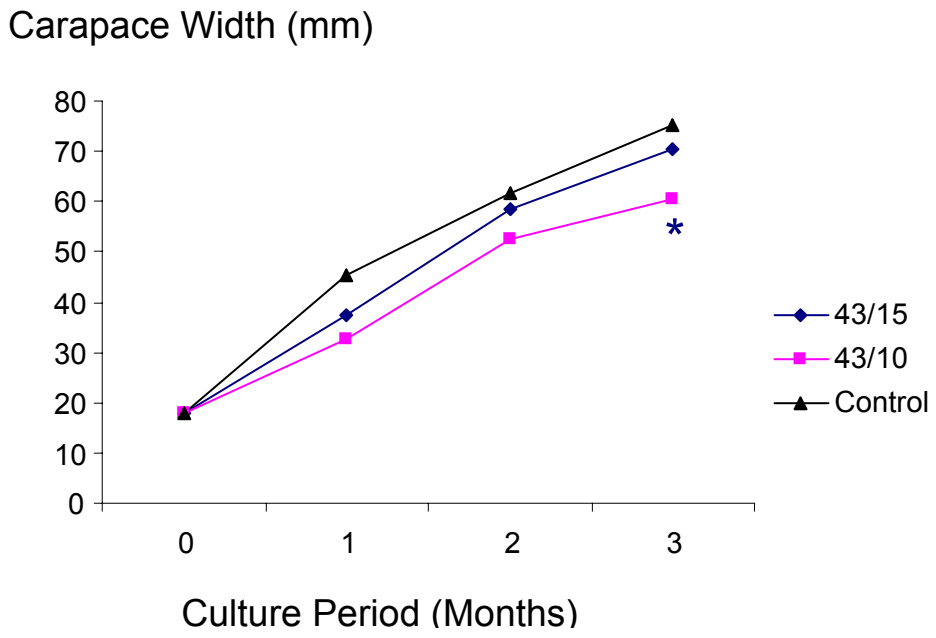

Fig. 7. Final carapace with of *S.paramamosain* juveniles fed experimental diets in pond-based trials at RIA3. . An asterisk indicates the data is significantly different to that obtained for the trash fish-based diet ($p<0.05$). n = 2 ponds/ treatment

Table 25. Food conversion ratio (FCR) and survival rate (%) of crabs in pond culture

Diet	FCR	Survival rate (%)
Control (trash fish)	2.08 ± 1.21	45
43% CP / 15% CL	1.79 ± 0.29	41
43% CP / 10% CL	1.84 ± 1.07	40

7.3 Assessment of the potential of digestible, low cost feed ingredients to replace fishmeal in formulated grow out diets for mud crabs.

7.3.1 Summary

As discussed previously, fishmeal has traditionally been utilised as a primary protein source for aquaculture diets for carnivorous or omnivorous species. In some countries with rapidly expanding aquaculture industries, however, locally manufactured fishmeal can be of poor quality and therefore unsuitable for aquafeed use (Edwards et al et al, 2004). As a consequence, there is often reliance on imported, expensive, high quality fishmeal for manufacture of formulated fish and crustacean feeds. With future demand for fishmeal expected to increase as aquaculture production expands, the identification and development of alternatives to fishmeal is viewed as an aquafeed industry priority (Glencross *et al*, 2007). In the current study, selected high protein feed meals were examined for their potential to replace fishmeal in diets formulated for mud crab species in Australia (*S.serrata),* Vietnam and Indonesia *(S.paramamosian).*

It was shown using laboratory-based systems that between 20% to 40% of fishmeal in formulated diets could be replaced by selected animal (poultry or krill), or plant-based (soybean or corn gluten) feed meals without significantly reducing growth performance. Furthermore, pilot studies conducted in earth ponds at RIA3 demonstrated that formulated diets containing 40% soybean meal could produce levels of growth that were equivalent to those obtained using trash fish.

Another major outcome of the study was the observation that crabs grew significantly faster in pond-based culture systems than in laboratory-based systems, regardless of the diet used. In extension to the current project it was subsequently demonstrated that increasing feeding rate or frequency above 5% BW/day or twice daily, respectively, produced no significant changes in crab growth performance in laboratory-based culture systems.

7.3.2 Results of fishmeal substitution trials conducted at partner institutions

7.3.2.1 Australia (*S.serrata*)

As shown in Table 26, initial analysis of data by ANOVA indicated that final body weights of crabs fed diets containing poultry meal, alone (diets 2-5) or in combination with soybean meal (diets 10-12), were not significantly less ($p>0.05$) than that obtained using the control fishmeal diet (diet 1). By contrast, using soybean meal to replace 80% of fishmeal in the dietary formulation (diet 9) resulted in a significant ($p<0.05$) reduction in final wet weight.

Analysis of FCR values revealed relatively little variation between dietary treatments (ranging from 2.3 to 1.56), although the FCR values obtained using diet 4 (60% fishmeal replaced by poultry meal) and the Turbo diet were significantly higher than those obtained for all other treatments. Survival rates for all treatments were generally high and ranged from 83% to 100%

Subsequent analysis of data was conducted using orthogonal polynomial regression which revealed a significant effect of diet on crab growth rate (Table 27). Specifically, LSD testing using the first order polynomial (ie. growth rate) showed that all experimental diets except diets 2 and 3 (20% and 40% poultry meal, respectively) and diet 7 (40% soybean meal) promoted significantly lower growth rates ($p<0.05$) than that obtained using the fishmeal-based control diet (diet 1)

Table 26. Mean final wet weights (adjusted for covariate; weight at t=0), feed conversion ratios (FCR) and survival of *S.serrata* fed experimental diets containing different proportions of fishmeal (FM), poultry meal (PM) or soybean meal (SBM) for 70days. n=12.

Diet	% FM replaced	FM replacement	Final Wt (g)	FCR	Survival
1	0		11.18 ± 1.04^{ab}	1.61 ± 0.23^{a}	100
2	20	PM	11.42 ± 0.9^{a}	1.56 ± 0.16^{a}	92
3	40	PM	10.2 ± 1.06^{ab}	1.73 ± 0.18^{a}	100
4	60	PM	7.44 ± 0.8^{bc}	2.01 ± 0.17^{b}	83
5	80	PM	8.06 ± 0.75^{abc}	1.92 ± 0.19^{a}	100
6	20	SBM	8.47 ± 0.77^{abc}	1.91 ± 0.15^{a}	100
7	40	SBM	10 ± 0.78^{ab}	1.64 ± 0.13^{a}	100
8	60	SBM	8.15 ± 0.73^{abc}	1.97 ± 0.17^{a}	100
9	80	SBM	6.03 ± 0.29^{c}	1.91 ± 0.09^{a}	100
10	40	PM/SBM	7.53 ± 0.76^{bc}	1.91 ± 0.21^{a}	92
11	60	PM/SBM	7.79 ± 0.69^{abc}	1.98 ± 0.15^{a}	100
12	80	PM/SBM	8.17 ± 0.66^{abc}	1.85 ± 0.19^{a}	100
Turbo			11.7 ± 1.2^{a}	2.3 ± 0.3^{b}	83

Within columns, results with different letter superscripts are significantly different from one other ($p<0.05$).

Table 27. Orthogonal polynomial regression showing effect of fishmeal substitution on weekly weights in juvenile mud crabs fed experimental feeds for 70 days.

Diet	% FM replaced	FM replacement	1st Polynomial average
1	0		1.073^{a}
2	20	PM	1.057^{a}
3	40	PM	0.994^{ab}
4	60	PM	0.757^{cde}
5	80	PM	0.727^{de}
6	20	SBM	0.830^{bcd}
7	40	SBM	0.920^{abc}
8	60	SBM	0.780^{cde}
9	80	SBM	0.592^{e}
10	40	PM/SBM	0.703^{de}
11	60	PM/SBM	0.748^{cde}
12	80	PM/SBM	0.773^{cde}

Data points with different letter superscripts are significantly different from one another ($p<0.05$).

7.3.2.2 Indonesia (*S.paramamosain*)

As shown in Table 28, final body weights ranged from 4.05g to 5.95g. Juvenile *S.paramamosain* fed diets where up to 20% or 40% of fishmeal was replaced by soybean or corn gluten meal, respectively, attained final body weights that were not significantly different from crabs fed the control high-fishmeal diet ($p>0.05$). By contrast, overall weight gain was significantly ($p<0.05$) reduced when 40% of fishmeal in formulated diets was replaced by soybean meal. Survival rates for all treatments were generally high and ranged from 91% to 100%.

Table 28. Initial weight, final weight, weight gain and survival rate of *S.paramamosain* fed experimental fishmeal-based diets containing different levels of soybean meal (SBM) or corn gluten meal (CGM) at GRIM for 9 weeks (n=18).

% Fishmeal replaced	Fishmeal replacement	Initial body weight (g)	Final body weight (g)	Weight gain (%)	Survival rate (%)
0		0.66 ± 0.03	5.95 ± 0.18	801.1 ± 54.48^{b}	96 ± 7.2
20	SBM	0.63 ± 0.02	4.46 ± 0.57	607.8 ± 109.17^{ab}	91 ± 7.8
40	SBM	0.64 ± 0.03	4.05 ± 0.23	531.9 ± 51.27^{a}	100 ± 0.0
20	CGM	0.66 ± 0.22	5.13 ± 0.40	680.2 ± 57.37^{ab}	92 ± 14.4
40	CGM	0.65 ± 0.03	5.14 ± 0.36	687.7 ± 68.70^{ab}	95 ± 8.2

Values in columns with the same letter superscript are not significantly different ($p>0.05$) from one another.

7.3.2.3 Vietnam (*S.paramamosain*)

Laboratory-based experiments

Results of growth trials conducted using juvenile *S.paramamosain* juveniles are shown in Tables 29-31. As demonstrated in Table 29, final body weights varied from 10.18g to 12.91 after 3 months. Analysis by ANOVA revealed that average weight gain and final body weights were significantly influenced by the extent of fishmeal substitution in experimental diets. Specifically, *S.paramamosain* fed diets where 20% of fishmeal was replaced by soybean meal or krill meal exhibited equivalent growth ($p>0.05$) to the treatment group fed the fishmeal-based control diet (D1). Increasing the fishmeal substitution level to 40%, however, significantly reduced final wet weight. Surprisingly, replacement of up to 40% of fishmeal with alternative feed meals appeared to have no significant ($p>0.05$) impact on carapace width (Table 30) or FCR values (Table 31). Survival rates for all treatments were generally high (82% to 93%) over the 3 month period (Table 31).

Table 29. Initial weight, final weight, and weight gain of *S.paramamosain* fed experimental fishmeal-based diets containing different levels of soybean meal (SBM) or krill meal (KM) at RIA3 for 3 months (n=24 crabs per treatment). Initial wt = 1.00g ± 0.25g

Ingredient replacing fishmeal	Weight (g)		
	First month	Second month	Third month
D1 (0% SBM or KM)	3.86 ± 0.33[a]	7.74 ± 0.20[a]	12.91 ± 0.75[a]
D2 (20% SBM)	3.54 ± 0.26[ab]	7.50 ± 0.91[ab]	12.57 ± 1.07[ab]
D3 (40% SBM)	3.15 ± 0.19[b]	5.75 ± 0.55[c]	10.36 ± 1.00[bc]
D3 (20% KM)	3.96 ± 0.80[a]	7.57 ± 0.73[a]	12.72 ± 1.03[ab]
D3 (40% KM)	3.00 ± 0.22[b]	5.97 ± 0.25[bc]	10.18 ± 0.70[c]

Values in columns with the same letter superscript are not significantly different (p>0.05) from one another.

Table 30. Average carapace width of *S.paramamosain* fed experimental fishmeal-based diets containing different levels of soybean meal (SBM) or krill meal (KM) at RIA3 for 3 months (n=24 crabs per treatment). Initial CW=17.05mm ± 2.3mm

Ingredients replacing fishmeal	Width of carapace (mm)		
	First month	Second month	Third month
D1 (0% SBM or KM)	26.19 ± 0.84	34.24 ± 0.95	40.38 ± 1.47
D2 (20% SBM)	25.65 ± 2.13	33.65 ± 2.37	39.50 ± 4.15
D3 (40% SBM)	24.52 ± 2.12	33.29 ± 1.97	36.36 ± 1.47
D3 (20% KM)	27.59 ± 2.62	35.40 ± 3.57	39.83 ± 2.15
D3 (40% KM)	24.19 ± 2.29	34.19 ± 1.90	36.19 ± 1.27

Table 31. Feed conversion ratio (FCR) values and survival rate of *S.paramamosain* fed experimental fishmeal-based diets containing different levels of soybean meal (SBM) or krill meal (KM) at RIA3 for 3 months (n=24).

Ingredients replacing fishmeal	FCR	Survival rate (%)
D1 (Fish meal)	1.66 ± 0.28	93.05
D2 (20% SBM)	1.47 ± 0.21	90.57
D3 (40% SBM)	2.28 ± 1.45	84.72
D3 (20% KM)	1.98 ± 1.06	92.50
D3 (40% KM)	2.45 ± 1.27	82.50

Pond-based experiments

As shown in Table 32, juvenile *S.paramamosain* fed pellet diets containing 15% or 40% soybean meal demonstrated increases in wet body weight and carapace width that were not significantly different (p>0.05) to those of crabs fed trash fish.

Table 32. Average carapace width and wet weight of *S.paramamosain* cultured in earth ponds for 90 days and fed trash fish or experimental diets containing soybean meal (SBM) as a fishmeal replacement. Crabs were cultured for 3 months (n=3 ponds with 15 crabs/pond sampled). Initial wet weight =1.1g ± 0.04g, initial carapace width = 12.6mm ± 0.17mm.

Diet	Carapace width (mm)	Wet Weight (g)
Trash Fish	52.8 + 1.7	83.6 + 6.3
Diet 1 (40% SBM)	52.2 + 1.6	82.4 + 5.5
Diet 2 (20% SBM)	51.9 + 1.7	78.6 + 5.6

7.3.5 Extension study: Determining optimum feeding rates and frequencies for mud crabs in laboratory-based culture systems.

As shown in Table 33, no significant differences in growth were observed when individual dietary treatments were compared.

It is important to note, that sample sizes for treatments (n=8) were only half those originally intended. The reduction in sample number is a consequence of a partial failure of the recirculation system at BIARC that led to the death or escape of half of the crabs within each treatment group. Although it was possible to replace lost crabs with sibling crabs from the original stock, the average weight of replacement crabs was significantly higher than the original treatment group (4g vs 0.7g). Nevertheless, the general trends observed in this second growth experiment were not significantly different to those of the current study (see appendix 1).

A three way analysis of variance was also conducted on the data used to generate Table 33. Analysis revealed that average weekly gain (AWG) of crablets (to and including the moult to C10) was significantly influenced by type of diet given ($F_{1, 63}$= 9.70, P=0.003). Specifically, fresh feed produced a significantly higher AWG value (1.582 g/wk) than was obtained for crabs fed the reference diet (1.400 g/wk). By contrast, the number of feeds per day ($F_{1,63}$=0.54, P=0.464) and the daily ration ($F_{2,63}$=0.37, P=0.691) did not influence AWG values, and there was no significant interactions between these two factors.

Since the three-way design did not fill all 64 cells in each tray, it was possible to fit a commercial prawn pellet as a third diet, but at two feeding rations (5% or 10% BW/day) and two feeding rates (2x or 3x). An analysis of variance showed that AWG for all crablets (excluding those fed 20% ration) was significantly influenced by the diet given ($F_{2, 61}$= 4.66, P=0.013), with fresh feed, as before, producing a significantly higher growth rate (1.574 g/wk) than either artificial diet (reference: 1.383 g/wk; Turbo: 1.408 g/wk). AWG values for crablets fed either type of artificial diet were not significantly different (P>0.05) from one other.

Table 33. Average final wet weight, average weight gain (AWG), daily growth coefficient (DGC) and survival rates for *S.serrata* fed experimental (reference), commercial prawn (Turbo) or fresh diets at different levels or frequencies. (n=8 crabs per treatment). Initial crab wet weight = 0.7g ± 0.02g

Ration % BW /day	Diet	Meals /day	Final wt. (g)	AWG g/wk	DGC	Survival (%)
5	Fresh	three	18.68 ± 1.34	1.54 ± 0.13	2.15 ± 0.13	87.5
		two	16.54 ± 2.14	1.29 ± 0.18	1.87 ± 0.20	100
	Reference	three	15.05 ± 1.24	1.30 ± 0.10	2.02 ± 0.11	100
		two	18.00 ± 1.11	1.39 ± 0.10	1.94 ± 0.08	100
10	Fresh	three	16.66 ± 1.28	1.35 ± 0.13	1.99 ± 0.15	100
		two	17.09 ± 1.73	1.39 ± 0.16	2.00 ± 0.16	100
	Reference	three	14.06 ± 1.85	1.01 ± 0.15	1.59 ± 0.14	100
		two	15.07 ± 1.32	1.17 ± 0.12	1.80 ± 0.13	100
20	Fresh	three	18.56 ± 1.52	1.58 ± 0.16	2.18 ± 0.16	100
		two	16.11 ± 1.28	1.28 ± 0.09	1.92 ± 0.10	87.5
	Reference	three	16.82 ± 1.11	1.33 ± 0.10	1.92 ± 0.09	100
		two	16.66 ± 0.91	1.37 ± 0.10	2.02 ± 0.11	100
5	Turbo	three	18.61 ± 0.61	1.48 ± 0.10	2.02 ± 0.11	100
		two	13.43 ± 1.55	1.03 ± 0.11	1.68 ± 0.11	100
10	Turbo	three	16.38 ± 1.09	1.34 ± 0.10	2.00 ± 0.10	100
		two	14.90 ± 1.65	1.18 ± 0.14	1.81 ± 0.13	100

The carapace width (mm) of crablets was also significantly influenced by the diet given ($F_{1, 63}$= 12.08, $P<0.001$) but not by the other main variables (ie. feed frequency or ration). Specifically, crabs fed the fresh diet had significantly wider carapaces at the C10 instar (49.54mm) than crabs fed the reference diet (47.83mm).

8 Impacts

8.1 Scientific impacts – now and in 5 years

Project scientists received extensive training in the design, conduct and analysis of crustacean nutrition research. In particular, world recognised crustacean nutritionists, Dr Kevin Williams and Dr David Smith (CSIRO) assumed mentoring roles, providing significant technical and theoretical expertise to all project scientists.

Resources and training provided in the current project has directly supported the development of several closely related postgraduate research projects at QUT investigating crustacean aquaculture nutrition or genetics. Specifically,

Postgraduate projects directly supported by the current project:

1. PhD Project: *Comparative analysis of the feeding behaviour and feeding responses in two species of cultured mud crabs, Scylla serrata and Scylla paramamosain*
Student: Mr Phuong Ha Truong
Status: PhD awarded 2008, subject to minor corrections to thesis

2. Masters Project: *Digestive profile and capacity of the mud crab (Scylla serrata)*
Student: Mr Marko Pavasovic
Status: Awarded 2005

Postgraduate projects indirectly supported by the current project:

1. PhD project: *An assessment of the genetic resources in wild and cultured stocks of mud crabs (Scylla paramamosain) in Vietnam*
Student: Mr. Le Van Chi (ACIAR Allwright Fellow).
Status: Project ongoing since 2006

2. PhD project: *Evaluation of the nutritional requirements of redclaw crayfish, Cherax quadricarinatus*
Student: Ms Ana Pavasovic
Status: Awarded 2008

8.2 Capacity impacts – now and in 5 years

The current project was primarily focussed on *basic* scientific research activities since there is a general lack of data about the nutritional requirements of mud crabs. As detailed in section 8.1, the research capacity of partner organisations was increased by the training provided to project scientists in methods of crustacean nutrition research.

In parallel to the current project, considerable investment has been made into building the capacity of facilities at RIA3 to support future crustacean research. Specifically, feed formulating equipment at RIA3 has recently undergone a four fold increase in capacity. Based on this increase we anticipate that it will be possible to support a follow on project in Vietnam directed at validating improved feeds and farming practices for use in

commercial production systems. The follow on crab nutrition project is contained within a large aquaculture feed project currently under development (FIS/2006/141) that will be coordinated by Dr Brett Glencross.

Further capacity building activities will also be incorporated into this follow on project including;

1. Training of scientists from RIA3 in the conduct and application of crustacean behaviour studies
2. Formation of linkages between research teams and local feed manufacturers to facilitate small scale commercial production of improved mud crab diets
3. Conduct of workshops in Nha Trang to train local mud crab farmers in the application of improved diets and husbandry practices.

8.3 Community impacts – now and in 5 years

Since the current project was focussed primarily on scientific research, it is difficult to identify immediate community benefits. Nevertheless, there are substantial long term benefits which may arise from the project outcomes that are elaborated upon below;

8.3.1 Economic impacts

Direct economic benefits to mud crab farmers from the current project are likely to arise from the identification of low cost feed ingredients that have the potential to be included in grow out diets. As described previously (3.0) replacing trash fish with formulated pellet feeds is regarded as an industry priority. Before this project, however, the best reported growth rates achieved for mud crabs using pellet diets were those fed commercial Kuruma prawn (*Penaeus japonicus*) feeds that are relatively expensive (approx. 5 AUD /kg). Widespread adoption of such expensive artificial diets for mud crab farming in Vietnam is unlikely, particularly with trash fish prices around 1.5 AUD/kg.

In the current project a range of low cost animal and plant based ingredients demonstrated potential for incorporation into mud crab diets. Data was also presented that superior feed conversion values were obtained using these artificial diets than were possible with trash fish. We suggest that the outcomes of the current project should help improve the profitability of the crab aquaculture industry by identifying opportunities to develop artificial feeds that are comparable in price (or cheaper) to trash fish or existing artificial prawn diets. In particular, diet costs for farmers will be reduced by minimising the inclusion of relatively expensive feed components (eg. fishmeal) via replacement with digestible low cost alternatives (eg. soybean meal).

8.3.2 Social impacts

Reducing poverty has been a major goal of the Government of Vietnam since unification and is one of the key objectives of the *Doi Moi* reforms launched in 1986. Aquaculture has been recognised by the Ministry of Fisheries and Vietnamese government as an industry with significant potential to generate income and alleviate poverty in rural communities. With increased availability of crab seed, there is now the opportunity to dramatically expand mud crab aquaculture in Vietnam. The availability of low cost formulated diets for mud crab aquaculture should help exploit the growth potential of this industry thereby improving income in many rural communities.

In Australia, the development of mud crab farming is viewed as an option for improving the economic prosperity of indigenous communities. In particular, the Federal Government

has funded projects initiated by indigenous communities in the Northern Territory such as the Gwalwa Daraniki Enterprise at the Mudla Crab Farm at Kulaluk near Darwin Harbour. We anticipate that excessive grow out feed costs will provide significant barriers to the adoption of mud crab aquaculture by indigenous communities. In the current project, however, a range of readily available, low-cost ingredients have been identified with the potential to be incorporated into mud crab diets. Such outcomes help improve feed resource security and lower production costs for the types of community ventures described above.

8.3.3 Environmental impacts

Many wild crab fisheries across the Asia-Pacific region are at, or close to, full exploitation. Current practices are considered unsustainable and there are already reports that some wild fisheries are experiencing stress from over fishing (Haddon, 2004). Expansion of mud crab aquaculture provides an opportunity to reduce reliance on natural mud crab stocks thereby avoiding further depletion of natural fisheries.

The development of formulated feeds for mud crab aquaculture should also help reduce the environmental impacts of this industry as it is currently practised in Vietnam. Specifically, the main supplemental feed currently used in crab aquaculture is trash fish which is recognised as a major environmental pollutant (Edwards *et al*, 2004). The superior FCR values achieved with artificial crab pellets in the current project indicate that it should be possible to reduce the total amount (by weight) of feed added to commercial production systems and reduce the likelihood of water fouling from uneaten food.

8.4 Communication and dissemination activities

Project results have been communicated via publication in *scientific journals* and *workshops* in Vietnam and Australia. Details are provided below:

8.4.1 Scientific publications (see also section 10.2)

Resources and training provided in the current project has directly or indirectly supported the production of several scientific publications investigating the nutrition or husbandry of crustacean species commonly cultured in Australia and South East Asia. Specifically;

- 4 publications on the nutrition or husbandry of *Scylla* species
- 2 publications on the nutrition or husbandry of the Blue swimmer crab *Portunus pelagicus*
- 3 publications on the nutrition of the redclaw crayfish *Cherax quadricarinatu*s

8.4.2 Workshops

Nha Trang Lodge, Nha Trang, Vietnam: June 2006

Upon completion of the first phase of the current project in 2006, a workshop was held in Nha Trang, Vietnam where scientists from partner institutions in Australia (QUT, BIARC), Indonesia (GRIM) and Vietnam (RIA3) presented their major project findings (see section 7.0). Also in attendance were representatives from the following stakeholder organisations;

- ACIAR
- Vietnamese Ministry of Fisheries
- RIA2
- Nha Trang Fisheries University

- QDPI & F

A series of open forums were also held to discuss future crab aquaculture research priorities and several technical problems associated with the current project. In particular, the findings of an ACIAR sponsored review of project FIS/2000/065 undertaken by Mike Heasman were presented. In this review, lower than expected growth rates of juvenile crabs were identified as major factors limiting the potential to apply data obtained using laboratory-based culture methods (see sections 7.2 and 7.3). In subsequent discussions it was recommended that project FIS/2000/065 be extended to determine if crab growth performance could be improved by further optimisation of feeding protocols.

Media coverage of this workshop was provided by a local television station.

BIARC, Australia: December 2007

As part of the extension to FIS/2000/065 in 2007, a workshop was held at the Joondooburri Conference Centre, Bribie Island where project participants and stakeholders discussed;

- the progress of experiments to optimise feeding protocols
- future crab aquaculture research priorities.

In attendance at this meeting were representatives from QUT, BIARC, RIA3, GRIM, CSIRO, ACIAR, QDPI & F and Coral Coast Mariculture.

Workshop presentations focussed on the status of mud crab aquaculture in partner countries and progress of research into the nutrition and husbandry of *Scylla* species. Open discussion forums were also held to identify research priorities relevant for an ACIAR aquaculture feed project (FIS/2006/141) currently under development.

9 Conclusions and recommendations

9.1 General Discussion

As described in the introduction, mud crabs in the genus *Scylla* are highly desired as a food source and provide income for many coastal communities in the Asia-Pacific region. With only limited potential for further expansion of wild fisheries, aquaculture is now regarded as the key strategy to meet future demand for mud crab product and reduce pressure on wild stocks. The development of low cost, but nutritionally adequate diets is of fundamental importance to this expanding aquaculture industry (Fielder, 2004). For this reason, a key objective of the current project was to examine the potential of readily available, low cost ingredients to be incorporated into aquafeeds designed for *Scylla* species cultivated in Australia, Indonesia and Vietnam.

9.1.1 Digestibility of selected feed ingredients in formulated mud crab diets.

Previously, it has been demonstrated that supplementary feeding is not required for mud crab aquaculture when crabs are held at a relatively low biomass densities of 0.05kg /m^2 (Christensen et al, 2004). Production of crustaceans at higher densities using semi-intensive or intensive culture methods offers the potential to increase yields, but requires supplemental feeding to support higher rates of growth and survival (Bautista, 1986). Until recently, there has been a general paucity of data on the digestive capacity or specific nutritional requirements of *Scylla* species in aquaculture. As a consequence, there are no formulated feeds developed specifically for mud crabs. Traditionally, farmers rely on trash fish or feeds formulated for other crustacean species (ie. marine prawns). The use of such feeds can compromise optimum productivity through the inclusion of unnecessary amounts of expensive ingredients such as fishmeal. As a consequence, a key objective of the current project was to assess the potential of mud crabs to digest a wide range of cheap but abundant plant, animal and single cell-based animal feed ingredients.

As described in section 7.1, high ADC values were observed for most test ingredients and corresponding diets in separate studies conducted in Indonesia, Australia and Vietnam. For example, many test ingredients had protein, dry matter or energy digestibility coefficient values in excess of 85%. An interpretation of this outcome is that a wide range of nutrient sources are readily digested by sub-adult mud crabs. Such findings also support the argument that these test ingredients should be further investigated for their potential to be used to replace increasingly scarce and expensive fish meal in formulated *Scylla* diets.

Digestibility of marine animal-based ingredients

Results of digestibility experiments conducted at partner institutions collectively demonstrated high ADC coefficient values for crude protein ($\geq$85%) for all marine-animal based meals. These findings are consistent with those reported by Catacutan *et al* (2003) and Tuan *et al* (2006) who demonstrated ADCP values above 90% for squid meal and

prawn meal incorporated into formulated *S.serrata* diets. The high crude protein digestibility of marine animal-based meals is not unexpected considering the preference of *Scylla* species for natural diets based on molluscs, crustaceans, and fish in the wild (Hill 1979). Furthermore, high ADCP values reported for marine animal-based meals in the current project are consistent with the high level of protease activity detected in the digestive system of *Scylla* species (Pavasovic *et al* 2004).

Comparison of digestibility trials conducted at RIA3 and GRIM using *S.paramamosian* revealed significant differences in the ADC coefficient values for gross energy. Specifically, AGED values obtained for all marine animal-based meals tested at GRIM were relatively high (>85%) and not significantly less than that obtained for fishmeal. By contrast, AGED values obtained for all marine animal meals at RIA 3 were 60% or less. Nevertheless, the AGED coefficient value obtained for prawn head meal at RIA3 was not significantly less than the corresponding value obtained using local fishmeal (Table 18).

The finding that ADC coefficients for prawn head meal were generally similar to those obtained for fishmeal, suggests that this ingredient should be further investigated for its potential to be incorporated into formulated *S.paramamosian* diets. Prawn head waste comprises approximately one third of the total prawn weight but is normally completely discarded by the fishery/aquaculture industry. The disposal of prawn head waste poses a serious disposal problem and contributes to the overall cost of the production. For example, in 2000 prawn heads represented 33% of total Brazilian prawn production of 25.0 metric tons (Nunes, 2001). Early attempts to incorporate prawn head flour into aquafeed formulations, however, have been confounded by high fibre and ash content which results in the formation of weak pellets (Meyers, 1986) with poor stability in water. In recent years, it has been shown that fibre and ash content in prawn head meal can be reduced by transformation into silage via the process of fermentation (Fagbenro and Bello-Olusoji, 1997). The potential of fermented prawn head meal to be incorporated into aquafeeds has since been demonstrated for fish Nwanna (2003) and crustacean species (Sudaryono *et al*, 1995).

Based on such findings, and the data obtained in the current study, we recommend that prawn head meal be further investigated for its potential to be incorporated into formulated *Scylla* diets.

Digestibility of terrestrial animal-based ingredients

Results of digestibility experiments conducted at BIARC and GRIM demonstrated high ADC coefficients ($\underline{\geq}$79%) for most nutrients in feed meals derived from poultry products. Moreover, these values were equivalent, or superior to, the corresponding values obtained for the locally available fishmeal. The only exception to this trend was the relatively low lipid digestibility value obtained for poultry feather meal.

Poultry based meals are increasingly viewed as promising alternatives to fishmeal in formulated animal feeds and there have several studies conducted to evaluate the potential of these products to replace fishmeal in crustacean diets. For example, Tan et al. (2003) and Cruz-Suarez (2007) demonstrated that up to 80% of fishmeal meal formulated for *Litopenaeus vannamei* diets could be replaced by poultry meal without significantly reducing growth performance. Likewise, Menasveta and Yu (2002) reported that *P. monodon* grew significantly faster when 60% of fishmeal was replaced with a poultry-based meal.

Based on such findings, and the data obtained in the current study, we recommend that poultry-based meals should be further examined for their potential to be incorporated into formulated mud crab diets.

A significant outcome of the experiments conducted at BIARC was the finding that crude protein in meat meal was effectively digested by sub-adult *S.serrata*. This finding is in general agreement with the study of Catacutan *et al* (2003) who demonstrated that crude protein in meat and bone meal was readily digested by *S.serrata*. By contrast, it was also shown in the current study that dry matter and gross energy digestibility coefficients for meat meal were significantly less than the corresponding values obtained for fishmeal. This finding is in general agreement with the study of Catacutan *et al* (2003) where it was shown that the dry matter and gross energy digestibility of meat and bone meal in *S.serrata* diets was significantly less the corresponding value obtained using fishmeal. Reduced digestibility coefficients have been associated with use of MBM or meat meal in aquatic species such as *C. destructor* (Jones and DeSilva, 1997), *P. setiferus* (Brunson et al., 1997), *Rachycentron canadum* (Zhou et al., 2004), *Bidyanus bidyanus* (Allan et al., 2000) and *Sparus aurata* (Robaina et al., 1997). Reduced digestibility related to use of MBM is often attributed to high levels of ash (Wu et al., 1999; Allan et al., 2000). Despite this, MBM has been used in crustacean diets where it has been used successfully to replace 60% (Tan et al., 2005) to 75% (Forster et al., 2003) of FM in the diet for *Litopenaeus vannamei*, and up to 50% of fishmeal in diets for *Macrobrachium nipponense* (Yang et al., 2004) without a significant negative effect on growth and survival. Further studies are required to determine if the relatively poor dry matter and gross energy digestibility of meat and bone meal observed in the current project is also indicative of a reduced capacity to promote growth of *S.serrata.*

Digestibility of plant and single cell-based ingredients

Overall, the digestibility of plant-based ingredients selected for the current study was relatively high and not significantly less than fishmeal. This outcome is in broad agreement with the findings of Catacutan *et al* (2003) and Tuan *et al* (2006) who observed high digestibility coefficients for several plant-based ingredients selected for *S.serrata* feeding trials. For example, Tuan et al (2006) reported apparent protein and energy digestibility coefficients for soybean meal that were equivalent to those obtained using fishmeal. Likewise Catacutan *et al* (2003), provided evidence that the apparent protein digestibility of five plant-based feed ingredients (copra meal, bread flour, rice bran, corn meal and soybean meal) was equivalent, or superior to, the corresponding value obtained for fishmeal. Nevertheless, based on the protein content of these test ingredients, only soybean meal contained sufficient protein (~40%) for it to be considered as a potential fishmeal replacement.

Interestingly, analysis of digestibility experiments from the current project and the available literature revealed substantial variation in nutrient digestibility coefficient values obtained for soybean meal. For example, AGED values ranged from 78% to 98% (Table 34). Such large discrepancies indicate the need to be aware of the nutrient digestibility of specific soybean meal and fishmeal preparations when formulating diets Nevertheless, in the current project digestibility coefficients for soybean meal were equivalent, or superior to, the corresponding values obtained for local fishmeal. The high nutrient digestibility of soybean meal observed here is consistent with similar reports using other crustacean species such as *Cherax quadricarinatus* (Pavasovic et al, 2007) and *Cherax destructor* (Jones and DeSilva, 1997). Likewise, several crustacean species including *Litopenaeus vannamei* (Amaya et al., 2007), *Penaeus setiferus* (Brunson et al., 1997) and *Macrobrachium rosenbergii* (Law et al., 1990), have been shown to effectively utilise soy products in their diet.

Table 34 Apparent dry matter (ADMD) crude protein (ACPD) and gross energy (AGED) digestibility coefficients for soybean incorporated into formulated *Scylla* diets.

	ADMD (%)	ACPD (%)	AGED (%)
BIARC	80.4	91.7	89.1
GRIM	89.2	96.05	98.5
RIA3	85.3	95.9	78.8
Tuan et al, 2006	95.7	97.1	97.9
Catacutan et al, 2003	90.9	95.5	

Consistent with the results observed using soybean meal, relatively high nutrient digestibility coefficients were obtained for yeast, canola meal, lupin meal and cotton seed meal in the current study. There is mounting evidence that many crustacean species have digestive systems which are well adapted to digest plant-based feed meals which typically contain relatively high levels of carbohydrate (Figueiredo *et al*, 2001; Lopez-Lopez *et al*, 2005; Pavasovic *et al*, 2007). For example, significant amylase and cellulase activity has been detected in the digestive system of *S.serrata* (Pavasovic *et al*, 2004). It is unclear, however, if a primary role of such enzymes is to allow plant-based carbohydrates to be utilised as nutrient sources, or if carbohydrase enzyme systems function to breakdown plant cell walls and allow access other nutrients (eg. protein) within the cell. Regardless, the presence of carbohydrase enzymes in digestive system suggests that mud crabs have the capacity to efficiently digest nutrients in plant-based meals. As such, these ingredients should be further investigated for their potential as nutrient sources, binders or bulk fillers for *Scylla* diets. In particular, based on its' high digestibility coefficients, the potential of defatted soybean meal to be incorporated into mud crab diet formulations should be further examined.

9.1.2 Evaluation of critical dietary nutrient requirements for mud crabs during grow out.

Laboratory-based growth trials: Dietary protein

Results of experiments conducted at all partner institutions collectively demonstrated that juvenile *Scylla* exhibit highest growth responses when fed diets containing 43% - 55% crude protein in laboratory-based culture systems. For example, final wet weight and SGR values for *S.serrata* fed diets containing 45% or 55% crude protein were not significantly different to the corresponding values obtained for the treatment group fed a commercial prawn diet (Turbo). Overall, the responses of juvenile *S.paramamosain* (GRIM or RIA3) to different levels of dietary protein in laboratory-based culture systems were similar to those observed for *S.serrata*. In particular, the highest growth responses were observed for the treatment groups fed experimental diets containing 43% to 50% crude protein.

In contrast to the results obtained at BIARC using *S.serrata,* growth responses obtained for *S.paramamosian* fed experimental diets at RIA3 were significantly lower than those observed for the control diet group. In this instance, trash fish was selected as the control diet since its use is representative of standard aquaculture practice in Vietnam. There are several possible reasons for the apparent discrepancies between the results obtained at RIA3 and BIARC. One possibility, is that there are species related differences in the response of mud crabs to artificial pellet diets. It should be noted, however, that

S.paramamosian was also selected as the candidate species for growth trials conducted at GRIM. A cursory comparison of results reveals that SGR values obtained at GRIM using best performing experimental diets were approximately double those obtained by the corresponding treatment group at RIA3 (Table 35). It should be noted, however, that there were substantial differences in the experimental protocols used at each institution (ie. initial weights of crabs, length of culture period). Nevertheless, the experimental results suggest that the artificial mud crab diets formulated at RIA3 were inferior to trash fish in terms of their capacity to promote growth in a laboratory-based culture system.

Another important finding revealed in the trials at GRIM was that dietary protein influenced the level of crude protein in the crab body. Specifically, as the level of crude protein in diets was increased from 26% (CP-25) to 46.8% (CP-45) there was a consistent and significant increase in whole body protein content. This result apparently contradicts that of Catacutan (2002) who demonstrated there was no significant change in the crude protein content of crab muscle as dietary protein was increased from 32% to 48%. At present, the reasons for the apparent discrepancy between these results are unclear. It is important to note, however, that the analysis of Catacutan (2002) determined protein content of muscle while in the current study assessed whole body protein content. The apparent disagreement between the two set of results may also be related to moult or development stage examined. For example, the initial weight of crabs analysed at GRIM was only 0.35g while Catacutan (2002) utilised crabs with an average initial weight of 9.15g. Age related differences in the body composition of crabs have been described elsewhere. For example, Prasad & Neelakantan (1988) reported that small *S.serrata* (51-80 mm carapace width) had higher body protein and lipid content than larger animals (81-130 mm of carapace width).

Table 35 Initial wet weight, final wet weight and specific growth rate values for *S.paramamosain* fed experimental best performing experimental diets and trash fish in study 7.2.

	GRIM[1]	RIA3[2]	RIA3[3]
Initial wet weight (g)	0.35	1.27	1.27
Final wet weight (g)	7.05	22.7	32.9
Days of culture	56	120	120
SGR	5.36	2.40	2.72

[1] CP-45 diet (Table 20)

[2] 43/15 diet (Table 24)

[3] Trash fish (Table 24)

Results of the current study collectively suggest that dietary protein levels in the range of 43%-55% are required to promote maximum crab growth in laboratory-based culture systems. This range is somewhat narrower than those reported by other workers who have investigated the crude protein requirements of *Scylla* species. For example, Catacutan (2002) reported that experimental diets containing 32% to 48% crude protein promoted equivalent levels of growth when fed to *S.serrata,* providing that dietary lipid was adjusted to 12%. Likewise, Trino and Rodriguez (2002) demonstrated maximum growth for *S.serrata* in mangrove pens fed diets with crude protein content in the range 32% to 47%. Similar results were obtained by Christensen *et al* (2004) who reported no significant difference in the growth performance of *S.paramamosain* or *S.olivacea* fed diets with crude protein content ranging from 38% to 59%. At present, the reasons for the apparent differences in dietary protein requirements for mud crabs are not known. One possibility is that these differences reflect ontogenic changes in dietary requirements.

Specifically, relatively small juveniles (0.35g – 1.27g) were used for the current growth trial while the other studies cited utilised much larger crabs (9g – 30g). An alternative explanation is that differences in dietary protein requirements may reflect differences in culture environments. Specifically, while the current study utilised a laboratory-based cell culture system, the investigations of Trino and Rodriguez (2002) and Christensen *et al* (2004) were conducted in ponds where crabs may have had access to natural sources of food (ie. other than provided by the experimental diets).

Laboratory-based growth trials: Dietary lipid

Results of experiments from all partner institutions indicated that growth of juvenile mud crabs was influenced by the level of lipid in the diet. For example, final weight values for *S.serrata* progressively increased as the level of dietary lipid was raised from 5% to 15%. Likewise, final weight values and whole body lipid content for *S.paramamosain* cultured at GRIM progressively increased as the level of dietary lipid was raised from 1.8% (CL-0) to 10.7% (CL-9). In contrast to the results obtained for *S.serrata*, increasing dietary lipid content beyond 10% failed to induce any further significant increases in crab growth responses.

Overall, the findings reported in the current study are in agreement with those reported by other workers who have investigated dietary lipid requirements of *Scylla* species in culture. For example, Sheen and Wu (1999) reported that dietary lipid levels ranging from 5.3% to 13.8% appeared to meet the nutritional requirements of *S.serrata*. Likewise, Catacutan (2002) demonstrated that *S.serrata* grew well when fed diets containing either 6% or 12% lipid. Based on results of the current study, we suggest that *S.paramamosain* juveniles require dietary lipid levels of 9% to 12% for optimal growth while *S.serrata* juveniles will demonstrate optimal growth in response to diets containing at least 15% lipid. Interestingly, Smith *et al*, (2005) have also demonstrated that optimal growth of the tropical spiny lobster *Panulirus ornatus* is obtained using a diet containing 12.8% lipid. By contrast, high levels of dietary lipid appear to depress growth or inhibit the digestibility of key feed ingredients in other crustacean species. For example, optimum dietary lipid levels for some prawn, lobster (*Homarus americanus*), and crayfish (*Procambanus acutus*) species are only 5% - 8% (Sheen & D'Abramo, 1991), 5% (Castel & Cowey, 1976) or 6% (Davis & Robinson, 1986), respectively. Findings such as these suggest that the common practise of using artificial prawn diets to support mud crab grow out in Australia may result in suboptimal growth performance if the nutritional specifications of the diet (ie. lipid levels) do not align with the nutritional requirements of the crab.

Extension study

Although the growth trials conducted in laboratory-based culture systems provided useful insights into the basic nutrient requirements of juvenile *Scylla*, there were two major problems with the experiments;

1. Due to an oversight in the original experimental design, apparent nutrient digestibility coefficients were unavailable for several key ingredients (eg. casein) incorporated into formulated diets. As a consequence, in some instances it was not possible to express diet composition in terms of digestible protein or digestible gross energy.

2. Crabs in the laboratory-based culture systems appeared to grow at rates which were significantly lower than those which could be achieved in semi-intensive pond-based production systems. An example of mud crab growth performance in semi-intensive pond-based culture is shown in Figure 8. The data was obtained in 1999 as a result of a FRDC

funded study conducted by researchers from BIARC. Juvenile *S.serrata* were stocked (0.6-0.5 crab/m^2) into 0.16ha ponds at Seafarm aquaculture production facilities (Cardwell, Queensland). Crabs were then fed a mixed diet based on cooked fish and prawns, salmon grower pellets and crocodile pellets. By the end of the culture survival rates ranged from 20%-47% and FCR values were approximately 3:1.

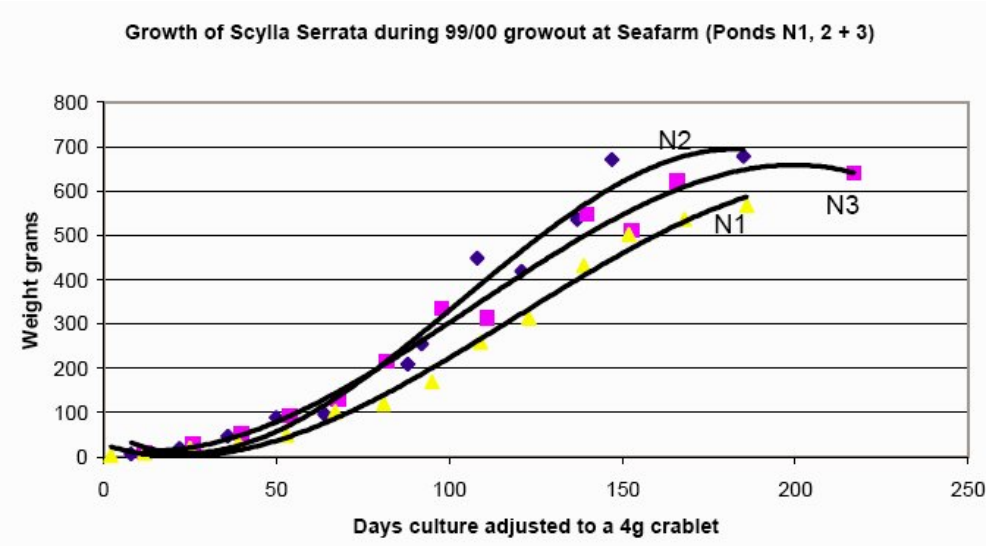

Fig 8. Weight gain of juvenile *S.serrata* cultured in earth ponds at Seafarm (1999)

As a consequence of the relatively poor growth performance of juvenile crabs observed in laboratory-based system, it was suggested in an ACIAR sponsored review (Heasman, 2006) of the current project that;

"Low growth performance achieved in the laboratory based dietary development experiments may be largely attributable to use of suboptimal fixed feeding rates of 3-5% body weight per day and to a once per day feeding frequencies that were applied over the full duration of all experiments."

To address this issue, an extension study was conducted to determine if growth responses could be in improved in laboratory-based culture systems via modifications to the feeding protocols. As detailed in section 7.3.5, however, increasing feed frequency or amount had no significant impact on crab growth performance. An interpretation of this outcome is that there are limitations in the capacity of laboratory-based culture systems (where crabs are held in individuals containers) to facilitate mud crab growth. It is interesting to note that Rodriguez et al (2007) also reported that *S.serrata* juveniles confined to small cages at the bottom of earth ponds demonstrated significantly lower growth than crabs which were permitted to range freely over the same ponds. However, the free ranging crabs suffered approximately double the mortalities of the caged crab treatment group. It will be important in future studies to determine if low growth responses observed in laboratory-based culture systems are a consequence of general environmental factors (eg. water quality, container size/colour) or inadequate provision of macro- or micronutrients in formulated diets.

Pond-based growth trials conducted at RIA3

A pilot study was conducted at RIA3 in earth ponds to determine the suitability of experimental diets for semi-intensive culture systems typically used by commercial mud crab farmers in South East Asia. As shown in Figs. 6 and 7, the treatment group fed an

experimental diet containing 43% crude protein and 15% lipid demonstrated growth responses (increase in weight and carapace width) that were equivalent to the treatment group fed trash fish. Moreover, growth responses for all treatment groups in semi-intensive pond production systems were dramatically higher (2.5-3 fold) that those observed for crabs fed equivalent diets in a laboratory-based culture system.

The outcomes of the pond trials at RIA3 are encouraging and help address one of the main objectives of the current project. Specifically, that formulated feeds may have the potential to replace traditional diets used in mud crab aquaculture. It is important to stress, however, that the pond-based growth trial conducted at RIA3 was only intended to test the feasibility of using experimental diets for more extensive trials in semi-intensive production systems. Any confirmation of the suitability of formulated diets for mud crab aquaculture will require the conduct of relatively large scale farm-based growth trials requiring resources in excess of those available for the current project. In particular, in a review of the current project conducted by Heasman (2006) it was recommended that such trials will require;

- adequate replication for each diet treatment group; in the current project only two ponds were available for each feed treatment. The use of free standing pens randomly positioned within ponds could be used as a low cost approach to increase treatment replicates.
- enclosures that are stocked at an initial seeding density of 0.5 crabs/m^2 to minimise stock losses due to cannibalism
- an unfed control treatment incorporated into the trial to assess the natural productivity of the culture environment
- a culture period of at least 120 days

9.1.3 Formulation and evaluation of improved mud crab diets.

Fishmeal substitution

A major consideration in the development of any formulated aquaculture diet is the provision of high quality dietary protein to support growth. Typically, diets for carnivorous aquatic animal species utilise fishmeal as the main source of protein. With future demand for fishmeal expected to increase as aquaculture production expands, there is concern that only species of very high market value (eg. lobster) will be able to compete for this critical feed ingredient. Similarly, in Australia, high international demand and cost are also expected to reduce the availability of fishmeal for diet development in all but the most valuable aquaculture commodities (Edwards et al et al, 2004). As a consequence, the replacement of fishmeal in formulated diets is recognised as an important research priority for many aquaculture industries.

In the current project it was shown that a variety of animal (krill and poultry meal) and plant-based (soybean and corn gluten meal) ingredients could be used to partially replace fishmeal in diets formulated for juvenile mud crabs in Australia, Indonesia and Vietnam. For example, in laboratory-based growth trials it was shown that 40% of fishmeal in *S.serrata* diets could be replaced by poultry or soybean meal without significantly reducing final wet weight. Likewise, soybean, krill and corn gluten meals all demonstrated the potential to replace at least 20% of fishmeal in diets formulated for juvenile *S.paramamosain.* Unfortunately, laboratory-based trials produced growth rates for *Scylla* species that were significantly lower than could be expected for pond-reared stock. To determine if data obtained in the laboratory-based fishmeal substitution experiments could

be applied to farm-scale production systems, a pilot study was conducted in earth ponds at RIA3 to assess the potential of soybean meal to replace fishmeal in formulated mud crab diets. There were three major outcomes of this pond-based pilot trial:

1. crabs held in semi-intensive culture environments (earth ponds) demonstrated far higher weight gain than crabs reared in laboratory-based culture systems,
2. there was no significant difference in the growth performance of crabs fed trash fish or the best performing experimental diet, and
3. a significant amount (40%) of fishmeal in diets formulated for *S.paramamosain* could be replaced by soybean meal without significantly reducing growth performance.

The outcomes of this pilot fishmeal substitution trial in earth ponds provides support for the argument that data obtained in laboratory-based culture systems has the potential to be applied to farm scale mud crab production systems.

The finding that plant-based ingredients have potential for incorporation into formulated *Scylla* diets is particularly encouraging. Moreover, these results are in general agreement with the findings of other workers who have investigated the potential of using plant-based ingredients to replace fishmeal in artificial crustacean diets. For example, Floreto, *et al* (2000) reported that 50% of fishmeal with in diets formulated for the American lobster *Homarus americanus* could be replaced by soybean meal providing there was also supplementation with selected amino acids. Likewise, Alvarez *et al*, (2007) demonstrated good growth of the white prawn *Litopenaeus schmitti* when soybean meal was used to replace up to 75% of fishmeal. Paripatananont (2001) reported that using soybean meal to replace 50% of fishmeal in diets for *P.monodon* did not significantly impact on growth or survival rates.

The discovery that soybean meal has the potential to partially replace fishmeal in mud crab diets has important implications for *Scylla* aquaculture. Soybean meal is widely viewed as a promising source of protein for fish feeds in terms of future availability (Hardy, 1995). Another major advantage of this product is that it is generally much less expensive than high quality (ie. Peruvian) fishmeal. For example, in the last few years the cost of soybean meal has been approximately half to a quarter that of high grade fishmeal (Figure 9a and 9b). Since feeds typically represent a major component of total production costs in crustacean aquaculture (Wee, 1992) the option to replace fishmeal with cheaper alternatives such as soybean meal offers the potential to significantly reduce production costs for *Scylla* aquaculture.

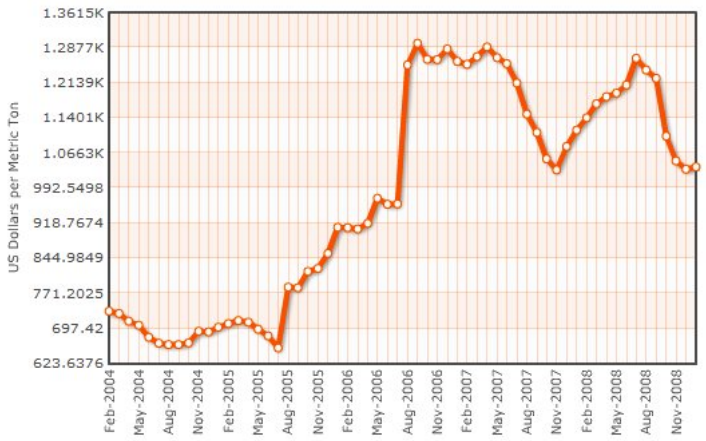

Fig 9a. Monthly price Peruvian fishmeal ($USD) per metric ton. Source: International Monetary Fund, Index Mundi (http://www.indexmundi.com/)

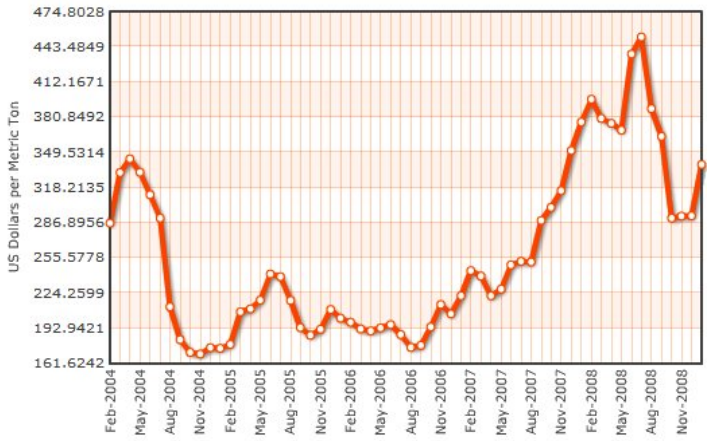

Fig 9b. Monthly price soybean meal ($USD) per metric ton. Source: International Monetary Fund, Index Mundi (http://www.indexmundi.com/)

The capacity of mud crabs to tolerate relatively high levels of plant-based ingredients in their diet was an unexpected outcome of the current project, based on their preference for animal-based diets in the wild. Nevertheless, it has been reported that *Scylla* species possess the necessary enzymes to digest plant-based carbohydrates such as starch and cellulose (Pavasovic *et al*, 2004). Although limited replacement of fishmeal in artificial *Scylla* diets by soybean meal does not appear to negatively impact on growth performance, further studies should be conducted to evaluate the impact of this ingredient on factors such as feed intake and palatability before it is routinely incorporated into mud crab diets. Consideration should also be given to reducing the potential impact of anti-nutritional factors present in soy products (NRC, 1993). For example, it has been shown that the addition of phytase is required to improve the nutritional value of diets containing soybean meal in diets formulated for fish aquaculture (Forster et al., 1999).

A surprising finding of the substitution study conducted at GRIM was that up to 40% of fishmeal in diets formulated for *S.paramamosain* could be replaced by corn gluten meal without significantly reducing growth performance. This result is perplexing considering the results of the digestibility studies which showed most digestibility coefficients for corn gluten meal were significantly lower than those obtained for soybean meal. There are a number of possible explanations for these apparent discrepancies. One possibility is that there are ontogenic changes in the capacity of *Scylla* species to digest corn gluten or soybean meals. Specifically, while the current growth studies utilised small juveniles (<0.3-1.2g/crab), previous digestibility determinations (7.1) were conducted using much larger individuals (90g – 125g/crab). It may be that juvenile *Scylla* have a much greater capacity to digest corn gluten meal (relative to soybean meal) than sub adult mud crabs and this capacity is reflected in higher growth in response to this ingredient. It may also be that the amino acid profile of corn gluten meal is better matched to the nutrient requirements of juvenile mud crabs than soybean meal. If corn gluten meal is to be considered as a potential fishmeal replacement, however, further studies should be conducted to ensure that carotenoids present in this ingredient do not impact negatively on flesh quality as reported by other workers (Cha *et al*, 2000).

9.1.4 Future Research Priorities

As described previously (8.4.2), during the term of this project workshops were held at Nha Trang and Bribie Island where project participants and stakeholders discussed the progress of project experiments and future crab aquaculture research priorities. In consultation with representatives from all stakeholders in project FIS/2000/065, the

following research priorities were identified for the mud crab aquaculture industry in Australia and SE Asia;

1. Refine diet formulation
   - Determine optimum protein and lipid levels
   - Minimise fishmeal content
   - Identify essential amino acid / lipid requirements
   - Develop diet formulations for mud crabs of different ages
   - Investigate effects of diets on shell colour and flesh quality

2. Improve feed presentation to crabs
   - Optimise pellet presentation (size / hard vs soft pellet / stability)
   - Investigate impact of different feeding strategies (eg. broadcast method vs central feed location)
   - Investigate feeding behaviour and shelter use in relation to crab size, cannibalism and aggression

3. Validate the use of formulated diets for commercial scale mud crab aquaculture production systems typically used in the Asia-Pacific region (eg. earth ponds or mangrove enclosures). Investigate impact of “natural” feeds in semi-intensive and/or co-culture systems

4. Investigate suitability of formulated mud crab diet for other crab species (eg. Blue swimmer crabs).
   - Altered diet formulations that influence moulting/ rate of hardening (for soft shell production)

9.2 Conclusions

1. Artificial pellet feeds demonstrated the potential to replace trash fish for mud crab grow out.
As shown in section 7.3.5, the growth performance of juvenile mud crabs fed artificial feed pellets in laboratory-based culture systems was only slightly less (~90%) than that obtained using a traditional diet based on marine animal flesh. Pilot studies conducted in earth ponds also demonstrated that formulated diets promoted levels of growth that were equivalent to those obtained using trash fish.

2. In laboratory-based culture systems, juvenile Scylla demonstrated best growth performance when fed artificial diets containing 43% - 55% protein and 9% - 15% lipid. Pilot studies conducted in earth ponds also demonstrated that formulated diets based on 43% crude protein (approx. 36% digestible protein) and 15% lipid promoted levels of growth that were equivalent to those obtained using trash fish.

3. A broad range of plant and animal-based feed ingredients were well digested by both Scylla species.

4. A significant amount (20%-40%) of fishmeal in artificial mud crab diets could be replaced by selected animal or plant-based meals (eg. soybean meal) without significantly reducing crab growth performance.
Evidence to support this statement was obtained in both laboratory and earth pond-based culture systems.

9.3 Recommendations

We recommend that;

1. *Consideration be given to using artificial diets that are specifically tailored to the nutritional requirements of local mud crab species in preference to the ad hoc use of diets developed for other crustacean species (ie. marine prawns).*

Evidence was presented in the current project that the crude protein and lipid requirements for mud crab diets may be substantially different to those found in commercial prawn feeds. As described previously, commercial prawn feeds are commonly used as supplemental feeds for mud crab grow out. While there is no evidence in the current project that artificial prawn feeds impact negatively on crab growth performance, the levels of expensive elements in these diets, such as crude protein, may be in excess of those required to effectively support grow out.

2. *A variety of animal and plant-based feed ingredients should be further investigated for their potential to replace fishmeal in artificial mud crab diets*.

Evidence from the current project indicates that a variety of animal and plant-based feed ingredients are readily digested by mud crabs. Moreover, several test ingredients (krill meal, poultry meal, corn gluten meal and soybean meal) demonstrated the potential to allow a significant reduction in fishmeal content in grow out diets.

3. *Improved feeds and husbandry practises be validated for farm-scale use.*

Pilot studies conducted in the current project indicate that under certain conditions (ie. semi-intensive culture in earth ponds), experimental diets offer equivalent performance to existing feeding practises. Future research should directed towards the development of practical diets for use in mud crab aquaculture industries of partner countries. Specifically, improved formulated feeds and husbandry practises should be tested in extensive pond-based feeding trials.

4. Alternatives to existing laboratory-based culture systems should be considered for future mud crab growth studies.

The significance of data obtained in laboratory-based culture systems can be difficult to interpret if it is confounded by growth rates that are significantly less than those routinely achieved by local farmers. We recommend that alternatives to the existing laboratory-based culture systems be explored for their potential to support future growth trials. One alternative may be the use of small floating cages to culture individual crabs in earth ponds. As shown in Fig. 10, this approach is commonly used in SE Asia to support the production of soft shell crabs. The potential advantage of such an approach is that it offers the opportunity to replicate the environment (eg. water quality) of current production systems in SE Asia more closely than laboratory-based systems. The used of individual containers for crab culture in earth ponds should also prevent stock losses from cannibalism.

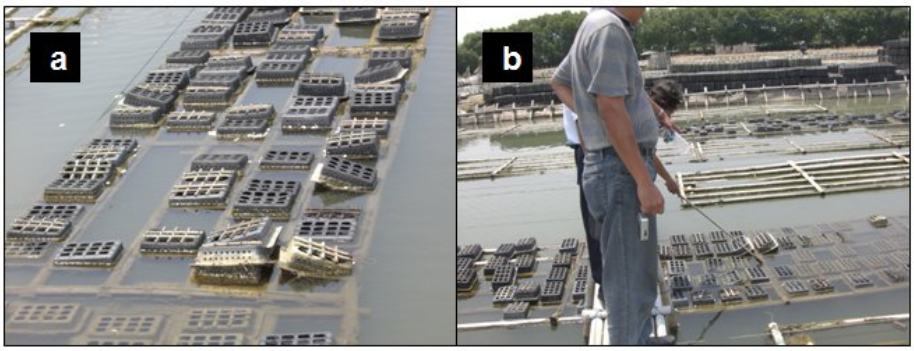

Fig 10. Floating plastic containers (a) used to culture individual mud crabs for soft shell production in an earth pond-based production system (b).

5. *Experiments should be conducted to observe the impact of diet processing and presentation on feed durability and crab feeding behaviour.*

The formulation and preparation of aquafeeds presents specific difficulties due to the aquatic medium in which the feed has to be delivered and ingested, and the relatively small size of the animals being cultivated when compared with animal species used in terrestrial agriculture. As a consequence, any feeds developed for mud crab aquaculture should be checked for pellet durability and water stability.

6. *Future dietary development research should be coupled with research aimed at mitigating cannibalism under semi intensive culture.*

A major bottleneck limiting the productivity of mud crab aquaculture is crop losses due to cannibalism. Research aimed at improving mud crab diets should be aligned with studies to determine the impact that different feeding practices, habitat provision and stocking density have on crab survival, limb loss and harvest biomass in semi-intensive culture.

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10.2 List of publications produced by the project

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11 Appendixes

11.1 Appendix 1: Results of second growth trial conducted in study 7.3.5: Confirming optimum feeding rates and frequencies for mud crabs in laboratory based systems

As described previously, (7.3.5) during the experimental phase of the extension project there was a partial failure of the recirculation system at BIARC which led to the death or escape of half of the crabs within each treatment group. Fortunately, it was possible to replace the lost crabs with sibling crabs from the original stock. The average initial weight of replacement crabs, however, was significantly higher than the original treatment group (4.03g vs 0.7g). As a consequence, the data obtained from this growth trial is presented separately to that described in section 7.3.5.

Table 35. Average final wet weight, average weight gain (AWG), daily growth coefficient (DGC) and survival rates for *S.serrata* fed experimental (reference), commercial prawn (Turbo) or fresh diets at different levels or frequencies. (n=8 crabs per treatment). Initial crab wet weight = 4.03g ± 0.031g

Ration % BW /day	Diet	Meals / day	Initial wt. (g)	Final wt. (g)	AWG g/wk	DGC
5	Fresh	three	4.12 ± 0.16	18.47 ± 1.00	1.90 ± 0.12	1.97 ± 0.10
		two	4.20 ± 0.18	17.33 ± 1.17	1.84 ± 0.33	1.93 ± 0.32
	Reference	three	3.95 ± 0.23	14.78 ± 1.10	1.50 ± 0.17	1.73 ± 0.19
		two	4.05 ± 0.24	18.23 ± 0.74	1.99 ± 0.21	2.07 ± 0.18
10	Fresh	three	4.09 ± 0.26	16.66 ± 2.18	1.97 ± 0.36	2.12 ± 0.38
		two	4.10 ± 0.23	16.96 ± 1.61	1.59 ± 0.12	1.71 ± 0.10
	Reference	three	4.04 ± 0.16	17.41 ± 1.55	1.88 ± 0.30	1.98 ± 0.27
		two	4.13 ± 0.30	15.71 ± 0.70	1.33 ± 0.11	1.48 ± 0.10
20	Fresh	three	3.92 ± 0.23	17.54 ± 1.09	1.67 ± 0.15	1.78 ± 0.10
		two	3.79 ± 0.19	14.73 ± 1.70	1.73 ± 0.43	1.94 ± 0.40
	Reference	three	3.79 ± 0.11	13.25 ± 1.85	1.17 ± 0.16	1.41 ± 0.13
		two	4.02 + 0.16	16.58 ± 1.46	1.95 ± 0.22	2.10 ± 0.21
5	Turbo	three	3.99 ± 0.20	18.62 ± 0.87	1.87 ± 0.16	1.96 ± 0.17
		two	4.23 ± 0.24	18.11 ± 0.86	1.56 ± 0.13	1.62 ± 0.11
10	Turbo	three	4.13 ± 0.20	15.78 ± 1.53	1.63 ± 0.36	1.73 ± 0.30
		two	3.99 ± 0.20	16.01 ± 0.77	1.48 ± 0.12	1.64 ± 0.10

11.2 Appendix 2: Pilot trials not reported in the results section of the current project

11.2.1 Evaluation of critical dietary nutrients for mud crabs during grow out: BIARC (Australia)

This study represented the first attempt to determine basic nutrient requirements (protein and lipid) for the growth of juvenile mud crabs in aquaculture in Australia (*S.serrata)*. Specifically, a growth trial was conducted to establish the level of dietary protein and lipid to support optimum growth of juvenile crabs under laboratory-based culture conditions. Dietary protein was derived primarily from Sea-Pep fishmeal obtained from local suppliers. Unfortunately, relatively poor growth was obtained using all experimental diets and a commercial redclaw (*Cherax quadricarinatus*) diet. By contrast, substantially higher growth was exhibited by crabs fed commercial *Penaeid* diets. The study was completed on 2nd February, 2005 at BIARC.

Methods

Nine fish meal-based experimental diets were formulated to contain three levels of crude protein (35%, 43% and 50%) and three levels of crude lipid (5%, 10% and 15%). The composition of experimental diets is shown in Table 36. As described previously, dietary protein was sourced primarily from Sea-Pep fishmeal. In addition to the experimental treatments, three commercial crustacean feeds were selected for this study, including a;

- *P.monodon* pellet diet (Turbo; 49.7% crude protein, 6.7% crude lipid)
- *P.japonicus* pellet diet (Ebistar No. 4; 61% crude protein, 11.1% lipid)
- *C.quadricarinatus* pellet diet (23% crude protein, 3.9% lipid)

Crabs were selected for this study with an initial weight of 0.35g + 0.01g and held in individual containers in a laboratory-based culture system. Crabs were fed 4% body weight/ day (over 2 daily feeds) for a period of 12 weeks

Unfortunately, upon completion of the growth trial it was discovered that data provided by the local supplier relating to the composition of the Sea-Pep fishmeal was incorrect. In particular, it was mistakenly believed that the composition of this fishmeal was equivalent to that of the high quality Peruvian fishmeal used for all other experiments conducted at BIARC (See Table 2). Subsequent analysis, however, revealed that the level of crude fat (18%) and ash (22%) in the Sea-Pep fishmeal was significantly higher than that in the Peruvian fishmeal. As a consequence, for all subsequent investigations the proximate composition of commercial feed meals used at BIARC was routinely confirmed by independent analysis conducted at the Animal Research Institute (Brisbane, Australia) according to AOAC protocols (1984). The corrected nutrient values for experimental diets incorporating Sea-Pep fishmeal are presented in Table 36.

Results

As shown in Table 37, relatively poor growth was exhibited by *S.serrata* juveniles fed any of the experimental diets containing Sea-Pep fishmeal or the commercial redclaw diet. For example, the average final wet weight of crabs fed the best performing experimental diet (Diet 1) was only 24% of that obtained for crabs fed the *P.japonicus* diet (Ebistar). Another unexpected result was the consistent decline in growth performance parameters (final wet

weight, SGR values and number of moults) that was observed as the level of Sea-Pep fishmeal incorporated into experimental diets was increased from 50% to 74%. Likewise, there were significant ($p<0.05$) increases in FCR values as the level of fishmeal in diets was raised.

Survival rates for experimental treatments varied widely, ranging from 100% to only 16% (Diet 9). By contrast, all crabs fed the *Penaeus* diets survived for the entire culture period.

Table 36. Composition (% dry weight) of experimental diets formulated to determine protein requirements of *S.serrata* at BIARC (n=12 crabs at commencement of trial)

Ingredients	Diet 1	2	3	4	5	6	7	8	9
Fishmeal[a]	50	50	50	63	63	63	74	74	74
Wheat Starch	24	26	24	11.4	13.3	11	-	1.6	-
Lipid[b]	0.3	5.3	10.35	-	4.1	9.1	-	3	7.9
Fullers earth	8.5	3.3	0.5	10.2	4.9	2.2	10.3	6.4	3
Common ingredients[c]	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Calculated[d]									
Crude protein (%)	34	34	34	42	42	42	47	47	47
Crude lipid (%)	11	14	19	11	15	20	13	16	21
Energy (Mj/kg)	16	17.5	18.8	16	17.5	18.8	16.4	17.5	18.9

[a] Sea-Pep, Australia (88.4% DM; 59% CP; 22.4% Ash; 18.3% CF).
[b] Cod liver oil; [c] Common ingredients (g/100g): gluten, 5; Vitamin mineral premix, 2.5; $CaHPO_4$, 3.
[d] represent calculations made once proximate analysis of Sea-Pep fishmeal was confirmed

Table 37. Mean values for final wet body weight, specific growth rate (SGR), feed conversion ratio (FCR) and survival for juvenile *S.serrata* fed formulated diets with different levels of crude protein (CP) and crude lipid (CL) at BIARC. Culture period: 84 days.

Diet	Final Wet Weight	SGR	FCR	Number of moults	Survival (%)
1	$4.73 + 0.24^{a}$	$3.24 + 0.06^{a}$	$1.57 + 0.06^{ab}$	$3.92 + 0.08^{abc}$	100
2	$4.24 + 0.22^{a}$	$3.1 + 0.08^{ab}$	$1.70 + 0.079^{abc}$	$3.83 + 0.11^{ab}$	100
3	$3.71 + 0.20^{a}$	$2.97 + 0.07^{abc}$	$1.57 + 0.17^{ab}$	$3.75 + 0.18^{ab}$	92
4	$4.24 + 0.31^{a}$	$3.02 + 0.11^{abc}$	$1.77 + 0.11^{abc}$	$3.83 + 0.11^{ab}$	100
5	$4.55 + 0.26^{a}$	$3.13 + 0.1^{ab}$	$1.67 + 0.11^{ab}$	$3.75 + 0.13^{ab}$	92
6	$3.0 + 0.31^{a}$	$2.68 + 0.15^{abcd}$	$2.08 + 0.15^{bcd}$	$3.33 + 0.22^{ad}$	75
7	$2.84 + 0.26^{a}$	$2.6 + 0.14^{bcd}$	$2.19 + 0.16^{bcd}$	$3.17 + 0.24^{ad}$	83
8	$2.28 + 0.27^{a}$	$2.42 + 0.24^{cd}$	$2.69 + 0.19^{d}$	$2.75 + 0.25^{de}$	58
9	$2.48 + 0.31^{a}$	$2.38 + 0.45^{cd}$	$3.02 + 0.18^{bcd}$	$1.83 + 0.24^{e}$	16
Redclaw diet	$1.27 + 0.30^{a}$	$2.24 + 0.08^{d}$	$2.39 + 0.1^{cd}$	$2.42 + 0.34^{de}$	66
P.japonicus diet	$19.6 + 1.42^{c}$	$4.94 + 0.1^{e}$	$1.15 + 0.09^{a}$	$4.83 + 0.11^{c}$	100
P.monodon diet	$15.57 + 1.14^{b}$	$4.63 + 0.13^{e}$	$1.24 + 0.08^{a}$	$4.67 + 0.14^{bc}$	100

Discussion

A consistent outcome of this growth trial was the relatively poor growth exhibited by *S.serrata* juveniles fed experimental diets. By contrast, the growth responses of crabs fed *Penaeus* diets were significantly (up to 5 times) higher than those fed any of the experimental diets. This outcome suggests that that the poor performance obtained using experimental diets was not a general problem associated with a suboptimal culture environment (eg. water quality, container size etc). Instead, we propose that the problems encountered in this study result from the incorporation of suboptimal ingredients into experimental diets. In particular, there appeared to be a direct correlation between the decline in growth performance of crabs fed experimental pellets and the level of Sea-Pep fishmeal incorporated into the dietary formulation. Based on these outcomes, the following changes were made to the experimental protocols;

- The proximate composition of all commercial feed meals used for diets formulated at BIARC was confirmed by independent analysis at the Animal Research Institute (Brisbane, Australia)
- The Sea-Pep fishmeal was not used in any subsequent experiments conducted at BIARC. Instead, high quality Peruvian fishmeal obtained from Ridleys Aquafeeds was selected.
- Advice was sought from feed nutrition experts Kevin Williams and David Smith (CSIRO, Cleveland laboratories) regarding selection of high quality feed meals and dietary supplements (eg. Stay-C, Lecithin) to incorporate into formulated crustacean diets.

Another interesting result of this study was that the growth responses of juvenile crabs to the *P.monodon* control diet (Turbo) were generally equivalent to those observed using the *P.japonicus* (Ebistar) commercial diet. It is important to note that the crude protein content of the Ebistar diet was 11% higher than that of the Turbo diet (61% vs 50%). Based on these findings, the Turbo diet was selected as a standard control diet for all further studies conducted at BIARC.

11.2.2 Evaluation of experimental diets for pond-based grow out using individual cage culture: RIA3 (Vietnam)

As described in section 7.3.2.3, it was demonstrated that at least 20% of fishmeal in diets formulated for juvenile *S.paramamosain* could be replaced by soybean meal without significantly reducing crab growth performance in a laboratory-based culture system. In this study, the potential of experimental diets containing soybean meal for pond-based production systems was examined. Crabs were held in individual cages to eliminate mortalities associated with cannibalism.

Methods

Two experimental diets were formulated to contain 43% crude protein and 15% lipid. These levels were selected on the basis of the outcomes presented in Table 24 where it was shown that the experimental diet containing 43% crude protein and 15% promoted significantly higher growth of crabs than the other experimental diets.

For Diet 1, protein was supplied primarily by the addition of equivalent quantities of plant-based and marine animal-based ingredients. By contrast, in Diet 2 the majority of crude protein was provided by marine animal-based meals. The specific composition of experimental diets is detailed in Table 38. A third treatment group was fed a diet based on trash fish.

Crabs were selected for this study with an average wet weight of 1.2g + .01g. These crabs were held individually in cages made of nylon mesh (20cm x 25cm x15cm) in earth ponds for the duration of the 70 day culture period.

Table 38 Composition of diets used for the pond-based grow out trial conducted at RIA3 using individually caged *S.paramamosain*

Ingredients	Diet1	Diet2
Fish meal	28.4	41
Krill meal	5.5	8
Squid	4.1	5
Defatted soybean meal	40.1	15
Rice bran	0	4
Wheat flour	6.4	11
Fish oil	2.8	3
Squid oil	5	5
Cholestine	0.7	1
Dicalci - P	1	1
Binder	3	3
Vi -Premix	3	3
Total	100	100
Calculated (%)		
Crude protein	43.3	43.4
Digestible protein	38.5	37.6
Crude lipid	14.8	15.4

Results

As shown in Table 39, crabs fed the diet containing 40% soybean meal (Diet 1) showed similar increases in wet weight to those crabs fed the trash fish diet. Unfortunately, the raw data relating to this study was unavailable preventing a more detailed statistical analysis of the data.

Table 39. Average final wet weight of individually caged *S.paramamosain* fed experimental diets containing different levels of soybean meal or marine animal meals in earth ponds at RIA3

DIET	Average wet weight of mud crabs (g)				
	15 days	30 days	45 days	60 days	75 days
Diet 1 (40% SBM)	3.0 + 0.1	5.6 + 0.5	12.9 + 1.3	15.2 + 2.0	25.5 + 2.1
Diet 2 (15% SBM)	2.4 + 0.1	3.7 + 0.3	6.8 + 0.5	9.2 + 0.9	15.8 + 1.1
Trash fish	3.4 + 0.1	7.0 + 0.5	14.1 + 1.5	20.8 + 1.9	27.9 + 1.5

11.2.3 Evaluation of experimental diets for pond-based grow out: GRIM (Indonesia)

In a previous study using a laboratory-based culture system, evidence was presented that a significant proportion (20% or 40%) of fishmeal in diets formulated juvenile *S.paramamosain* could be replaced by corn gluten meal or soybean meal without significantly reducing growth performance (7.3.2.2). The objective of the current study was to use these findings as a basis to investigate the potential of experimental diets to promote mud crab growth in farm-scale production systems.

Methods

Three diets were employed for these investigations;

- A commercial *P.monodon* prawn diet
- A commercial *Litopenaeus vanamei* diet
- An experimental formulated diet where protein was sourced from a blend of soybean meal and various marine animal-based meals. The composition of this experimental diet is detailed in Table 40

Crablets were selected from the crab hatchery at GRIM with an average wet weight of 0.46g ± 0.006g. These crablets were then stocked into net enclosures measuring 3m x 5m x 2m at a density of 15 crabs/m2 (Fig. 11). Crablets in each net enclosure were then fed with one of the three feeds detailed above (4 replicate enclosures per feed treatment) for a period of four months.

Fig. 11. Net enclosures used for pilot pond-based growth trials at GRIM

Table 40. Composition (% dry weight) of the formulated diets prepared for the pond-based growth trials at GRIM

Ingredients	%
Fishmeal	24.1
Prawn head meal	12.0
Squid liver meal	8.0
Soybean meal	30.0
Wheat flour meal	11.7
Soybean oil	2.5
Fish oil	1.2
Lecithin	2.0
Vitamin mix	2.0
Mineral mix	2.5
Dextrin	5.2
CM - cellulose	2.0
Gluten	2.0
Total	100

Results

A comparison of final wet weight (Table 41) values obtained after four months of culture revealed no significant difference ($p>0.05$) between the values obtained for crabs fed the experimental diet and crabs fed the commercial prawn feeds. Survival rates for each treatment ranged from 20% to 30%.

Table 41 Initial weight and final weight of *S.paramamosain* fed commercial crustacean diets or an experimental diets for 4 months at GRIM

Diets	Initial body weight (g)	Final body weight (g)
L.vanamei diet	0.46 ± 0.006	118.56 ± 16.91
P.monodon diet	0.46 ± 0.006	132.21 ± 24.92
Experimental diet	0.46 ± 0.006	146.33 ± 13.08

Values represent means + SD

Discussion

As shown in Table 41, comparison of wet weight values obtained after three months of culture revealed that there were no significant differences between any of the dietary treatments. Although these results were encouraging, closer analysis revealed several significant problems with the experimental protocols. Specifically;

- To reduce experimental costs, net enclosures were not established free standing and separate from one another. Instead, they were established as two groups of six pens which often shared common walls (Fig. 8). This design was fundamentally

flawed since it imposed considerable inter-pen variation in the seawater exchange characteristics.

- Net enclosures were stocked with crabs at a density of 15 crablets/m^2. This is approximately thirty times above the maximum threshold needed to keep cannibalism losses low in semi intensive culture environments.
- There was no unfed control group to assess the natural productivity of the net enclosures.

As a consequence of these flaws in experimental design, no further analysis of the study outcomes were considered.

11.3 Appendix 3: Published manuscripts directly related to the current project

Aquaculture Research

Aquaculture Research, 2008, **39**, 1778–1786 doi: 10.1111/j.1365-2109.2008.02056.x

Effect of selected feed meals and starches on diet digestibility in the mud crab, *Scylla serrata*

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Effect of selected feed meals and starches on diet digestibility in the mud crab, *Scylla serrata*

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Running title: Digestibility of mud crab feed ingredients

Key words: Mud crab, *Scylla serrata*, digestibility, starch

Abstract

The present study examined the capacity of the mud crab, *Scylla serrata* to digest experimental diets that contained different animal and plant-based feed meals or different levels or types of starch. The apparent dry matter digestibility (ADMD) coefficients for all feed meals tested in the first part of this study, except meat meal, were similar (78% to 88%). Crude protein digestibility (ACPD) coefficients for all feed meals were relatively high, with values ranging from 86% to 96%. Cotton seed meal, poultry meal, canola meal, fishmeal, soybean meal and lupin meal had similar gross energy digestibility (AGED) values ($p>0.05$) ranging from 84% to 89%. In the second part of this study, the impact of selected starches on the digestibility of fishmeal-based formulated diets was assessed. The apparent starch digestibility (ASD) of wheat starch decreased significantly as the inclusion level was increased from 15% to 60%, however, there was no significant effect on ACPD values. At a 30% inclusion level, the ASD of diets containing different starches decreased in the order corn > wheat > potato = rice. Moreover, ACPD values were significantly higher ($p<0.05$) in the diets containing corn or rice starch than in those containing wheat or potato starches.

1. Introduction

Mud crabs of the genus *Scylla* are a valuable source of nutrition and provide income for many coastal communities in the Asia-Pacific region. In recent years, however, many mud crab fisheries have experienced over harvesting which has threatened capacity to meet future demand from local and export markets. Aquaculture is now widely regarded as a key strategy for meeting increased demand for mud crab product and reducing pressure on wild stocks.

Typically, mud crabs kept in culture have been fed natural diets based on marine animals such as “trash fish”. The collection and use of such natural feeds has many disadvantages including depletion of local marine communities, fouling of culture production systems, variable feed availability and cost. It is now widely recognised that the future viability and expansion of mud crab aquaculture is dependant upon the development of low cost but nutritionally adequate formulated diets (Fielder 2004).

Formulated feeds designed for penaeid prawns have been used with some success for mud crab grow out (Mann and Paterson 2004). Many of these penaeid diets, however, are relatively expensive and there is little data available to indicate how effectively they meet the nutritional requirements of mud crabs. In particular, there is evidence that the nutritional requirements of mud crabs may not be as stringent as those reported for penaeid prawns and that the levels of some relatively expensive components in mud crab diets, such as protein, may be reduced. For example, many artificial prawn diets contain between 50% - 60% protein (Teshima and Kanazawa 1984) yet good growth rates for mud crabs in culture have been reported using substantially lower levels of dietary protein (Catacutan 2002; Tuan, Anderson, Luong-van, Shelley and Allan 2006). Another

disadvantage of using artificial prawn diets for crab aquaculture is that most are based on marine animal meals, such as fishmeal. Typically, fishmeal is one of the most expensive ingredients incorporated into aquaculture diets (Hardy and Tacon 2002). It has been predicted that with future demand for fishmeal expected to increase as aquaculture production expands, only species of very high market value will be able to compete for this critical feed ingredient (Edwards, Tuan and Allan 2004). Reducing reliance on fishmeal is now recognised as a priority for reducing aquaculture feed cost.

Identification of feed ingredients with the potential to replace fishmeal in formulated aquaculture diets requires data on the nutritional requirements and digestive capacity of candidate species. Traditionally, mud crabs have been viewed as carnivores that show a preference for natural diets containing molluscs, crustaceans, and fish (Hill 1979). Interestingly, however, the presence of significant amounts of plant-based material in the mud crab digestive system has been reported (Hill 1976; Tacon and Akiyama 1997). Moreover, Prasad and Neelakantan (1988) demonstrated that detritus was the main food source for crabs less than 70mm carapace width. In recent years, evidence has emerged that the mud crab digestive system possesses a significant capacity to digest plant-based materials. Investigations into the digestive physiology of *S. serrata* have confirmed that this species possesses the necessary enzymes to digest many plant-based carbohydrates. For example, Pavasovic, Richardson, Anderson, Mann and Mather (2004) detected significant amylase, cellulase and xylanase activity in soluble extracts from the mid gut gland. Findings such as these provide a rationale to investigate the use of relatively cheap plant-based ingredients in mud crab diets.

Supplying nutrients from plant-based sources also offers potential opportunities to reduce reliance on fishmeal and therefore lower diet costs.

One of the first steps in estimating the potential of a new ingredient for use in formulated aquaculture diets is to test its digestibility in candidate species. Recent digestibility studies have confirmed that the mud crab has a significant capacity to utilise feed ingredients from a variety of terrestrial animal or plant-based sources. For example, Catacutan, Uusebio and Teshima (2003) showed that plant-based feed ingredients such as soybean meal, corn meal and copra meal were all highly digestible to mud crabs while Tuan *et al.* (2006) reported that the protein digestibility of blood meal and soybean meal was not significantly different to that of fishmeal.

In a recent study, Pavasovic *et al.* (2004) demonstrated that mud crabs have a relatively high capacity to digest purified carbohydrates derived from plants. Specifically, high digestibility coefficients were obtained for soluble cellulose derivatives and starch in formulated feeds. The inclusion of digestible carbohydrate has already been recommended for diets formulated for carnivorous fish and some crustacean species (Wilson 1994; Catacutan and Coloso 1997; Kaushik 2001). Nevertheless, the digestibility of plant based materials such as starch can vary with botanical origin and treatment. Furthermore, the inclusion of starch in aquafeeds can affect the digestibility of other dietary elements, such as protein, which are essential for growth (Cousin, Cuzon and Guillaume 1996: Sales and Britz 2002; Stone, Allan and Anderson 2003). As a consequence, digestibility coefficients for plant-based carbohydrates should be determined before they are included routinely in formulated diets for candidate species.

The objective of the current study was to evaluate the potential of a range of feed ingredients for use in diets formulated for *S. serrata*. In the first part of this study, digestibility coefficients for selected commercial animal feed meals were determined. Feed meals tested were derived from either terrestrial animal (meat or

poultry) plant (soybean, lupin, canola or cotton seed) or single cell (yeast) sources. The aim of the second part of the study was to examine the digestibility of experimental diets containing selected plant-based starches. Specifically, digestibility coefficients were determined for fishmeal-based formulated diets containing different levels (15%, 30%, 45% or 60%) or types (wheat, potato, rice or corn) of purified starch.

2. Materials and Methods

2.1 Animals

Experimental animals were supplied by Bribie Island Aquaculture Research Centre Station (BIARC), Bribie Island, QLD Australia. For all experimental treatments, crabs were supplied with recirculated, aerated seawater that was gravity fed through an electrically heated overhead tank. Water temperature was maintained within a range (27.5 + 0.5^{o}C) suggested for optimal growth of mud crabs.

2.2 Study 1: Digestibility of Selected Feed Meals

A digestibility trial was conducted to evaluate the digestibility coefficients of Brewer's yeast (BY) (Swift and Co, Australia) and selected animal feed meals: South American fish meal (FM) (Ridley Aqua Feed, Australia), meat and bone meal (MBM) (Southern Meats, Australia), poultry meal (PM) (AJ Bush, Australia), lupin meal (LM) (MC Croker, Australia), soybean meal (SBM) and canola meal (CM) (Radford Park Aquafeed, Australia). The reference diet was used as a control which consisted of a commercial *P.monodon* diet (Turbo, Thailand). Experimental diets were formulated by combining test ingredients and the reference diet in a 30%:70% ratio, on a dry weight basis. A complete list of ingredients for all test diets is presented in Table 1. The proximate nutrient content of the reference diet and test ingredients shown in Table 2. Proximate composition of diets

and faecal material were determined at the Animal Research Institute (Brisbane, Australia) according to AOAC protocols (1984).

Diets used in the experiment were prepared by thoroughly mixing dry ingredients, followed by wet ingredients, until a crumbly dough consistency was achieved. Diet mixture was pressure pelleted using a meat grinder with a 3mm die. Pellets were steamed in a rice steamer in a microwave oven (Sanyo) for 10 min, prior to drying at 50°C in a drying oven, overnight. All experimental diets were stored at -20°C until required. All diets contained 0.5% Chromic oxide (Cr_2O_3) as an inert indicator to allow the calculation of digestibility coefficients for dry matter (ADMD), crude protein (ACPD) and energy (AGED) using formulae suggested by Jones and De Silva (1997). Pilot studies determined that there was no significant loss of detectable chromium (Cr) in feed pellets immersed in water at 26^{o}C for 1h (data not shown).

At the commencement of this study, crabs were selected that had an average body weight of 89g $\pm$ 18g. These crabs were then assigned randomly into ten groups and housed individually in plastic containers (19.5cm x 28cm x 22cm). Crabs were kept in these containers for one week to acclimate to the culture conditions prior to the start of the experiment and fed a commercial prawn diet (Turbo; for analysis see Table 2). Nine dietary treatments (n=12 crabs / treatment) were utilised in this study (Table 1). Crabs were fed experimental diets twice daily at a feeding rate of 5% body weight (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected. A daily record was kept of mortalities in each test group. To allow crabs sufficient time to acclimate to experimental diets, faecal collection did not commence until day 7 of the experiment. Faecal material at the bottom of the tank was collected by syphoning into a plastic sieve and gently rinsed for one minute in distilled water before removing individually using forceps. To collect sufficient material for analysis, faecal material from three crabs in each treatment was pooled (n=4 / treatment). All samples were lyophilized and stored at -20°C until required for analysis.

The indirect method of Furukawa and Tsukahara (1966) was used to calculate the digestibility coefficients of all diets tested. Briefly, 0.5g of feed or faecal material was added to 4.0mL of concentrated nitric acid (AnalaR grade, 16 M HNO_3) and incubated overnight at room temperature. Samples were then heated to 150^oC for an additional 60 min. After cooling, samples were mixed with 5.0mL of concentrated perchloric acid (AnalaR grade, 70% $HClO_4$) then heated to 220^oC for 30 min and 245^oC for a further 30 min. After cooling, the absorbance of each sample was read at 346.5nm. For calibration purposes, the above protocol was repeated using known quantities of Cr_2O_3.

Coefficients for ADMD were determined using the formula:

ADMD = 100 – 100 (%Cr_2O_3 in feed / % Cr_2O_3 in faeces).

Coefficients for crude protein (ACPD) or gross energy (AGED) were determined using the formula:

APD = 100 – 100 [(%Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% protein or Mj/kg energy in faeces/ % protein or Mj/kg energy in feed)].

Apparent digestibility coefficients (ADC) of the test ingredients were calculated using the following equations described by Bureau, Harris and Cho (1999).

$ADC_I = ADC_T + ((1 – s)\ D_R / s\ D_I)\ (ADC_T – ADC_R)$; where: ADC_I = apparent digestibility coefficient of test ingredient; ADC_T = apparent digestibility coefficient of test diet; ADC_R = apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; *s* = proportion of test ingredient in test diet mash (i.e. 0.3 in this study); (1 – *s*) = proportion of reference diet mash in test diet mash (i.e. 0.7 in this study).

2.3 Study 2: Impact of starch on diet digestibility

As described previously, other workers have recommended the inclusion of plant-based carbohydrates in formulated diets to supply energy for aquatic animal

species. A second digestibility trial was therefore conducted in this study to assess the impact of starch included in formulated mud crab diets based on fishmeal. Adult sibling crabs (164.3 ± 27.7g) of the population used for study 1 were selected from a grow out recirculating cellular system at Bribie Island Aquaculture Research Centre for this trial. Each individual crab was weighed at the start and end of each experiment. Crabs were assigned randomly into nine groups with twelve crabs in each group and held in individual containers (19.5cm x 28cm x 22cm) which were covered by plastic net lids. Crabs were allowed to acclimate to experimental conditions and diets as described previously (2.2).

To ensure dietary protein levels in all test diets were set above those reported to promote good growth rates in culture (Catacutan 2002), a reference diet based on high quality South American fishmeal was formulated (RF Diet; Table 3). Eight other diets were also formulated where fishmeal in the reference diet was replaced with different amounts (15%, 30%, 45% or 60%) or types (wheat, corn, rice and potato) of purified starch (Sigma product). Diets used in the experiment were prepared as described previously (2.2), with the exception of the WSU45 Diet (45% wheat starch) which was not steam cooked (uncooked) prior to drying. All diets contained 0.5% Cr_2O_3 as an inert indicator to allow calculation of apparent nutrient digestibility coefficients.

Faecal material was collected as described previously (2.2) after which proximate composition of diets and faecal material was determined at the Animal Research Institute (Brisbane, Australia) according to AOAC protocols (1984). Proximate composition of diets is shown in Table 4. A total starch assay (amyloglucosidase/ α-amylase method, AOAC method 996.11) was also performed on all diets and faecal material collected. Briefly, 100mg of sample was

wetted with 0.2mL of aqueous ethanol (80%v/v) to aid dispersion, stirred on a vortex mixer and then immediately 3mL of thermostable α-amylase (300Units) in MOPS buffer (50mM, pH 7.0) was added; vigorously stirred on a vortex mixer. The tube was then incubated in a boiling water bath for 6 minutes, placed in a bath at 50^{o}C, then sodium acetate buffer (4mL, 200mM, pH 4.5) was added. This was followed by addition of amaloglucosidase (0.1mL, 20U), stirred on a vortex mixer and incubated at 50^{o}C for 30 min. The test tube was mixed thoroughly and the volume adjusted to 10mL with distilled water, then samples centrifuged at 3000g for 10 minutes. Duplicate aliquots (0.1mL) of this solution were transferred to the bottom of glass test tubes and 3.0mL of glucose oxidase-peroxidase (GOPOD) reagent was added to each tube (including the glucose controls and reagent blanks). The tube was then incubated at 50^{o}C for 20 min. A glucose control consisted of 0.1mL of glucose standard solution (1gL-1) and 3.0mL of GOPOD reagent. A reagent blank solution consisted of 0.1mL of distilled water and 3.0mL of GOPOD reagent. Absorbance of the supernatant solution and the glucose control was read against the reagent blank at 510nm using a Novospec spectrophotometer (LKB). Values of starch contained in diets and faecal were calculated according to the following formula:

Calculation of starch = Δ E x F x 1000 x 1/1000 x 100/W x 162/180

= Δ E x F/W x 90

Starch % (Dry weight basis) = Starch % (as is) x 100/ [100 – moisture content (%)]

Where:

Δ E = Absorbance (reaction) read against the reagent blank

F = 100 (µg of glucose)/ absorbance of 100µg of glucose

1000 = Volume correction (0.1mL taken from 100mL)

1/1000 = Conversion from micrograms to milligrams

100/W = Factor to express “starch” as a percentage of sample weight

W = Weight in milligrams (“as is” basis) of the sample

162/180 = Adjustment from free glucose to anhydro glucose (as occurs in starch)

2.4 Statistical analyses

The significance of data were determined by one- way ANOVA (SPSS version 13.0) and *post hoc* comparison by Tukey’s HSD. For all analysis the significance level of $p<0.05$ was used as standard.

3. Results

3.1 Digestibility of Selected Feed Meals

Table 5 displays the apparent digestibility coefficients for dry matter, crude protein and gross energy obtained using selected animal feed meals. Overall, the ADMD coefficients for most feed meals tested were not significantly different from the value obtained for fishmeal, ranging from 79% to 88%. The ADMD value of meat meal, however, was significantly lower ($p<0.05$) than those obtained for all other test ingredients. ACPD coefficients for all feed meals tested were relatively high, with values ranging from 86% to 97%. Interestingly, the ACPD value for yeast was significantly higher ($p<0.05$) than those obtained for all other test ingredients. Cotton seed meal, poultry meal, canola meal, fishmeal, soybean meal and lupin meal had AGED values that were not significantly different ($p>0.05$) from one another, ranging from 84% to 89%. The AGED coefficient for meat meal, however, was significantly less ($p<0.05$) than values obtained for all other ingredients except cotton seed meal and poultry meal. By contrast, the highest value for AGED was obtained for yeast which was significantly higher ($p<0.05$) than those obtained for meat meal, cotton seed meal and poultry meal. Survival rates for all treatments were high, ranging from 92% to 100% (data not shown).

3.2 Impact of starch on diet digestibility

Apparent digestibility coefficients for dry matter, crude protein, gross energy and starch for diets tested in this study are presented in Table 6. ADMD values ranged from 72% to 85.3%. Inclusion of wheat, potato, rice and corn starch in cooked diets had no significant impact on dry matter digestibility when compared with the reference diet (RF). By contrast, the inclusion of 45% wheat starch in the uncooked diet (WSU45) significantly increased dry matter digestibility. Apparent digestibility of crude protein was relatively high for all treatments, ranging from 86 % to 92%. Interestingly, ACPD values for diets containing rice starch (RS30), corn starch (CS30) or uncooked wheat starch (WSU45) were significantly higher ($p<0.05$) than the values obtained for all other experimental diets

containing between 15% and 60% starch. AGED values for test diets ranged from 73% to 92%. The highest value obtained was for the uncooked diet containing wheat starch (WSU45) which was significantly higher (p<0.05) than for all other experimental diets, excepting diets RF and WS30. By contrast, the AGED value obtained for the diet containing potato starch (PS30) was significantly lower (p<0.05) than the values obtained for all other test diets. A consistent and significant (p<0.05) decline in starch digestibility in cooked diets was recorded as the level of wheat starch included was progressively raised to a maximum of 60% (WS60). The type of starch incorporated into test diets also appeared to significantly impact on ASD values. Specifically, at a 30% inclusion level in cooked diets the apparent digestibility of starch was in the following order (from most to least digestible) corn > wheat > rice = potato. Interestingly, for all parameters tested, significantly higher (p<0.05) ASD values were obtained for the diet containing 45% wheat starch which was not cooked (WSU45) than was obtained using the same amount of wheat starch in the cooked diet (WS45).

4. Discussion

Many plants and animal-based feed ingredients have been reported to have potential as replacement components for fishmeal in formulated aquafeeds (Tacon 1994). In the present study, we observed relatively high digestibility coefficients for a broad range of animal, plant and single sell- based ingredients, reflecting the ability of mud crabs to utilize a wide range of nutrient sources. These findings are consistent with other studies reporting high digestibility coefficients for a wide variety of animal and plant-based ingredients in mud crab diets (Catacutan *et al*. 2003, Tuan *et al*. 2006). In the current study, the ADMD coefficients for most high protein feed meals tested were similar, with the exception of meat meal. Similar results using some terrestrial animal-based meals in diets for mud crab have been reported by Catacutan *et al.* (2003) and Tuan *et al.* (2006). Reduced digestibility coefficients have also been associated with use of meat meal in *C. destructor* (Jones and De Silva 1997) and *P. setiferus* (Brunson, Romaire and Reigh 1997).

Protein digestibility for all feed meals was relatively high, with values over 85%. This finding is in general agreement with the findings of Catacutan *et al.* (2003) who observed high ACPD in adult mud crab fed a range of animal and plant-based ingredients. The high capacity of mud crabs to digest protein is not surprising considering the high level of protease in the digestive system of this species (Pavasovic *et al*. 2004). Based on the findings of Pavasovic *et al.* (2004) and the current study we argue that the mud crab has a high capacity to digest protein in a wide range of single cell, terrestrial animal and plant-based ingredients. Further studies which attempt to exploit the potential of these feed ingredients to replace fishmeal as a source of dietary protein may help reduce feed costs for this species.

In the second part of this study the digestibility of different types and amount of starches in formulated mud crab diets was tested. Overall, digestibility coefficients for starch and the associated diets were high. This finding is in close agreement with many other investigations which have reported that cereals containing high levels of starch are readily digested by crustaceans (Shi-Yen and Chu-Yang 1992; Cousin *et al*. 1996). The high digestibility of starch demonstrated in this study is not surprising considering the detection of significant carbohydrase activity in the mud crab digestive system (Pavasovic *et al*. 2004). Specifically, amylase, cellulase and xylanase activity has been detected in extracts prepared from the mud crab midgut gland suggesting a significant capacity to digest plant-based nutrients. Furthermore, plant-based material and detritus has been demonstrated in the digestive system of mud crab juveniles sampled from the wild (Hill 1976; Prasad and Neelakantan 1988).

Another important finding of the current study was that diet digestibility was affected significantly by the origin of starch. These results are in close agreement with other study that have shown the digestibility of starch varies with botanical origin (Cousin *et al*. 1996; Sales and Britz 2002; Stone *et al*. 2003). The cooking process also had a significant impact on the digestibility of mud crab diets in the current study. Specifically, the wheat starch diet prepared without steam cooking (WSU45) demonstrated higher digestibility coefficients than the equivalent diet that was steam cooked (WS45). Such differences are not unexpected since there is abundant evidence that cooking processes can have a dramatic impact on the performance of artificial diets for vertebrates (cf. Singh et al, 2007). We suggest that the steam cooking process used in the current study may increase the degree of starch gelatinisation in mud crab diets. Elsewhere it has been shown that

changes in the gelatinisation of carbohydrate-rich ingredients in artificial diets can significantly alter diet performance parameters such as apparent total tract digestibility (Vicente et al, 2008) intestinal viscosity, feed intake and body weight gain (Garcia et al, 2008). Based on such findings, we recommend that further studies be conducted to assess how different diet processing methods effect the gelatinisation of starch in formulated mud crab diets and if the degree of starch gelatinisation impacts on growth performance.

In the current study it was shown that the digestibility of corn starch was generally higher than that for wheat starch (at 30% inclusion level). This contradicts the findings of Davis and Arnold (1993) who reported that in other crustacean species wheat was more efficiently digested than corn. The reason for this apparent discrepancy is unclear but it may reflect species specific differences in the capacity to digest carbohydrates from different sources or differences in the purity or preparation of carbohydrates incorporated into crustacean feeds.

The findings of the second part of this study suggest that starch should be considered as a potential feed ingredient in formulated mud crab diets. Overall, most diets containing starch were readily digested. In particular, there were no negative impacts on the digestibility of major nutrients (e.g. protein) observed following the inclusion of wheat, rice or corn starch in formulated feeds. These results argue that further studies are warranted to investigate the potential of starch to supply energy in mud crab diets and reduce the requirements for more expensive feed ingredients such as fishmeal.

Acknowledgements

The authors would like to thank David Mann, Marko Pavasovic, Tom Asakawa and Beverly Kelly at BIARC for their technical assistance. This study was supported by Ministry of Education and Training, Vietnam and ACIAR Mud Crab Project FIS/2000/065, Australia. Astaxanthin was kindly provided by Dr Linda Browning of DSM Nutritional Products (Australia). Our gratitude is also extended to Dr Richard Smullen of Ridley Aqua-Feed (Australia) for supplying the fishmeal and John Harsant of Radford Park Aquafeeds (Australia) for supplying the soybean, canola and cotton seed meals. This manuscript is written in partial fulfillment of the Doctor of Philosophy degree in aquaculture of Phuong Ha Truong, at the Queensland University of Technology, Brisbane, Australia.

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Table 1: Composition (% dry matter of the diet) of the formulated diets for the digestibility trial using commercial feed ingredients.

	Diet								
Ingredient	1	2	3	4	5	6	7	8	9
Basal Diet (Turbo)	62.4	62.4	62.4	62.4	62.4	62.4	62.4	62.4	92.4
Fishmeal	30								
Meat meal		30							
Poultry meal			30						
Soybean meal				30					
Canola meal					30				
Lupin meal						30			
Cotton seed meal							30		
Yeast								30	
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Common ingredients[a]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6

[a] Common ingredients (g/100g): mineral and vitamin premix (2%), (kg $^{-1}$ of total diet - 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1750 mg riboflavin; 1125 mg pyridoxine hydrochloride; 3750 mg cyanocobalamin; 25000 mg ascorbic acid; 500 000 mg colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin); astaxanthin (0.1%); chromic oxide (Cr_2O_3) (0.5%).

Table 2: Proximate nutrient composition (%) in dry matter of basal diet and test ingredients used in the digestibility trial.

Ingredient	Dry Matter	Crude Protein (N x 6.25)	Crude Fat	Ash	Energy (Mj/kg)
Basal Diet; Turbo *P.monodon* feed	90.4	49.7	6.7	15.3	19.1
Fishmeal	91.7	75.5	8.7	17.2	15.9
Meat meal	97.7	59.6	13.4	20.4	16.9
Poultry meal	96.7	69.2	13.1	14.1	20.9
Soybean meal	88.3	53.2	1.9	7.2	20.6
Canola meal	90	44.1	3.8	7.4	21.5
Lupin meal	86.1	30.8	9.4	3.6	15.9
Cotton seed meal	89.2	48.4	2.4	7.3	19.3
Yeast	95.1	48.6	0.4	9.6	18.3

Table 3: Composition (% dry matter of the diet) of the formulated diets for the digestibility trial using different starches.

Ingredient (%)	Diet								
	RF[a]	WS15	WS30	WS45	WSU45	WS60	PS30	RS30	CS30
Fish meal	87.4	72.4	57.4	42.4	42.4	27.4	57.4	57.4	57.4
Wheat starch (WS)	0	15	30	45	45[b]	60			
Potato starch (PS)							30		
Rice starch (RS)								30	
Corn starch (CS)									30
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Common ingredients [c]	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6

[a] Reference diet

[b] Prepared without steam cooking (Uncooked)

[c] Common ingredients (g/100g): mineral and vitamin premix (2%), (kg $^{-1}$ of total diet - 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1750 mg riboflavin; 1125 mg pyridoxine hydrochloride; 3750 mg cyanocobalamin; 25000 mg ascorbic acid; 500 000 mg colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin); dried squid (5%); astaxanthin (0.1%); chromic oxide (Cr_2O_3) (0.5%).

Table 4: Proximate nutrient composition (%) in dry matter of experimental diet used in the starch digestibility trial.

Diet	Dry matter	Protein (Nx6.25)	Crude fat	Ash	Starch	Gross Energy ($MJkg^{-1}$)	P/E ratio
RF	93.7	70.5	8.7	15.4	1.3	20.6	3.42
WS15	93.3	63.6	7.5	13.0	14.9	20.6	3.09
WS30	92.8	53.2	6.3	10.6	31.2	19.9	2.67
WS45	90.8	41.8	5.1	8.2	48.5	19.2	2.18
WSU45	92.3	41.9	5.1	8.2	47.6	19.3	2.17
WS60	87.6	33.9	3.9	5.8	60	19.0	1.78
PS30	89.0	49.1	6.3	10.6	33.2	19.7	2.49
RS30	93.4	53.3	6.3	10.6	28.6	19.9	2.68
CS30	93.8	53.6	6.3	10.6	29.8	19.9	2.69

Table 5: The apparent digestibility coefficients (%) of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) for yeast and selected animal feed meals.

Ingredient	ADMD	ACPD	AGED
Basal Diet; Turbo prawn feed	83.2 + 0.5 ^{b}	90.4 + 0.5 ^{ab}	89.3 + 0.9 ^{cd}
Fishmeal	85.4 $\pm$ 1.7^{b}	88.3 $\pm$ 0.7^{ab}	87.8 $\pm$ 1.3^{cd}
Meat meal	67.0 $\pm$ 1.3^{a}	86.3 $\pm$ 0.9^{ab}	78.2 $\pm$ 0.8^{ab}
Poultry meal	78.9 $\pm$ 3.0^{b}	88.2 $\pm$ 2.1^{ab}	85.2 $\pm$ 1.9^{bc}
Soybean meal	80.4 $\pm$ 1.2^{b}	91.7 $\pm$ 0.5^{bc}	89.1 $\pm$ 0.9^{cd}
Canola meal	83.5 $\pm$ 4.7^{b}	87.6 $\pm$ 2.7^{ab}	87.5 $\pm$ 2.9^{cd}
Lupin meal	88.1 $\pm$ 1.6^{b}	89.1 $\pm$ 0.9^{ab}	89.9 $\pm$ 1.4^{cd}
Cotton seed meal	80.5 $\pm$ 0.8^{b}	86.8 $\pm$ 0.6^{ab}	83.9 $\pm$ 0.4^{abc}
Yeast	85.7 $\pm$ 3.2^{b}	96.8 $\pm$ 1.6^{c}	93.5 $\pm$ 1.7^{d}

Values are means ± standard error (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different (p>0.05) from one another

Table 6: Impact of starch on apparent digestibility coefficients (%) for dry matter (ADMD), crude protein (ACPD), gross energy (AGED) and starch (ASD) in fishmeal-based formulated mud crab diets

Diet	ADMD	ACPD	AGED	ASD
RF	75.4 ± 1.9^{ab}	89.2 ± 0.8^{abcd}	86.0 ± 1.1^{def}	97.0 ± 0.2^{h}
WS15	72.0 ± 2.8^{ab}	86.5. ± 1.1^{ab}	83.7 ± 1.4^{de}	92.8 ± 0.1^{f}
WS30	75.2 ± 1.2^{ab}	88.0 ± 0.8^{abc}	87.7 ± 0.7^{ef}	90.0 ± 0.1^{de}
WS45	77.6 ± 0.3^{bc}	86.7 ± 0.1^{ab}	81.4 ± 0.7^{bcd}	87.2 ± 0.2^{b}
WSU45	85.3 ± 0.4^{d}	91.9 ± 0.5^{d}	91.8 ± 2.0^{f}	91.1 ± 0.1^{e}
WS60	80.7 ± 0.6^{bcd}	86.9 ± 0.4^{ab}	82.7 ± 0.9^{cde}	84.4 ± 0.4^{a}
PS30	76.4 ± 1.2^{abc}	88.0 ± 0.9^{abc}	73.1 ± 1.6^{a}	88.5 ± 0.1^{c}
RS30	83.0 ± 2.5^{bcd}	91.6 ± 1.3^{d}	84.8 ± 1.5^{de}	88.5 ± 0.1^{c}
CS30	83.4 ± 0.9^{bcd}	91.6 ± 0.6^{d}	85.2 ± 1.1^{de}	92.5 ± 0.1^{f}

Values are means ± standard error (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different (p>0.05) from one another

Aquaculture Research

Aquaculture Research, 2009, **40**, 322–328 doi: 10.1111/j.1365-2109.2008.02095.x

Apparent digestibility of selected feed ingredients in diets formulated for the sub-adult mud crab, *Scylla paramamosain*, in Vietnam

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Apparent digestibility of selected feed ingredients in diets formulated for the sub-adult mud crab, *Scylla paramamosain* in Vietnam

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Key words: Mud crab, *Scylla paramamosain*, digestibility, plant ingredients

Abstract

The present study was conducted to explore the potential to incorporate local plant-based feed ingredients into diets formulated for the mud crab species, *Scylla paramamosain* commonly exploited for aquaculture in Southeast Asia. Four test ingredients (defatted soybean meal, rice bran, cassava meal and corn flour) were incorporated at 30% or 45% inclusion levels in a fishmeal-based reference diet, and used in digestibility trials where apparent digestibility coefficients (ADCs) for experimental diets and test ingredients were determined. Generally, high ADC values were obtained using diets containing 30% soybean meal or rice bran. By contrast, the lowest ADC values were obtained for the diet containing 45% cassava meal (70.9% ADMD; 77.1% ACPD; 80.2% AGED). Similar trends were observed when ADC ingredient (I) digestibilities were compared. Specifically, the highest ADCI values were obtained for soybean meal when used at a 30% inclusion level (87.6% ADMDI; 98.4% ACPDI; 95.6% AGEDI) while the lowest ADCI values were obtained using cassava meal at a 45% inclusion level (53.8% ADMDI; 60.2% ACPDI; 67.3% AGEDI). Based on the current findings we propose that soybean meal and rice bran could be considered for incorporation into formulated diets for *S.paramamosain*.

Introduction

Mud crabs of the genus *Scylla* are commercially important in several Indo-Pacific countries and they provide an important source of income and fresh food for many coastal fishing communities (Keenan 1999). Currently, mud crab farming is well established throughout Southeast Asia with most mud crab farmers using trash fish, bivalve meats or animal by-products as feeds. This traditional feeding practice, however, is now considered unsustainable and the development of formulated low cost grow out diets is widely viewed as a priority issue for mud crab aquaculture (William & Abdullah 1999; Edwards, Tuan & Allan 2004; Christensen, Macintosh & Phuong 2004; Fielder 2004; Tuan, Anderson, Luong-van, Shelley & Allan 2006).

Consideration of any feed ingredient for incorporation into aquafeeds requires data on the target species capacity to digest and absorb it. Several recent studies have confirmed that the mud crab species *Scylla serrata* has a significant capacity to utilise feed ingredients from a variety of terrestrial animal or plant-based sources. In particular, many plant-based ingredients have been evaluated for their potential to be incorporated into aquafeeds for this species. For example, Catacutan, Uusebio & Teshima (2003) demonstrated that soybean meal, corn meal and copra meal were all highly digestible in diets formulated for *S. serrata*. Tuan *et al.* (2006) also reported apparent digestibility values for soybean meal in diets formulated for *S. serrata* which were not significantly different to those obtained using fishmeal. Likewise, Truong, Anderson, Mather, Paterson & Richardson (2008a) reported high apparent digestibility coefficients for soybean, canola, lupin and cottonseed meals incorporated into diets formulated for this species. In addition, the study of Truong *et al.* (2008a) demonstrated that the inclusion of wheat, corn or rice starch did not significantly reduce the apparent protein and energy digestibility values of formulated *S.serrata* diets.

The present study was carried out to determine the capacity of the mud crab species commonly cultured in Vietnam, *Scylla paramamosain,* to digest formulated diets

containing selected, locally available plant-based ingredients (defatted soybean meal, rice bran, cassava meal and corn flour). Apparent digestibility coefficients for dry matter, crude protein and gross energy were determined for test ingredients and diets containing test ingredients. The effect of varying the inclusion level of test ingredients on nutrient digestibilities was also examined.

Materials and Methods

Experimental site and animals

The experiment was carried out at Research Institute for Aquaculture No 3 (RIA3), Nha Trang, Vietnam from 20 November 2006 to 4 January 2007 with hatchery reared sub-adult mud crabs (94.1± 1.1g) collected from a pond at RIA3. Crabs were fed a commercial diet (Turbo, C-P Feeds, Thailand) twice daily at a feeding rate of 3% body weight for a week to acclimate to experimental conditions.

Culture system

Crabs were assigned randomly into nine groups with twelve crabs in each group and held in black individual containers (19cm x 28cm x 21cm) which were covered by plastic net lids. For all experimental treatments, crabs were supplied with recirculated, aerated seawater. During the experimental period, temperature, salinity, pH, and dissolved oxygen of water were maintained at 27.5 ± 0.5^{o}C, 27.5 ± 1.5g L^{-1}, 7.67±0.09 and 4.26±0.18mg L^{-1}, respectively.

Diet preparation

Diets used in the experiment were prepared by thoroughly mixing dry ingredients, followed by wet ingredients, until a crumbly dough consistency was achieved. All diets contained 0.5% Chromic oxide (Cr_2O_3) as an inert indicator to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED). Pilot studies determined that there was no significant loss of detectable chromium (Cr) in feed pellets immersed in water at 26^{o}C for 1h (data not shown).

Diet mixture was pressure pelleted using an electronic mincer with a 3mm die. Pellets were steamed in a rice steamer in a microwave oven (LG) for 5 min, prior to drying overnight at 50 °C in a drying oven. After drying, diet strands were cut into 8-10mm strands. All experimental diets were stored at -20°C until required.

To ensure dietary protein levels in all test diets were set above those reported to promote good growth rates in culture (Catacutan 2002), a reference diet (RF) based on high quality South American fishmeal was formulated (Table 1). Eight other diets were also formulated where fishmeal in the reference diet was replaced with different amounts (30% and 45%) of rice bran, cassava meal, corn flour or defatted soybean meal.

Feeding and faecal collection

Crabs were fed experimental diets twice daily at a feeding rate of 3% body weight (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected. A daily record was kept of mortalities in each test group. Faecal material at the bottom of the tank was collected by syphoning into a plastic sieve, then rinsed gently for one minute in distilled water and removed individually using forceps. To collect sufficient material for analysis, faecal matter from three crabs in each treatment was pooled (n=4 / treatment). All samples were lyophilized and stored at -20°C until required for analysis.

Chemical analysis and calculations

The proximate nutrient content of experimental diets is shown in Table 2. Proximate composition of diets and faecal material were determined at Nha Trang Fisheries University, Vietnam, following AOAC standards (1984). Cr content of diets and faecal material used in calculating apparent digestibility values were determined using the method described by Furukawa & Tsukahara (1966). Apparent dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) digestibilities were calculated using equations described by Jones & De Silva (1998):

ADMD = 100 – 100 (%Cr_2O_3 in feed / % Cr_2O_3 in faeces).

Digestibilities of crude protein (ACPD) or gross energy (AGED) were determined using the formula:

APD = 100 – 100 [(%Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% protein or MJ kg^{-1} energy in faeces/ % protein or MJ kg^{-1} energy in feed)].

Apparent digestibility coefficients (ADC) of test ingredients were calculated using equations described by Bureau, Harris & Cho (1999).

$ADC_I = ADC_T + ((1 - s)\ D_R\ /\ s\ D_I)\ (ADC_T - ADC_R)$; where: ADC_I = apparent digestibility coefficient of test ingredient; ADC_T = apparent digestibility coefficient of test diet; ADC_R = apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; *s* = proportion of test ingredient in test diet mash (i.e. 0.3 and 0.45 in this study); (1 – *s*) = proportion of reference diet mash in test diet mash (i.e. 0.7 and 0.55 in this study).

Statistical analyses

The significance of data were determined by one- way ANOVA (SPSS version 13.0) and *post hoc* comparison by Tukey's HSD. For all analysis the significance level of $p<0.05$ was used as standard.

Results

Digestibility determinations: Experimental diets

Apparent digestibility coefficients for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) obtained using experimental diets are presented in Table 3. ADMD coefficients ranged from 70.9% to 85.7%. The highest ADMD value was obtained using the diet containing 30% soybean meal (SBM30) which was significantly higher ($p<0.05$) than those obtained using any other diet except that containing 30% rice bran (RB30) or the reference (RF) diet. By contrast, the lowest ADMD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly less ($p<0.05$) than those obtained for all other experimental diets. It was also shown that incorporation of more than 30% soybean or cassava meal into experimental diets resulted in a significant reduction ($p<0.05$) in ADMD values.

ACPD coefficients obtained using experimental diets ranged from 77.1% to 93.2%. ACPD coefficients for diets containing soybean meal (SBM30, SBM45) or 30% rice bran (RB30) were either significantly higher ($p<0.05$) or equivalent to those obtained using the fishmeal-based reference (RF) diet. By contrast, the lowest ACPD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly lower ($p<0.05$) than those obtained using any other experimental diet. Furthermore, it was demonstrated that if the level of any test ingredient incorporated into experimental diets was increased from 30% to 45%, there was a significant reduction ($p<0.05$) in the ACPD value of the diet.

AGED coefficients for experimental diets were generally high and ranged from 80.2% to 92.2%. The highest AGED value was obtained using the diet containing 30% soybean meal (SBM30) which was significantly higher ($p<0.05$) than those obtained using other experimental diets except the diet containing 30% rice bran (RB30) or the reference

(RF) diet. By contrast, the lowest ACPD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly lower ($p<0.05$) than that obtained from other experimental diets. It was also demonstrated that if the level of rice bran, cassava meal or soybean meal incorporated into diets was increased from 30% to 45% there was a significant reduction ($p<0.05$) in the AGED value of the diet.

Digestibility determinations: Test ingredients

Apparent dry matter (ADMDI), crude protein (ACPDI) and gross energy (AGEDI) digestibility coefficients calculated for specific feed ingredients are presented in Table 4. The highest ADMDI value (87.6%) was obtained for soybean meal (at 30% inclusion level) which was significantly higher ($p<0.05$) than values obtained for all other test ingredients. By contrast, ADMDI values obtained for cassava meal were significantly lower than those obtained for all other test ingredients. ACPDI values were obtained for soybean meal (at any inclusion level) and rice bran (at a 30% inclusion level) that were significantly higher ($p<0.05$) than those obtained for any other test ingredient. . By contrast, the ACPDI value obtained for cassava meal (at 45% inclusion level) was significantly lower than those obtained for all other test ingredients. The ingredient with the highest AGEDI value (95.6%) was soybean meal, when used at a 30% inclusion level, while values obtained using cassava meal were significantly lower ($p<0.05$) than those obtained for any other test ingredient.

Discussion

In a previous study, we demonstrated that *S. serrata* has a high capacity to digest a range of plant based feed ingredients (Truong *et al*. 2008a). In the present study we have extended these investigations and examined the potential to incorporate local plant based feed ingredients into diets formulated for the mud crab species commonly cultured in Southeast Asia (*S.paramamosain)*. In agreement with our previous finding on *S. serrata*, *S.paramamosain* demonstrated a high capacity to digest soybean meal at inclusion levels up to 45%. These results are consistent with the findings of Catacutan *et al*. (2003) who reported that ADC values for dry matter and crude protein in diets formulated for *S.serrata* were 90.9% an 95.5%, respectively. Likewise, Tuan *et al.* (2006) reported that ADC coefficients for dry matter, energy and crude protein for soybean meal were relatively high with values of 95.7%, 97.1% and 97.9% respectively.

In this study, at a 30% inclusion level rice bran demonstrated generally high ADC values. This finding is in close agreement with the observations of Catacutan *et al*. (2003) who demonstrated that dry matter and crude protein digestibility coefficients for rice bran in *S.serrata* were relatively high (89% and 94% respectively). In a related study, Truong *et al.* (2008a) also reported high ADC coefficients for rice-based ingredients incorporated into diets formulated for *S.serrata.* Furthermore, incorporation of rice starch into fishmeal-based diets formulated for juvenile *S.serrata* did not appear to significantly reduce growth performance (Truong, Anderson, Mather, Paterson & Richardson. 2008b). Based on the above findings, and those of the current investigation, we suggest that rice-based products, such as bran and starch, are generally well digested and should be investigated further for their potential to be incorporated into aquafeeds formulated for *Scylla* species.

A key finding of this study was that several of the ADC values for corn flour were significantly less than those obtained for rice bran or soybean meal. This result is surprising considering that Truong *et al.* (2008a) reported that *S.serrata* demonstrated a high capacity to digest diets containing corn starch. Likewise, Catacutan *et al.* (2003)

reported high ACPD (96.4%) and ADMD (93.2%) coefficient values for *S.serrata* diets containing 30% corn flour. A possible explanation for these apparent discrepancies is that there may be significant differences in the capacity of the various *Scylla* species to digest corn-based ingredients. Alternatively, the capacity of mud crabs to digest corn-based ingredients may be influenced by the source or preparation of the corn-based ingredient.

Relatively poor ADC values were obtained using diets containing cassava meal. This result is in contrast to other studies which have examined the potential of cassava meal for incorporation into crustacean aquafeeds. For example, Gomes & Pena (1997) reported that inclusion of 30% heated cassava meal in diets formulated for *Macrobrachium rosenbergii* did not significantly reduce digestibility coefficients for protein and energy. The relatively poor digestibility of cassava meal in diets formulated for mud crabs might be a consequence of the presence of toxic factors in this ingredient. Specifically, Oboh & Akindahunsi (2005) reported that rats fed a diet containing 40% cassava meal had a significant rise in the serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase activity indicating possible damage to the liver and/or heart. Further studies will be required to determine what factors may be inhibiting the digestion of cassava meal in *S.paramamosain* and if the relatively poor digestibility of this ingredient is consistent across other *Scylla* species.

An important observation of the current study was that several ADC values for diets and test ingredients were significantly reduced when ingredient inclusion levels were raised. For example, the ACPDI values for rice bran and cassava meal were reduced by approximately 9% and 15%, respectively, when the dietary inclusion level was raised from 30% to 45%. These findings are consistent with those of other workers who have reported increased incorporation of plant-based materials can impact negatively on the digestibility of aquaculture diets. For example, Hansen, Rosenlund, Karlsen, Olsvik & Hemre (2006) reported that plant-based ingredients reduced protein and fat digestibility in formulated cod diets. Likewise, alpha-cellulose reduced the digestibility of diets formulated for the

shrimp *Macrobrachium rosenbergii* (Gonzalez-Pena, Anderson, Smith & Moreira 2002). By contrast, other workers have demonstrated that increased incorporation of plant-based materials can improve aquaculture diet digestibility. For example, Bautista-Teruel, Eusebio & Welsh (2003) reported that higher ADC values for dry matter and crude protein were achieved if the level of feed pea meal incorporated into practical diets for *Penaeus monodon* was increased. Likewise, cornstarch has been used to improve the digestibility of diets formulated for the white shrimp, *Litopenaeus vannamei* (Guo, Liu, Tian & Huang 2006).

At present, it is unclear why there are differences in the impacts of plant-based ingredients on aquaculture diets. Such variations may reflect species-related differences in the capacity of candidate organisms to digest plant-based materials. For example, in these laboratories it has been shown that enzymes, such as cellulase, which are required for the breakdown of plant-based materials are present at much higher levels in the digestive system of the omnivorous redclaw crayfish *Cherax quadricarinatus*, than in digestive tissues from the carnivorous mud crab *S.serrata* (Pavasovic, Richardson, Anderson, Mann & Mather 2004; Pavasovic, Anderson, Mather. & Richardson 2007). It is also possible that differences in the digestibility of plant-based materials may reflect how the addition of these materials has affected the levels of other dietary components. For example, Guo *et al* (2006) speculated that adding corn starch to shrimp diets helped improve digestibility by permitting a reduction in the level of less digestible dietary components, such as cellulose.

In conclusion, the current investigation has shown that at a 30% inclusion level, soybean meal and rice bran did not impact negatively on the digestibility of fishmeal-based artificial diets. Moreover, even at an inclusion level of 45%, crude protein and gross energy digestibility of experimental diets was not significantly reduced by the presence of soybean meal. This suggests that soybean meal and rice bran are ingredients with high potential for inclusion into diets formulated for *S. paramamosain.* By contrast, corn flour

demonstrated only limited potential for inclusion into diets formulated for *S.paramamosain.* In particular ADC values were generally less than those observed in similar studies performed using *S.serrata*. Another important finding of the current study was that cassava meal demonstrated poor potential for inclusion in diets formulated for *S.paramamosain*, at least over the inclusion range tested.

Acknowledgements

The authors would like to thank Nguyen Co Thach, Truong Quoc Thai, Nguyen T. X. Thu, and Nguyen Xuan Nam at Research Institute for Aquaculture No3 for their technical assistance. This study was supported by Ministry of Education and Training, Vietnam and ACIAR Mud Crab Project FIS/2000/065, Australia. Our gratitude is also extended to Tran Dinh Thanh of SEAPRODEX Company (Da Nang, Viet Nam) for supplying the fishmeal.

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Table 1: Composition of diets formulated for the digestibility trial using different local ingredients (% dry weight basis).

Ingredient (%)	Diet								
$g100g^{-1}$	RF[1]	RB30	RB45	CM30	CM45	CF30	CF45	SBM30	SBM45
Fish meal[2]	81.5	51.5	36.5	51.5	36.5	51.5	36.5	51.5	36.5
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Cod liver oil[3]	3	3	3	3	3	3	3	3	3
CaHPO4	3	3	3	3	3	3	3	3	3
Common Ingredients[4]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Rice bran (RB)[5]	-	30	45	-	-	-	-	-	-
Cassava meal (CM)[5]	-	-	-	30	45	-	-	-	-
Corn flour (CF)[5]	-	-	-	-	-	30	45	-	-
Soybean meal (SBM)[5]	-	-	-	-	-	-	-	30	45

[1] Reference diet

[2] Peruvian fish meal

[3] Healthier of Australia (Healtheries of New Zealand, Auckland, New Zealand)

[4] Common ingredients ($g100g^{-1}$): dried squid (5%); chromic oxide (Cr_2O_3) (0.5%); mineral and vitamin premix (2%), (active ingredients per kg^{-1} of premix 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1.75g riboflavin; 1.125g 1125 mg pyridoxine hydrochloride; 3.75g cyanocobalamin; 25g ascorbic acid; 50g colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin).

[5] Products of Vietnam

Table 2: Dry matter (DM), gross energy (GE MJ kg^{-1}), crude fat (CF), crude protein (CP) and ash of experimental diets and test ingredients used in the formulated diets (% dry weight basis)

Sources	DM (%)	CP (%)	CF (%)	Ash (%)	GE[1] (MJ kg^{-1})
Experimental diets					
RF	93.6	65.3	11.2	16.4	20.4
RB30	91.2	48.5	11.7	11.7	18.9
RB45	90.1	40.1	11.4	9.3	18.6
CM30	91.1	46.5	10.8	11.7	18.6
CM45	92.4	37.1	10.0	9.3	18.0
CF30	89.0	46.6	11.2	14.4	18.8
CF45	90.9	37.2	10.6	13.3	18.4
SBM30	93.4	56.0	10.2	13.8	19.6
SBM45	93.8	51.4	9.4	12.4	19.6
Test ingredients					
Fish meal	93.6	71.2	8.7	16.8	21.0
Defatted soybean	91.2	40.1	1.4	7.4	20.1
Rice bran	90.2	15.1	6.8	8.1	18.8
Cassava meal	93.6	8.2	3.6	9.2	17.4
Corn flour	92.2	8.6	4.9	4.6	18.2

[1] Determined using a bomb calorimeter at Nha Trang University

Table 3: Apparent digestibilities (%) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) in experimental diets

Diet	ADMD	ACPD	AGED
RF	84.9 ± 0.5^{e}	90.9 ± 0.5^{d}	90.7 ± 0.7^{de}
RB30	82.6 ± 0.2^{de}	91.8 ± 0.4^{d}	90.6 ± 0.4^{de}
RB45	80.6 ± 0.4^{cd}	87.8 ± 0.4^{c}	87.3 ± 0.7^{c}
CM30	75.8 ± 0.9^{b}	84.7 ± 0.8^{b}	83.8 ± 1.1^{b}
CM45	70.9 ± 0.6^{a}	77.1 ± 0.7^{a}	80.2 ± 0.5^{a}
CF30	80.2 ± 0.1^{cd}	87.8 ± 0.2^{c}	88.7 ± 0.5^{cd}
CF45	78.9 ± 0.3^{c}	83.6 ± 0.3^{b}	85.6 ± 0.37^{bc}
SBM30	85.7 ± 0.3^{e}	93.2 ± 0.4^{e}	92.2 ± 0.4^{e}
SBM45	81.6 ± 0.2^{cd}	92.1 ± 0.9^{d}	88.6 ± 0.3^{cd}

Values are means ± SE (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different (p>0.05) from one another

Table 4: Apparent digestibility coefficients (%) for dry matter (ADMDI), crude protein (ACPDI) and gross energy (AGEDI) of test ingredients used in formulated diets

Sources	Inclusion (%)	ADMDI (%)	ACPDI (%)	AGEDI (%)
Rice bran	30	77.3±1.7^{c}	93.8±0.4^{d}	90.2±1.8^{cd}
	45	75.3±1.2^{bc}	84.0±0.7^{c}	83.1±1.6^{bc}
Cassava meal	30	54.8±0.9^{a}	70.3±2.6^{b}	67.8±0.9^{a}
	45	53.8±1.1^{a}	60.2±1.3^{a}	67.3±1.1^{a}
Corn flour	30	69.2±1.2^{b}	80.6±0.9^{c}	83.9±1.9^{bc}
	45	71.5±0.8^{bc}	74.7±0.5^{b}	79.4±1.4^{b}
Defatted soy bean	30	87.6±2.4^{d}	98.4±0.4^{d}	95.6±2.9^{d}
	45	77.5±0.5^{c}	93.5±0.2^{d}	86.1±0.7^{bc}

Values are means ± SE (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different (p>0.05) from one another