Development of diets for soft-shell mangrove crabs (*Scylla* spp)

by

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Abstract

The objective of this research was to develop practical formulated diets for soft-shell mangrove crab farming. Although the large-scale farming of soft-shell crab has been going for more than 20 years, little research has been conducted regarding formulated feeds. Presently, there is an urgent need to replace "trash fish" with manufactured diets. In the first study, we evaluated three compounded feeds for their acceptance. A total of 600 crabs were placed into individual boxes with 150-boxed crabs stocked into one PVC pontoon raft all in the same pond. Crabs were fed 3% of their body weight split between two feedings a day over a 45-day feeding trial. The growth of crabs fed the trash fish control was not significantly different from the high fishmeal content diet, both near 0.80 g/day. The growth rate when fed a high soy content diet was significantly lower, 0.71g/day. However, the soy-based diet had the highest survival rate (98%) versus crab feed trash fish (94%) and high fishmeal diets (91%). The results of the present study demonstrate that mangrove crabs will accept formulated feeds using either simple laboratory process or commercial extrusion. Hence, the development of specific diets for crabs is technically possible.

To address pellet stability and durability issues we modified the high soy basal diets and evaluated the efficacy of various binders both under laboratory pelleting conditions as well as using a laboratory extruder. We hypothesize a binder would provide much longer stability in the water and the pellets would not fall apart as quickly even with aggressive handling by the crab. Two different binders: methylcellulose (CMC) and methylhydroxypropyl cellulose (MHPC) were used at inclusion levels of 0%, 0.15%, 0.3%, 0.6%, 0.9%, 1.2%, and 2.4% and pellet stability was

tested at one, two and three hours. Results showed that MHPC was not significantly different at reducing dry matter leaching than CMC, except at the highest inclusion level of 2.4%. The level of binder in the diet did not seem to have a significant effect within the range tested, except for MHPC at 2.4% when all the times were included.

In the third study, a series floating extruded basal diets were formulated to contain 40% protein and 10% lipid. The basal diet was modified to produce three additional diets each using 1% of a binder (Sodium alginate, methylhdroxypropyl cellulose and methylcellulose). A total of 600 wild caught crabs, placed into individual boxes with 150-boxes randomly assigned to each treatment. Crabs were fed 3% of body weight and fed once a day in a 45-day feeding trial. The result showed that diet-using binder methylhdroxypropyl cellouse MHPC produced crabs with better survival and weight gain than sodium alginate SA or methylcellulose CMC. However, molting took longer for the crab fed with reference diet (35 days) as compare to other treatment groups less than 34 days. We also observed that crabs struggled to grasp and hold the floating feed with their claws indicating that a floating feed is inappropriate.

In my final experiment, we compared traditional trash fish to three (sinking, slow sinking and floating), extruded diets to consider the preferred physical form of formulated feed by mangrove crabs. Diet were randomly assigned to the crabs (150 per treatment) across one raft. Crabs were fed 3% of body weight once a day in a 45-day feeding trial. The result showed that there is no significant difference in average weight gain or survival between crabs fed sinking pellets compared to trash fish in term of weight gain. However, crabs fed with slow slinking pellet have the shortest average number of days to molting (29 days) and crabs fed with sinking diet have the longest average period to molting of 38 days. These experiments appear to be the first on-farm research that focused on formulated diets for soft-shell mangrove crabs. The results of these trials will contribute to the industry by demonstrating that formulated diets could be used for soft-shell crab farming and reduce the demand for “trash” fish.

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List of Abbreviations

BW	Body weight
CMC	Methylcellulose
CP	Crude protein
CW	Carapace width
h	hours
kj	kilojoules
MHPC	Methylhdroxypropyl cellulose
MJ	Megajoules
PVC	Polyvinylchloride
SA	Sodium alginate

Chapter 1. **Introduction**

Aquaculture is an important sector of agriculture with global production of 76.6 million metric tons in 2015 and more than 50% of fish and crustaceans consumed nowadays being farmed raised (FAO, 2016). It is one of the fastest growing sectors in food production valued at USD 157.9 billion in 2015. Currently, 567 aquatic species are cultured around the world although 25 species represent 90% of production (FAO, 2016; Tacon, 2003). Aquaculture production is expected to double in the next 10-20 years due to static capture fisheries with increasing human population and improved standards of living resulting in increased demand for seafood (FAO, 2016). Crustacean aquaculture is the second largest production sector of aquaculture with 6.9 million tons of 2016 (US$ 36.2billion) and marine shrimp as the dominant species group (FAO, 2016).

Mud crabs, or mangrove crabs, are one of the most valuable groups of crab species in the world. Mud crab, genus *Scylla*, aquaculture is expected to continue to grow in the future (Azra and Ikhwanuddin, 2015). Production of mud crabs in 2015 was around 226,390 metric tons (FAO, 2015) with a farm-gate value of US$1.06 billion. Commercial markets are primarily driven by live or frozen soft-shell crab sales (Quinitio and Lwin, 2009). Mud crab aquaculture has been practiced for many years in Asia and especially the Southeast Asia region, but is based primarily on captured animals and fattening prior to sale.

There are four species of mangrove crabs used for commercial aquaculture, *Scylla serrata*, *S. tranquebarica*, *S. paramamosain*, and *S. olivacea* (Keenan and Blackshaw, 1999; Allan and Fielder, 2003; Fratini and Vannini, 2002; Fushimi and Watanabe, 1999). These four species have slightly different life cycles and their habitats are mangrove forests where they get their other common name of mangrove crab. Mud crabs live along tropical, sub-tropical and warm temperate coastlines from Eastern Africa to the southern tip of Japan and northeast Australia (Allan and

Fielder, 2003; Chandrasekaran and Natarajan, 1994). These four different species can be found together in many countries, for example in locations in the Philippines and Indonesia (Keenan and Blackshaw, 1999). Among the four species, *S. paramamosain* is the most important species in aquaculture because it is the dominant species in China and Vietnam where mud crab farming is most successful (Lucas and Southgate, 2012).

The life cycle of the mangrove, crab starts with courtship and mating which occur in brackish water after which the fertilized female migrates offshore to spawn (Baylon and Failaman, 1999; Marichamy and Rajapackiam, 1991; Keenan, 2004). Depend on species, a mature female can produce 1 to 6 million eggs. After mating, females retain sperm and without further intervention of a male, 2 or 3 egg masses can be produced (Shelley and Lovatelli, 2011). Spawned eggs attach to the pleopod hairs of the abdominal flap (berried female) and eggs hatch into zoea 1 (Quinitio and Parado-Estepa, 2008). *Scylla spp*. have a complex life cycle, where larvae go through five zoea stages (3–5 days each) and one megalopa stage (7–10 days). The zoea and megalopa feed on zooplankton (Thach, 2009). The megalopa molts once more and it takes on a more crab like appearance as a crablet or benthic juvenile (Raja, 1955). Megalopa can swim in the plankton but resemble crabs and become increasingly benthic (Lucas and Southgate, 2012). Small crabs are found in estuaries, tidal flats and mangroves where they burrow in mud or sand, or hide under fallen leaves and shaded areas during the day (Shelley and Lovatelli, 2011). Crab in-star and juveniles undergo several more molts until full maturity. Mud crab can reach sexual maturity within 1 year (Lucas and Southgate, 2012).

Mangrove crabs are largely omnivores and scavengers with diets including a wide range of plant materials, mollusks, small crustaceans, polychaetes and detritus (Lucas and Southgate, 2012). Mangrove crabs have broad tolerances to a wide range of salinity and will forage into freshwater streams and euryhaline estuaries. Among the four species, *S. olivacea* is generally the most aggressive and *S. serrata* has the strongest osmo-regulatory ability (3 ppt – 35 ppt seawater (Keenan and Blackshaw, 1999).

Wild caught crab is the major source of stock for crab farming and experienced fishermen using traps can collect hundreds per day (Shelley and Lovatelli, 2011). The development of soft-shell and value added mud crab products; both for domestic and international markets induce more farmers to culture these species. There is increasingly high demand from export markets making this farming even more popular.

Farming of soft-shell mangrove crabs also has been practiced for some time now in a number of Asian countries such as Thailand, Myanmar, Vietnam, Malaysia, Indonesia, and more recently, the Philippines, Bangladesh, Brunei and India (Shelley and Lovatelli, 2011). Large-scale soft-shell mangrove crab farming in Asia started during the early 1990's when there was a strong demand for soft-shell crabs in domestic and international markets (Quinitio and Lwin, 2009). The soft-shell mud crab can be eaten whole (carapace and all limbs) when cooked. Soft-shell crabs are available year round. Because of its profitability, there is an increasing interest to engage in this type of crab farming business venture in Asia. Thailand is one of the leading soft-shell crab producers in the world with the greatest concentration of soft-shell mangrove crab farms in Ranong, where farmers can market the crabs daily at the numerous seafood-processing plants in the area. Fresh soft-shell mangrove crabs are sold to local restaurants and frozen crabs are exported to Hong Kong, Singapore, China, South Korea, Japan, Taiwan, Australia, Europe and the United States. Income is generated daily from the harvest of soft-shell mangrove crabs (Quinitio and Lwin, 2009).

The most common system for soft-shell crab production used in Ranong is individual rearing where healthy intact crabs are held in individual perforated plastic boxes having detachable covers. Boxes are positioned on floating platforms (pontoons) made of wood or PVC pipes that can hold up to sixteen hundred boxes. Each crab stocked in these boxes is checked every 3 to 4 hours for molting and fed every other day. In order for the mud crab to grow larger, crabs must periodically shed or molt through a process known as ecdysis (Quinitio and Parado, 2008). The hard outer shell cannot stretch with growth, so the crab must molt or shed its old exoskeleton in order to grow. Before molting, a new exoskeleton is formed below the old and hard exoskeleton. During the molting process, the old shell splits and the crab backs out of it. The new shell develops and hardens in 4-8 hours. The exoskeleton is soft immediately after molting. The crab expands its body and limbs by taking in water before the new shell hardens and usually increases in size by 30-50%. The molting frequency is greater when crabs are small and less often when bigger. If the crab is removed from the brackish water shortly after molting (during the first four hours), it will remain in the soft-shell condition when held in freshwater. Then it can be sold as a soft-shell crab. Regular monitoring and retrieval of soft-shell crabs is laborious. Individual boxes in the pontoons are examined by workers sitting on a bridge with the pontoon passes below with a pulley system. Experienced staff can easily identify soft-shell crabs while observing hundreds of crabs.

Figure 1.1. Soft-shell crab farming in Ranong, Thailand

Despite the industrial scale of mangrove crab aquaculture, compared to many other species, mud crab farming can be considered to be in an early stage of development. The use of formulated feed is still in its infancy (Anderson et al., 2003) and little work has been done to improve performance through breeding programs. There are no commercial feeds available for commercial production of any type of crab farming, which includes soft-shell mud crab production (Holme et al., 2009). Production of soft-shell mud crab presently is conducted using trash (low value forage) fish as feed (Quinitio and Lwin, 2009). The prospect of decreased trash fish availability and affordability is becoming a major issue in the industry. The other issues are the storage of trash fish, water pollution, and the fish as potential vectors of parasites and pathogens. Feed is one of the expensive inputs in the culture of mud crabs. The market prices of mud crabs are dependent on the body content or fullness of flesh attained when crabs molt. Although the soft-shell crab industry has been going for more than 20 years, little research has been done regarding feed and nutrition and there is an urgent need to replace trash fish with formulated diets.

Traditionally, crabs were viewed as carnivores with preferences in their natural diet for mollusks, crustaceans and fishes that have relatively high protein content (Hill, 1979). It is known that the nutritional requirements of the mangrove crabs change during their life cycle (Lucas and Southgate, 2012; Heasman and Fielder, 1983). Several studies have been conducted to describe the nutrient requirements of broodstock mangrove crabs, only preliminary studies have been conducted to define the nutritional requirements for grow out diets. For soft-shell crab farming, crabs are held only a short period yet molting requires considerable energy and is a very stressful process for the animals. Efficient nutrition is vital to support growth, which in turn supports the process of molting (Suprayudi et al., 2004). Hence, there is a need for nutritionally complete feed formulations.

Dietary requirements for mud crabs have been demonstrated to not be as stringent as those of most penaeid shrimp, with good growth occurring over a wide range of protein and lipid levels (Catacutan, 2002). Sheen and Wu (1999) reported that *S. serrata* demonstrate better utilization of lipids than shrimp following a study in which they measured the growth response of juvenile crabs that had been fed diets with a range of inclusion levels (2 to 13.8% lipid) of a mixture of cod liver oil and corn oil. The result showed that using dietary lipid levels ranging from 5.3% to 13.8% appeared to meet the lipid requirement of juvenile crabs (Sheen and Wu, 1999). Sheen

(2000) indicated the importance of a dietary source of cholesterol and polyunsaturated fatty acids (22:6n-3, 20:4n-6, 18:3n-3) for the healthy growth of juvenile crabs and the results showed that mud crab juveniles required 22:6n-3, 20:4n-6, 18:3n-3 to support acceptable weight gain. Research conducted by Sheen (2000) reported that juvenile crabs had a cholesterol requirement of 0.51% in the diet and lower levels would result in low weight gain. While Anderson et al., (2003) reported that adding over 1.12% of dietary cholesterol level had an adverse effect on growth.

Dietary protein supply is one of the major factors that influence the productivity of cultured species (Davis and Sorgeloos, 2005). Based on research by Unnikrishnan and Paulraj (2009), juvenile mud crabs of *S. serrata*, demonstrated their best growth performance with dietary protein level of 45% (experiment tested a range of 14.7-55% crude protein (CP) with 5 % intervals) and required dietary protein of 14.7% to 16.2% for the bare minimum to maintain metabolism in their 63-day trial. Researched conducted by Catacutan (2002), with six test diets (with three protein levels of 32%, 40% and 48% at two lipid levels of 6% and 12% and protein: energy ratio from 20.5 to 31.1 mg protein/kj) found that *S. serrata*, juveniles grew faster when fed diets containing 32 and 40% dietary protein with either 6% or 12% lipid and dietary energy ranging from 14.7–17.6 MJ/kg. Research conducted by Catacutan et al., (2003), reported that juvenile mangrove crabs have elevated crude protein requirements of more than 40%. We presume juvenile crab diets must have the right composition of minerals to sustain the rapid molting cycles, but we did not find any research detailing the mineral nutritional requirements for juvenile *Scylla* crabs.

A series of studies have been carried out to measure digestive enzyme activity and the digestibility of several potential ingredients for formulated feed for crabs (Catacutan et al., 2003; Truong et al., 2009). Mangrove crabs have digestive enzymes that effectively digest protein, carbohydrate, and lipids (Pavasovic, 2004; Pavasovic et al, 2004). Several studies showed that crabs were able to digest many different ingredients, in particular protein from plants (Catacutan et al., 2003; Truong et al., 2009; Nguyen et al., 2014). It was also reported that crabs have capacity to digest several starches (Catacutan et al., 2003). Same researchers reported that, crude fat in plant-based ingredients are more digestible for crabs than crude fats of animal origin (Catacutan et al., 2003).

Several authors (Marichamy et al., 1986; Marichamy and Rajapackiam, 1991; Marichamy, 1996; Samarasinghe et al., 1991; Fortes, 1999; Saha et al., 2000; Triño et al., 2001; Christensen et al., 2004; Alava et al., 2007; Manivannan et al., 2010; Azra and Ikhwanuddin, 2015) have conducted brood stock nutrition studies. In the review by Azra and Ikhwanuddin (2015), comparisons were drawn between maturation brood stock diets based on natural diets (squid, fish, and mussel), formulated diets (shrimp brood stock diets) or mixed diets of natural and formulated feeds. The review concluded that the mixed diets generally provided the superior reproductive results. Using mixed feeding, 50% of formulated diet (protein 58% and lipid 10%) with natural feed 50% gave better survival, fecundity, spawning and hatching rate (Millamena and Quinitio, 2000). In addition, nutrition research conducted by Djunaidah et al., (2003) reported that a formulated diet performed the same as fresh fish in terms of reproductive performance of crabs sized 200g- 300g. According to Alava et al. (2007), using both a formulated diet containing 58% protein and 18% lipid in combination with natural feed gave the shortest number of days from spawning to hatching and better survival of broodstock. Thus, there is a limited amount of nutritional information for grow out diets for sub-adult and adult mud crabs (Holme et al., 2009) and virtually all of the trials have been conducted in the lab.

Mangrove crabs typically grasp their food items with their claws before they eat and they are slow eaters with the characteristic of using mouth appendages to shred the food item before ingestion (Barker and Gibson, 1978). The feed should be water stable to minimize leaching of water-soluble nutrients and allow the feed matrix to retain its "shape" while the crabs are nibbling on the feed. Hence, a good binder and or specialized processing may be required to produce an appropriate physical characteristic of the pellet. In general, the stability of shrimp or crab feeds in water can be achieved either by pelletization with binders or by extrusion processes, triggering the gelatinizing activity of starches, especially those of plant origin to develop a water stable matrix is likely to be essential.

Today extrusion technology is the method of choice to process many aquatic feeds because extrusion processing results in improved physical properties of the feed, inactivates some anti-nutritional factors, destroys many pathogenic microorganisms in the feed and thus provide benefits to both manufacturers and farmers (Williams, 1991; Riaz,1997; Rokey and Platter, 2004). The extrusion cooking system has become popular, despite higher capital costs, because of its great versatility, and high thermodynamic efficiency (Rokey and Plattrer, 2004).

However, if pellet water stability during manufacturing process is insufficient, the inclusion of a binding agent is necessary to reduce dry matter loss (Argüello and Poveda, 2013). Binders are used in aquaculture feed production to improve feed manufacturing, reduce feed wastage, increase water stability of diets, stabilize ingredients, and minimize leaching of nutrients. Cruz-Suarez et al. (1999), suggest the true nutritional value of feed depends not only on the amount of nutrients, but also on their availability and quality. Water stability of aquatic feed depends on both quantity and quality of binding material used (Ali, 1982). Polysaccharide sources such as starch and cellulose are abundant and cellulose is a relatively cheap source of biopolymers to be employed as binders (Volpe et al., 2010). A cellulose molecule has three-hydroxyl groups in each repeating glucose unit, which gives the molecule strong hydrophilic properties (Boulos et al., 2000). Cellulose is indigestible for most monogastric animals due to their lack of the enzymes required to break it down (Hansen and Storebakken, 2007). However, certain amount of cellulose has been used in feeds for many aquaculture species including European sea bass (Dias et al., 1998) and Nile tilapia (Schaeffer, 2010) with minimal effects on digestibility of the primary nutrients. Typically, binders such as wheat flour, wheat gluten, gelatin, carboxymethylcellulose and sodium alginate are used to stabilize fish and shrimp feeds (Akiyama et al. 1992; Cuzon et al. 1994; Argüello and Poveda, 2013).

Development of a good stable, nutritionally complete mangrove crab feed will reduce the total dependence of the crab aquaculture industry on unsustainable resources such as "trash fish" and fishmeal. Despite the industrial scale of mangrove crab aquaculture, there is need for compound feeds to substitute for trash fish. These days we cannot afford to feed the whole trash fish and now many farms are feeding only the head of the fish. Not only are the trash fish expensive but also hard to get year-round. It is becoming a major issue for soft-shell crab growers. Therefore, it is essential to develop complete diets for the crab, which must start with confirming the efficacy of a commercially produced feed and determining appropriate physical characteristics of the feed.

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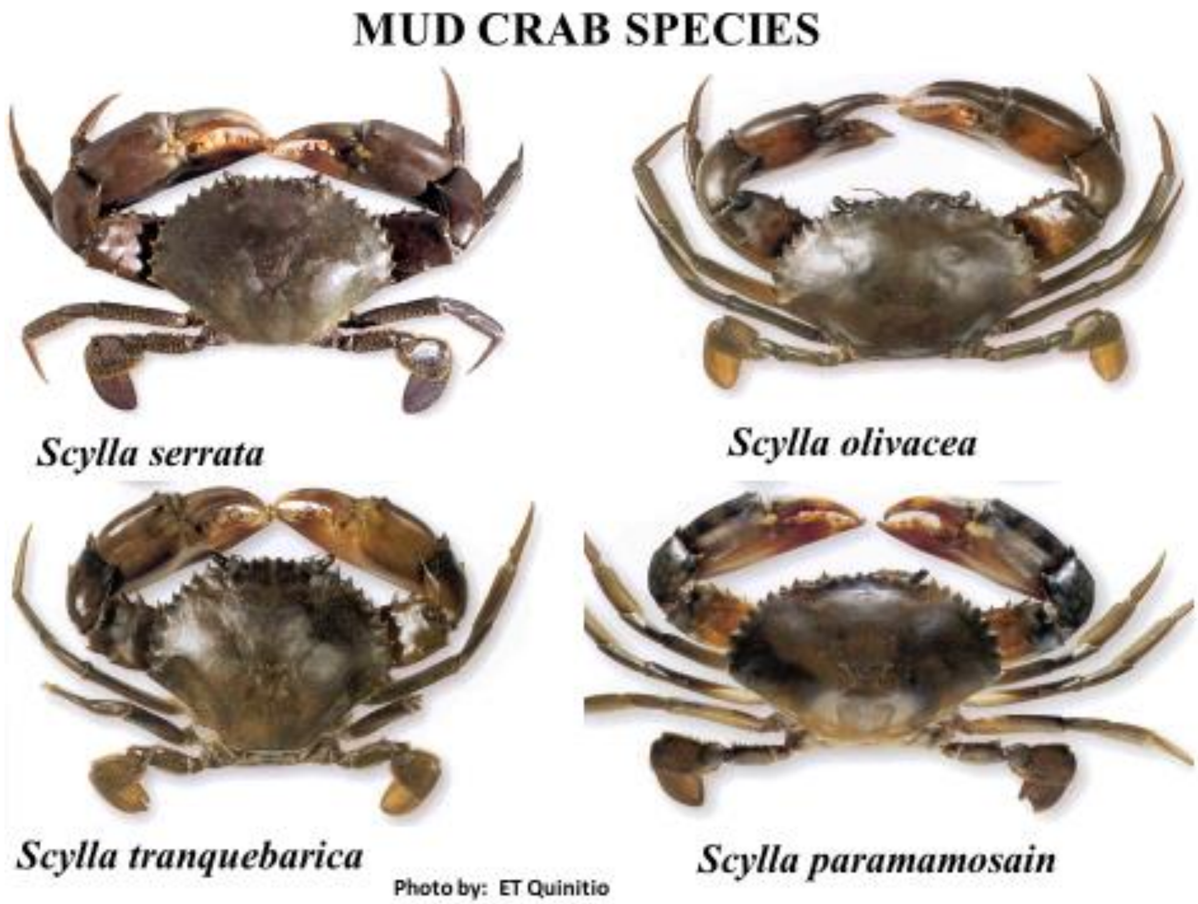
Chapter 2. **Review of literature**

2.1. **Biology of Mangrove Crab**

Mangrove crabs, also commonly called mud crabs, are marine, estuarine portunid crabs (Keenan and Blackshaw, 1999) that are mainly distributed in the warmer regions of the Indo-Pacific. The preferred habitats of these four crabs, in the genus *Scylla*, are intertidal mud flat river mouths generally covered by mangroves (Pavasovic et al., 2004) thus the common name of mangrove crabs. Mangrove crabs have a flattened, broad body covered by a fan-shaped carapace. Along the front margin of the carapace are six spines between the eyes and nine spines on either side (anterolateral margin) **(**Meyer et al., 2009, 2013). Each has one pair of chelipeds and three pairs of walking legs. The fourth pair of legs are flattened and used for swimming. In males, the walking legs are used for clasping a female during copulation; females use these walking legs for scratching the eggs off just prior to hatching (Quinitio and Parado-Estepa, 2008).

The chelipeds consist of enlarged segments (merus, carpus, propodus, dactylus, and pollex) and are used for crushing shells and holding and bringing food to the mouth. The mouthparts are responsible for collection and processing food (Barker and Gibson, 1978). The eyes, antennules, antennae, dactylus and maxilipeds are used to sensory perception. Mud crabs have separate sexes, immature females have a triangular-shaped abdomen or abdominal flap and mature females have a broader, semi-circular abdomen. Males have a T-shaped abdomen. Mature males have larger chelipeds than females of similar carapace size (Pavasovic, 2004).

Figure 2.1. Four different species of mangrove crab, *Scylla* spp.



Keenan and Blackshaw, (1999).

Figure 2.1.Abdominal flaps of mangrove crab of differnet sex and maturity **(**Shelley and Lovatelli, 2011).

Immature female	Female	Male

2.2. **Anatomy and physiology of the digestive tract**

Like other crustaceans, mangrove crab's digestive tract has three parts: foregut, midgut and hindgut. The foregut has three parts; a mouth, esophagus and stomach. The mid gut is a tube with an interior and posterior caecum and mid gut gland called the hepatopancreas. The hindgut is a simple straight tube that finishes at the anus (Pavasovic et al., 2004). Crabs grasp feed with their chelipeds and bring the feed into the mouth. Then food taken into the mouth is passed though the esophagus to the anterior (cardiac) part of the stomach. Digestive liquid (fluid) from the hepatopancreas flows forward through the posterior (pyloric) part into the anterior part of the stomach where food is mixed with digestive liquid and ground (tritutation) in the gastric mill (Barker and Gibson, 1978). The mass of digestive liquid and food process through the ventral section of the anterior part of the stomach and goes back into the posterior part of the stomach, which excludes all the particles larger than 1 micron. The filtered mass of the digestive liquid and food then continues into the hepatopancreas (Dall and Moriarty, 1983; Ceccaldi, 1997).

In the process of food digestion, the hepatopancreas secretes digestive enzymes and some of these enzymes include protease enzymes, which include trypsin, and chymotrypsin to hydrolyze protein, whereas, amylase and maltase will be secreted to hydrolyze carbohydrates. Lipase (triglyceride lipase or phospholipase) is secreted to hydrolyze lipids. Protein, carbohydrates, and lipids are converted into smaller molecules of amino acids, glucose and fatty acids, respectively, which are easily absorbed. Absorption of nutrients takes place in the mid-gut gland and anterior and posterior caeca (NRC, 2011).

After the absorption process, a portion of digestible nutrients are retained in the cultured species supporting growth and maintenance (standard metabolism, motion, swimming, etc.), and stored nutrients are transported to the muscles, gonads, and other tissues. The rest are excreted in the form of dissolved products such as ammonia and carbon dioxide, by metabolic processes. Water absorption and formation of feces takes places in the hindgut. In the food digestion process, highly digestible nutrients in feed can increase absorption and retention processes and reduce waste of nutrients in excretion. Indigestible nutrients are excreted in the form of feces (solid waste) and released into the water. Thus, fecal production depends on the feed composition especially dietary fiber content.

Table 2.1. Taxonomy and identification of mangrove (mud) crabs.

Scientific Classification	
Phylum	Arthropoda
Class	Crustacea
Subclass	Malacostraca
Order	Decapoda
Infraorder	Brachyura
Family	Portunidae
Genus	*Scylla*
Species	*S. serrata* *S. tranquebarica* *S. olivacea* *S. paramamosain*

Sarower et al., (2013)

Figure 2.2. Life cycle of mangrove crabs

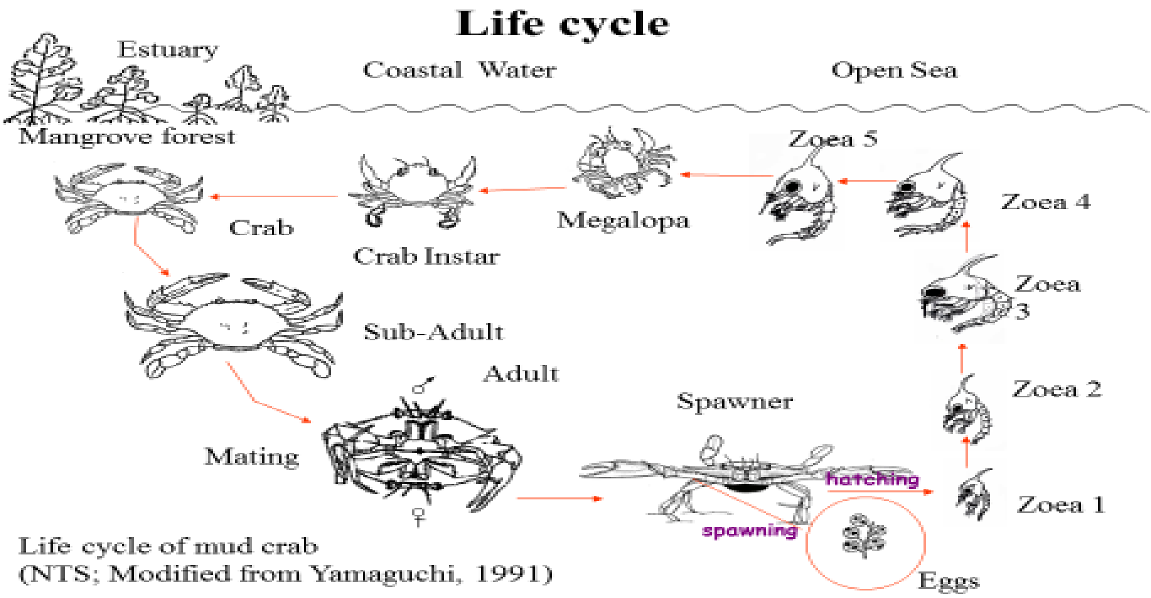
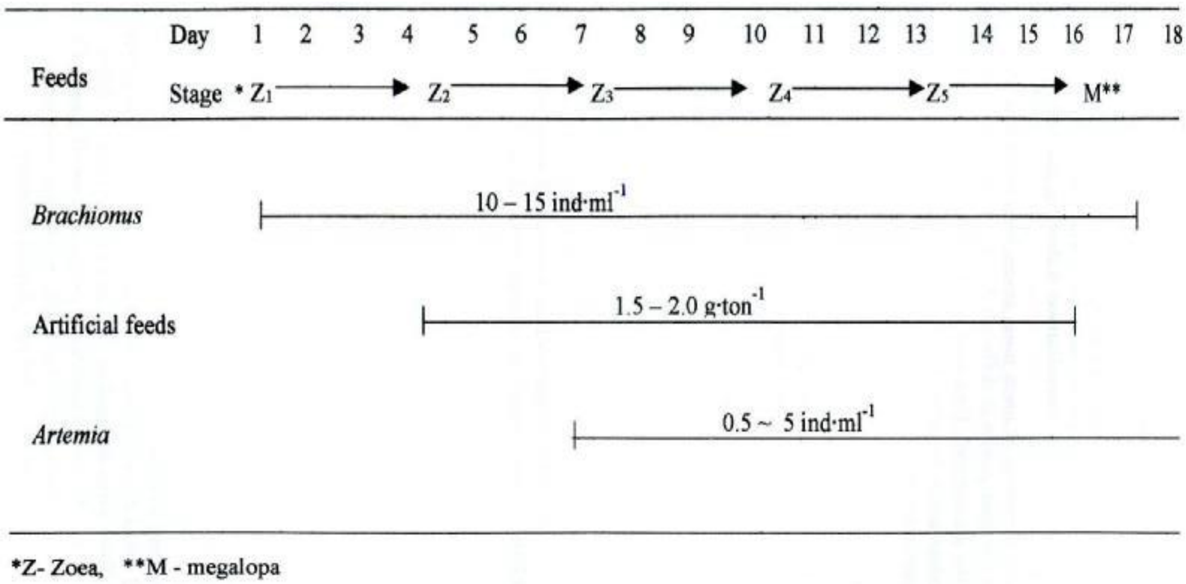


Table 2.2. Percentage composition of natural food of *Scylla serrata* of different ontogenetic stages, Indonesia

Food items	Juveniles	Subadults	Adults
Unidentified organic matter – meat	42.09	46.30	41.09
Inorganic sand-shell	9.46	15.04	17.18
Plant, algae and sea grasses	13.05	12.88	16.00
Fish meat and hard parts	17.41	12.18	10.24
Mollusc	9.21	7.18	8.58
Crustacea	8.20	5.16	4.56
Unidentified organic matter – wood	0.57	1.26	2.24

Source: La Sara *et al*., 2007.

Figure 2.3. Feeding rate for mangrove crabs based on age for hatchery production.



Quinitio and Parado-Estepa, (2008).

2.3. **Molting**

In order for the mangrove crab to grow larger, crabs must periodically shed or molt through a process known as molting or ecdysis (Quinitio and Parado-Estepa, 2008). The exoskeleton is soft immediately after molting. The crab expands its body and limbs by taking in water before the new shell hardens and usually increases in size by 30-50%. The molting frequency is greater when crabs are small but less often when bigger. At the first zoeal stage, the animal is just over 1 mm. long but then increases in size by molting four times over the next 12 to 15 days (Parado-Estepa and Quinitio, 2011). During the fifth molt, the larval crab transforms into a megalopa with large (relative to its size), functional claws (Nguyen et al., 2014). The megalopa later molts into a stage one crab. The newly molted crab is especially vulnerable to cannibalism. The number of days between molts increases with size. Daily increase in carapace of mud crab stops but the daily weight gain continuously increases, noticeably for adult crabs. Daily average weight gain can be 3.4g in the grow-out phase (Nguyen et al., 2014).

Crabs with an initial body weight of about 0.7 g grow to marketable size of >300g after 4-6 months (Catacutan, 2002). With each molt, the size increase 25 to 50% (Huong et al., 2010). A majority of crabs molt during nighttime and for crabs in the size range of 80g-160g, the next molt would normally occur within 25 days (Ganesh et al., 2015). It is thought that wild mud crabs reach 100-mm carapace width in about a year and sexual maturity in about two years. Maturation time varies according to water temperature, with higher temperatures accelerating the growth and decreasing the time taken to reach maturity (Azra and Ikhwanuddin, 2016). The typical life span of a mud crab is thought to be three to four years (Keenan, 2004). Factors that trigger molting are both environmental such as light, temperature, salinity, nutrients, microorganisms and water quality, and within the body such as metabolism and hormone and enzyme systems

The major stages of an individual molt cycle are as follows:

2.3.1. Pre-molt or pre-ecdysis stage

Molting hormones are released to start ecdysis. The outer hard layer of the shell separates from the membranous layer and causes an evident mottled appearance of the old shed carapace (Ceccaldi, 1997). Activity is reduced and feeding stops as the crab loses it muscle insertion. Prior to molting, a crab reabsorbs some of the calcium carbonate from the old exoskeleton, and then secretes enzymes to separate the old shell from the underlying skin (or epidermis). Then, the epidermis secretes a new soft, paper-like shell beneath the old one. When this new shell has fully formed, the crab will be ready to molt.

2.3.2. Ecdysis

A day before molting, the crab starts to absorb seawater, begins to swell and stops eating. This helps to expand the old shell and causes it to come apart at a special seam that runs around the body. The carapace then opens up like a lid and the crab backs out of the old shell and takes up water rapidly. The crab extracts itself from its old shell by pushing and compressing all of its appendages repeatedly. First, it backs out, then pulls out its hind legs, then its front legs, and finally comes completely out of the old shell. This process takes 5-20 minutes, depending on the health of the crab, and is the most stressful part of the molt cycle.

2.3.3. Post Molt

The newly molted crab is soft and inactive. The crab expands its body and limbs by taking in water before the new shell hardens. The newly molted crab pumps water into its tissues in order to inflate the shell to its new size. The crab usually increases in size by 30-50%. Feeding may start when part of the shell becomes hard. The salvaged inorganic salts are rapidly redeposited to help thicken and harden the new shell. Following the molt, some crabs will eat the exoskeleton it has just shed. Ingesting this calcium rich shell allows the animal to stock up on nutrients needed to synthesize the next shell (Tavares et al., 2017).

2.3.4. Inter molt

At the early part of the inter-molt stage, the carapace is almost hard but the walking legs are still flexible depending on the crab's size. The shell hardens completely after several days. The inter-molt stage is the longest among the molt stages. Before molting occurs, crabs begin to accumulate food for energy and nutrient storage. The following are the signs that indicate the stage between molts of the crabs held for molting to become soft-shell. Soft-shell crab farmers (portunid and swimming) have come up with their own terminology to describe these stages (Chamchuen, et al., 2014; Keenan and Blackshaw, 1999).

- Normal hard shell crabs- 14-50 days prior to molt, depend on crab size and hard shell
- Song kadon (Rank peeler) - one week prior to molt
- Buster - in the process of shedding its old shell
- Soft-shell – Immediately following molt

In general, it is more difficult to identify the stage of molting by touching or handling mangrove crabs compared to blue crabs. However, experienced mangrove crab farmers can predict whether a mangrove crab is ready to molt or not. Molting takes a lot of metabolic energy; so, larger animals must store far more nutrients for molting than smaller juveniles. The larger the crab, the more difficult to store energy and nutrients for molting. The crude protein requirement in mud crabs ranges from 35-40% for adult crabs and 45% for juvenile crabs 0.25g – 3g (Catacutan, 2002).

Lipid requirements range from 5.3 to 13.8% (Sheen and Wu, 2011) and dietary cholesterol is 0.5-0.79% (Sheen, 2000). Specifically, for the molting process, the likely minerals of greatest need would be calcium for shell formation and potassium and magnesium to help with osmoregulation (Catcutan, 2002). Of course, lipids stored for energy will be important to accumulate before the process begins.

2.4. **Soft-shell mangrove crab farming in Southeast Asia**

In recent years, soft-shell crab farming has become popular because it is a profitable business. The crabs used are mostly wild-sourced *Scylla* species and some blue swimming crabs, *Portunus pelagicus*. The soft-shell crab can be eaten completely when cooked, including the carapace and all limbs. Thailand, Myanmar, Vietnam, Malaysia, Indonesia, and recently the Philippines are producing soft-shell crabs. The greatest concentation of soft-shell crab farms is in Ranong, Thailand, where farmers can market the crabs daily at the numerous seafood-processing plants in the area. Income is generated daily from the harvest of soft-shell crabs. Processed crabs are sold to local restaurants and exported to Hong Kong, Singapore, South Korea, Japan, Taiwan, Europe and the United States.

2.4.1. Pond site requirements for setting up soft-shell mangrove crab farms

According to a soft-shell crab farming manual (Quinitio and Lwin, 2009), a typical brackish water earthen pond for fish or shrimp farming can be used for farming of soft-shell mangrove crabs. The pond area for soft-shell farming may range from 0.5 to 1.0 ha. The three sides of the pond should have at least 3 meters free space from the pontoon and crab boxes to the dikes. The fourth side where the gate (water inlet/outlet) is situated should have at least 5 meters free space from the pontoon and crab boxes to the dike for better water circulation. Fish are often stocked in the pond in a polyculture to maximize the use of the area. A 0.5 ha farm can accommodate at least 15 pontoons that can hold 24,000 crab boxes. The criteria of a suitable pond site are the following (Quinitio and Lwin, 2009):

- Site - The site should be protected from typhoon, flood and siltation.
- Accessibility - The farm should be near the roads, source of crab supply, processing plant and market.
- Soil – The pond soil should be clay or clay-loam to retain the water.
- Seawater / brackish water supply - Adequate supply of clean seawater or brackish water should always be available.
- Availability of electric power - Electricity is important in managing the farm especially during daily harvest of soft-shell crabs where these are held in buckets with freshwater and aeration, freezer for storing the feeds and crabs, illumination for inspection for soft-shell crabs at nighttime and other activities.
- Freshwater supply - This is used for holding of soft-shell crabs, washing of plastic containers, and domestic use.

2.4.2. Pontoons or floating platforms

Pontoons support the plastic boxes where crabs are held. A pontoon of polyvinylchloride (PVC) pipes (4 cm in diameter) is constructed (with 4 m length x 8 units pipe) connected to each other and having 4 columns. Both ends of the pipes are covered with PVC caps to prevent the entry of water. The length of the pontoon depends on the pond size. The pontoon is divided into several compartments measuring 110 x 110 cm each, separated by a narrow piece of wood (2.5 cm width x 2.0 cm thickness x 110 cm length) or PVC pipe with smaller diameter tied across the main PVC pipes. One compartment can hold 24 boxes arranged in 4 rows, that is 6 boxes in each row. One pontoon may have 25 compartments that can hold 600-boxes (25 x 24 = 600) depending on box size. More than 4 rows in each compartment are not advisable since it is difficult to inspect the boxes across at one glance. Polyethylene rope is tied to a wooden pole and inserted in the rings installed in the mid-section of the pontoon. The other end of the rope is tied to the opposite pole. Rings to guide the rope for pulling are installed every 5 compartments in the mid-section of the pontoon. Another rope is tied from the end of the PVC pipe and inserted under the wood support of the bridge going to the opposite end of the pontoon.

2.4.3. Maintenance of water quality

Pond water deteriorates due to accumulated wastes; hence, it is important to maintain good water quality. This is especially important as feeding with trash fish or crushed molluscs adds a tremendous load of high nitrogen content organic matter to the pond.

1. Maintain water depth of not less than 80 cm and water parameters as follows:

Parameter	Value
Temperature	27-32 °C
Salinity	16-30 ppt
Dissolved oxygen	≥ 5ppm
pH	7.5-8.5
Transparency	30 cm (secchi disk)

2. Change 10-20% of the total water volume each day for 2-3 days during spring tide.

2.4.4. Sources of crabs

Mud crabs captured in mangrove areas are sold by collectors to traders who sell the crabs to the farmers. In countries where the quantity of the desired size for soft-shell crab farming is a problem, crabs may be obtained from ponds in which seed stock comes from the hatchery. From the hatchery, crablets less than 1.0 cm CW (carapace width) are grown in nursery cages or pens for 3-4 weeks, then in grow-out ponds for another 1-2 months before they could be used for soft-shell crab farming.

1. Select 50-130 g body weight (BW) of any mud crab species sourced from either mangroves, estuaries or pond.
2. Select immature hard-shelled and not newly molted female and male crabs.

2.4.5. Feeding of soft-shell crabs

In soft-shell crab farming, feeding is not done daily because it is laborious and expensive. When we have tried to feed crabs daily, many boxes contain uneaten food, which needs to be removed to prevent the growth of bacteria, fungus and other disease-causing microorganisms. Removal of excess food is laborious and time consuming. Therefore, at our farm and most of our neighbors, we feed the crabs early morning or afternoon once every 2-3 days. We feed the crabs in each container with fish, fish ball or other cheap protein source at roughly 8% of the biomass using the formula:

Amount of food (g) = <u>average body weight (g) x 0.08</u>

So for a typical pond stocked with 20,000 crabs

Example: 80 g x 0.08 x 20,000 crabs = 128,000 g or 128 kg (6.4 g per crab)

At my farm, we would purchase 350-400 kg of forage fish every second or third day to feed the three ponds of crabs. After we bring the fish to the farm, we wash the fish and chop to size according to ration for the size of crabs in a particular part of the pontoon. The staff then use the pulley system to slide the pontoon under the bridge and place the food items through the holes of each crab box. About four hours later, during the next inspection, the staff remove any uneaten food and molted and dead crabs. (Quinitio and Lwin, 2009).

2.4.6. Inspection of crab

It is most common that crabs molt during the night and early morning. The peak of molting for 80-100 g crabs in the inter-molt stage is normally within the 3rd week after stocking. The newly molted crabs must be retrieved immediately after molting because the shell starts to harden in less than 5 hours. Once the shell hardens, the crab cannot be sold to the processor. The next molting will take longer since crabs have grown bigger.

2.4.7. Processing and packing of soft-shell crabs

In Thailand where soft-shell crab farming is well established, many seafood processing plants buy the soft-shell crabs directly from the farmers. It is very convenient for the small-scale farmers to bring the products to these processing plants. In other countries, big farms set-up their own freezing facilities. Soft-shell crabs are classified and processed prior to packing based on the requirements of the buyer. Crabs may be packed whole, or without one or more body parts (eyes, mouthparts, gills and abdominal flap). Crabs are sorted according to the following size ranges.

Classification	Size range (g)
Extra large or jumbo	Over 200
Large	151-200
Medium	101-150
Small	50-100

2.4.8. Advantages of soft-shell crab farming

Soft-shell crab farming can be profitable but is labor intensive. The advantage of this business venture is that income can be generated daily. The market price depends on the size and condition of the soft-shell crab. The highest prices are for the largest crabs complete with legs (over 200 g). Lowest prices are for crabs that lack legs regardless of the size, and those with slightly hardened shell. In 2017, our farms in Ranong are paid Baht 250 (US$ 7.3) for one kilogram (8-10 pcs/kg) of soft-shell crabs. Soft-shell crabs are marketed locally or exported to Hong Kong, Singapore, South Korea, Japan, Taiwan, Europe and the United States. The wholesale price ranges from US$ 7.5 – 10 /kg (5-15 pcs/kg). A 0.5 ha pond farm with 30,000-35,000 crabs requires at least six regular staff and two laborers, hired on a daily basis to wash the plastic boxes and assist in the stocking of crabs. Since inspection is done every four hours, two work shifts (night and daytime) are necessary. About 50% of the initial investment goes to purchase boxes (Blackshaw, et al., 1999).

2.4.9. Protein requirement of mangrove crabs

In general, crabs are viewed as carnivores as gut analyses and feeding observations in their natural habitats including mollusks, crustaceans and fishes that contain relatively high protein content (Hill, 1979). Dietary protein supply is one of the major factors that influences the productivity of cultured species (Davis et al., 2014). Proteins are complex biomolecules found in the cell and tissues of the crabs. Proteins are made up of linear sequences of amino acids. Twenty naturally occurring amino acids are present in all living organisms (NRC, 2011). Crustaceans require ten kinds of essential amino acids, such as arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and tryptophan. Researchers in the Philippines, Vietnam and Australia reported that crabs have protein requirements of 35-55% based on their age and size (Catacuan et al., 2002; Tuan et al., 2006; Truong, 2008; Truong et al., 2008; Anderson et al., 2004; Holme et al, 2006). Millamena and Quinitio (2000) examined the effects of diet on reproductive performance of female mud crabs. By using three dietary treatments, a natural diet (meat from squid, mussel and fish), a formulated artificial diet, and a 50:50 mixture of the two, they concluded that a mixture of the two diets gave the best results overall in terms of fecundity, egg hatchability and larval survival. Catacutan (2002) reported that juvenile mangrove crabs have elevated crude protein requirements of more than 40%. Similar research also reported that mangrove crabs have similar dietary requirements to *Penaeus* shrimp species (Catacutan et al., 2003). Table 2.3 shows the various essential amino acid profiles used in typical shrimp diets.

Table 2.3. Comparison of various essential amino acid profiles used to formulate shrimp diets.

Essential amino acids	Amino acids as % of protein					
	Tissue amino acid profile				Average for all species	Published amount in feeds[5]
	P. aztecus[1]	*P. japonicus*[2]	*P. monodon*[3]	*P. sp.* [4]		
Arg	5.17	5.32	6.34	6.50	5.83	5.8
His	3.15	1.57	2.31	1.10	2.03	2.1
Iso	4.47	3.01	4.60	4.90	4.24	3.5
Leu	9.75	5.25	7.76	9.90	8.16	5.4
Lys	6.09	5.53	6.54	6.40	6.14	5.3
Met and Cys	4.85	2.55	2.80	3.60	3.45	3.6
Phe and Tyr	8.43	5.85	5.75	8.80	7.21	7.1
Thr	5.38	2.87	4.79	4.40	4.36	3.6
Try	1.10	-	-	1.10	1.10	0.8
Val	5.07	2.90	5.69	3.40	4.26	4.0

Shewbart et al., 1972[1], Tacon, 1990[2], Marsden et al., 1997[3], Boghen and Castell, 1981[4], Akiyama et al.,1991[5]

2.4.10. Lipid requirements of mangrove crabs

Alava et al., (2007) and Ali et al., (2011) reported that *S. serrata* could utilize vegetable oil or rendered animal fats as supplements in the formulated diets as a partial replacement (50%) of cod liver oil without compromising growth and survival. Partial substitution of marine fish oil with suitable vegetable oils or animal fats can reduce the feed cost considerably, especially in the context of rapidly rising fish oil prices. Sheen (2000) reported a requirement for dietary cholesterol of 0.5 to 0.79%, which must be incorporated into any formulated feed. Table 2.4 describes the percent cholesterol found in common marine and terrestrial animals that might be used as ingredients in a formulated crab diet. Tables 2.5 and 2.6 describe the mineral levels suggested for shrimp feeds, Table 2.7 lists the suggested vitamin levels each of which would be a guideline for a formulated crab diet. Table 2.8 provides guidelines for protein and energy levels and ratios for different levels of intensification of shrimp farming, which again provides a guide for intensive crab farming.

Table 2.4 . Cholesterol content in common marine lipids and animal fats.

Adapted from NRC, 2011	Lipid source	% Cholesterol in oil
Marine oils	Squid oil	1.3
	Herring oil, Pacific	0.8
	Cod liver oil	0.6
	Salmon oil, wild	0.5
	Menhaden oil	0.5
Rendered animal fats	Poultry fat	0.1
	Pork lard	0.1
	Tallow	0.1

Table 2.5.Recommended mineral levels in commercial shrimp feeds.

Mineral	Quantity per kg of feed
Calcium	maximum 2.3%
Phosphorus – available	0.8%
Magnesium	0.2%
Potassium	0.9%
Iron	maximum 200 mg
Copper	35 mg
Zinc	150 mg
Manganese	20 mg
Selenium	1 mg
Cobalt	0.05 mg

Adapted from: Akiyama *et al*, 1991 and Davis and Gatlin, 1991.

Table 2.6. Mineral supplementation for practical diets (American Soybean Association International Market Program shrimp mineral premix).

Element	Units	Amount in premix	Amount in feed
Manganese	mg / kg	2000	500
Copper	mg / kg	8000	2000
Zinc	mg / kg	40000	10000
Iodine	mg / kg	20	5
Selenium	mg / kg	280	70

Table 2.7. Recommended vitamin content in shrimp feed by American Soybean Association International Marketing Program**.**

Vitamin	Units	Amount in premix	Amount in feed
Vitamin A	IU/kg	1,500,000	600,000
Vitamin D3	IU/kg	500,000	200,000
Vitamin E	IU/kg	20,000	8,000
Biotin	mg/kg	50	20
Folic acid	mg/kg	1,875	750
Niacin	mg/kg	25,000	10,000
Pantothenate	mg/kg	18,750	7,500
Pyridoxine (B6)	mg/kg	7,500	3,000
Riboflavin (B2)	mg/kg	7,500	3,000
Thiamin (B1)	mg/kg	10,000	4,000

Table 2.8. Dietary requirements for shrimp based on culture system

Culture System	Crude protein (%)	Digestible protein (DP) %	Metabolized energy (ME) Kcal/100g feed	DE/DP ratio (mg/kcal)
Extensive	23	18-19	257-271	70
	25	20-21	286-300	70
	30	24-26	253-274	95
Semi-intensive	35	28-30	295-316	95
	40	32-34	337-358	95
Intensive	40	32-34	291-309	110
	45	36-38	327-345	110

2.5. **Aquaculture Feed Ingredients**

The raw materials that will be selected for feed production must be sustainable, cost effective and with steady availability. Raw materials from aquatic origin, such as fisheries by-products and fish meal, are suitable for feeding of marine organisms, including mangrove crabs. However, the availability of these raw materials is becoming more restricted and the prices are increasing accordingly. Therefore, these raw materials must be spared and replaced by meals from sustainable sources. The alternative sources may include rendered products from the animal production industry (including fish meals produced from fish processing by-products) and plant materials.

Raw materials from plant origin include numerous types, such as soybean meal (SBM) and other oil seed by-products and glutens. The global availability of such raw materials is good and their market price is significantly lower compared to the raw materials from aquatic origin. It has been shown in several research works that the digestibility of some vegetable raw materials is high (above 85%) when fed to mangrove crabs (Azra and Ikhwanuddin, 2016; Nguyen et al., 2014; Pavasovic et al., 2004; Catacutan et al., 2003), implying they have a potential to be ingredients in mangrove crab feeds. One of the goals of the present project is to find ways to include plant materials into the crab diet (the use of these raw materials is not straight-forward). The plant raw materials might contain anti-nutritional factors that can decrease the digestibility of the feed and reduce the palatability when fed to aquatic organisms

Poultry by-products are another type of promising ingredient in compounded feeds for crabs. This group of raw materials includes several products, such as poultry meal, feather meal and hydrolyzed feather meal. Being rendered products from the poultry industry, the availability and quality of these raw materials are good and they are suitable for the aqua-feed industry. The nutritional quality is expected to be high for feeding of mangrove crabs, though the price of the poultry by-product meals is higher compared to the plant raw materials. Meat, blood and bone meals are available as by-products of cattle and hog processing. These are also widely used ingredients in aquaculture diets as they have good nutrient profiles, are readily digested by shrimp and are cost effective compared to fish meal.

2.5.1. Feed engineering

The technical properties of the feeds are pivotal to their suitability for mangrove crab feeding. The feed should be stable in water for long time periods, the leaching rate of water soluble nutrients should be low and minimal amount of feed should be wasted while the crabs are nibbling on the feed. Two main options are available to manufacture the feed for the crabs: to produce dry pellets (either extruded or compression pelletized) or to produce semi-moist feeds. Dry pellets can be produced either in an extrusion system or in a compression pellet machine. The advantage of producing feeds in these methods is that the machinery is available in many places around the world and the technology is established. Another advantage is that the feeds produced in these methods have long shelf life, they can be transported long distances and stored for long time periods. The main disadvantages of the dry pellets are that significant amount of feed material may be wasted due to the feeding habits of the crabs and potentially low palatability. Poor feed utilization efficacy would result in elevated production costs (due to high feed conversion ratio) and pollution of the water.

Semi-moist feeds can be produced in several techniques. Some are “homemade” methods that require relatively simple and inexpensive equipment; however, the production capacity is low. Recently some new equipment for production of semi-moist pellets has been introduced into the market, permitting an industrialized scale production of semi-moist pellets. In preliminary work that had been carried out in Singapore (Ali et al., 2011) it was found that the soft (semi-moist feeds) feeds are very suitable for mud crab feeding. The crabs ingested the semi-moist feeds very well resulting in low level of waste released to the water and good growth and survival rates. The semi-moist feeds production system permits the use of fresh and locally available raw materials, such as fresh fish offal or protein hydrolysates. The use of these raw materials not only reduces the cost of the products but also improves the digestibility and palatability of the produced feeds. Shelf life and physical durability (to enable transportation of the products) are among the limitations of the soft, semi-moist feeds. However, research conducted by Ali et al., (2011) conclude d that using dry pellets are preferred over the semi-moist feed for feeding mud crabs

A recent study (Catacutan, et al., 2003) showed that nutrients in feedstuffs of animal and plant origin were digested well by *S. serrata*. The digestibility of lipid in protein-rich animal feedstuffs was low compared to carbohydrate-rich plant feedstuffs. This study indicated that plant feedstuffs can be utilized as the major source of nutrients for cheaper and effective diet. *S. serrata* grows well when fed diets containing 32–42% dietary protein with 6% or 12% lipid at dietary energy ranging from 14.7–17.6 mega-joules/kg (MJ/kg). Triño et al., (2001) showed that the survival, growth and economic viability of using a diet with or without vitamin and mineral supplements for grow-out culture in ponds were comparable. Selective harvesting is normally practiced partly due to multiple sizes of crabs in ponds. Mangrove crab culture in ponds can give a return on investment (ROI) of 49–66% and a payback period of 1.2–2 years (Tobias-Quinitio, 2015).

2.5.2. Crab feed manufacturing

When preparing a formulated diet, raw materials need to be stored in dry cool and clean conditions. If the ingredients have a shelf life, the expiry dates should be recorded. Use first in and first out (FIFO) if possible, but be careful to avoid storing any ingredients too long. Raw materials nearing their expiry dates should be checked by quality assurance (QA) to confirm they meet quality criteria and are still useable. After the ingredients are measured and mixed they are passed to the pre-conditioner. From the pre-conditioner, feed mash is put into the extruder barrel inlet. The inside of the extruder is like a screw conveyor, and pre-conditioned feed mash is pushed towards the die plate. The starches are gelatinized in the mix due to built-up heat and pressure (Table 2.9). For floating feed, it requires higher starch levels and lower fat levels that a sinking feed. The starch minimum requirement for a floating pellet is more than 20% while a sinking feed can be made with less than 8% starch. As feed pellets exit the extruder, the feed passing through the die is cut to length by a rapid spinning knife. After the extruder die, the pellets are conveyed to the dryer. Drying is an important step in an extruded feed because it is necessary to remove 12 to 15% of moisture from every pellet. Two common types of dryers are horizontal bed dryers and vertical dryers. A horizontal dryer was used for this experimental feed production. Feed coming out of a dryer, especially warm feed at temperatures of 50 °C to 70 °C is easy to spray coat with oil which better penetrates the surfaces of the pellet. Two types of coaters are rotary spin coaters and drum coaters, but for this experimental feed, a rotary spin coater was used. Rotary spin coaters have compact design and are easy for simple liquid application. We weighed feed into a twin-shaft paddle mixer mounted on load cell. Then oil was weighed and sprayed into the mixer filled with feed. Once feed passed through the dryer and spray-coating steps, cooling is the next step. We used a forced air, counter flow design, with cool air coming into the cooler from the bottom while warm pellets entered from the top. The feed was then loaded into bags for transport to the farm (Figure 2.4). The most important thing in cooling is that the final pellet temperature should be within 5°C ambient temperature to prevent moisture condensation in the sealed bag.

Figure 2.4.Crab feed manufacturing process

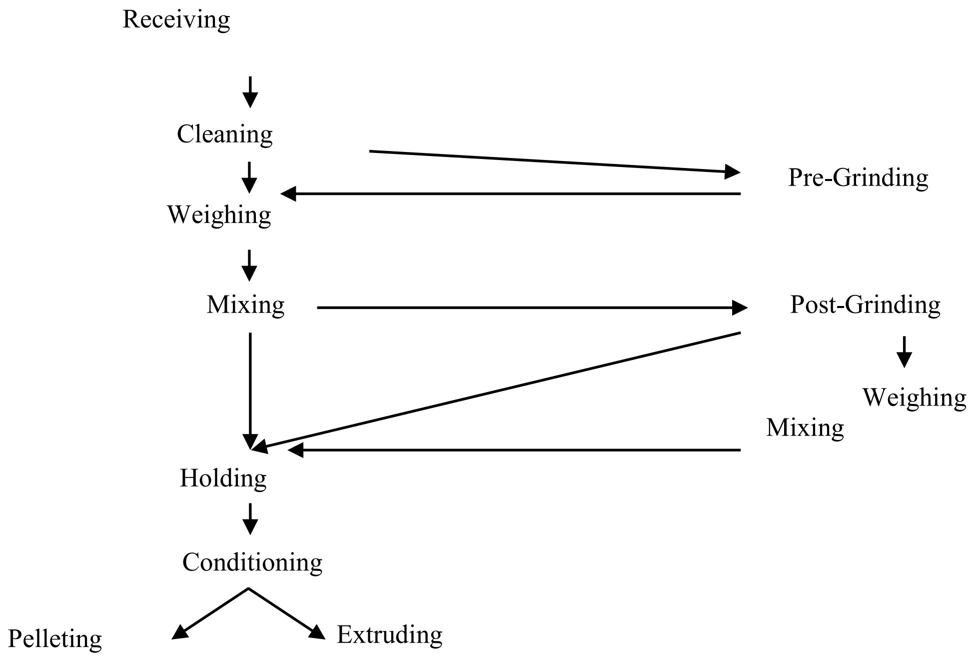


Table 2.9. Crab feed extrusion process for experimental feed

BHULER DNDL 44 Twin Screw Extruder **10 mm pellets (use 8 mm 1 hole dies for all diets)**			
Name	Slow Sinking	Sinking	Floating
Solid quantity	55.000 kg/h	55.000 kg/h	55.000 kg/h
K-Ton Feeder 1	55.000kg/h	55.000kg/h	55.000kg/h
Oil Feeder 3	0.000kg/h	0.000kg/h	0.000kg/h
Water feeder 4	14.990kg/h	15.981 kg/h	14.984 kg/h
Pressure endplate	41.973 bar	41.580 bar	37.074 bar
Drive power	5.608 KW	5.085 KW	4.932 KW
SME	80.124 Wh/kg	71.641 Wh/kg	70.467 Wh/kg
Through-put	69.990 kg/h	70.981 kg/h	69.984 kg/h
Temperature Barrel 3 (Add measurement 1)	85.363 C	78.395C	87.867C
Temperature Barrel 4 (Add measurement 3)	78.507 C	72.247 C	83.161 C
Temperature Barrel 5 (Add measurement 4)	85.199 C	80.278 C	89.310 C
Temperature Barrel 6 (Add measurement 5)	98.631 C	91.566 C	102.875 C
Revolution screws	450.2 RpM	400.2 RpM	400.2 RpM
Torque absolute	118.6 Nm	121.3 Nm	118.1 Nm
Torque relative	26%	26%	25.5%
Temp Endplate	102° C	94.5 °C	101.0 °C
Revolution cutter	178.8 RpM	178.5 RpM	178.2 PpM
Mokon 1- Temperature of barrels	180	170	200
Mokon 2-Temperature of die plate	180	170	200

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Chapter 3. **Proof of principle: Practical diets for soft-shell mangrove crabs**

3.1. **Abstract**

Large-scale farming of soft-shell crab has been going for more than 20 years, yet little research has been conducted regarding formulated feeds. In order to ensure the sustainability of this industry there is an urgent need to replace "trash fish" with manufactured diets. The present study evaluated three compounded feeds for their acceptance and attempted to understand the preferred physical form of the formulated feed by mangrove crabs, also commonly known as mud crabs. Wild caught crabs, sized 60-80g, were collected from one estuary and delivered to a farm in Ranong, Thailand. A total of 600 crabs were placed into individual boxes in one PVC pontoon raft each with 150-boxed crabs. Three experimental diets and a trash fish reference diet were randomly assigned to 150 crabs per treatment across one raft. The crabs were fed at 3% of their body weight per day with two feedings over a 45-day period. Crabs reared on trash fish were not significantly different from those reared on the high fishmeal diet, both near 0.80 g/day. The high soy content diet produced significantly lower growth (0.71g/day) in the crabs. However, the soy-based diet had the highest survival rate (98%) versus commercial crab feed at 94% and trash fish and high fishmeal at 91% each. The results of the present study demonstrate that mangrove crabs will accept formulated feeds using either simple laboratory process or commercial extrusion. Hence, the development of specific diets for crabs is technically possible. Further work on nutritional requirements are encouraged. Based on observation in the field pellet size and stability

of the feed should be improved to minimize pellets from falling out of the containers and allow handling by the crab.

3.2. Introduction

Scylla spp. mangrove crabs are one of the fastest growing of all commercially farm-raised crustaceans (Keenan and Blackshaw, 1999). Juvenile crabs typically double their weight each time they molt (Nguyen et al., 2014; Truong et al., 2008, 2009; Anderson et al., 2004). Due to their fast growth, high prices and both domestic and international market demand, mangrove crab farming has become popular especially in Southeast Asia. Mangrove crabs are sold as live, chilled and frozen products. There are three different kinds of crab farming in Asia namely crab fattening, grow-out farming and soft-shell mangrove crab farming. Among mangrove crab farmers, soft-shell crab is the most profitable farming because the soft-shell mangrove crab can be eaten whole (carapace and all limbs) when cooked and are available year round (Tobias-Quinitio, 2015). Because of its profitability, there is an increasing interest to engage in this type of business venture in Asia (Quinitio and Lwin, 2009).

Farming of soft-shell mangrove crab has been practiced for many years in a number of Asian countries such as Thailand, Myanmar, Vietnam, Malaysia, Indonesia, and recently, the Philippines, Bangladesh and India (Quinitio and Lwin, 2009). Soft-shell mangrove crab farming in Asia started during the early 1990's when there was a strong demand for soft-shell crabs in domestic and international markets. Thailand is the leading soft-shell crab producer in the world. One of the greatest concentrations of soft-shell mangrove crab farms is in Ranong, Thailand, where farmers can market the crabs daily at the numerous seafood-processing plants in the area (Quinitio and Lwin, 2009).

The most common system for soft-shell crab production used in Ranong is individual rearing where healthy intact crabs are held in perforated plastic boxes having detachable covers. Boxes are positioned on floating platforms made of wood or PVC pipes that can hold several hundred boxes. Each crab stocked in these boxes is checked for molting every 3 to 4 hours. Mangrove crabs of the genus *Scylla* collected from mangrove swamps are the major source of stocks for farming (Quinitio and Lwin, 2009.) The crabs must be stocked in individual containers to avoid cannibalism and typically are fed every day or every other day. Crabs grow through repeated molting (Shelley and Lovatelli , 2011). The hard outer shell cannot stretch with growth, so the crab must molt, or shed, its old exoskeleton in order to grow (Allan and Fielder, 2004). Before molting, a new exoskeleton is formed below the old and hard exoskeleton. During the molting process, the old shell splits and the crab backs out of it. The new shell develops and hardens in 4-8 hours. If the crab is removed from the brackish water shortly after molting (during the first four hours), it will remain in the soft-shell condition when held in freshwater (Parado-Estepa and Quinitio, 2011). Fresh water does not have the concentrations of minerals that are needed for hardening of the shell (Tobias-Quinitio, 2015). Then it can be sold as a live soft-shell crab. Regular monitoring and retrieval of soft-shell crabs is laborious. However, experienced staff can quickly identify soft-shell crabs from hundreds of crabs in the stock.

Currently, production of all types of crab farming, including soft-shell mangrove crabs, relies on trash fish as feed (Ali et al., 2011). The prospect of decreased trash fish availability and affordability is becoming a major issue in the industry. Related issues are the storage of trash fish, water fouling, and the potential of trash fish as vectors of parasites and pathogens. The development of a cost-effective, nutritionally adequate formulated diet is a major need for sustainable development of soft-shell mangrove crab farming. Efficient nutrition is vital for the process of molting (Suprayudi et al., 2004), and market prices are dependent on the body content or fullness of flesh attained when crabs molt. Although the soft-shell crab industry has been going for more than 20 years, little research has been published regarding compound feeds and the urgent need to substitute for trash fish with formulated diets (Kar et al., 2017; Quinitio and Lwin, 2009). The Southeast Asia Fisheries Development and Education Center (SEAFDEC) has developed technology for crab breeding and the survival rate of juvenile crabs is increasing every year. However, there is no widely available commercial feed specifically for soft-shell mangrove crab production, while there are papers describing the nutritional requirements of brood stock and juvenile mangrove crabs (Allan and Fiedler, 2003).

Dietary requirements for mud crabs have been demonstrated to not be as stringent as those of most penaeid shrimp, with good growth occurring over a wide range of protein and lipid levels (Catacutan, 2002). Sheen and Wu (1999) reported that *S. serrata* demonstrate better utilization of lipids than shrimp following a study in which they measured the growth response of juvenile crabs that had been fed diets with a range of inclusion levels (2 to 13.8% lipid) of a mixture of cod liver oil and corn oil. The result showed that using dietary lipid levels ranging from 5.3% to 13.8% appeared to meet the lipid requirement of juvenile crabs (Sheen and Wu, 1999). According to (Alava et al., 2007), using both formulated diet protein 58% and lipid 18% and natural feed gave the shortest number of days from spawning to hatching and better survival of broodstock. Based on research by Unnikrishnan and Paulraj (2009), juvenile mud crabs of *S. serrata,* demonstrated their best growth performance with dietary protein level of 45% (experiment tested a range of 14.7-55% CP with 5 % intervals) and required dietary protein of 14.7% to 16.2% for the bare minimum to maintain metabolism in their 63-day trial. Researched conducted by Catacutan (2002), with six test diets (with three protein levels of 32%, 40% and 48% at two lipid levels of

6% and 12% and protein:energy ratio from 20.5 to 31.1 mg protein/kj) found that *S. serrata*, juveniles grew faster when fed diets containing 32 and 40% dietary protein with either 6% or 12% lipid and dietary energy ranging from 14.7–17.6 MJ/kg. Research conducted by Catacutan et al., (2003), reported that juvenile mangrove crabs have elevated crude protein requirements of more than 40%.

Brood stock mangrove crab nutrition studies have been conducted by several authors (Marichamy et al., 1986; Marichamy, 1996; Samarasinghe et al., 1999; Fortes, 1999; Saha et al., 2000; Triño et al., 2001; Christensen et al., 2004; Alava et al., 2007; Manivannan et al., 2010; Azra and Ikhwanuddin, 2015). In the review by Azra and Ikhwanuddin (2015) comparisons were drawn between maturation brood stock diets based on natural diets (squid, fish, mussel), formulated diet (shrimp brood stock diets) or mixed diets of natural and formulated feeds. The review concluded that the mixed diets generally provided the superior reproductive results. Using mixed feeding, 50% of formulated diet of protein (58% and lipid 10%) with natural feed 50% gave better survival, fecundity, spawning and hatching rate (Millamena and Quinitio, 2000). In addition, nutrition research conducted by Djunaidah et al., (2003) reported that a formulated diet performed the same as fresh fish in terms of reproductive performance of crabs sized 200g- 300g.

However, there is a limited amount of nutritional information for growout diets for sub-adult and adult mud crabs (Holme et al., 2009) and virtually all of the trials have been conducted in the lab. This project will address the need for a practical formulation feed for grow-out crab farming by testing diets on-farm under normal production conditions.

This industry is creating many jobs and contributing a considerable amount of economic development. These days many farmers cannot afford to feed the whole trash fish and now they are feeding only the head of the fish. Not only are the “trash” fish expensive but also they are hard to get the whole year around. Especially in Southeast Asia, the crab farming industry is competing for the trash fish with human consumption, pet feed and fishmeal production. The present study evaluated a compounded feed for its acceptance and attempted to understand the preferred physical form of formulated feed by mangrove crab for better-feed utilization.

3.3. **Materials and Methods**

3.3.1. Feed preparation

Both high fishmeal and high soybean meal diets were designed and produced at Aquatic Animal Nutrition laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, U.S.A.). Both diets were formulated to be iso-nitrogenous (40% of protein and 7% of fat) and iso-lipidic (Table 3.1). Pre-ground dry ingredients and oil were mixed in a food-mixer (Hobart Corporation, Troy, Oh, USA-) for 15 minutes then hot water was blended into the mixture to a consistence appropriate for making pellets using a meat grinder (Hobart Corporation, Troy, Oh, USA). The mash was placed into a meat grinder with the resulting strands then cut into pellets. The pellets were approximately 4 mm in diameter and were dried in a forced air dryer (≈50° C). A sample of each diet, after production, was collected and sent to Eurofins Laboratory, Ho Chi Minh City, Vietnam for analysis of proximate composition (g 100 g^{-1} as is) and amino acid (g 100 g^{-1} as is) following AOAC (1995) procedure by the lab (Table 3.3). The rest of the pellets to be fed in the experiment were stored in re-sealable plastic bags and air shipped to Thailand for feeding at a commercial soft-shell crab farm in Ranong.

A commercial crab fattening feed to be used in the trial was manufactured in Thailand, and is custom prepared only for their regular customers. It is an extruded diet with 40% protein and 10% lipid in a sinking feed. The “trash fish” were primarily composed of one pelagic fish species, the smooth-scale rainbow sardine (*Dussmieria elopsoides*). On each day, fish were cut into portions, such that the estimated dry weight of the portion was equivalent to the dry weight of the commercial and experimental feeds to be supplied. This was determined by drying several of the trash fish and determining the wet weight and dry weight.

3.3.2. Feeding Trial

The crabs for the trial were all collected by one supplier from an estuary in Kyun Su Township, Myeik district, of Southern Myanmar. Thus, the population was expected to be somewhat homogenous. Several thousand crabs were collected on one night and delivered to the farms the following morning for stocking. A total of 600 crabs, with (initial weight 70±5.57 g) and a range of 60 to 80 g, were selected from that day's catch. The most popular design for pontoon rafts to hold crab boxes in Thailand and Myanmar is a 4-by-400-box array. Thus, each pontoon can hold up to 1,600 crabs at a time. The 600 experimental crabs (150 crabs per diet) were stocked in one PVC pontoon raft. The pontoon array is moved with a pulley mechanism under the center of the pond bridge so that the farmer can assess the condition of the crabs every four hours and feed on a selected feeding schedule.

Crabs were stocked into the boxes on 28 May 2016 and the feeding trial was started on 1 June 2016. Boxes within one raft were randomly assigned, with a random number table such that 150 crabs were fed with the high fishmeal formulated diet, another 150 with high soybean meal diet, 150 with commercial crab fattening feed and 150 with traditional trash fish diet as reference. The crabs were fed 3% of body weight per day, split between two feedings. Trash fish were fed on a dry weight basis. Several fish were sun dried to a constant weight to determine the wet weight – dry weight ratio. Water temperature and salinity were measured twice a day, at 0800 h and 2000 h, using an alcohol thermometer and a hand-held refractometer (Master by Atago, Thailand). pH and alkalinity were determined every week at 0800 h (portable pH tester and saltwater alkalinity colorimeter, both by Hanna, United Kingdom) and water in the pond was exchanged with every spring tide (approximately 10- 20% exchange as normal farming practice). Other pontoons in the pond were stocked and monitored using normal commercial farms processes and only one pontoon was used (PVC raft) for the experiment.

Mortalities were noted, removed, and discarded when the crabs were checked every four hours for molting during the 45-days of trial. During the experiment, the date and inspection time of any crab who had molted was noted. The crab was weighed individually as it was removed from the box as a newly molted soft-shell crab and recorded as final weight in grams. The old shell or exoskeleton were removed and discarded. The researcher recorded both initial weight at the time of stocking into the box and final body weight of newly molted crabs out of the box. Carapace length was measured but no analyses have been conducted. The trial terminated on 17 July 2016 when all the surviving crabs were counted. Percentage weight gain was calculated as follows:

Percentage weight gain (%) = (Final weight- Initial weight) / Initial weight x 100

3.3.3. Environmental conditions

1 June 2016 was the end of the hot and dry season and beginning of the monsoon. May, June, and July are typical molting season of mangrove crabs in Thailand. Water temperature and salinity were measured two times a day (0800 h and 2000 h) each day. The temperature ranged from 27-34^{o} C and the salinity ranged from 15-28 ppt. This period normally has very good environmental conditions to encourage fast growth and frequent molting with minimal conditions that would induce stress (Table 3.2).

During the feeding trial, the crabs were checked for molting and mortality every four hours. If a crab was found to have died, it was removed and discarded. If a crab was found to have molted, the date and time of inspection was noted. Live crab or newly molted crab after taking out of the box was weighed and recorded it as final weight for experiment data analysis. Then molted crab or soft shell crab were put individually into oxygenated fresh water for 30 minutes. Soft shell crab was weighed and recorded it again after putting in fresh water for 30 minutes which is normal practice by the farmers. Live soft shell crabs were put into tray with a wet cloth with a lot of fresh water and sell it next day as live soft shell crab to the processing plant. On July 17, 2016, all remaining live crabs were counted for survival. The average weights and percentage weight gains for the individual crabs were determined. For those crabs that molted during the 45-day feeding trial, the average number of days from stocking until molting was also determined.

3.3.4. Statistical Analysis

Final body weight was defined as the weight in grams of the newly molted soft-shell crab as it came out of the box. The final weight gain was evaluated using initial weight at the time of stocking into the box. If a crab did not molt within 45 days, it was not included in the weight gain or molting statistical analyses. Data from percent weight gain trial were analyzed using analysis of covariance (ANCOVA) to determine significant differences among the treatments, followed by Tukey's multiple comparison test to compare difference among treatment means. A Kaplan-Meier estimator (time-to-event analysis) was used to measure time to molting (mean number of days at which 50% of crabs in a treatment had molted) between groups to determine significance. If any crab molted within seven days of stocking, for any treatment group, they were not included in the data as it is doubtful that there could be any nutritional or diet effect that quickly. Mortality (or survival) was analyzed by a generalized linear model with logistic regression. All data were analyzed using SAS (V 9.4. SAS Institute, Cary, NC, USA) software with significance level set at $\alpha \leq 0.05$ (unless otherwise stated).

Results

The crabs fed the trash fish (mean percentage weight gain 57.79 %) had a significantly higher mean final weight and mean weight gain than crabs offered the other diets. Final weights of crabs offered the high fishmeal feed (mean percentage weight gain 51.58 %) mean was then significantly higher than the high soybean meal (mean percentage weight gain 45.71%). The commercial feed and the high fishmeal content diet provided for mean weight gains higher than the high soy content diet 51.2% (Table 3.4). However, the high soybean content diet had not a survival rate (98%) significantly greater than the other three diets. Trash fish still have the shortest number of days to molt, 50% of crab molt within 29 days compared to 32 days for each of the formulated diets.

3.4. **Discussion**

In this study, the experimental diets were designed to contain 40% crude protein and 7% lipid. The experimental diets were formulated based on previous nutritional studies on juvenile and brood stock mangrove crabs. As expected, the high fishmeal diet yielded better weight gain compared to a high soybean meal content diet although both diets were accepted by the crabs. The high soybean meal content diet achieved the highest survival, possibly due to a healthy balance between both animal and plant-based feed ingredients in the formulation. Prasad and Neelakantan (1988) reported that significant amounts of plant-based material and detritus have been identified in the digestive system of juvenile mangrove crab samples from the wild. This experiment confirmed that plant-derived ingredients could be used in formulation of diets for soft-shell crab farming in agreement with Tuan et al., (2006).

Each treatment had a high number of crabs molting. The beginning of the monsoon season in May, June, and July every year is the peak molting season for the crab farms and a good number of crabs molted in all treatment groups. Crabs fed with trash fish were not significantly different from others in terms of number of crabs (138 crabs) to molt.

The commercial diet from the feed mill in Thailand, which had 40% crude protein, 10% lipid, 10% moisture, and 3% ash was an extruded feed and, in terms of water stability, performed better compared to the test diets especially handing by the crabs with their claws. In terms of pellet size, both length and width of 1 cm made it more advantageous because plastic crab boxes have holes that are 1 cm in both width and length. However, in terms of water stability, all the manufactured diets (extruded commercial and meat grinder pellets) had the same 1 hour of stability in the water. In Ranong, most farmers continue to use trash fish and are not aware that pelleted feed is effective. It has been reported that the commercial crab fattening diet is no longer produced in Thailand. The feed used for this experiment was the last production for crab feed due to low market demand. The price for the crab fattening feed was around US$2 per kg.

The increasing costs of forage fishes and the difficulties of storing and feeding these fish to farmed crabs leads us to believe that compounded diets will be the preferred method of feeding crabs in the near future. There is limited research work on diets for soft-shell mangrove crabs and the result of mangrove crabs' acceptance of formulated feeds using either simple laboratory process or commercial extrusion can be considered a significant contribution to industry. A study in India (Ali, et al., 2011) found a similar result that the crabs on an experimental fattening diet (mean initial weight 151.7 g demonstrated acceptability of the compounded feed and achieved a weight gain of 36.7% in their 30-day trial with a diet containing 41.6% of crude protein. The same researchers reported that crabs fed with dry pellets achieved a higher weight gain than semi-moist pellets.

The research also observed that mangrove crabs eat trash fish straight away when offered compared to 3-7 days to train crabs to eat compound feed. The present results reinforce conclusions of research conducted by Keenan (2004) that using trash fish achieved better weight gain than a formulated diet for grow-out crab culture. Another disadvantage of formulated feeds is the size of the pellets because, crabs are slow eaters, and like to hold their prey with their powerful claws. Two of the experimental diets had small pellet size of 4 mm and easily broke apart from handling which might be one of the reasons that crabs had less weight gain compared to trash fish.

Soft-shell crab farming is somewhat different compared to grow-out crab farming or crab fattening. For grow-out crab farming, juvenile crabs are stocked into the pond for a couple months until they reach market size and are fed mainly trash fish, fish processing wastes, crushed mollusks, or in the rare case, some formulated diets, usually shrimp pellets. It may be easier to train juvenile crabs to eat formulated diets than wild caught sub-adult crabs. In the case of crab fattening in Southeast Asia, crabs are put in the mangrove area with fences or earthen ponds, and there is an opportunity for the crabs to get feed from the ponds or mangrove grounds. For soft-shell crab farming, the farmers hold the sub-adult crabs only for a very short period until they molt and they are sold as live soft-shell crabs to the processing plants in Thailand. All commercial soft-shell crab production uses crabs caught from the wild. One advantage of the soft-shell crab farming system that was used in the experiment was that crabs were in individual plastic boxes or cages so it was easier to observe and starve the crabs. Since no other food was available for crabs inside the boxes, the crabs were forced to eat experimental diets or any other food provided by the farmers. However, it was not sure that crabs would eat the formulated diets. However, the results showed that after a few days of training, formulated diets could be fed to mangrove crabs.

During the course of the experiment, it was also observed that crabs ate more in the evenings than mornings. In the reality of crab farming, it is hard for the workers to feed in the evening hours due to mosquitos in tropical climates. Feeding at soft shell crab farms is somewhat different from feeding shrimp and fish farms in that it requires putting feed into the individual boxes, one by one and that takes a considerable amount of time to feed all the animals. Additional research is needed to consider how best to improve the water stability of pellets by using different binders and extrusion methods. Another focus of research should be an examination of pellet size and the use of different extrusion methods to document the physical properties of the pellets and the preferences of the mangrove crabs. Considering the limited research work on mangrove crab diets, the fact that this trial was conducted at a farm-scale on a commercial farm should provide the most practical information available.

3.5. **Conclusion**

Results of the present study demonstrate that mangrove crabs will accept formulated feeds using either simple laboratory process or commercial extrusion. Hence, the development of a specific diet for crabs is technically possible. Therefore, further works on nutritional requirements are encouraged. Based on observations in the field physical properties of these feed must also be improved This should include both size of the pellet so that it does not easily fall from the container and have suitable handling characteristics to allow the crab to hold the feed.

Table 3.1. Ingredient composition of diets formulated to contain protein 40% and lipid 7% with high fishmeal and high soybean meal formulas

Ingredient (As is basis g kg^{-1} feed)	Inclusion rate in feed (%)	
	High fish meal diet	High Soybean meal diet
Menhadenfishmeal[1]	30.00	15.00
Soybeanmeal[2]	32.50	56.40
Gelatin [8]	1.00	1.00
Menhaden fish oil [1]	2.74	4.09
Corn Starch[5]	0.46	0.01
Whole wheat[6]	30.00	19.20
Trace Mineral premix – shrimp [3]	0.50	0.50
Vitamin premix w/o choline [4]	1.80	1.80
Choline chloride [4]	0.20	0.20
Stay C 35% [6]	0.10	0.10
CaP-dibasic [4]		0.10
Lecithin (soy commercial) [7]	0.50	0.50
Cholesterol [5]	0.20	0.20

[1] Omega Protein Inc., Houston, TX, USA.
[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[3] Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.
[4]Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.
[5]MP Biomedicals Inc., Solon, OH, USA.
[6]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[7]The Solae Company, St. Louis, MO, USA
[8] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

Table 3.2. Summary of water quality parameters which were measured twice daily (0800 h and 2000 h) over a 45 day cultured perido. Values represent mean and standard deviation together with minimum and maximum.

Water Parameter	Mean ± SD	Minimum	Maximum
Temperature (°C)	30.8 ± 1.66	27.0	34.0
Salinity (g/L)	9.8 ± 2.9	5.0	16.0
pH	7.1 ± 0.04	7.1	7.2

Table 3.3. Proximate composition and amino acid profile (% as is) of the test diets used in the growth trial. (LOD – Below the limit of detection)

Proximate composition[1]	High fishmeal	High soybean meal
Crude protein	39.90	39.30
Moisture	9.50	9.80
Crude fat	7.55	7.38
Crude fiber	1.18	1.08
Ash	9.53	9.62
Amino acids		
Alanine	2.11	2.26
Aspartic acid	3.28	3.70
Cystine/ Cysteine	0.25	0.19
Glutamic acid	6.38	6.70
Glycine	2.51	2.67
Histidine	0.90	0.96
Hydroxyproline	0.80	0.74
Isoleucine	1.58	1.67
Leucine	3.11	3.29
Lysine	2.90	3.13
Methionine	0.30	0.28
Phenylalanine	1.89	2.01
Proline	2.72	2.92
Serine	2.27	2.38
Threonine	1.89	1.98
Tyrosine	1.36	1.45
Valine	1.80	1.91
Sum of Amino acids	36.0	38.30

[1] Diets were analyzed at Eurofins Lab, Ho Chi Minh City, Vietnam

Table 3.4. Response of mangrove crabs fed experimental diets over a 45-day period. (150 initial crabs per treatment)

Diet	[1]Initial weight (g)	[2] Final weight (g)	[3]Weight gain (g)	Number of crabs molting	% weight gain	Days to 50% molt (days)	Survival Ratio
Trash Fish	69.39	97.79	40.10	138	57.79[a]	29[a]	142/150[ab] (95%)
High fish meal	69.79	91.53	36.00	131	51.58[ab]	32[b]	136/150[c] (89.5%)
High soybean meal	70.00	94.65	32.00	144	45.71[c]	32[b]	147/150[a] (98%)
Crab fattening	69.56	91.05	35.90	130	51.61[b]	32[b]	138/150[bc] (92%)
PSE					3.317		
p value					<0.0001	<0.0713	<0.0007

[1]Initial weight= crab weight at stocking
[2]Final weight = Soft shell crab or newly molted crab weight (i.e. body weight in grams of the newly molted soft-shell crab as it came out of the box)
[3]Weight gain (g) = Final weight (g) -initial weight (g)
[4]% Weight gain= (Final weight - Initial weight)/ Initial weight *100
[5]Day to molt= Average number of days 50% of population molted within 45 days of trial
[9]Initial crab per treatment- 150 crabs
[7] Values with different superscripts within a row are significantly different based on Tukey's multiple range test
[8]PSE- Pool Standard Error

Figure 3.1. . Mean and standard error days to 50% molt for each treatment of mangrove crabs fed with different diets over the 45 day trial. The number of crabs which molted (n) for each treatment were 138,131,144,130 for trash fish, high fishmeal, high soybean meal, crab fattening feed respectively.

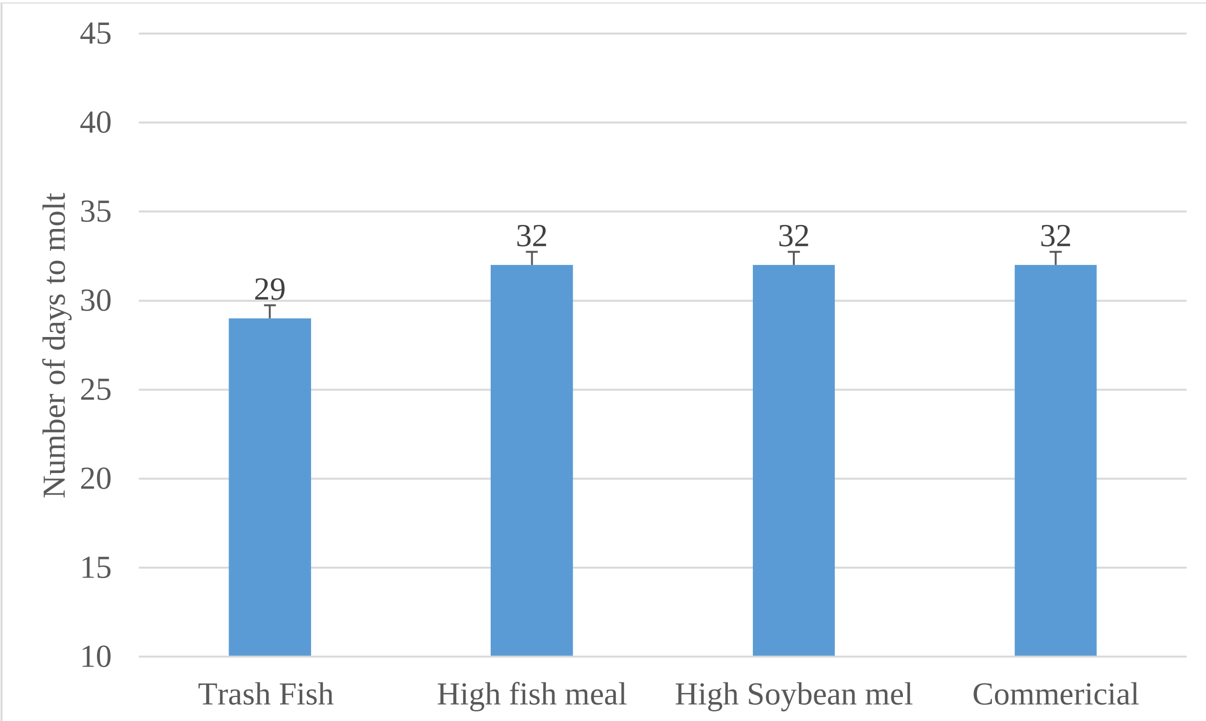


Figure 3.2. Mean response percentage weight gain (with standard error) per each treatment of mangrove crabs fed with different diets during 45 day trial.

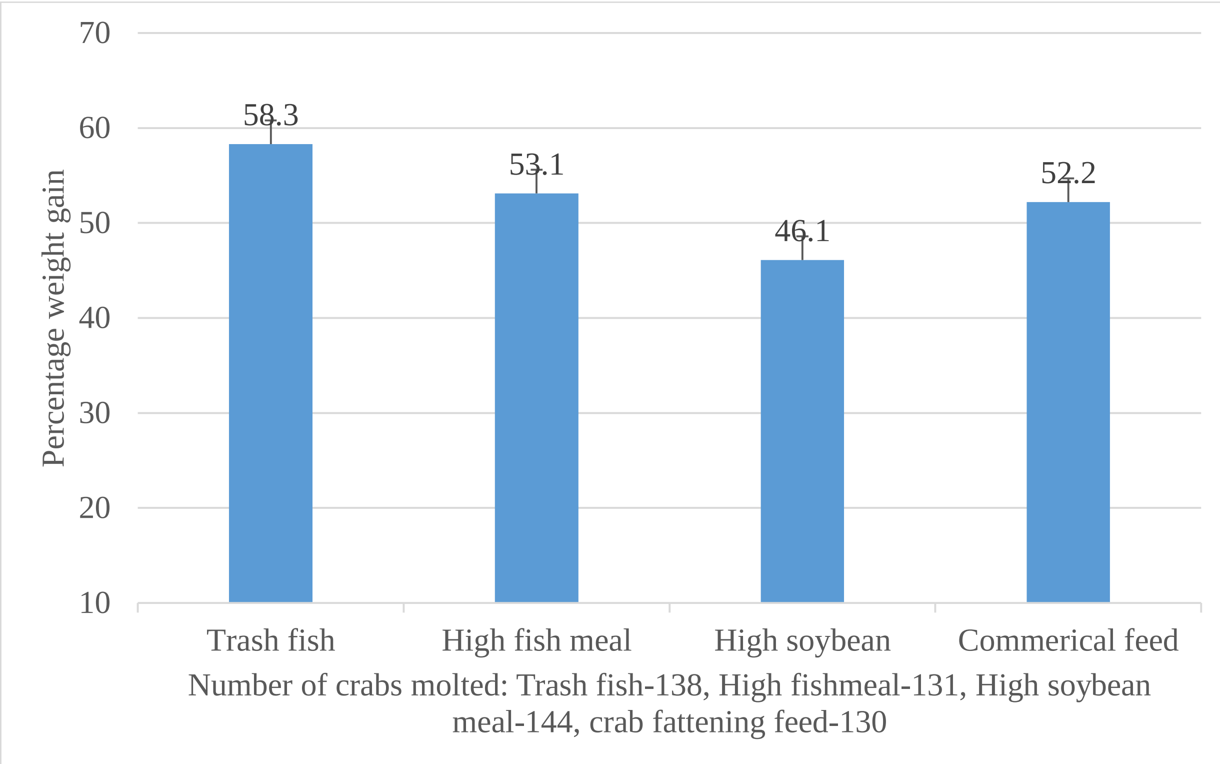
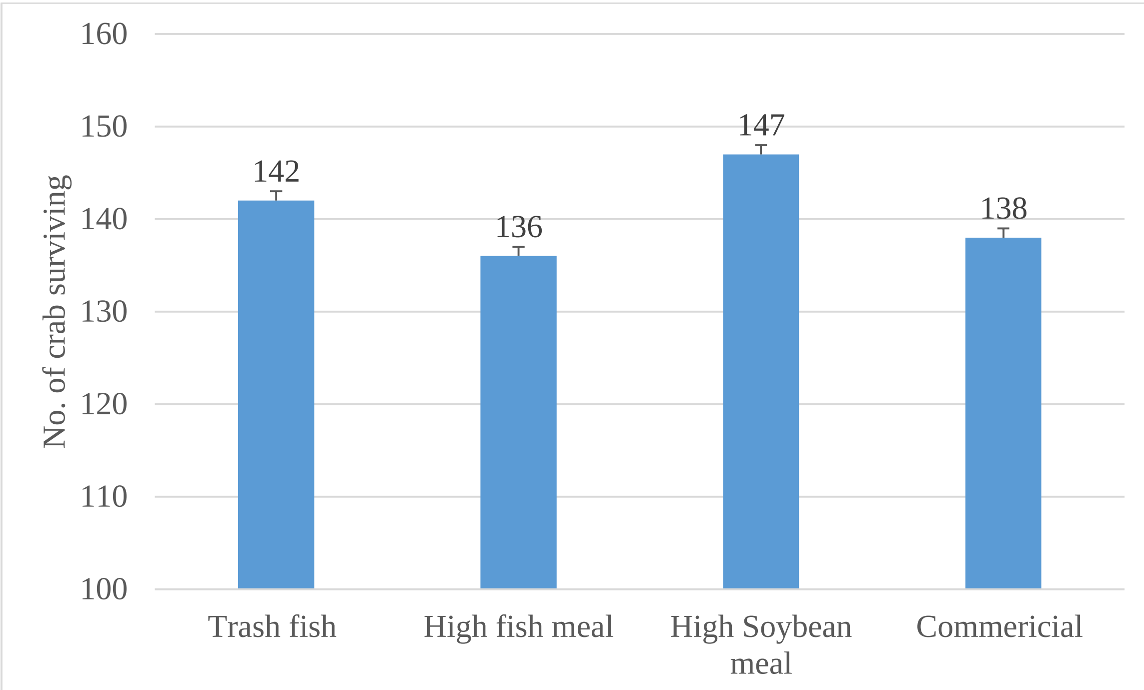


Figure 3.3. Total number crabs surviving per each treatment of crabs fed with different diets in 45 days of trial (n=150)



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Chapter 4. **Effects of different level of methylcellulose binders on pellet stability as measured by retention of dry matter in soft-shell mangrove crab diets**

4.1. **Abstract**

Prior research demonstrated that wild-caught mangrove crabs will accept pelleted formulated feeds after only a couple of days of training. However, the pellets created under simple laboratory conditions (e.g. meat grinding equipment) did not have sufficient stability to stay intact for an extended period of time with handling by the crabs exacerbating the situation. Therefore, this experiment examined using various levels of methylcellulose as a binder for additional pellet stability. Methylcellulose (CMC) and methylhydroxypropylcellulose (MHPC) are processed products derived from natural gums that are used in the food industry as binders, thickeners and, texturizing agents. Inclusion of small amounts of CMC and MHPC in the formulation should provide improved pellet stability. Five levels of supplementation (0.15%, 0.3%, 0.6%, 1.2%, and 2.4%) of each of the binders was supplemented to the basal diet producing a total of 11 diets. The water stability was then determined with immersion times of 60 min, 120 min and 180 min and salinity of 15 ppt at 25°C. Undissolved solids were filtered and the dry matter (DM) of each feed was determined by drying it at 95°C to constant weight. Lab results showed that MHPC was only significantly different as a binder compared to CMC or the basal diet, with no binder, at the 2.4% inclusion level. The level of binder in the diet did not seem to have a significant effect within the range tested otherwise. When all the times and inclusion levels were considered together, the MHPC had a slightly better arithmetic regression line, but not enough for the rate of loss of dry matter to be significantly different.

4.2. **Introduction**

Soft-shell crab farming is a billion-dollar industry in Southeast Asia, primarily using several closely related species of the genus *Scylla*, commonly called mangrove or mud crabs. The crabs are reared in individual boxes to avoid cannibalism, with the boxes placed into a pontoon array. Multiple pontoons, controlled with a pulley system allowing the staff access to all of the boxes, float in ponds under a bridge spanning the middle of the pond, which is used to stock, feed and harvest. The mangrove crabs are normally fed forage fish once a day until they molt. The freshly molted crabs are placed in fresh water so the shell will not harden and then quickly sold for local live markets or frozen for export markets. Feeding forage fish is quickly becoming uneconomical as prices, handling and storage costs are rising quickly. Feeding fish also causes fouling problems in the ponds and increases opportunities for spread of parasites and disease. Converting the crabs to pelleted feeds is needed to just maintain the present industry and critical if it is to expand.

Traditionally, crabs were viewed as carnivores with preferences in their natural diet containing mollusks, crustaceans and fishes that have relatively high protein content (Hill, 1979). However, research conducted by Hill (1976) and Prasad and Neelakantan (1988) reported that significant amounts of plant-based material and detritus have been identified in the digestive system of juvenile mangrove crab samples from the wild. Pavasovic (2004) demonstrated that crabs have the necessary digestive enzymes and that they can achieve high digestibility of carbohydrates derived from plants. During the last ten years, several research efforts have been focused on mangrove crab broodstock and juvenile nutrition, especially the role and effects of various components of lipids, cholesterol, and proteins (Azra and Ikhwannudin, 2015). Moreover, other research focused on digestibility of animal and plant-based feed ingredients by mangrove crabs. Researchers in the Philippines, Vietnam and Australia reported that crabs have protein requirements of 35-55 % based on their age and size (Catacuan et al., 2002; Truong, 2008; Truong et al., 2009; Anderson et al., 2004). The level of lipids required for mangrove crab juveniles to reach maximum growth was reported to be in the range of 5.3-13.8% (Sheen and D'Abramo, 1991; Sheen and Wu, 1999). Using dietary lipid at 13.8% did not show any reduction of growth; thus, mangrove crabs appear to tolerate or require a higher level of dietary lipid than *Penaeus monodon* (Glencross et al., 2002). The cholesterol requirement of mangrove crab was studied by Sheen (2000) based on growth, molt frequency and survival and then reported that the optimal cholesterol requirement for mangrove crab is 0.5% or 5.1 g/kg. However, a level of 1.12% cholesterol in the diet did have a negative effect on growth of mangrove crab. Research conducted by Catacutan et al. (2003) reported that juvenile mangrove crabs have elevated crude protein requirements of more than 40%. The paper also reported that crude fat in plant-based ingredients are more digestible for crabs than crude fats of animal origin

Initial experiments demonstrated that wild-caught mangrove crabs would accept pelleted formulated feeds after only a few days of training. Results from the trials provided similar growth rates achieved by feeding low value forage fish. However, pellets created on meat grinding equipment did not have sufficient water stability and quickly broke apart in the cages. Handling by the crabs exacerbated the situation. Water stability plays an important role in physical property of feed for culture species and high water stability feed can prevent increased cost of feed and nutrient availability to the crabs (Obaldo et al., 2002). Stability is defined as the retention of the pellet's physical integrity with minimal disintegration and nutrient leaching while it is immersed in the water and until animals consume it. The formulated diets used for the first experiment contained gelatin and corn starch which are natural binders yet, feeds were only stable for a short period of time. Hence to improve the feed we may need to add a more effective binder.

Binders are used in aquaculture feed production to improve feed manufacturing, reduce feed wastage, increase water stability of diets, stabilize ingredients, and minimize leaching of nutrients (Ali, 1982; Storebakken, 1985; Storebakken and Austrong, 1987). Binder types also plan an important role in determining the attractiveness and digestibility of certain nutrients (Holme et al., 2006). Cruz-Suarez et al. (2007) suggested the true nutritional value of feed depends not only on the amount of nutrients, but also on their availability and quality. Polysaccharides sources, such as starch and cellulose, are abundant and cellulose is a relatively cheap source of biopolymers to be employed as binders (Volpe et al., 2010). A cellulose molecule has three-hydroxyl groups in each repeating glucose unit, which gives the molecule strong hydrophilic properties (Boulos et al., 2000). Cellulose is indigestible for most monogastric animals due to their lack of the enzyme cellulase (Hansen and Storebakken, 2007). However, cellulose has been used in feeds for many aquaculture species including European sea bass (Dias et al., 1998) and Nile tilapia (Amirkolaie et al., 2005) with minimal effects on digestibility of the primary nutrients.

A good binder can enhance gelling power to produce a more stable pellet. Mangrove crabs like to hold their food item with their claws before they eat and they are slow eaters with the characteristic of using mouth appendages before ingestion. Therefore, it is critical that the pellet has a good physical quality preventing attrition and fragmentation of the pellet.

The objective of this research was to determine if two binding agents could improve the water stability of a laboratory produced crab feed.

4.3. **Materials and Methods**

Experimental diets were formulated and prepared at the Auburn University Aquaculture Nutrition lab. The basal was supplemented with five levels of CMC or MHPC (0.15%, 0.3%, 0.6%, 1.2%, or 2.4%) producing a total of 11 diets, 10 experimental diets and a basal diet with no CMC or MHPC binder used as a control (Table 4.1). Seven kilograms of the diet were prepared. From that diet mix, 650 g were removed and 0.975 g of methylcellulose (0.15%) was added to the dry mix. This was followed by 5.85 g (0.6%), 7.8 g (1.2%) and 15.6 g (2.4%) of CMC. The procedure was repeated by adding the same inclusion rates of MHPC. The diets were then remixed and then fed into a meat grinder to prepare strands that were subsequently cut to a common size. The meat grinder was thoroughly cleaned between each diet. The pellet diameter was 4 mm for all experimental diets with proximate composition of 40% crude protein and 7% fat.

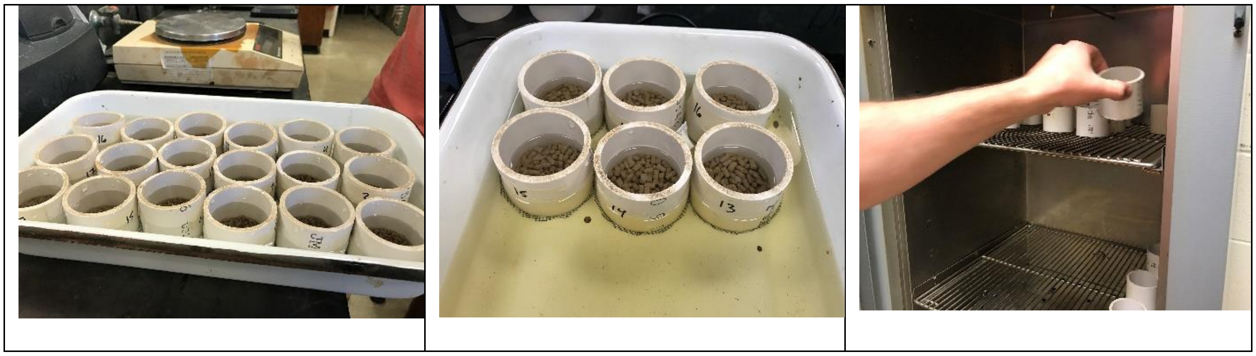
For the experimental procedure, three replicates of each diet (10 experimental and 1 basal control) were tested. The trial was started with two grams of pellets (4 mm diameter) of each diet weighed and put into 33 separate PVC rings which were placed into one of two pans each containing 2,000 mL of brackish water (15 ppt) at 25^{o}C. The pans with rings were placed on a lab counter in a static condition for the assigned time period. Each test run consisted of determining beginning and final dry weights of pellets submerged at one of three intervals of immersion time (60, 120 and 180 minutes). Pellet stability was determined by using the method of Ruscoe et al. (2005) and is expressed as the percentage of dry matter lost or leached (DML) after periods of immersion in brackish water salinity 15 ppt. Feed stability was calculated in terms of dry matter lost (DML) using the following formula:

$$DML\ (\%) = 100\ \frac{DWbi - DW\ ad}{DWbi}\ X100$$

DW_{bi}= Dry weight of diet before water immersion

DW_{ad} = Dry weight of diet after immersion followed by drying to a constant weight

Figure 4.1. Experimental proceedure



The PVC rings (50 mm height and 75 mm diameter) containing the pellets had a screen bottom with a 1.3-mm mesh. At 60 minutes, the pellets were collected and dried at 95°C for 10 hours to get a constant weight then placed in a desiccator. Desiccation continued to be certain the feed pellet reached a constant weight. Dried feed samples were weighed and analyzed for dry matter retention. Pellet stability was calculated as the ratio of dry matter lost (DML) after leaching and dry matter of original samples expressed as a percentage. The procedure was repeated on subsequent days for 120 minutes and 180 minutes, respectively. Photos and descriptions were taken at 60, 120 and 180 minutes, respectively.

4.3.1. Statistical Analysis

All data were analyzed using SAS 9.4 (SAS Institute, PLACE) software. One and two-way analysis of variance was used to determine significant differences ($p \leq 0.05$) among the treatments which was followed by Tukey' multiple comparison test to determine differences among inclusion levels and source binder. Logical regression was used to determine differences between dry mass loss with the different inclusion levels of binders at different immersion times.

4.4. **Results**

The experimental results showed that there was a significant increase of dry matter loss with time for all of the diets tested. However, with the diet prepared in this experiment, binders CMC and MHPC provided no significant difference of pellet stability in water (Tables 4.2) compared to the basal diet without any binder, except at the 2.4 percent inclusion of MHPC when all three time frames were considered together (Table 4.3). Nor was there any significant difference in dry matter loss between inclusion levels of CMC or MHPC in the diets except for MHPC at the 2.4 % inclusion level. Evaluation of the regression lines developed by plotting the dry matter leaching or loss, showed almost identical regression curves between CMC and MHPC (Figures 4.2, 4.3 and 4.4).

4.5. **Discussion**

Binders are used in aquaculture feed production to improve feed manufacturing, reduce feed wastage, increase the water stability of diets, provide stabilization of ingredients, and minimize leaching of nutrients. Cruz-Suarez et al. (1999) suggested that the true nutritional value of feed depends not only on the amount of nutrients, but also on their availability and quality. Water stability of aquatic feed depends on both quantity and quality of binding material used (Ali et al., 2005; Ahamad et al., 2011). Polysaccharide sources, such as starch and cellulose, are abundant. Cellulose is a relatively cheap source of biopolymers to be employed as binders (Volpe et al., 2010). A cellulose molecule has three hydroxyl groups in each repeating glucose unit, which gives the molecule strong hydrophilic properties (Boulos et al., 2000). Using cellulose in trout diets resulted in increased pellet durability and hardness of the pellet (Hansen and Storebakken, 2007). Brown et al. (2015) used five non-starch polysaccharide binder (guar, wheat gluten, and carboxymethylcellulose (CMC)) and two exopolysaccharide gums (xanthan and pullulan) to test binders for extruded floating feeds formulated for, but not fed to yellow perch. The xanthan and pullulan gums were reported to improve the pellet stability in water more than the other binders. Partridge and Southgate (1999) worked with barramundi using alginate, gelatin, carrageenan and zein as binders in different combinations to create microbound diets for larvae and juveniles. Yamamoto and Akiyama (1995) described research in which CMC, α- starch (from potato) and wheat gluten were used to prepare diets for Japanese flounder (*Paralichthys olivaceus*) on a compression (California) pellet mill. Each binder was used at a 5% inclusion level and fed for 4 weeks. The water stability of pellets was not reported but the CMC diet provided significantly less growth in the fish compared to the other binders. It appeared that the CMC may have inhibited the activity of proteolytic enzymes in the fishes' digestion of the feed. Castille and Lawrence (1995) reported on the use of polymethylolcarbamide (urea formaldehyde resin) as a pellet binder for white leg shrimp (*Penaeus vannamei*). Levels of inclusion from 0 to 8% were tested on the shrimp. Although the binder did perform well increasing pellet stability, any levels above 0.5% inclusion significantly decreased growth rate of the shrimp. The metabolic release of urea and/or formaldehyde were considered to be the cause of reduced growth.

A good binder can enhance gelling power to produce a stable pellet. Mangrove crabs frequently hold their food items with their claws before they eventually consume the food. They are slow eaters with a characteristic of using mouth appendages before ingestion. Therefore, it is critical to include a good binder providing a good physical pellet and preventing attrition and fragmentation of the pellet. Moreover, it should also be acceptable to the mangrove crabs. Binders commonly used for aquafeeds include starch, carboxy-cellulose, guar gum, hemicellulose, molasses, whey, sodium alginate, and gelatin due to their good adhesive abilities.

In this experiment, it was found that the binder MHPC was equivalent to binder CMC with the exception of a level of 2.4 % inclusion, where there was a reduction in Dry Matter Loss with MHPC. We used 60, 120 and 180 minutes as target times, which is longer than most other trials of nutrient leaching or dry matter studies. The target was to get at least two hours of water stability for the pellets. Although the highest level of inclusion resulted in the least Dry Matter Leaching, in terms of pellet physical appearance, little difference occurred between the levels of inclusion. The suggested temperature for gelatinization was between 58°C to 67°C according to the TIC Gum Company. In hindsight, lab conditions of temperature and pressure may not have been adequate to generate good gelatinization of either MHPC or CMC. Or possibly that only the 2.4% level reached the adequate level.

Although MHPC and CMC apparently had not been used as a binder in crab feed, the result showed that MHPC might be an adequate binder. Another technology which might produce an improved pellet would be high pressure extrusion. Incorporating a binder and the extrusion process might produce a more stable pellet with good nutritional characteristics.

4.6. **Conclusion**

The objectives of this study were to assess and compare the effect of methylhdroxypropyl cellulose and methylcellosue in dry matter leaching and water stability of an aquafeed pellet. It appeared that there was in increase in dry matter leaching with time, but there was little impact from binder type or the level of inclusion on the dray matter (pellet) stability. It showed that the binder methylhdroxypropyl cellulose, MHPC, could be a better binder than methylcellulose CMC, at the 2.4% level of inclusion, for this application. It appeared that this highest level of inclusion tested provided less dry matter loss in the pellet. Additional testing is needed, especially using a higher temperature in feed preparation in order to reach the recommended temperature for best binder activation.

Table 4.1. Formulation and proximate composition of mangrove crab feed used in leaching experiment.

Ingredient (As is basis $g\ kg^{-1}$ feed)	Diet code										
	1	2	3	4	5	6	7	8	9	10	11
	Basal	M1	M2	M3	M4	M5	MH1	MH2	MH3	MH4	MH5
Menhaden fishmeal[1]	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Soybean meal[2]	43.38	43.38	43.38	43.38	43.38	43.38	43.38	43.38	43.38	43.38	43.38
Menhaden fish oil[2]	2.81	2.81	2.81	2.81	2.81	2.81	2.81	2.81	2.81	2.81	2.81
Lecithin (Soy Commercial) [8]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cholesterol[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Whole wheat [4]	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
***Methylcellulose* [9]**	***0***	***0.15***	***0.30***	***0.60***	***1.20***	***2.40***					
***Methylhdroxypropylcellulose* [9]**	***0***						***0.15***	***0.30***	***0.60***	***1.20***	***2.40***
Mineral premix (shrimp)[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix (shrimp)[6]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride [4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Rovimix Stay-C 35% [7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Cellufill - nonnutritive bulk[4]	**2.21**	**2.06**	**1.91**	**1.61**	**1.01**	**0.19**	**2.06**	**1.91**	**1.61**	**1.01**	**0.19**
Total	100	100	100	100	100	100	100	100	100	100	100

[1] Omega Protein Inc., Houston, TX, USA.
[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[3] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
[4] MP Biomedicals Inc., Solon, OH, USA.
[5] Trace mineral premix(g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.

[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.
[7] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[8] The Solae Company, St. Louis, MO, USA
[9] TIC GUM Company, White March MD, USA

Table 4.2. Percent mean dry matter loss at different times

	60 minutes	120 minutes	180 minutes
Basal	$16.94^{a} \pm 0.015$	$19.00^{b} \pm 0.010$	$21.03^{b} \pm 0.004$
CMC 0.15%	$15.94^{a} \pm 0.022$	$21.12^{b} \pm 0.021$	$21.06^{b} \pm 0.005$
CMC 0.30%	$14.95^{a} \pm 0.005$	$22.67^{b} \pm 0.015$	$21.15^{b} \pm 0.010$
CMC 0.60%	$15.28^{a} \pm 0.019$	$21.33^{b} \pm 0.015$	$19.80^{b} \pm 0.005$
CMC 1.20%	$15.33^{a} \pm 0.014$	$24.79^{b} \pm 0.027$	$23.16^{b} \pm 0.023$
CMC 2.40%	$15.56^{a} \pm 0.004$	$21.10^{b} \pm 0.023$	$21.98^{b} \pm 0.017$
MHPC 0.15%	$17.14^{a} \pm 0.023$	$21.43^{b} \pm 0.001$	$22.33^{b} \pm 0.006$
MHPC 0.30%	$18.33^{a} \pm 0.003$	$22.75^{b} \pm 0.016$	$22.00^{b} \pm 0.030$
MHPC 0.60%	$14.81^{a} \pm 0.008$	$22.80^{b} \pm 0.013$	$21.97^{b} \pm 0.010$
MHPC 1.20%	$16.08^{a} \pm 0.010$	$22.92^{b} \pm 0.022$	$24.77^{b} \pm 0.038$
MHPC 2.40%	$13.10^{a} \pm 0.007$	$18.33^{b} \pm 0.010$	$19.17^{b} \pm 0.006$

Mean values with different superscripts are significantly different at a $p<0.05$.

Table 4.3. Mean and standard deviation of dry matter loss at different inclusion level of binders for soft-shell mangrove crab diets including all time periods.

Binder Inclusion Level	0% (Basal)	0.15%	0.30%	0.60%	1.20%	2.4%
CMC	18.99 [a] ±2.04	19.37 [a] ±2.97	19.59 [a] ±4.09	18.80 [a] ±3.14	21.09 [a] ±5.05	19.55 [a] ±3.48
MHPC	18.99 [a] ±2.04	20.30 [a] ±2.77	21.03 [a] ±2.36	19.86 [a] ±4.39	21.26 [a] ±4.57	16.87 [b] ±3.28

Mean values with different superscripts are significantly different at a $p<0.05$.

Figure 4.2. Linear regression of percent dry matter loss over 180 minutes with MHPC binder.

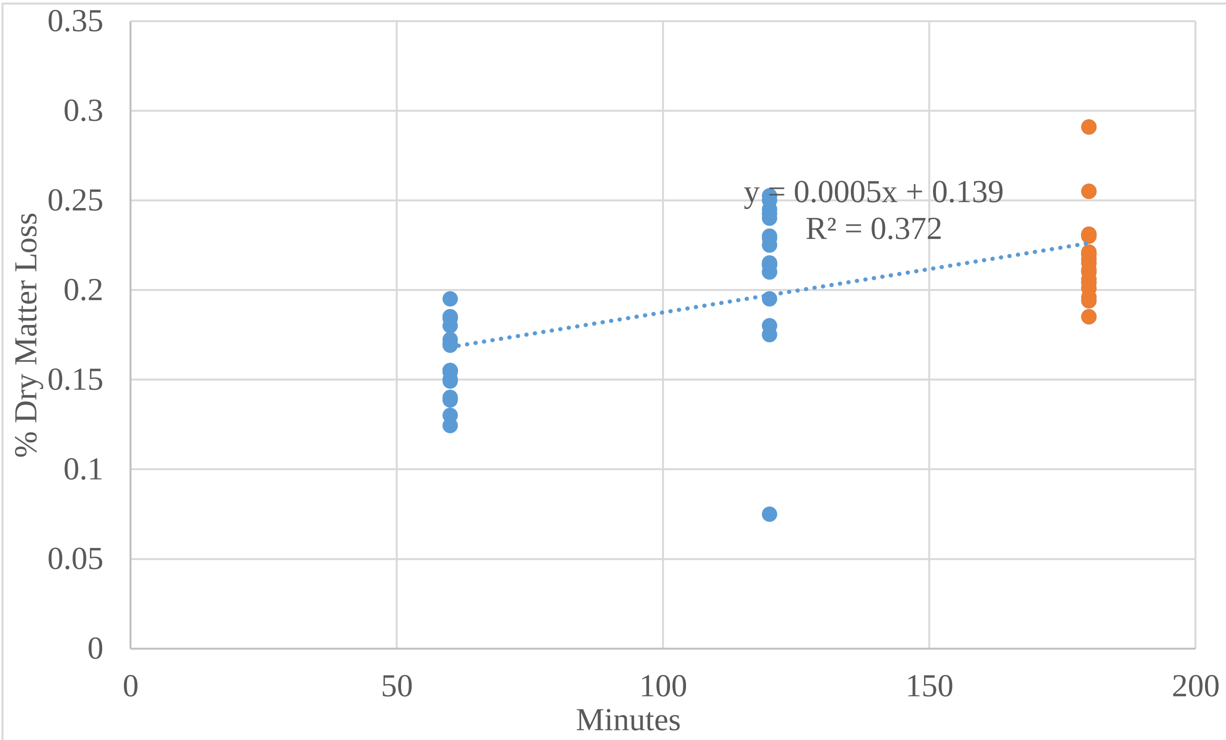


Figure 4.3. Linear regression of percent dry matter loss over 180 minutes with CMC binder.

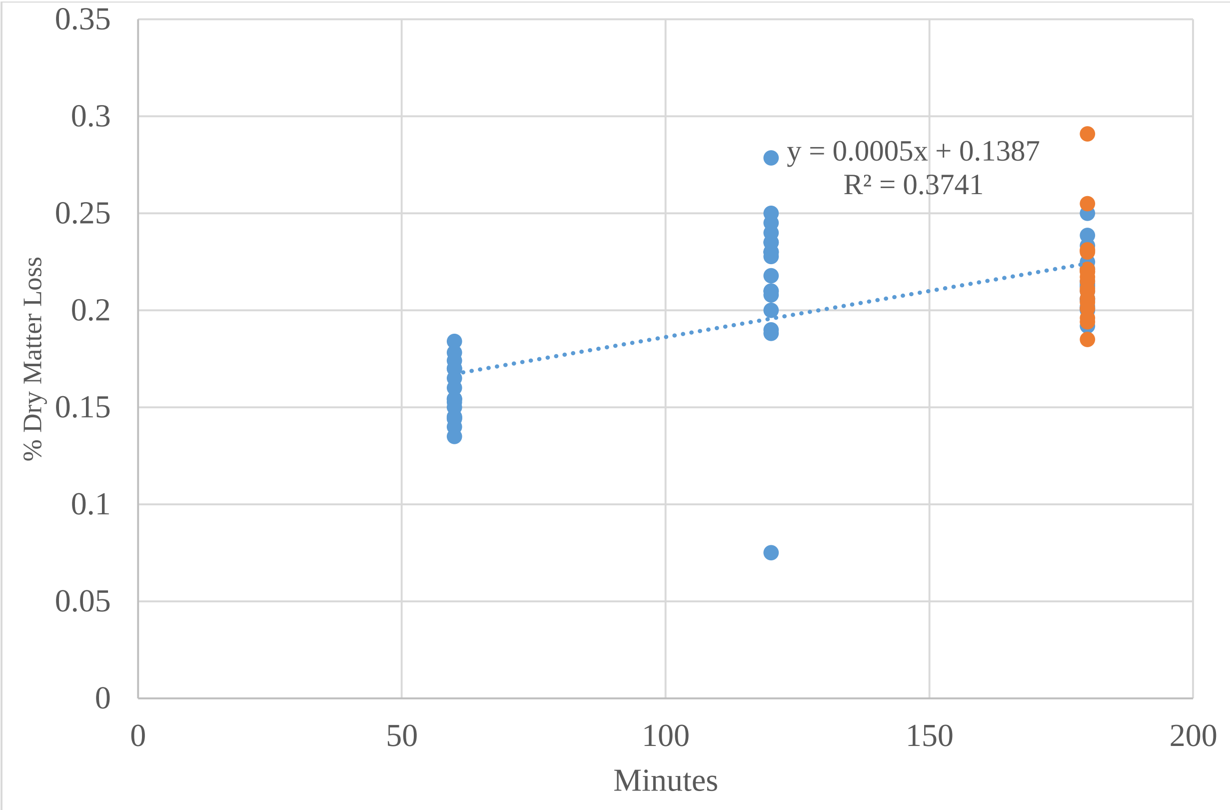
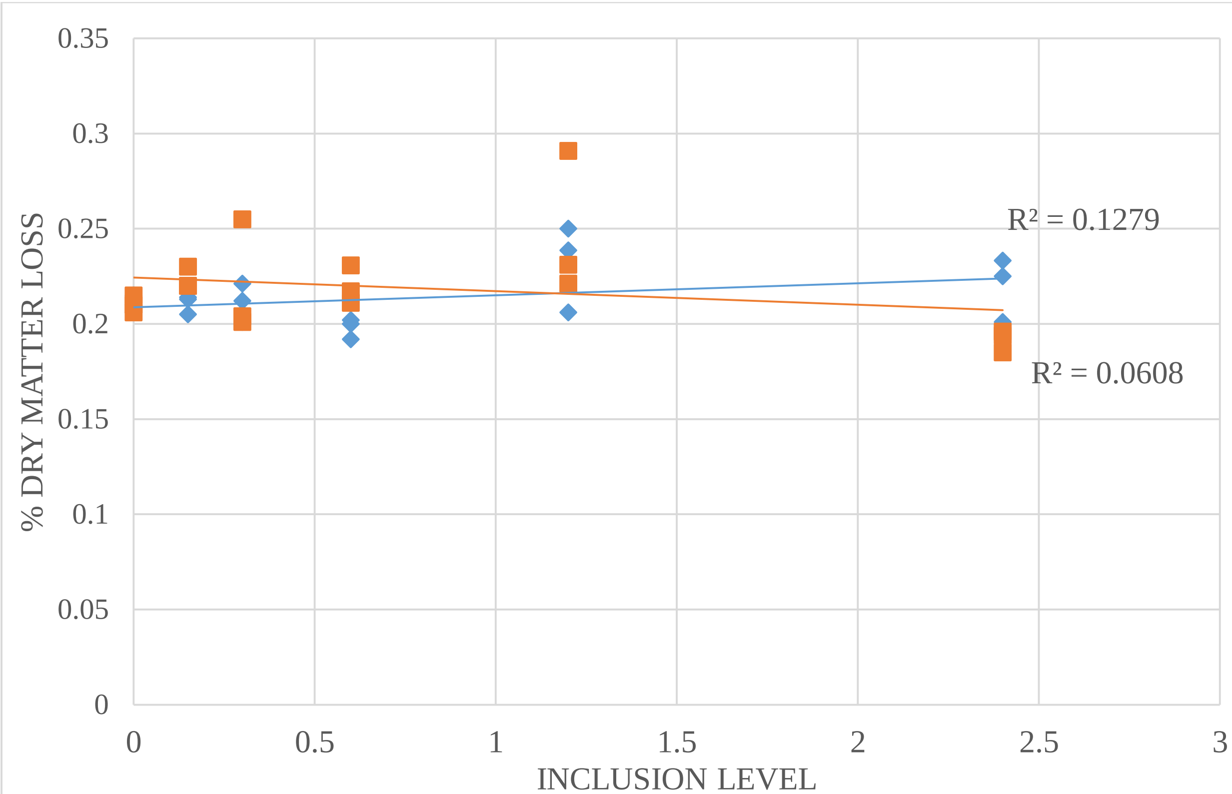


Figure 4.4. Linear regression of dry matter loss at 180 min.
(0 inclusion level = Basal, squares = MHPC, diamonds CMC)



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Chapter 5. **Comparison of different types of binders on pellet stability for commercial production of soft-shell mangrove crabs**

5.1. **Abstract**

This present study evaluated the effects of three types of binders, sodium alginate (SA), methylhdroxypropyl cellulose (MHPC), and methylcellulose (CMC) to improve water stability for the soft-shell mangrove crab's diets and acceptance by the crabs. The basal diet and three test diets, each supplemented with 1% binder, were formulated to contain 40% protein and 10% lipid. A total of 600 crabs in the range of 60-80g were randomly selected and placed into individual boxes housed in a PVC pontoon raft. Each dietary treatment was offered to 150 crabs that were randomly selected. Crabs were fed 3% of body weight once per day at 1600 h over a 45-day feeding trial. The crabs were checked every 4 hours (24/7) for mortality and molting. The current results demonstrated that the crabs reared on the control diet had the lowest survival (85 of 150) compared to crabs maintained on the other diets.

The diet with added sodium alginate binder had the lowest number of molting crabs, 77 crabs compared to other diets at 92 to105 of the 150 crabs in the treatment. MHPC diet had not only the highest number of molting crabs (105) but also, along with the control diet, had the highest average weight gain 27.6%. The results demonstrated that using different binders could have an effect on mangrove crab survival, weight gain as well as days to molt. The data demonstrates a clear advantage of the use of binders in extruded crab diets.

5.2. **Introduction**

Mangrove crabs are commercially important species in Southeast Asia due to their fast growth, and high market price and demand from both local and international markets (Paterson and Mann, 2011). Mangrove crabs are sold as live, chilled and frozen products. Three different kinds of crab farming are used in Asia, namely grow-out farming, crab fattening, and soft-shell mangrove crab farming. Soft-shell crab farming is the most popular due to their higher prices and consumer preference as the entire crab can be consumed (Tobias-Quinitio, 2015). Crabs grow by repeated molting and most of the crab's life is spent in inter-molt stages (Allan and Fielder, 2004). In the case of *Scylla serrata* juvenile crablets, they can molt 15 times, from new crablet to reach a size of 150 mm (Shelly and Lovatelli, 2011). Soft-shell crab farming has been practiced in Southeast Asia for more than 20 years. Typically, wild-caught crabs, 60-100 g, are used for commercial-scale soft-shell crab production. Crabs were held in individual plastic boxes and trash fish are fed once a day. The crabs are checked every 4 hours, day and night, to determine whether crabs have molted (Quinitio and Lwin, 2009).

Soft-shell crab farming is a multi-million dollar business in Southeast Asia. A Few researchers have publications focused on hatchery production of mangrove crabs. SEAFDEC published a rearing guide for soft-shell mangrove crab production and it reports promising results (Tobias-Quinitio., 2015). However, little or no research work has focused on replacing trash fish with formulated diets for the commercial-scale soft-shell crab production. Traditionally, most of the crab farming operations use trash fish as feed (Christensen, et al., 2004; Truong et al., 2008, 2009; Troung, 2008). Understanding the nutritional needs to properly replace trash fish with formulated diets is a prime bottleneck of the crab industry's development to the next level (Shelly and Lovatelli, 2011).

Previous experiments demonstrated that wild-caught mangrove crabs would accept pelleted formulated feeds after only a few days of training (Catacutan, 2002; Catacutan et al., (2003; Alava et al., 2007; Mirera and Moksnes, 2014; Ganesh, 2015). However, issues were identified including poor water stability of the pellet. Crabs like to hold their prey with their powerful claws when they eat and there is considerable handling and shredding of the feed prior to consumption. Previous diets had limited water stability and broke down easily when handled by crabs. Using binders and extrusion are known to enhance stability and durability of the pellet.

Feed stability is affected by binder type (Genodepa, et al., 2007). In this research, three different binders (methylhdroxypropyl cellulose, sodium alginate and methylcellulose) were used. Methyl cellulose (CMC) is a chemical compound derived from cellulose. CMC is normally produced as a white powder that is hydrophilic and dissolves in cold water, where it forms a clear viscous solution or gel. Methylhydroxypropyl cellulose (MHPC) is a further processed form of cellulose which is soluble in both water and organic liquids. CMC and MHPC are commonly used as alternatives to gelatin and gluten in vegan-friendly products. Alginate has been used extensively in other crustacean feeds providing improved water stability (AQUACOP 1976). Current research evaluated the effects of these three types of binders to improve water stability for the soft-shell mangrove crab's diets and acceptance by the crabs.

5.3. **Materials and methods**

In this trial, the feed formulation, ingredient selection, and processing were conducted at the US Fish and Wildlife Service Bozeman Fish Technology Center, in Bozeman, Montana. All the ingredients were analyzed for proximate composition and then the diets were formulated. The target for the experimental diets was iso-nitrogenous (40% of protein and 10% of fat) and iso-lipidic (Table 5.1). All the dry ingredients for the basal diet and three different diets (with 1% of methylhdroxypropyl cellulose, 1% methyl cellulose, or 1% sodium alginate binders), including vitamin and mineral premix were mixed in a paddle mixer for 20 minutes separately (Model # 6369 - Marion Mixers, Inc, Marion, Iowa) then pulverized for fine grinding. Water was added and mixed, with the resulting mash placed into the pre-conditioner. From the pre-conditioner the mash was fed into the extruder (DNDL-44, Buhler Twin Screw Extruder, AG, Uzwil, Switzerland) with 18-second exposure to an average temperature of 103°C in the sixth extruder barrel section. Die head pressure varied from 200- 400 psi. The mash was cooked and extruded to be a floating pellet by using different pressure and temperature (Table 5.2) and cut to approximately 1 cm lengths. The pellets were dried in a pulse bed dryer (Buhler Ag, Uzwil, Switzerland) for 25 minutes at 102°C with a 10- minute cooling period with a final moisture level of less than 10%. The dried pellets were then sprayed with fish oil (2% by weight) in a vacuum coater (A.J. Mixing, Ontario, Canada). A sample of each diet after production was collected and sent to the Eurofins Laboratory, Ho Chi Minh City, Vietnam for analysis of proximate composition (g 100 g^{-1} as is) and amino acid (g 100 g^{-1} as is) and fatty acids (g 100 g^{-1} as is) profiles following AOAC (1995) procedure by the lab. (Table 5.3 and 5.4). The rest of the pellets were stored in re-sealable plastic bags and shipped to Thailand for use in the experiment at a commercial soft-shell crab farm in Ranong.

5.3.1. Feeding Trial

One supplier collected all the crabs for the trial from an estuary in Kyun Su Township, Myeik district, of Southern Myanmar. Thus, the population was expected to be somewhat homogenous. Several thousand crabs were collected on one night and delivered to the farm the following morning for stocking. A total of 600 crabs with an average weight of 70 grams and a range of 60 to 80 grams were selected from that day's catch. For each treatment, 150 crabs were stocked into individual boxes in the same PVC pontoon raft. The most popular design for pontoon rafts to hold crab boxes in Thailand and Myanmar is a 4-by-400 box array. Thus, each pontoon could hold up to 1,600 crabs at a time. The array is moved with a pulley mechanism under the bridge so that the farmer can assess the condition of the crab every four hours and feed every day.

Crabs were stocked into the cages on July 29, 2017 and the feeding trial was started on August 1, 2017. Boxes within one raft were randomly assigned, with a random numbers table, such that 150 crabs were fed extruded floating pellets with sodium alginate binder, another 150 with methylcellulose feed, 150 with methylhydroxypropyl cellulose feed and 150 with the basal diet with no binder added as a reference group. The crabs were fed 3% of body weight per time once a day.

Water temperature and salinity were measured twice a day, at 0800 h and 2000 h, using an alcohol thermometer and a hand-held refractometer (Master by Atago, Thailand). pH and alkalinity were determined every week at 0800 h (portable pH tester and saltwater alkalinity colorimeter, both by Hanna, United Kingdom) and water in the pond was exchanged with every spring tide (approximately 10- 20% exchange as normal farming practice). Other pontoons in the pond were stocked and monitored using normal commercial farm processes and only one pontoon (PVC raft) was used for the experiment. The experiment trial was conducted in the middle of the monsoon season and typically farmers observe fewer molting crabs.

Every day during the feeding trial, crabs were checked for molting and mortality every four hours. If a crab was found to have died, it was removed, noted in the logbook and discarded. If a crab was found to have molted, the date and time of inspection was noted. The newly molted crab removed from the box was weighed individually as final weight and then placed into a bucket of aerated fresh water for 30 minutes, so that the shell would not harden. The crab was weighed again after 30 minutes, which determines the weight for sale to the processor. The soft-shell crabs are then packed closely together and covered with a soaking wet cloth in a cooler or bench style refrigerator for daily sale as still live soft-shell crabs to the processing plant. For the purpose of the experiment, we used the weight of the crabs when first removed from the box. The weight after 30 minutes in freshwater is increased considerably as the crab swells with water during that time. The other data recorded at harvest besides body weight, was carapace width, and harvest time and date. The carapace width and weight after soaking in freshwater are not reported here as they did not seem to have any direct relevance. The trial concluded on September 16, 2017 when all remaining live crabs were counted for survival. The average weights and weight gains for the surviving crabs were determined, but not used in the analyses for two reasons. First, because these crabs are not marketable and their hard-shell weights would not be meaningful for a commercial farm. Second, the weights including the hard shell, would likely skew the data in some manner we could not predict. For those crabs that molted during the 45-day feeding trial, the average number of days from stocking until molting was also determined.

5.3.2. Statistical Analysis

Final body weight was defined as the weight in grams of the newly molted soft-shell crab as it came out of the box. The final weight gain was evaluated using initial weight at the time of stocking into the box. If a crab did not molt within 45 days, it was not included in the weight gain or molting statistical analyses. Data from percent weight gain trial were analyzed using analysis of covariance (ANCOVA) to determine significant differences among the treatments, followed by Tukey's multiple comparison test to compare difference among treatment means. A Kaplan-Meier estimator (time-to-event analysis) was used to measure time to molting (mean number of days at which 50% of crabs in a treatment had molted) between groups to determine significance. If any crab molted within seven days of stocking, for any treatment group, they were not included in the data as it is doubtful that there could be any nutritional or diet effect that quickly. Mortality (or survival) was analyzed by a generalized linear model with logistic regression. All data were analyzed using SAS (V 9.4. SAS Institute, Cary, NC, USA) software with significance level set at $\alpha \leq 0.05$ (unless otherwise stated).

5.4. **Results**

During the trial the temperature ranged from 25-34°C and the salinity ranged from 10-20 ppt. pH was very stable between 7.1 and 7.2 during the entire 45-day period, at the time it was sampled at 0800 once a week. In hindsight, diurnal measurements would have been useful. There were frequent heavy rains during the 45 days and there were two spring tides with water exchanges (10-20% water exchange each time).

The results showed that crabs fed with MHPC had greater percentage weight gain (41.17%) and significantly greater final weight than crabs maintained on diets with sodium alginate (36.02%) or MC (30.89%) (Table 5.5). Crabs fed diets with CMC and MHPC had a significantly shorter number of days to molt (31 and 32 days) than those of the SA or reference diet (34 and 35 days). Significant differences occurred for mean survival rates between treatments with binders and that of the reference diet, which had the lowest survival (57%). Crabs fed the diet with sodium alginate binder SA had the fewest number of crabs molting and longer average days to molt, at 34 days, than the CMC or MHPC (31 and 32 days). Although a rigorous test of the diets' stability in water was not conducted at the farm, a quick test was done by putting all the experimental diets into four different 1-L glasses for 120 min and 180 min and observing the dry matter leaching, the color of the water, and shape of the pellets. It showed that MHPC still had better water stability and less dry matter leaching. The reference diet with no binder lost its shape after two hours.

Furthermore, based on observation, the reference diet appeared to break down more rapidly when handling by crabs with their claws.

The results demonstrated that the binder can have an effect and binder MHPC might be the preferable ingredient as compared to CMC and SA. The results demonstrated that using different binders could have an effect on mangrove crab diets, their acceptance by the crabs, and the eventual growth of the animals.

5.5. **Discussion**

General observation as well as the results of the experiment indicated that using a binder and extrusion processing improved water stability of the diet resulting in improved performance. The diet with the binder MHPC was superior to the other diets as it resulted in significantly higher final weight and percentage weight gain of the crabs as compared to those offered diets with CMC and SA binders. This is beneficial to the farmer as value at the processing plant is based on weight of the crabs. The MHPC diet was also superior to the basal diet and SA feed, as it provided a shorter number of days for the population of crabs to molt. This is also important to the farmer as the quicker the crab molts, the less feed and labor inputs are used and the more quickly the box can be restocked to restart the process.

CMC is sometimes used as a binder in aquafeed production, while MHPC is relatively new to the aquafeed industry. This current research indicated that using binder MHPC not only improved pellet water stability but also acceptance by the crab because it achieved the highest weight gain. The diet with binder CMC demonstrated the lowest weight gain and previous lab analysis predicted a similar result.

When using laboratory based feeds that are not extruded the stability in water is not very long (one to two hours). In previous work when this type of feed was offered to crabs on farm, some of the pellet fell apart before the crab could consume it. Previous research (Genodepa et al. 2004, 2007; Ali et al., 2011; Azra and Ikhwanuddin, 2016) identified similar issues related with water stability of crab diets. Hence, for this work we used a DNDL-44, Buhler twin-screw extruder and various binders to enhance stability. All the extruded test diets with binders in this trial had 4 hours of water stability, which is considerably longer than the 1 h achieved in the earlier trial using a simple laboratory process (meat grinder). The current results also supported research findings from India that extrusion cooking produced a good water stable shrimp diet with whole-wheat flour as binder (Rout and Bandyopadhyay, 1999). Depending on formula, the extrusion process can achieve water stability for 24 h (Munoz, 2011).

Quite often binders are added to improve stability and produce more consistent pellets. Sodium alginate has been used as a binder in many crustacean research diets as an alternative or addition to gelatin and wheat gluten. Argüello-Guevara and Poveda (2013) used a mixture of sodium alginate and wheat gluten and achieved good weight gain and survival in white shrimp broodstock. Research conducted by Saleela et al. (2015) suggested using sodium alginate (3%) in combination with a commercial phytochemical (11%) and agar (1%) for spiny lobster diets. Research in Australia by (Ruscoe et al. 2005) with freshwater crayfish, *Cherax quadricarinatus*, research diets, reported that using 5% CMC binders and sodium alginate with normal compression pellets gave better water stability of the pellets in the lab. An earlier study (Ruscoe et al., 2002) compared dry and moist diets also fed to freshwater crayfish, with no significant differences reported.

In this experiment, sodium alginate did not give the best results in terms of mean weight gain, survival and days to molt. The poor response in crabs could be due to a number of reasons including species, diet matrix and inclusion levels all affecting results. As a natural binder, work on various levels of alginate is warranted.

In this experiment, a higher mortality rate occurred compared to a previous trial conducted in May 2016. This trial was conducted in July and August, in the middle of the monsoon season, and there were strong winds and heavy rain during many of the days during the experiment. Ranong has average rain fall of 300 mm per month during the rainy season, one of the highest totals in Thailand. The survival rates in the current trial are typical for the rainy season which results in a lot of fresh water falling into the pond stressing the animals by reducing the temperature and salinity (Parado-Estepa and Quinitio, 2011). Crab boxes are at the surface of the pond water and temperature fluctuation is quite high (26 – 35°C). Every year the farmers face the same increase in mortalities which appears to be related to environmental as opposed to nutritional stress. Based on these observations, additional research should be conducted to determine if environmental variation can be reduced during the monsoon season possibly with paddlewheels or submerged aerators to improve mixing and stabilize temperatures.

In this experiment, it appeared that the extruded floating pellets were much more stable as compared to feeds made on a meat grinder used in the first trial in May 2016. However, it was observed that the floating pellets caused the crabs to struggle to grasp them with their claws, and then they had difficulty holding the pellet near their mouth. The crabs often held the pellets under their walking legs due to buoyancy of the pellets. When fed, they do grab and eventually eat the pellets. One advantage was that when floating pellets were broken into pieces by the crab claws, the pieces did not fall down through crab box holes and then to the pond bottom.

In this trial, across dietary treatments, it took longer for the crabs to molt as compared to an initial trial. This could clearly be due to differences in environmental parameters between the seasons, populations of crabs or due to delays in learning to feed. Hence, this may be in part due to a delay in feeding as the crabs learn to consume the floating pellets. Diet treatments produced a similar number of days to molting, which appeared to be more synchronized than in the previous trial. Given the feeding habits of the crabs, additional research is needed to consider crab preferences of physical properties of diet.

5.6. **Conclusion**

The result of this study confirmed that using different types of binders have effects on the water stability of pellets and acceptance by the crabs. It showed that the binder methylhdroxypropyl cellulose (MHPC) contributed to better weight gain of the crabs than other binders including sodium alginate SA and methylcellulose CMC. As expected, the basal diet with no binder had the lowest survival and longest average number of days to molt (38 days). This research showed that using binder methylhdroxypropyl cellulose (MHPC) provided better water stability of pellets and acceptance by the crabs and that continued research into the preferred physical properties of crab feeds is warranted.

Table 5.1. Ingredient composition of diets formulated to contain 40% protein and 10% lipid without supplemental binder (Basal) or 1% methylcellulose, 1% sodium alginate, and 1% methylhdroxypropyl cellulose binders.

Ingredient	Inclusion rate in feed (% as is)			
	Basal diet	CMC	SA	MHPC
Menhaden fish meal[1]	28.00	28.00	28.00	28.00
Soybeanmeal[2]	32.50	32.50	32.50	32.50
Squid [3]	2.50	2.50	2.50	2.50
Menhaden fish oil[1] Top coat	5.75	5.75	5.75	5.75
Spirulina [4]	2.50	2.50	2.50	2.50
Whole flour	23.30	23.30	23.30	23.30
Trace mineral premix[5]	0.20	0.20	0.20	0.20
Vitamin premix[6]	2.00	2.00	2.00	2.00
Choline chloride[7]	1.00	1.00	1.00	1.00
Sodium alginate [8]			**1.00**	
Methylhdroxypropyl cellulose [8]				**1.00**
Methyl cellulose [8]		**1.00**		
Stay C 35% [9]	0.15	0.15	0.15	0.15
Lecithin (soy commercial) [10]	1.00	1.00	1.00	1.00
Cholesterol [7]	0.10	0.10	0.10	0.10

[1] Omega Protein Inc., Houston, TX, USA.
[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[3] Foodcorp S. A., Chile
[4] Earthrise Nutritionals, Irvine, CA
[5] Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.
[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.
[7] MP Biomedicals Inc., Solon, OH, USA.
[8] TIC GUM Company, White March MD, USA
[9] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[10] The Solae Company, St. Louis, MO, USA.

Table 5.2. Summary of water quality parameters which were measured twice daily (0800 hrs and 2000hrs) over a 45 day culture period. Values represent mean and standard deviation together with minimum and maximum.

Water Parameter	Mean ± SD	Minimum	Maximum
Temperature (°C)	31.5 ± 1.86	26.0	35.0
Salinity (g/L)	8.6 ± 3.06	5.0	16.0
pH	7.1 ± 0.037	7.1	7.2

Table 5.3. Proximate composition and amino acid profile (% as is) of the test diets used in the growth trial. (CMC - methylcellulose, SA - sodium alginate, and MHPC – Methylhdroxypropyl cellulose) (LOD – Below the limit of detection)

Proximate composition[1] (% as is g kg^{-1})	Basal diet	CMC	SA	MHPC
Crude protein	38.70	37.80	37.70	37.80
Moisture	7.71	8.52	7.36	8.46
Crude fat	10.60	9.48	10.40	10.20
Crude fiber	0.91	1.02	1.12	1.07
Ash	9.44	9.30	9.65	9.23
Amino acids				
Alanine	2.02	2.15	2.04	2.16
Aspartic acid	3.29	3.29	3.53	3.59
Cystine / Cysteine	0.22	0.22	0.18	LOD=0.02
Glutamic acid	6.57	6.15	6.55	5.81
Glycine	2.39	2.47	2.36	2.71
Histidine	0.87	0.87	0.82	0.90
Hydroxyproline	0.60	0.78	0.70	0.77
Isoleucine	1.59	1.58	1.50	1.67
Leucine	2.94	3.13	2.95	3.22
Lysine (Total)	2.86	3.01	2.96	2.75
Methionine	0.59	0.28	0.55	0.38
Phenylalanine	1.81	1.85	1.79	1.99
Proline (Total)	2.53	2.65	2.55	2.92
Serine (Total)	2.08	2.32	2.17	2.36
Threonine (Total)	1.72	1.88	1.80	1.94
Tyrosine	1.37	1.39	1.38	1.46
Valine (Total)	1.74	1.80	1.70	1.92
Sum of Amino acids	35.20	35.80	35.50	36.50

[1]Diets were analyzed at Eurofins Lab, Ho Chi Minh City, Vietnam

Table 5.4. Fatty acids profile (% as is) of the test diets used in the growth trial. (CMC - methylcellulose, SA - sodium alginate, and MHPC – Methylhdroxypropyl cellulose) [1]Diets were analyzed at Eurofins Lab, Ho Chi Minh City, Vietnam.

Fatty acids profile (As is %)	Reference diet	CMC	SA	MHPC
Lauric acid (12:0)	0.022	<0.01	<0.01	<0.01
Tridecanoic acid (13:0)	<0.01	<0.01	<0.01	<0.01
Myristic acid (14:0)	0.391	0.354	0.339	0.369
Myristoleic acid (14:1)	<0.01	<0.01	<0.01	<0.01
Pentadecanoic acid (15:0)	0.040	0.036	0.036	0.039
Palmitic acid (16:0)	1.81	1.63	1.77	1.74
Palmitoleic acid (16:1)	0.730	0.667	0.675	0.708
Margaric acid (17:0)	0.044	0.038	0.033	0.042
Stearic acid (18:0)	0.471	0.410	0.452	0.439
Trans-Elaidic acid (18:0)	<0.01	<0.01	<0.01	<0.01
Cis-oleic acid (18:1)	3.52	3.14	3.39	3.39
Cis-linoleic acid (18:2)	1.67	1.48	1.76	1.61
Alpha-linolenic acid (18:3)	0.342	0.308	0.342	0.333
Arachidic acid (20:0)	0.027	0.024	0.026	0.026
Eicosenoic acid (20:1)	0.181	0.164	0.175	0.176
Cis-11,14,17- Eicosatrienoic acid (20:3)	0.018	0.017	0.018	0.017
Arachidonic (20:4)	0.083	0.076	0.018	0.081
Cis-5,8,11,14,17-Eicosapentaenoic acid (20:5)	0.457	0.416	0.439	0.449
Heneicosanoic acid (21:0)	0.058	0.053	0.058	0.056
Behenic acid (22:0)	0.018	0.015	0.017	0.016
Cis-13,16-docosahexaenoic acid (22:6)	<0.01	Non detected	<0.01	<0.01
Cis-4,7,10,13,16,19-Docosahexaenoic acid (22:6)	0.528	0.482	0.585	0.520
Lignoceric acid (24:0)	0.029	0.026	0.028	0.028
Nervonic acid (24:1)	0.028	0.026	0.025	0.027
Omega-6 (Gamma-Linolenic acid) (18:3)	0.041	0.036	0.053	0.040
N6-cis-DGLA (18:3)	0.037	0.034	0.036	0.037
1n-9 Erucic (22:1)	0.025	0.023	0.024	0.024
Monounsaturated fatty acids in product	4.50	4.03	4.31	4.34
Omega -3 fatty acids in the product	1.35	1.22	1.38	1.32
Omega-6 fatty acids in the product	1.83	1.63	1.93	1.77
Polyunsaturated fatty acids in products	3.18	2.85	3.32	3.09
Saturated fatty acids in the product	2.92	2.60	2.77	2.77
Unsaturated fatty acids in the products	7.68	6.88	7.63	7.43

Table 5.5. Response of mangrove crabs fed experimental diets over a 45-day period. (150 initial crabs per treatment)

Diet	Initial weight (g)	Final weight (g)	Weight gain (g)	Number of crabs molting	% weight gain	Days to 50% molt (days)	Survival Ratio
Basal diet	69.39	97.79	28.40^{a}	94	40.92^{ab}	35^{b}	$85/150^{b}$ (57%)
CMC	69.56	91.05	21.49^{b}	92	30.89^{c}	31^{a}	$113/150^{a}$ (75%)
SA	68.59	93.30	24.71^{ab}	77	36.02^{b}	34^{b}	$107/150^{a\,(}$71%)
MHPC	68.44	96.62	28.18^{a}	105	41.17^{a}	32^{a}	$110/150^{a}$ (73%)
PSE	1.81	3.41	3.523		3.523		
p value					<0.0001	<0.0001	<0.0001

[1]MHPC- Methylhdroxypropyl cellulose
[2]CMC- Methyl cellulose
[3]SA- Sodium alginate
[4]Initial weight= crab weight at stocking
[5]Final weight = Soft shell crab or newly molted crab weight (i.e. body weight in grams of the newly molted soft-shell crab as it came out of the box)
[6]Weight gain (g) = Final weight (g) -initial weight (g)
[7]% Weight gain= (Final weight - Initial weight)/ Initial weight *100
[8]Day to molt= Average number of days 50% of population molted within 45 days of trial
[9] Initial crab per treatment- 150 crabs
[10] Values with different superscripts within a row are significantly different based on Tukey's multiple range test
[11]PSE- Pool Standard Error

Figure 5.1. Mean and standard error days to 50% molt for each treatment of mangrove crabs fed with different diets over the 45 day trial. The number of crabs which molted (n) for each treatment were 94, 92, 77 and 105 for the basal, CMC, SA and MHPC, diets respectively.

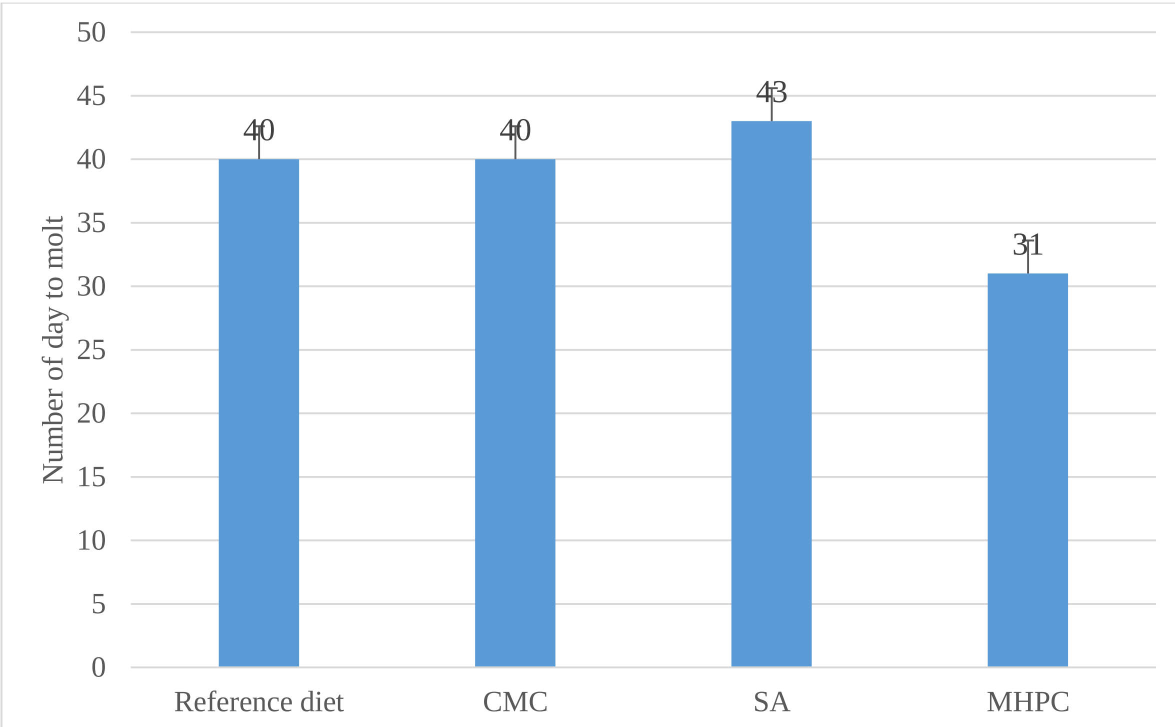


Figure 5.2. Mean response percentage of weight gain (with standard error) per each treatment of mangrove crabs fed with different diets in 45 day trial. The number of crabs which molted (n) for each treatment were 94, 92, 77 and 105 for the basal, CMC, SA and MHPC, diets respectively.

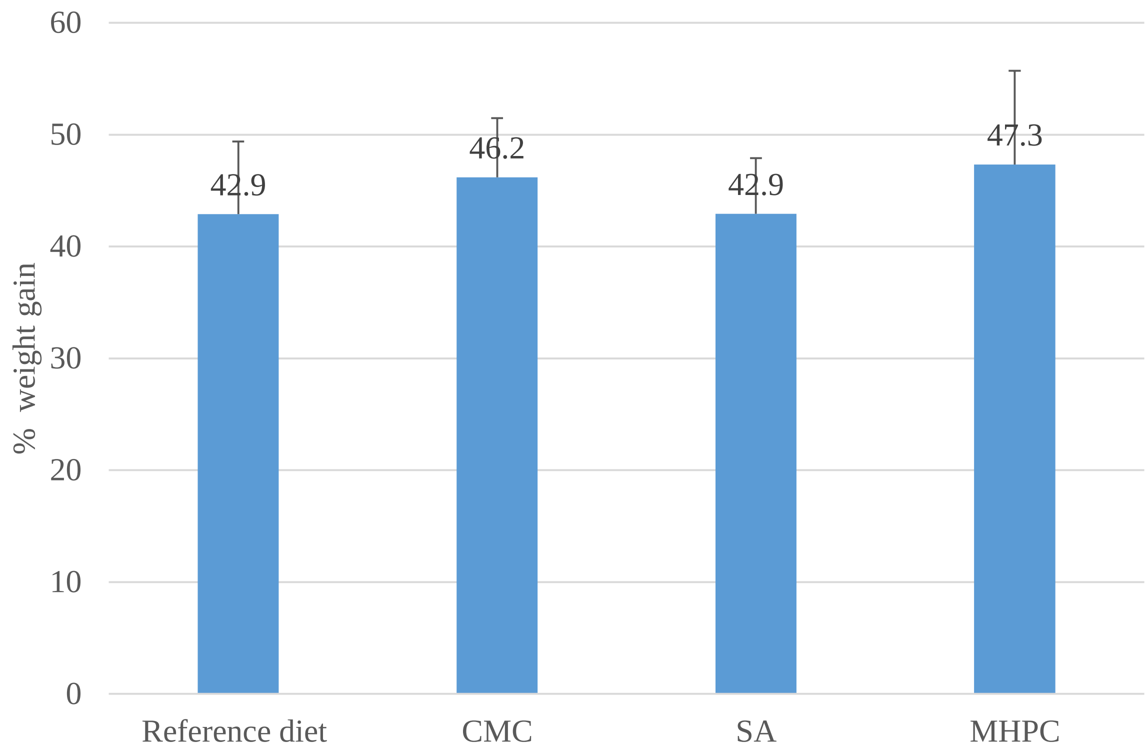
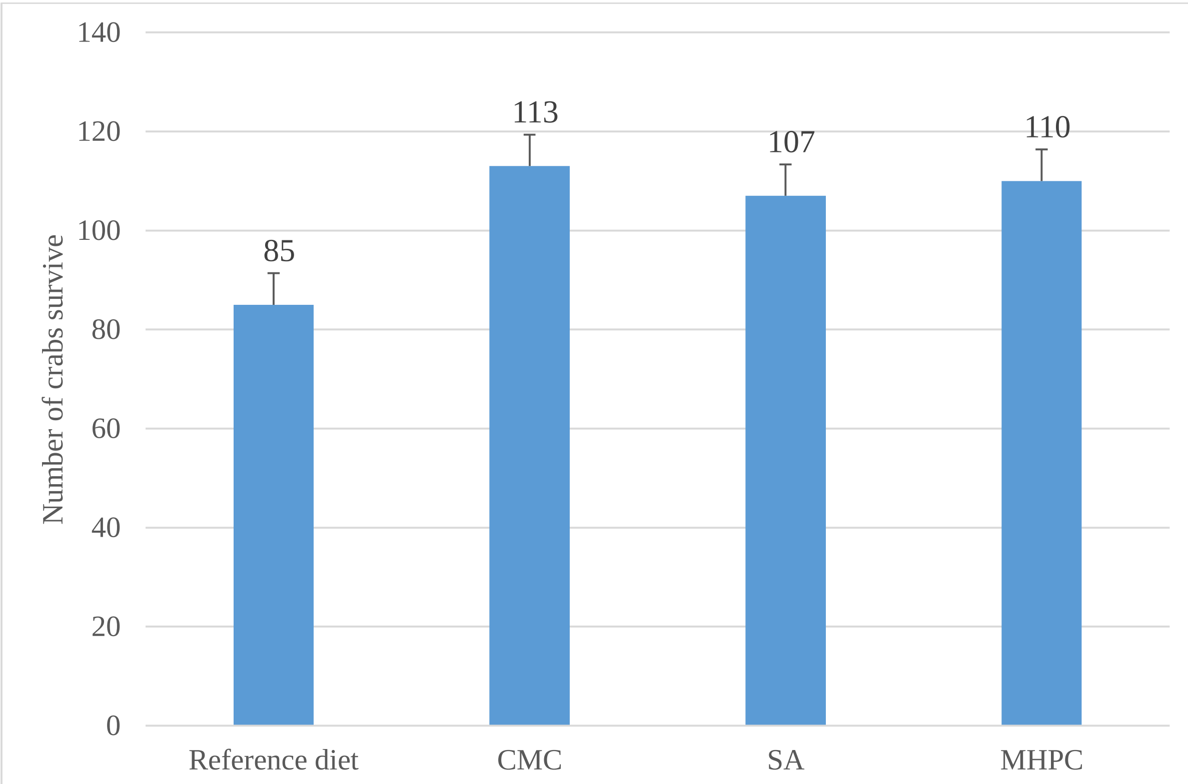


Figure 5.3. Mean resposne to number crabs surviving per each treatment of crabs fed with different diets in 45 day trial (n=150).



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Chapter 6. **Comparison of different types of extruded feeds for commercial production of soft-shell mangrove crabs**

6.1. **Abstract**

This study evaluated different extrusion methods to consider the preferred physical form of formulated pelleted feed by mangrove crabs and its acceptance. From previous experiment, it took longer for all the crabs to molt when using extruded floating feed. For the present study, three diets were formulated containing the presumed optimum 40% protein, 7% lipid using the same ingredients from earlier trials. Three experimental diets and one reference diet were randomly assigned to the crabs across the one raft. One group of 150 crabs were fed with an extruded sinking diet, another 150 with extruded floating diet, 150 with a slow-sinking extruded diet and 150 crabs were fed a traditional "trash fish" diet as the reference group. Crabs were fed 3% of body weight once a day in a 45-day feeding trial. The results showed no significant differences in mean percent weight gain of crabs fed sinking pellets compared to that of those fed trash fish. However, crabs fed with slow sinking pellets had the shortest average number of days to molting (29 days) and crabs fed with sinking diet had the longest average period to molting of 38 days.

6.2. **Introduction**

Mangrove crabs have emerged as a valuable aquaculture species due to their high prices, growing export market demand and ready availability of wild-caught seed crabs (Paterson and Mann, 2011). Soft-shell mangrove crab farming is a booming industry in Southeast Asia especially Thailand, Myanmar, Indonesia, Vietnam, the Philippines, and Malaysia. Currently, soft-shell mangrove crab farming is an emerging aquaculture crop in Bangladesh. Another reason that soft-shell mangrove crab farming is more popular is due to disease issues in shrimp farming with many farmers in Southeast Asia are looking for alternatives where they could use their existing marine coastal infrastructure. For example, in Myanmar's Kyauk Tan aquaculture zone, which is 2 hours away from Yangon, economic capital city of Myanmar, the whole aquaculture zone was once devoted to shrimp farming. However, the area was hit by white spot disease many years ago and now 80% of the area has converted into soft-shell mangrove crab farming.

In the current commercial soft-shell mangrove crab farming practice in Southeast Asia, sub-adult crabs, and either in inter-molt or pre-molt stage of 60-80 g are collected from the wild. Crabs are stocked individually into plastic perforated boxes sized 10 cm x 15 cm x 15cm. In one pond of 6,000 m^2, one can stock 40,000 crabs with a monthly production of 2.5 to 3 tons with a continuous harvest size of 100-120 g per crab. In order for the crab to grow, they have to molt and with each molt, the size increases 25 to 50% (Tobias-Quinitio, 2015). Sixty to seventy percent of crabs molt during nighttime and for crabs sized 80-160 g, the next molt can occur within 25 days (Ganesh et al., 2015).

In recent years, mangrove crab hatchery technology has developed in Australia, the Philippines, Vietnam and India. In Vietnam, there are a number of commercial crab hatcheries. Some successful research work at the SEAFDEC, in Iloilo, the Philippines and at the Rajiv Gandhi Center for Aquaculture, India, has used hatchery-reared crab for soft-shell crab production and the results are promising (Tobias-Quinitio., 2015; Ganesh et al., 2015). However, little research has been conducted using formulated diets for soft-shell mangrove crab farming.

Traditionally, crabs were viewed as carnivores with preferences in their natural diet containing mollusks, crustaceans and fishes that have relatively high protein content (Hill, 1979). Although the soft-shell crab industry has been going for more than 20 years, little research has been published regarding compounded feeds and the urgent need to replace trash fish with formulated diets (Quinitio and Lwin, 2009; Genodepa and Failaman, 2016). Development of alternative feed to trash fish is emerging as a bottleneck to this industry (Paterson and Mann, 2011). These days the farmers cannot afford to feed the whole trash fish and now they are feeding only the head of the fish. Not only are the “trash” fish expensive, but also they are hard to get the whole year around. Especially in the Southeast Asian region, the crab farming industry is competing for trash fish with human consumption, pet feed and fishmeal production.

The previous research showed that a formulated diet could be used for soft-shell crab farming after a few days of training. The research also addressed issues related to water stability of pellets, pellet hardness and pellet size by using binders and extrusion methods. In this current research, all the experiment diets were produced by using extrusion technology. Extruded cooking has many advantages. All the ingredients are cooked under high temperature and pressure and it reduces certain toxin levels in some feed ingredients and destroys many pathogens. Extrusion allows buoyancy control to make floating, sinking or slow sinking feed by controlling processing parameters. Extrusion cooking is widely used in aquaculture feed industry due to flexibility during processing and ability to produce different shape and texture products (Merciers et al., 1989). In this experiment, the same formulation for three different types of extruded diet was used. By controlling temperature, moisture, bulk density and pressure, different diets were produced using the same formulation with same ingredients. In this research, diets produced with different extrusion methods were evaluated to consider the preferred physical form of formulated feed by mangrove crabs and its acceptance.

6.3. **Materials and methods**

In this trial, the feed formulation, ingredient selection, and processing were conducted at the US Fish and Wildlife Service Bozeman Fish Technology Center, in Bozeman, Montana. The ingredients were in general nominally the same as those used in Auburn, Alabama, but from different sources. All the ingredients were tested or analyzed before inputting nutrient values into the nutrient database. The target for the experimental diets was iso-nitrogenous (40% of protein and 7 % of fat) and iso-lipidic (Table 6.1). All the dry ingredients for each diet, including vitamin and minerals premix and 1% methylhdroxypropyl cellulose were mixed in a paddle mixer for 20 minutes (Model# 6369- Marion Mixers, Inc, Marion, Iowa) then pulverized for fine grinding. Water was added and mixed, with the resulting mash placed into the pre-conditioner. From the pre-conditioner the mash was fed into the extruder (DNDL-44, Buhler Twin Screw extruder, AG, Uzwil, Switzerland) with 18-s exposure to an average temperature 90-103°C in the sixth extruder barrel section. Die head pressure varied from 200- 400 psi. The pellets were extruded as floating, sinking and slow sinking pellets approximately 1 cm by using different pressure and temperature (Table 6.2). The pellets were dried in pulse bed dryer (Bhuler Ag, Uzwil, Switzerland) for 25 minutes at 102°C with a 10-minute cooling period reaching a final moisture level of less than 10%. The dry pellets were then sprayed with fish oil in a vacuum coater (A.J. Mixing, Ontario, and Canada). The proximate analysis was estimated using a spreadsheet program analysis. A sample of each diet after production was also collected and sent to Eurofins Laboratory, Ho Chi Minh City, Vietnam for analysis of proximate composition (g 100 g^{-1} as is) and amino acid (g 100 g^{-1} as is) and fatty acids (g 100 g^{-1} as is) profiles following AOAC (1995) procedure by the lab. (Table 5.3 and 5.4). The rest of the pellets were stored in re-sealable plastic bags and shipped to Thailand for use in the experiment at a commercial soft-shell crab farm in Ranong.

The “trash fish” were primarily composed of a pelagic fish species, a smooth scale rainbow sardine (*Dussmieria elopsoides*). On the feeding days, fish were cut into portions such that the estimated dry weight of the portion was equivalent to the dry weight of the commercial and experimental feeds to be supplied. This was determined by drying several of the trash fish and determining the average weight and dry weights.

6.3.1. Feeding Trial

One supplier collected all the crabs for the trial from an estuary in Kyun Su Township, Myeik district, of Southern Myanmar. Thus, the population was expected to be somewhat homogenous. Several thousand crabs were collected on one night and delivered to the farm the following morning for stocking. A total of 600 crabs with an average weight of 70 grams and a range of 60 to 80 grams were selected from that day’s catch. For each treatment, 150 crabs were stocked into individual boxes in the same PVC pontoon raft. The most popular design for pontoon rafts to hold crab boxes in Thailand and Myanmar is a 4-by-400 box array. Thus, each pontoon could hold up to 1,600 crabs at a time. The array is moved with a pulley mechanism under the bridge so that the farmer can assess the condition of the crab every four hours and feed every day.

Crabs were stocked into the cages on 20 August 2017 and the feeding trial was started on 21 August 2017. Boxes within the four rafts were randomly assigned with a random number table such that 150 crabs were fed with extruded sinking diet, another 150 with slow sinking extruded pellet, 150 with extruded floating feed and 150 crabs traditional trash fish diet as control group. The crabs were fed 3% of body weight and fed at 1500 h once a day.

Water temperature and salinity were measured twice a day, at 0800 h and 2000 h, using an alcohol thermometer and a hand-held refractometer (Master by Atago, Thailand). pH and alkalinity were determined every week at 0800 h (portable pH tester and saltwater alkalinity colorimeter, both by Hanna, United Kingdom) and water in the pond was exchanged with every spring tide (approximately 10- 20% exchange as normal farming practice). Other pontoons in the pond were stocked and monitored using normal commercial farm processes and only one pontoon (PVC raft) was used for the experiment. The experiment trial was conducted in the end of the monsoon season and typically, farmers observe fewer molting crabs. They attribute slower or reduced molting to the high winds and continuous rain some days and sudden changes of temperature and salinity that occur with heavy rains. Crab boxes are suspended right on the surface of the pond and temperature and salinity fluctuations are high. However, this is the normal farming practice by the farmers.

Every day during the feeding trial, crabs were checked for molting and mortality every four hours. If a crab was found to have died, it was removed, noted in the logbook and discarded. If a crab was found to have molted, the date and time of inspection was noted. The newly molted crab removed from the box was weighed individually as final weight and then placed into a bucket of aerated fresh water for 30 minutes, so that the shell would not harden. The crab was weighed again after 30 minutes, which determines the weight for sale to the processor. The soft-shell crabs are then packed closely together and covered with a soaking wet cloth in a cooler or bench style refrigerator for daily sale as still live soft-shell crabs to the processing plant. For the purpose of the experiment, we used the weight of the crabs when first removed from the box. The weight after 30 minutes in freshwater is increased considerably as the crab swells with water during that time. The other data recorded at harvest besides body weight, was carapace width, and harvest time and date. The carapace width and weight after soaking in freshwater are not reported here as they did not seem to have any direct relevance. The trial concluded on October 5, 2017 when all remaining live crabs were counted for survival. The average weights and weight gains for the surviving crabs were determined, but not used in the analyses for two reasons. First, because these crabs are not marketable and their hard-shell weights would not be meaningful for a commercial farm. Second, the weights including the hard shell would likely skew the data in some manner we could not predict. For those crabs that molted during the 45-day feeding trial, the average number of days from stocking until molting was also determined.

6.3.2. Statistical Analysis

Final body weight was defined as the weight in grams of the newly molted soft-shell crab as it came out of the box. The final weight gain was evaluated using initial weight at the time of stocking into the box. If a crab did not molt within 45 days, it was not included in the weight gain or molting statistical analyses.

Data from percent weight gain trial were analyzed using analysis of covariance (ANCOVA) to determine significant differences among the treatments, followed by Tukey's multiple comparison test to compare difference among treatment means. A Kaplan-Meier estimator (time-to-event analysis) was used to measure time to molting (mean number of days at which 50% of crabs in a treatment had molted) between groups to determine significance. If any crab molted within seven days of stocking, for any treatment group, they were not included in the data as it is doubtful that there could be any nutritional or diet effect that quickly. Mortality (or survival) was analyzed by a generalized linear model with logistic regression. All data were analyzed using SAS (V 9.4. SAS Institute, Cary, NC, USA) software with significance level set at $\alpha \leq 0.05$ (unless otherwise stated).

6.4. **Results**

The crabs fed the trash fish control diet (mean weight gain of 29.9g and percentage weight gain 46.09%) had a significantly greater final weight and mean weight gain than the experimental floating and slow sinking diets. The sinking feed diet (percentage weight gain 40.82%) was not significantly different from the trash fish diet or the slow-sinking diet. The sinking feed had significantly greater weight gains than the floating diet (mean of 22.1 g or 34.0%) (Table 6.4). No statistical differences occurred between mean survival rates or days to molt among all treatments. However, significant differences in the number of crabs to molt during the 45 days with the trash fish being the highest followed by the sinking diet and then the floating and slow sinking diets. The results demonstrated that the mangrove crabs accepted different types of extruded compound feed but that the sinking feed seemed to offer the most similar results to the trash fish control in growth rate and number of crabs to molt in during the 45-day experimental period.

6.5. **Discussion**

From the final experiment, the results showed that sinking pellets provided significantly better weight gain compared to slow sinking and floating pellets. Again, trash fish achieved significantly higher weight gain compared to the experimental diets slow sinking and floating feed. Several potential reasons explain why the trash fish performed better than the pelleted diets. One reason could be that the pelleted feed was missing some limiting nutrient or that the digestibility of some of the ingredients are not sufficient to make the nutrients available to the crabs. In addition, a possibility of some anti-nutritional factor at work may have been in play. Another reason could be that it takes a couple days to a week for the crabs to completely adjust to eating and digesting pelleted feed. From the start of the feeding of pellets, crabs would hold the pellet with their claws, but they would not eat it straight away unlike trash fish. Similar results were reported by Keenan (2004) that using trash fish gave better growth rate than formulated diets with vitamins and without vitamins designed for mangrove crab grow-out culture.

Current research demonstrated promising results in that different types of extruded formulated diets could be used for soft-shell crab production because experimental diets improved weight gain in every trial with good survival and in one case similar number of crabs molting in the same time period. In this experiment, it was observed that crabs typically use their right hand claw for crushing and left claw as a cutter (Figure 6.1). By using both a binder and an extrusion cooking process, it improved not only water stability of the pellet, but also made the pellet hard enough for the crab to handle with their powerful claws. With the sinking pellet, achievement of 40.9% average weight gain was possible in less than 45 days with once-a-day feeding and still get very good survival in this trial.

In terms of taste or flavor for human consumers, no scientific taste test was conducted. However, the soft-shell crabs from the experiment were sold directly to the processing plant. They were aware of experimental formulated diets being fed to the crabs. They tested taste and could not detect any difference. It showed tentative market acceptance for the crabs fed by formulated diets and they may not have any detectable difference.

For each of these on-farm trials, the pontoons were used for regular daily farm activities of stocking, feeding, and checking every 4 hours. The only difference was provision of different feed treatments and record keeping of individual crabs when they molted and their weight after molting. In terms of water quality, the experimental groups did not get any special or specific water treatment or conditioning. We conducted the normal daily farming activities of changing water every 2 weeks at 10-20% of pond volume exchange. It was also observed that although it took several days for the crabs to adjust to the formulated pelleted diet, the feeds achieved very good survival and a majority of crabs molted within a few days of the trash fish fed crabs.

It was also observed that after two to three weeks of feeding formulated diets, the normal color of the crab carapace changed and it had slightly darker color than crab fed with trash fish. It might be due to fishmeal and fish oil and other ingredient pigmentation in the diets. However, when the crabs molt, the soft shell crabs are the same normal color. The soft-shell market is not like salmon and pigmentation may not be important since the consumer probably has no color preference.

Using extra attractant(s) in the formulated diets might increase palatability or consumption. A fish oil top coating for the experimental diets was used but still it takes time for the crab to adjust to the formulated diet. Crab will eat trash fish straight away but for formulated diets, they have to be trained for at least a couple days. Crabs locate their food by contact chemoreception through dactyls of their walking legs and maybe experimental diets did not give enough stimulation.

When feeding the floating pellet, it was observed that crabs struggled to maintain the floating feed inside their mouths, claws and walking legs. Crabs hold their food before they eat. Floating feed comes out from below the walking legs and sometimes from the mouth. In Ranong, some farmers previously attempted to feed fish meatballs, which are produced for human consumption, to crabs for soft-shell production. But, the crabs had no interest in those fish balls and the farmers stop using it. Naturally, crabs are bottom eaters and like to eat slow moving things instead of floating prey. However, floating feeds are not sinking down to the bottom holes of the crab boxes after crabs crush them or making new small pieces unlike sinking pellets. Crabs like to eat slow moving things in nature and it was thought that slow sinking feed might be a preference of the crabs (Tuan et al., 2006). The current results showed that some differences in growth and number of crabs molting between the feeds existed. Additional trials should be conducted to confirm these differences.

The results of these trials may contribute to the industry by demonstrating that formulated diets could be used for soft-shell crab farming and reduce the demand for "trash" fish. For the research community, these findings will provide some basic formulation and methodology with which to further additional lines of experimentation for improvement of formulated pelleted diets. The unique aspect of this research was that we conducted the work at the commercial farm level, on an operating farm in a non-controlled environment. However, it was hard to compare with other research results due to most other research having a focus on juvenile crabs or brood stock crabs conducted in a controlled lab environment. Some of the normal laboratory level trials were skipped in favor of a closer and immediate application to industry.

Figure 6.1. Crab eating experimental diet

6.6. **Conclusion**

The results of this study confirmed that different extruded formulated feeds are acceptable for mangrove crabs. However, trash fish still provided better weight gain than experiment diets in part due to 3-7 days of training for formulated diets. All the treatments had very good survival. The current feed formulation with 40% protein and 7% lipid can be used for commercial scale soft-shell crab farming. The current experiment demonstrated that trash fish could be replaced or supplemented with extruded formulated diet.

Table 6.1. Ingredient composition of diets formulated to contain 40% protein and 7% lipid prepared with different extrusion processes.

Ingredient (As is basis g kg^{-1} feed)	Inclusion rate in feed (%)		
	Sinking	Floating	Slow sinking
Menhaden fishmeal[1]	29.40	29.40	29.40
U.S soybean meal[2]	43.34	43.34	43.34
Squid [3]	2.50	2.50	2.50
Menhaden fish oil[1] Top coat	2.75	2.75	2.75
Spirulina[4]	2.50	2.50	2.50
Whole flour	14.00	14.00	14.00
Trace Mineral premix[5]	0.20	0.20	0.20
Vitamin premix [6]	2.00	2.00	2.00
Choline chloride [7]	1.00	1.00	1.00
Stay C 35% [9]	0.15	0.15	0.15
Lecithin (soy commercial) [10]	1.00	1.00	1.00
Cholesterol[7]	0.10	0.10	0.10
Methylhdroxypropyl cellulose [8]	1.00	1.00	1.00

[1] Omega Protein Inc., Houston, TX, USA.
[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[3] Foodcorp S. A., Chile
[4] Earthrise Nutritionals, Irvine, CA
[5] Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.
[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.
[7] MP Biomedicals Inc., Solon, OH, USA.
[8]TIC GUM Company, White March MD, USA
[9]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[10] The Solae Company, St. Louis, MO, USA.

Table 6.2. Summary of water quality parameters which were measured twice daily (0800 h and 2000 h). Values represent mean and standard deviation together with minimum and maximum.

Water parameter	Mean ± SD	Minimum	Maximum
Temperature (°C)	30.8 ± 1.7	26.0	34.0
Salinity (g/L)	9.8 ± 2.9	5.0	16.0
pH	7.1 ± 0.037	7.1	7.2

Table 6.3. Crab feed extrusion production data for experimental feed

BHULER DNDL 44 Twin Screw Extruder 10 mm pellets use 8 mm 1 hole die for all diets			
Name	Slow Sinking	Sinking	Floating
Solid quantity	55.000 kg/h	55.000 kg/h	55.000 kg/h
K-Ton Feeder 1	55.000kg/h	55.000kg/h	55.000kg/h
Oil Feeder 3	0.000kg/h	0.000kg/h	0.000kg/h
Water feeder 4	14.990kg/h	15.981 kg/h	14.984 kg/h
Pressure endplate	41.973 bar	41.580 bar	37.074 bar
Drive power	5.608 KW	5.085 KW	4.932 KW
SME	80.124 Wh/kg	71.641 Wh/kg	70.467 Wh/kg
Throughout	69.990 kg/h	70.981 kg/h	69.984 kg/h
Temperature Barrel 3 (Add measurement 1)	85.363 C	78.395C	87.867C
Temperature Barrel 4 (Add measurement 3)	78.507 C	72.247 C	83.161 C
Temperature Barrel 5 (Add measurement 4)	85.199 C	80.278 C	89.310 C
Temperature Barrel 6 (Add measurement 5)	98.631 C	91.566 C	102.875 C
Revolution screws	450.2 RpM	400.2 RpM	400.2 RpM
Torque absolute	118.6 Nm	121.3 Nm	118.1 Nm
Torque relative	26%	26%	25.5%
Temp Endplate	102° C	94.5 °C	101.0 °C
Revolution cutter	178.8 RpM	178.5 RpM	178.2 PpM
Mokon 1- Temperature of barrels	180	170	200
Mokon 2-Temperature of die plate	180	170	200

Table 6.4. Proximate composition and amino acid profile (% as is) of the test diets used in the growth trial.

Proximate composition[1] (% as is g kg^{-1})	Sinking	Floating	Slow sinking
Crude protein	39.9	39.8	39.3
Moisture	5.4	5.5	5.3
Crude fat	7.5	8.0	7.4
Crude fiber	1.2	0.8	1.1
Ash	9.5	9.6	9.6
Amino acids			
Alanine	2.11	2.34	2.26
Aspartic acid	3.28	3.44	3.70
Cystine / Cysteine	0.25	0.18	0.19
Glutamic acid	6.38	6.13	6.70
Glycine	2.51	2.73	2.67
Histidine	0.90	0.94	0.96
Hydroxyproline	0.80	0.83	0.74
Isoleucine	1.58	1.78	1.67
Leucine	3.11	3.32	3.29
Lysine	2.90	2.93	3.13
Methionine	0.30	0.40	0.28
Phenylalanine	1.89	1.91	2.01
Proline	2.72	2.87	2.92
Serine	2.27	2.44	2.38
Threonine	1.89	1.96	1.98
Tyrosine	1.36	1.33	1.45
Valine	1.80	2.01	1.91
Sum of amino acids	36.0	37.50	38.30

[1] Diets were analyzed at Eurofins Lab, Ho Chi Minh City, Vietnam

Table 6.5. Fatty acids profile (% as is) of the test diets used in the extruded feeds trial.
[1]Diets were analyzed at Eurofins Lab , Ho Chi Minh City, Vietnam

Fatty acids profile (As is %)	Sinking	Floating	Slow sinking
Lauric acid (12:0)	0.012	0.021	0.015
Tridecanoic acid (13:0)	<0.01	<0.01	<0.01
Myristic acid (14:0)	0.345	0.364	0.349
Myristoleic acid (14:1)	<0.01	<0.01	<0.01
Pentadecanoic acid (15:0)	0.036	0.039	0.037
Palmitic acid (16:0)	1.41	1.50	1.40
Palmitoleic acid (16:1)	0.540	0.570	0.533
Margaric acid (17:0)	0.035	0.038	0.034
Stearic acid (18:0)	0.336	0.359	0.327
Trans-Elaidic acid (18:0)	<0.01	<0.01	<0.01
Cis-oleic acid (18:1)	2.06	2.19	1.96
Cis-linoleic acid (18:2)	1.35	1.42	1.32
Alpha-linolenic acid (18:3)	0.257	0.274	0.251
Arachidic acid (20:0)	0.021	0.025	0.021
Eicosenoic acid (20:1)	0.107	0.113	0.102
Cis-11,14,17- Eicosatrienoic acid (20:3)	0.012	0.068	0.012
Arachidonic (20:4)	Not detected	Not detected	0.063
Cis-5,8,11,14,17-Eicosapentaenoic acid (20:5)	0.064	0.415	0.382
Heneicosanoic acid (21:0)	0.388	0.034	0.030
Behenic acid (22:0)	0.032	0.018	0.015
Cis-13,16-docosahexaenoic acid (22:6)	0.015	Not detected	Not detected
Cis-4,7,10,13,16,19-Docosahexaenoic acid (22:6)	Not detected	0.436	0.394
Lignoceric acid (24:0)	Not detected	0.025	0.022
Nervonic acid (24:1)	0.023	0.023	0.022
Omega-6 (Gamma-Linolenic acid) (18:3)	0.031	0.036	0.031
N6-cis-DGLA (18:3)	0.023	0.025	0.023
1n-9 Erucic (22:1)	0.015	0.016	0.015
Medium Chain Triglyceride	0.014	0.028	0.019
Monounsaturated fatty acids in product	2.75	2.92	2.64
Omega -3 fatty acids in the product	1.06	1.14	1.04
Omega-6 fatty acids in the product	1.47	1.55	1.44
Omega-9 fatty acids	2.08	2.21	1.98
Polyunsaturated fatty acids in products	2.53	2.69	2.47
Saturated fatty acids in the product	2.27	2.43	2.26
Unsaturated fatty acids in the products	5.28	5.61	5.12

Table 6.6. Response of mangrove crabs fed experimental diets over a 45-day period. (150 initial crabs per treatment)

Diet	Initial weight (g)	Final weight (g)	Weight gain (g)	Number of crabs molting	% weight gain	Days to 50% molt (days)	Survival Ratio
Trash Fish	64.9	94.9	29.9[a]	71[a]	46.1[a]	34[b]	141/150 [a] (94%)
Sinking	64.7	91.2	26.4[ab]	56[b]	40.8[ab]	38[c]	137/150[a] (92%)
Floating	65.0	87.1	22.1[c]	37[c]	34.0[c]	27[a]	141/150 [a] (94%)
Slow Sinking	66.0	91.5	25.5[b]	34[c]	38.6[bc]	28[a]	141/150 [a] (94%)
PSE			3.331		3.331		
p value					<0.0001	<0.0713	<0.0007

[1]Initial weight= crab weight at stocking

[2]Final weight = Soft shell crab or newly molted crab weight (i.e. body weight in grams of the newly molted soft-shell crab as it came out of the box)

[3]Weight gain (g) = Final weight (g) -initial weight (g)

[4]% Weight gain= (Final weight - Initial weight)/ Initial weight *100

[5]Day to molt= Average number of days 50% of population molted within 45 days of trial

[6]Initial crab per treatment- 150 crabs

[7] Values with different superscripts within a row are significantly different based on Tukey's multiple range test

[8] PSE- Pool Standard Error

Figure 6.2. Mean and standard error days to 50% molt for each treatment of mangrove crabs fed with different diets over the 45 day trial. The number of crabs which molted (n) for each treatment were 71, 56, 37 and 34 for the trash, sinking, floating and slow sinking diets respectively.

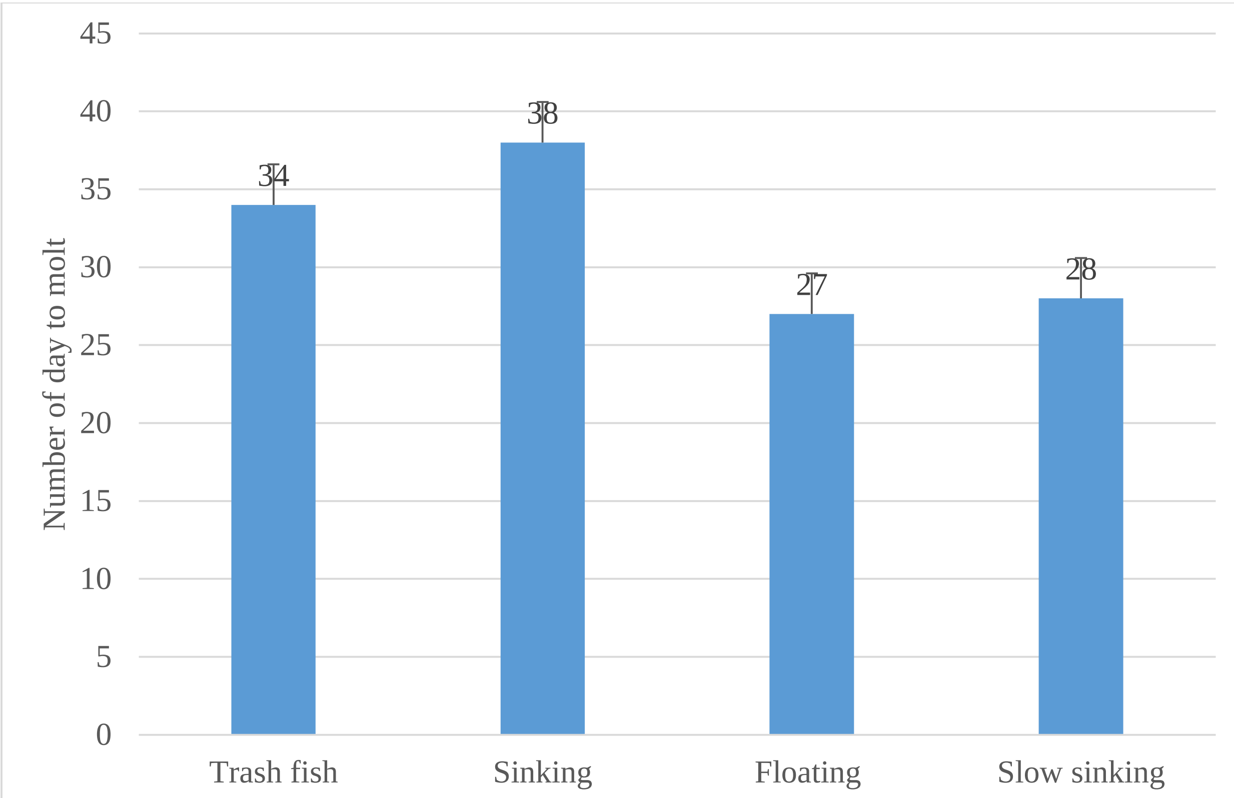


Figure 6.3. Mean response percentage of weight gain (with standard error) per each treatment of mangrove crabs fed with different diets over the 45 day trial. The number of crabs which molted (n) for each treatment were 71, 56, 37 and 34 for the trash, sinking, floating and slow sinking diets respectively.

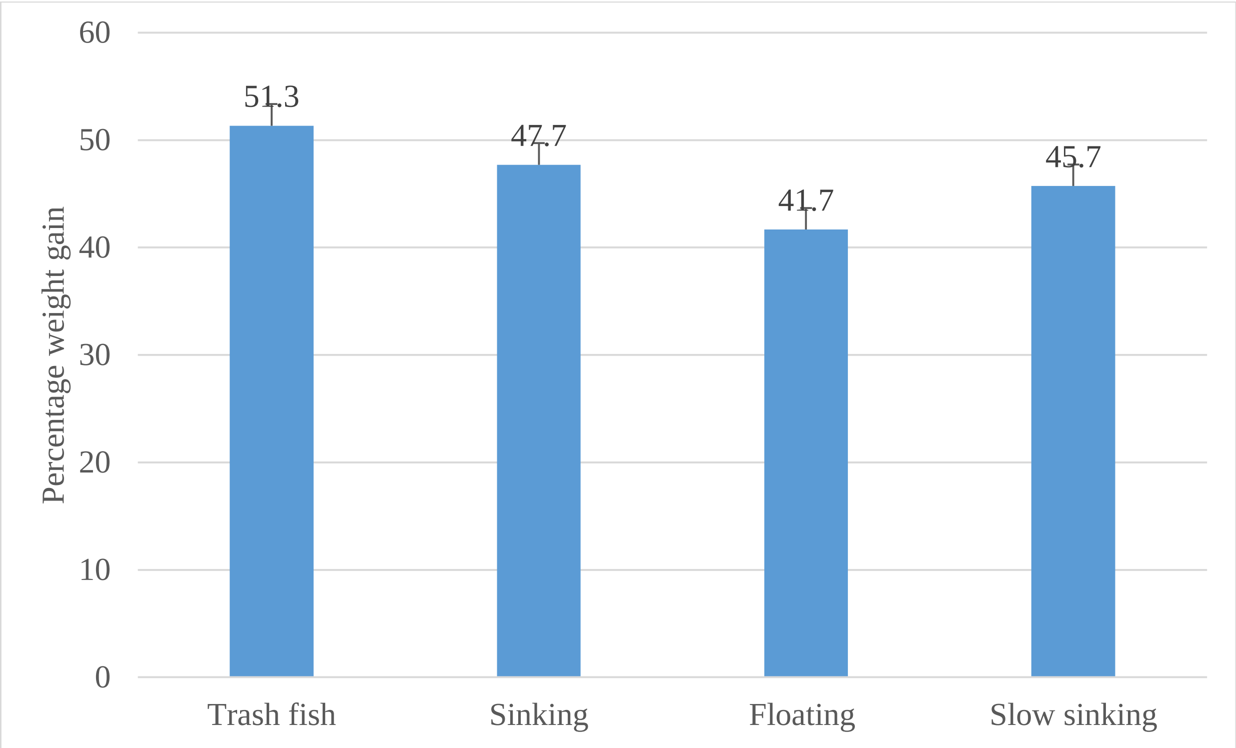
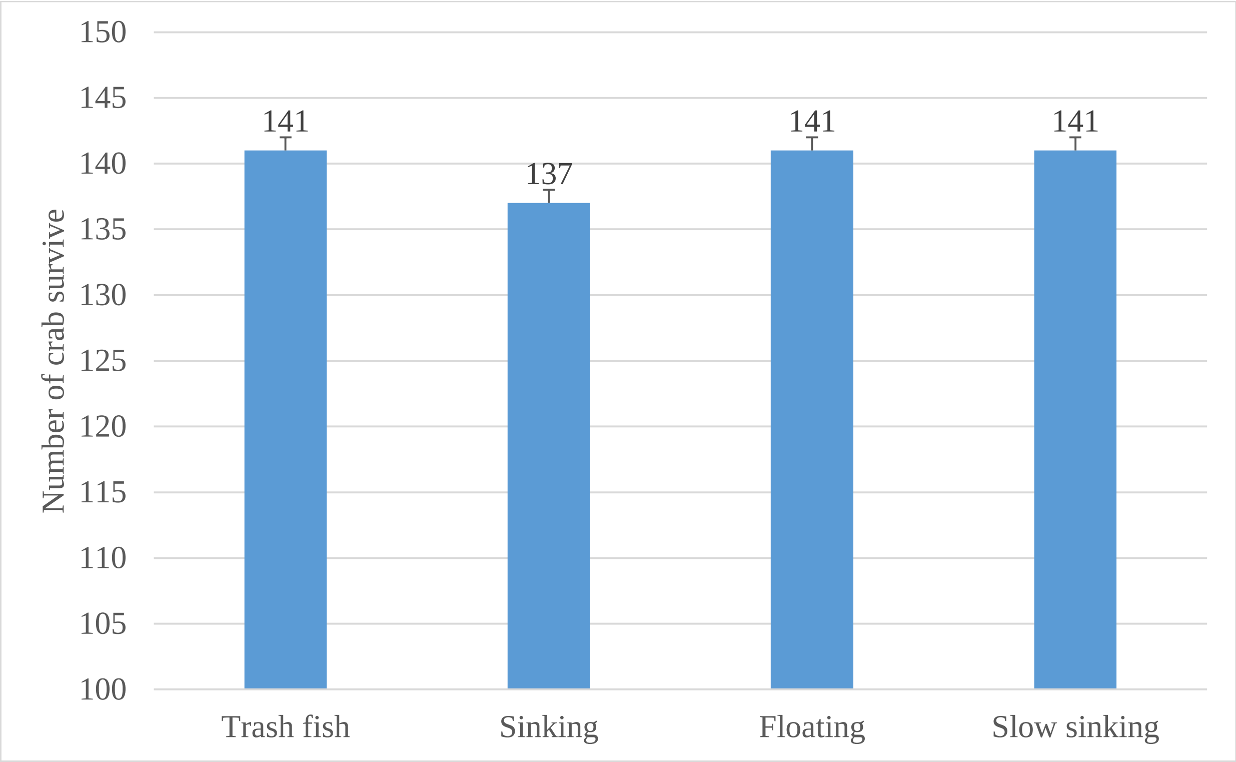


Figure 6.4. Mean resposne to number crabs surviving per each treatment of crabs fed with different diets over 45 day trial (n=150).



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Chapter 7. **Summary**

Soft-shell crab farming is a billion dollar industry in Southeast Asia, that is currently based on the use of low value forage (trash) fish as the primary source of feed. Rapidly decreasing supplies and increasing prices, necessitate developing low or no fishmeal content diets. A few studies have been conducted on larval and juvenile feeds and a few more on broodstock nutrition. However, there are very few studies on nutritional needs or growout / production diets for soft-shell farming. The focus of this research was to examine some of the basic parameters necessary to develop and manufacture a practical farm diet for crab farming. These parameters included shape of the pellet, stability in water, growth rate of the crabs and survival, time to moult.

The first trial was designed to manufacture a basic diet based on published literature values. These diets were prepared using a meat grinder to make sinking pellets that were transported to Thailand for an on-farm trial. From this trial we learned that wild-caught crabs would not immediately accept the pelleted feed, but needed several days of exposure to the pellets before they learned to consume the feed. The other important finding was that the crabs' handling of the feed reduced the integrity of the pellet and increased the leaching of nutrients and dry matter.

The second trial was a laboratory study utilizing two binders that have proven successful in preparing other aquatic diets in the past. Diets containing levels of methylcellulose and methylhydroxypropyl cellulose were prepared and then tested for water stability. Specifically, the diets were soaked for periods of up to three hours in warm saline water mimicking the condition on farm. The results indicated that adding binders to mash turned into pellets on a meat grinder sis not significantly improve water stability of diets as determined by leaching of dry matter from the pellets. Thus a decision was made that adding a binder to a formulation that would be prepared using heat and extrusion would be more likely to provide the nutritional and mechanical benefits desired.

In this trial, feeds were prepared on steam extrusion machinery at the Bozeman fish technology Center in Bozeman, and transported to Thailand for more on-farm testing. The diet formulations included three different binders and a control diet with formulations that were otherwise similar to those from the prior experiments. In this trial the diet containing MHPC binder provided the best growth and the greatest number of crabs moulting in the shortest time, two of the key parameters of most interest to soft-shell crab farmers who sell on the basis of number and weight of newly moulted animals. One observation from this study was that many of the crabs had difficulty grasping the floating pellets as they had a tendency to float away from the chelipeds.

In the final trial we prepared three diets on the same extruder but adjusted cooking conditions in order to create sinking, slow-sinking and floating versions of the diet. Again these were tested on a commercial farm in Ranong, Thailand. There were no significant differences in growth between the different diet forms. However, the slow sinking diet had the shortest number of days to moulting while the sinking diet had the longest period to moulting.

Overall, I believe that we have shown several useful results of interest to the industry. First, we demonstrated that wild-caught crabs can be trained to consume pelleted feed within just a few days and experience growth and time to moulting that is not significantly different from crabs fed a traditional diet of “trash” fish. Second, we learned that adding binders to an extruded diet would provide great water stability while not sacrificing any potential growth or speed to market. Third, we demonstrated that survival was not significantly different between the pelleted feeds and trash fish fed crabs.

The current situation is that virtually all soft-shell crab farms feed only “trash” fish or fresh by-products. There are some farms in Indonesia who feed crushed mollusks and some in Myanmar who feed trimmings from processed fish. Nevertheless, in all cases the feed is getting more expensive, provides a potential vector for pathogens and parasites, and requires cooling for storage. It is clear the industry must adopt compound feeds that will provide the necessary nutrients in a more convenient manner at a reasonable cost with fewer environmental issues.

Defining and answering the real needs of the industry is a key for the advancement of aquaculture. Hence, the research goal was to improve the feed quality for the industry and initiate the development of compounded feeds. Each feeding trial was conducted at a commercial farm providing research results under commercial conditions. The first experiment demonstrated that compound diets could be used for soft-shell crab farming production. However, it took a couple days to train the crabs to eat the formulated diet. Our protocol was to feed two feedings with more in the evening because crabs are night scavengers and most of their feeding activities are at night. In the first trial, two laboratory produced feeds and a commercial crab fattening diet were compared against the traditional trash fish diet. Trash fish provided a significantly higher weight gain of the crabs than the formulated experimental diets. The high soybean diet produced the highest survival rate but least weight gain in the crabs. Therefore, a farmer would need to balance the reduced growth rate compared to the higher population survival, that is - more crabs at lower weight to sell. Our high fishmeal diet was not statistically significantly different from trash fish in terms of weight gain, but the survival rate was lower than the crabs on the high soybean diets. These results confirmed that compounded diets can be used successfully.

Observations in the first experiment showed that crabs use their powerful claws to grab the feed pellets, and after a few minutes of manipulation, the pellets created under laboratory conditions were falling apart. Hence, the second trial was a test to use binders to determine if we could improve the stability of the laboratory produced feeds. The second trial was conducted in the laboratory to simply evaluate stability or dry matter loss with the presence of binders. The experiment tested two grams of pellets placed in a small arena (5 cm high PVC pipe with a screen bottom) which allowed submersion in ambient temperature water for 60, 120 and 180 minutes. It was assumed that feeds with better water stability would facilitate stability when the crabs handled the feeds. We evaluated two binders at multiple levels in our high soybean meal diet.

We determined that the binder methylhdroxypropyl cellulose (MHPC) provided significantly better water stability to the pellets than binder Methylcellulose (CMC). In terms of inclusion level, there was no significant difference between the levels tested (0.15%, 0.3%, 0.6%, 1.2%, 2.4%).

From the laboratory trials we chose to use a fixed level of 1% and evaluated three binders to produce a floating extruded feed. A practical diet similar to that previously evaluated was formulated at 40% protein and 7% lipid and the binders at 1%. In this third experiment, only one feeding per day, at 3 pm, was used, which is a typical farm practice. Feeding crabs is somewhat different compared to fish and shrimp as the farmers or workers need to put feed into individual crab boxes piece by piece. It is very labor intensive and time consuming. Due to late afternoon rains in the wet season and threat of mosquito borne diseases, feed is not delivered in the evening even though the farmers know that crabs will eat more under darker conditions. Ranong, Thailand has rainfall of over 300 mm per month during the rainy season. This is another critical reason for conducting these trials at an on-farm scale and location.

The results of this experiment demonstrated that diets containing the binder methylhdroxypropyl cellulose (MHPC) produced significantly higher weight gain in the crabs than sodium alginate SA, methylcellulose CMC and the reference diet with no binder. However, the mortality was high for all diets compared to the normal survival rate expected on the farm. During the rainy season, farmers expect higher mortality each year due to sudden changes of water temperature and other stress associated with the heavy rains. Crabs are held in plastic boxes which are on the surface of the water and the depth of the crab boxes is only 10 to 12 cm, so temperature, salinity fluctuations are high. The diet with no binder produced the lowest number of crabs molting. As soft-shell crabs are the primary products of the farm, delays or lack of molting crabs, is a negative factor. We noted that it took longer for all the crabs on the experimental diets to molt compared to the previous trial. It was also observed that floating feed required a longer period for the crabs to be trained and accept the pellets. It is possible that there will be no need to use binder at all, or use only 0.5% MHPC inclusion. It may be that extrusion alone with the proper ingredient starch content may provide sufficient binding capability and can improve pellet stability in water and handling by crabs.

From the final experiment, we evaluated three diet types under commercial condition. Results indicated that sinking pellets provided significantly better weight gain of the crabs as compared to slow sinking and floating pellets. Sinking pellets had the longest average number of days to molt, 38 days. There was statically no significant in percentage weight gain between trash fish and sinking pellet confirmed the efficacy of sinking extruded feeds. However, trash fish achieved slightly higher weight gain compared to the experimental diets. The reason could be that it takes a couple days to a week for the crab to completely adjust to eating pelleted feed. Although there is an adjustment period to pelleted feeds, this research demonstrated promising results showing that formulated diets could be used for soft-shell crab production.

The results of these trials will contribute to the industry by demonstrating that formulated diets could be used for soft-shell crab farming and reduce the demand for “trash” fish. We purchased the best quality of trash fish available as control diet for all the experimental trials. In the reality of farming, the farmers may not get or use the best quality trash fish for their daily feeding. Variable quality of trash fish is an increasing problem which also implies that nutritional value may be different on a day to day basis. Whereas formulated diets have a more reliable, nutritionally complete feed based on the requirement of the mangrove crabs. For the research community, these findings will provide some basic formulation and methodology with which to further additional lines of experimentation for improvement of formulated pelleted diets. The unique aspect of this research was that it was conducted at a commercial farm level, on an operating farm in a non-controlled environment.

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