Commercial mud crab Scylla Serrata : Study on growth, energy and protein requirement of juveniles in the view to develop peleted feed for crab farming in New Caledonia

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COMMERCIAL MUD CRAB *SCYLLA SERRATA*: STUDY ON GROWTH, ENERGY AND PROTEIN REQUIREMENT OF JUVENILES IN THE VIEW TO DEVELOP PELETED FEED FOR CRAB FARMING IN NEW CALEDONIA

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Résumé - En Nouvelle-Calédonie il y a une forte volonté politique pour diversifier l'aquaculture qui repose encore aujourd'hui sur la crevetticulture. Dans ce contexte le crabe de palétuvier est considéré comme une espèce à fort potentiel. Un des principaux verrous au développement de la carcinoculture en Nouvelle-Calédonie est la disponibilité d'un aliment granulé commercial. Ainsi le principal objectif de cette thèse est d'améliorer notre connaissance des besoins nutritionnels du crabe de palétuvier afin d'être en mesure de formuler un aliment équilibré pour son élevage. Cependant avant d'aborder les études nutritionnelles nous avons vérifié le nombre d'espèces de crabes de palétuvier présentes en Nouvelle-Calédonie. Nos résultats d'études morphologiques et génétiques de 63 individus provenant de 9 sites des côtes Ouest et Nord-Est de la Nouvelle-Calédonie ont confirmé l'existence d'une unique espèce commercialisée: *Scylla serrata*. C'est donc sur cette espèce que nous avons travaillé en nutrition avec deux séries expérimentales ayant pour objectifs : i) d'évaluer le concentré protéique de soja (CPS) en comparaison avec la farine de poisson comme principale source en protéines et ii) de déterminer le taux optimum d'incorporation du CPS pour la mue et la croissance tissulaire des animaux. Nous avons ainsi observé deux phases de croissance tissulaire au cours d'un cycle de mue (CM): une phase rapide (CTR) qui démarre après la mue et dure jusqu'au début de l'intermue (elle représente 30% du CM) suivi d'une phase de croissante lente (CTL) sur toute la durée de l'intermue et jusqu'à la mue suivante (elle représente 70% du CM). L'accumulation des protéines et des lipides au cours du CM a suivi le même profil que la croissance tissulaire contrairement aux cendres qui ont augmenté de façon rapide durant les 5 jours suivant l'ecdysis pour atteindre un plateau jusqu'à la prochaine mue. Les deux phases de croissance étaient corrélées avec une prise de l'aliment par les animaux maximale pendant les deux premières semaines suivant la mue. Elle a diminué de moitié sur les 5 semaines suivantes et s'est maintenue ensuite à un niveau de base jusqu'à la prochaine mue. L'énergie ingérée était allouée principalement à la croissance et à la l'entretien respectivement durant les périodes CTR et CTL. Durant la phase de croissance lente, 28% de l'énergie ingérée étaient mis en réserve en prévision de la prochaine mue. Le remplacement de la farine de poisson par le CPS n'a pas modifié la croissance tissulaire, l'efficience de l'aliment et le bilan énergétique des animaux quelque soit la phase de croissance considérée. Le taux d'incorporation dans l'aliment de 42% de CPS a permis la meilleure croissance (fréquence de mue et croissance tissulaire), efficience de l'aliment et la rétention de l'énergie des protéines et des lipides. L'hypothèse d'une toxicité de l'ammonium issu de la dégradation des protéines en excès ou des facteurs antinutritionnels du soja est avancée pour expliquer les effets négatifs observés avec les aliments renfermant des taux d'incorporation élevés en CPS. En conclusion, nos travaux apportent des informations originales sur la croissance tissulaire et les dépenses énergétiques durant un cycle de mue et la capacité du crabe juvénile d'utiliser le CPS comme principale source de protéines. Sur ces bases nous sommes en mesure de préconiser des contraintes nutritionnelles permettant de formuler un aliment équilibré sans farine de poissons pour l'élevage du crabe de palétuvier *S. serrata*.

Abstract - In New Caledonia, there is the strong political will to diversify aquaculture which is mainly based on shrimp farming. In this context, mud crabs have been considered as a potential species for aquaculture development. One of the main constraints to develop crab farming is the availability of formulated feed. Thus, the main purpose of this thesis is to get information on the crab nutritional requirements in order to formulate a balanced diet. However, we had to clarify first how many species of mud crab were present in New-Caledonia. The result of our morphological and genetic investigations carried out on 63 specimens from 9 areas of the west and northeast coast of New-Caledonia confirmed that only one species, *Scylla serrata,* is commercialized in this country. Consequently, *S. serrata* was used in our nutritional study based on two experiments to: i) evaluate the soy protein concentrate (SPC) compared with the fishmeal as the main protein source and ii) determine the optimum level of SPC in the diet for molting and tissue growth. We observed two tissue growth phases within one molt cycle (MC): a fast tissue growth (FTG) occurred after ecdysis until early intermolt stage (30% of MC) which is followed by a slow tissue growth (STG) period from intermolt to ecdysis (70% of MC). Protein and lipid deposition followed the same trend than tissue growth while ash level increased quickly during five days after molt and then remained stable until the next molt. The two growth phases were correlated with the voluntary feed intakes (VFI) which was maximum during 2 weeks after ecdysis and then decreased by 50% over the five following weeks to reach a baseline until the next molt. Intake energy was allocated mainly for growth during FTG period and for maintenance during STG period. During STG, 28% of the ingested energy was accumulated for the next ecdysis. Replacement of fishmeal by SPC as main protein source did not affect tissue growth, efficiency of feed utilization and energy budget of crabs whatever the tissue growth period considered. The dietary SPC inclusion of 42% in the diet promoted growth (molt frequency and tissue growth), feed efficiency and retention of energy, protein and lipid. Hypothesis related to ammonia toxicity from catabolism of proteins in excess or anti-nutritional factors from soybean could explain the negative effects of higher inclusion of SPC in the diet for juvenile crabs. In conclusion, our work brings novel information on tissue growth, energy budget during a molt cycle and the ability of juvenile crab to use SPC as a main source of protein. On this basis we suggest to formulate nutritionally balanced diet without fishmeal to farm juvenile mud crabs *S. serrata*.

Key words: Juvenile mud crab, *Scylla serrata*, tissue growth, molt cycle, molt frequency, ecdysis, voluntary feed intake, feed efficiency, fishmeal, soy protein concentrate, energy budget.

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1 - CHAPTER I: GENERAL INTRODUCTION

1.1 - BACKGROUND AND GENERAL OBJECTIVES

Background

Big size, high meat yield and delicate flavor mean that mud crabs are valuable seafood. As they are easily caught, they can be kept alive for long period after capture and they are of high value, mud crabs are important sources of income for small scale fishers throughout the Asia Pacific and South Pacific regions, including New Caledonia. Aquaculture of mud crab has been conducted for at least the past 100 years in China (Yalin & Qingsheng, 1994) and for the past 30 years throughout Asia (Keenan, 1999). Crab aquaculture production in the recent past relied on wild-caught stock. However techniques for captive breeding and larval rearing have been recently developed and allowed aquaculture production of mud crab to grow considerably, particularly in Vietnam (RIA3, pers. com.).

However, some technological bottlenecks still prevent the development of large-scale crab farming:

i. Diseases affecting the larval phase. The larvae are very susceptible to fungal attack and vibriosis.

ii. Cannibalism affects overall survival and prevents intensification of farming.

iii. In Asia, mud crabs are still mainly fed on trash fish although manufactured diets have been available in some countries (e.g. China and the Philippines). This practice introduces biosecurity problems leading to the development of disease and mortality in farms.

General objectives

In New Caledonia no genetic studies have yet been carried out to identify the mud crab species. Therefore, a unique local species has been considered until now, *Scylla Serrata*. However, since the review by Keenan et al. (1998) on the genus *Scylla*, it is necessary to determine how many and which species are now present in New Caledonia. This step is fundamental as breeding techniques will depend on the biology and the behavior of the species.

Mud crab fisheries in New Caledonia are experiencing an increase in fishing efforts and a risk of resource depletion in some areas (ADECAL, 2006). However, the stock of crabs

and the maximum sustainable yield are not known in New Caledonia and the risk of overfishing is very difficult to assess.

As mentioned above, one of the technological bottlenecks to the development of commercial farming of mud crabs, is the availability of an effective pelleted feed to enhance growth. In Asian countries (Vietnam, Philippines, Indonesia) crab farming relies on trash fish for feed. This traditional practice of feeding is strongly discouraged today and the development of a pelleted feed is widely seen as a priority for the expansion of mud crab farming (FAO, 2011). Unprocessed fish waste cannot be used for aquaculture in New Caledonia, as its use poses bio-security (disease transmission and degradation of pond bottoms) and logistical difficulties (chilling facilities for conservation and transportation).

In this background, a research program on mud crabs has started as part of a scientific and technical cooperation between RIA3 (Research Institute for Aquaculture No.3, Vietnam), the University of New Caledonia and IFREMER supported by the Southern Province of New Caledonia. This thesis is a part of this research program and has two goals: to identify the crab species in New Caledonia and to study the mechanism of growth according to the nutrition requirement of the species. This work will bring forth fundamental information on the biology of the mud crab and contribute to developing its potential farming in New Caledonia and providing nutritional solutions.

1.2 - BIOLOGY OF MUD CRABS

1.2.1 - Taxonomy and distribution

Mud crabs are commonly known as either mangrove crabs, serrated swimming crabs or edible mud crabs (Keenan et al., 1998; Chainy, 2012). The taxonomy of the mud crab genus, *Scylla* de Haan, 1833, has been unclear for a long time. Mud crabs were considered as one species but with different names (Keenan et al., 1998; Overton, 1999). Subsequently, the study of Estampador (1949) recognized three species: *Scylla serrata* (Forskål, 1775), *Scylla oceanica* (Dana, 1852), *Scylla tranquebarica* (Fabricus, 1798), and also one new variety *Scylla serrata* var. *paramamosain*. These identifications were supported by Serène (1952) based on samples collected in Vietnam, who recognized four forms but applied only two species' names. However, the validity of the different species was called into question (Holthuis, 1978; Fuseya & Watanabe, 1996; Overton et al., 1997) and all taxa continued to be considered as one species, *S. serrata* (Stepheson & Campbell, 1960; Fortes, 1999). The

confused taxonomy of the genus *Scylla* was a major constraint for the development of the mud crab aquaculture as well as the management of wild fishery (Infofish, 1992; Brown, 1994). In the past, identification of the mud crab genus, *Scylla* was based on traditional morphological keys built from relatively few samples collected in restricted areas (Overton, 1999). Recently the development of the genetic techniques such as: allozyme electrophoresis and mitochondrial DNA (mtDNA) sequencing of cytochrome oxidase I, 12S and 16S rRNA genes, was applied to a large number of specimens collected in various areas of the globe to clarify the identification of species in the *Scylla* genus (Keenan et al., 1998; Imai et al., 2002). More recently, Imai et al. (2004) used the first internal transcribed spacer (ITS-1) and 16S rRNA and identified four *Scylla* species at early larval and juvenile stages. At the present state of knowledge, there are four valid *Scylla* species (Fig. 1.1): *S. serrata* (Forskål, 1775), *S. olivacea* (Herbst, 1796), *S. tranquebarica* (Fabricius, 1798) and *S. paramamosain* (Estampador, 1949). The morphological key for the genus *Scylla* was also updated in the revision of Keenan et al. (1998).

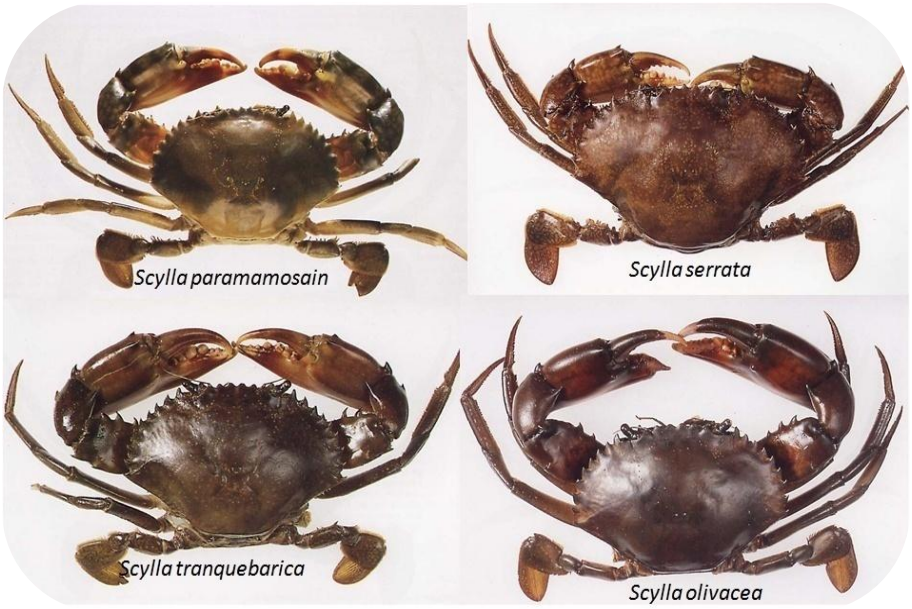

Figure 1.1. Photographs of the four mud crab species of genus *Scylla* de Hann: *S. paramamosain, S. serrata, S. tranquebarica and S. olivacea* (Keenan et al., 1998).

Mud crabs in the genus *Scylla* inhabits mangrove areas across the Pacific and the Indian Ocean (Dai & Yang, 1991; Keenan, 1999; Fao, 2011). The species, *S. serrata* has the most widespread distribution being found as far west as South Africa, east as Hawai, as far

north as Okinawa, Japan, and south to Sydney, Australia (FAO, 2011).

This species is strongly associated with mangrove forests with high salinity as natural oceanic salinity (Keenan et al., 1998). In addition, the mud bottoms of the mangrove forest with its fluctuating salinity are the favored habitat for the *S. serrata* (Vay, 2001). Keenan et al. (1998) also investigated that *S. tranquebarica* and *S. olivacea* have narrower distributions and can be found in the South China Sea, across the Indian Ocean and also the western Pacific region. The distribution of *S. tranquebarica* is often associated with *S. olivacea* and found at lower salinities of mangrove forest habitats and coastal areas, rather than areas where *S. paramamosain* is distributed (Keenan et al., 1998; Moser et al., 2002; Walton et al., 2006). *S. paramamosain* is an abundant species within its distribution range and found in estuarine and mangrove forest areas with shallow littoral zones, from Java to the South China Sea (Keenan et al., 1998; Macintosh et al., 2002; Obata et al., 2006; FAO, 2011).

In New Caledonia and other countries of the Indo-Pacific region, the mud crab, genus *Scylla*, is a major resource for many local populations located nearby mangroves (Angell, 1991; Brown, 1993). Commercial mud crabs are distributed all along the coastal areas of the main island. One species, *S. serrata*, was identified by Keenan et al. (1997). However, only 6 specimens from New Caledonia were collected for this study, while it is widely recognized that mud crabs of the Indo-Pacific region belong in more than one species of the genus *Scylla* (BOBP, 1992) and that these species can be sympatric (Keenan, 1997).

1.2.2 - Life history

Mud crabs are an euryhaline species, which spends its adulthood in brackish watery areas, where they will grow and then migrate offshore to spawn (Srinivasagam et al., 2000; Webley et al., 2009). Adult males and females can be recognized by the shape of their abdominal flap, triangular for males and semi-circular for females.

Sexual maturity

In the wild, the size at maturity of mud crabs depends on the species and the sex. For example, the carapace width size at maturity is between 125-133 mm and between 129-135 mm for the males and females of *S. tranquebarica* respectively, between 80-89 mm and

between 85-96 mm for *S. serrata* (Srinivasagam et al., 2000). It may also vary according to geographic location (Vay, 2001). Before molting, females secrete a hormone to attract males (Thach, 2009). Crab sexual maturation is a step-wise process, where they pass through an apparent physiological maturation before becoming functionally mature; for example, a sudden change in the chela height to carapace width (CW) ratio has been linked to functional maturation of males in *S. paramamosain* (FAO, 2011).

Copulation and spawning

Copulation of mud crabs typically follows the change of the abdomen from the more triangular immature female to the more rounded broad form (FAO, 2011). First the couple is constituted, the male grabs the female dorsally then they move together for 3 to 4 days until the female goes into molting (Fig. 1.2A). Then the copulation follows the ecdysis of female, the male turns over the female, and then it opens and presses the female's abdomen (Fig. 1.2B). The copulation lasts from 7 to 12 h, where the male's spermatozoa is transferred into the female's seminal receptacle, which is located behind her heart (Bhavanishankar & Subramoniam, 1998; Thach, 2009). After copulation, the male continues to carry the female for a few days until her shell hardens. Sperm stored inside the female receptacle can survive between 9 to 12 months, with repeated extrusion of 2 to 3 egg batches in laboratory conditions (Srinivasagam et al., 2000). The fertilization takes place in the spermathecae inside the female before eggs are extruded. The color of the immature ovaries is translucent to yellow and then changes to a darker yellow, then into a dark orange when the ovaries mature (Fig. 1.3). This is due to the accumulation of yolk in the oocytes which serves as a source of nutrition for the developing embryo (Quinitio et al., 2007). The major phases of oogenesis in crabs include, the previtellogenic phase, which contains essentially primary oocytes, and the vitellogenic phase, in which oocytes increase in size as yolk is incorporated into the cytoplasm (Cheng, 2003). Crabs with fully mature ovaries spawn within 3 weeks when brought to the hatchery but those ovaries that are not fully mature (light orange colour) may take more than 4 weeks to spawn (Quinitio & Parado-Estepa, 2003).

The eggs pass through the oviducts and attach to the hairs of the abdominal flap. The breeding season of the mud crab is highly correlated with temperature (Thach, 2009). Therefore, it varies in different areas; for example, mud crabs can spawn across the year but the peak spawning season occurs from November to February in New Caledonia and from April to July in central and south of Vietnam.

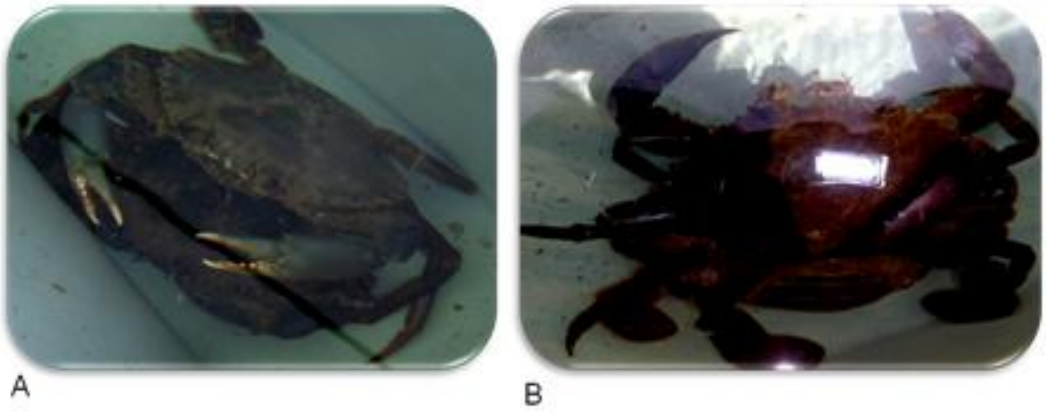

Figure 1.2. Photographs of (a) a crab couple and (b) crab's copulation.

Figure 1.3. Ovary color changes in relation to the progress of vitellogenesis of the mud crab. Immature ovaries are translucent to yellow (A,B) and mature ovaries become a darker yellow to a dark orange (C,D,E).

Fecundity

The fecundity of the crab, *Scylla,* depends largely on the size (body weight and carapace width) of the female (Zeng et al., 1991; Lin et al., 1994). Each female of *S. paramamosain* may spawn 1-2 million eggs (Thach, 2009), while *S. serrata* can bear from 1 to 5 million eggs (Hill, 1994; Srinivasagam et al., 2000).

Embryonic development

Once eggs have been spawned, the time to hatching and releasing larvae is temperature dependent, with a shorter time at higher temperatures and longer time at lower temperatures (FAO, 2011). Embryonic development can occur successfully between 20 and 30 °C, but temperatures above 26 °C are preferred (Li et al., 1999; Quinitio et al., 2001). Depending on the temperature, in captive mud crabs, egg incubation usually takes between 9-12 days after extrusion (Quinitio et al., 2001).

Larval stages and feeding

At hatching, a planktonic zoea larvae (Z1) emerges from the egg. Zoea molt up to four times during a period of about three weeks (Z2 to Z5), during this period they are transported by the currents back to the coastal environment (Brown 1993). The final zoea stage changes into a megalopa, which settles out on the suitable and available substrates, such as: reed beds, aquatic macrophytes, under stones or in the mud and sandy sediments (Ong, 1964; Delathière, 1990; FAO, 2011) (Fig. 1.4). Megalopa larvae prefer a complex habitat rich with refuges and food while juveniles are located in the sea grass and algal beds linked to the mangrove forest (Webley et al., 2009; FAO, 2011).

Zoea stages are identified by the appearance of pereiopods and the development of pleopods. Megalopa have chelipeds originating from the 1st working leg, a broader carapace and segmented abdomen which is folded (Thach, 2009). The duration of each larval stage is negatively related to the temperature and their survival rate depends on both temperature and salinity (FAO, 2011). Thus, the length of the five zoea stages and of the megalopa stage can vary considerably before the first crablet stage is attained (C1). In New Caledonia, zoea stages lasted 16 days at a salinity of 31.75 ‰ and temperatures between 28.5 °C and 29.0 °C (Delathière, 1990).

In aquaculture, Z1 and Z2 are usually fed on rotifers (*Branchionus*) or umbrella-stage *Artemia* (Li et al., 1999; Mann et al., 1999; Takeuchi et al., 2000; FAO, 2011). Late Z2 or Z3 to megalopa are commonly fed on *Artemia* (FAO, 2011). Along with rotifers and *Artemia*, the larvae are also given supplementary diets, such as microbound diets, minced fish or bivalves. Several studies have determined the digestive enzyme activities of crab larvae. High protease was found in Z1 larvae which indicate that the crab larvae have the ability to digest food immediately after hatching (Li et al.., 1999). The levels of larval digestive enzyme activities

(protease, amylase and cellulase) were correlated with larval development stage (Tang et al., 1995). The accumulation of glycogen, lipid and protein in the digestive tract is the highest for the Z5 and megalopa stage, and showing a significant increase appearing at the Z3 stage (Li & Li, 1995).

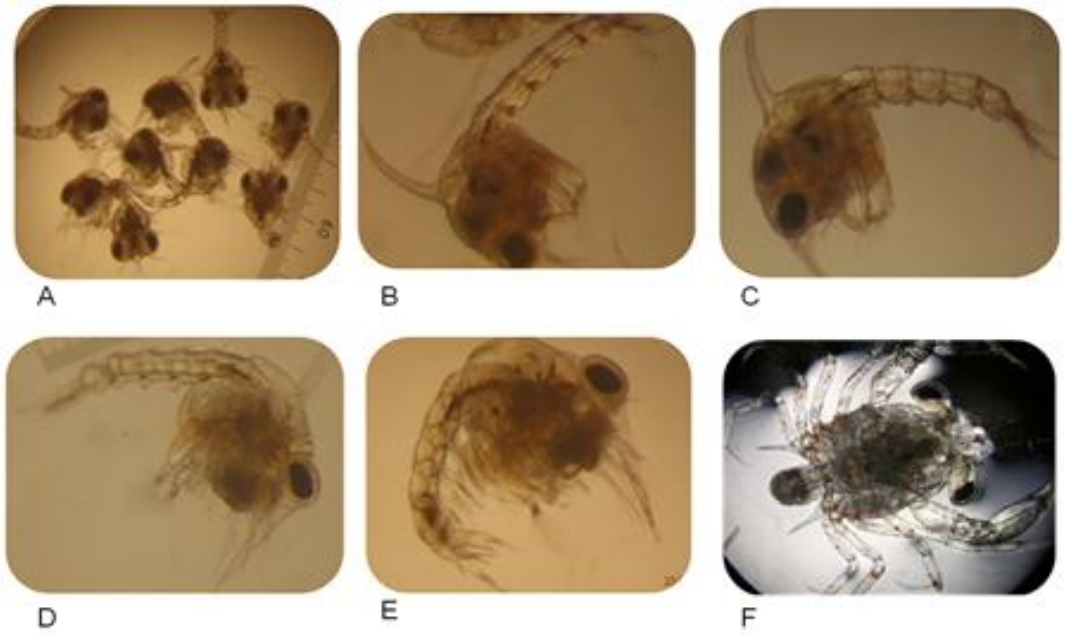

Figure 1.4. Photographs of larval stages of *S. serrata*; Z1through Z5 (A-E) and megalopa (F).

Molt and growth

Molting in crustaceans consists of shedding the old exoskeleton and regenerating a new one. Drach's (1939) original molt staging scheme has been adapted for a wide range of decapods, including species in brachyuran families (Passano, 1960). The molt cycle of *Scylla serrata* has 3 distinct morphological stages: i) postmolt, which is further divided into A, B, C_1, C_2, C_3 sub-stages; ii) intermolt, which then divided into C_4, D_1 sub-stages and iii) premolt, which consisted of D2, D3, D4 sub-stages (Heasman, 1980). The main characteristics of each sub-stage are presented in Table 2.1. The most widely used technique to identify the molt stages of crabs is based on external changes in the exoskeleton and the internal setal development of the appendages (Freman et al., 1987; Tian et al., 2012). The duration of the molt cycle stages in crabs is the net result of complex interactions between many endogenous

(genetic) and exogenous factors, such as: temperature, nutrition, autotomy, regeneration, size, sex and sexual maturity (Heasman, 1980).

Once the crabs molt, the carapace width (CW) and body weight (BW) increase. However, the molt frequency decreases as the crab gets older and the increase rate in body weight is higher, than in the carapace width during the succeeding molts (Thach, 2009). Thus, in crab farms, daily increase in the carapace width slows down gradually until the end of the crop, while the daily weight gain continuously increases noticeably, when crabs reaches the marketable size (Thach, 2009).

At the crablet stage, *S. serrata* can molt up to 15 times to reach 150-200 mm in CW, and only two further molts may occur before death, with their maximum recorded size being 280 mm (Carpenter & Niem, 1998). *S. tranquebarica* reaches 80-120 mm in CW within the first year and 140-150 mm in the second year. Maximum CW of *S. paramamosain* is 200 mm (Carpenter & Niem, 1998). *Scylla olivacea* tends to be the smallest (maximum CW is 180 mm; Carpenter & Niem, 1998) of the four species of the mangrove crabs. However, mud crabs of genus *Scylla* almost attains the maximum growth within one or two year of their hatching in their natural habitats (Chainy, 2012).

As the crabs grow in connection with the molt, fresh weight follows a large and abrupt increase associated with rapid water uptake at ecdysis. Then moderate gains are associated with mineralization of the integument and the accumulation of organic matter during the late molt stages. Finally, a relative stabilization of fresh weight occurs during intermolt until the onset of the successive ecdysis (Heasman, 1980). The majority of the fresh weight gain (almost 90%) of *S. serrata* occurs in the very brief period of rapid water uptake (Heasman, 1980). On the other hand, an increase in tissue mass is a continuous process that occurs throughout the molting cycle (Frank et al., 1975; Sulkin et al., 1975; Anger et al., 1989).

Feeding

In the wild, the diet composition of the mud crab depends entirely on their location. Usually, the diet consists of marine detritus, mangrove plants, sea-grass, molluscs, crustaceans and fish. For example, 39% of mangrove clams were found in the mid gut of *Scylla serrata* in Pohnpei-Micronesia (FAO, 2011) while only less than 10% of mollusc was ingested by *S. serrata* in Indonesia (La Sara et al., 2007). However, the percentage of natural food composition of *S. serrata* ingested appeared to be similar across the different growth stages at

the same location (Fig. 1.5). Therefore, mud crabs are best described as opportunistic feeders; they are herbivores, carnivores, scavengers and cannibals (Hutching & Saenger, 1987; Jayamane & Jinadasa, 1991). They will eat just about anything that they encounter. Experimental studies confirmed that the capacity of *S. serrata* to digest fibers well in all the plant feedstuffs (Catacutan et al., 2003). This may indicate that the crabs have some capacity to break down plant cellular structures, and liberate the cell contents for better digestion (Anderson et al., 2004).

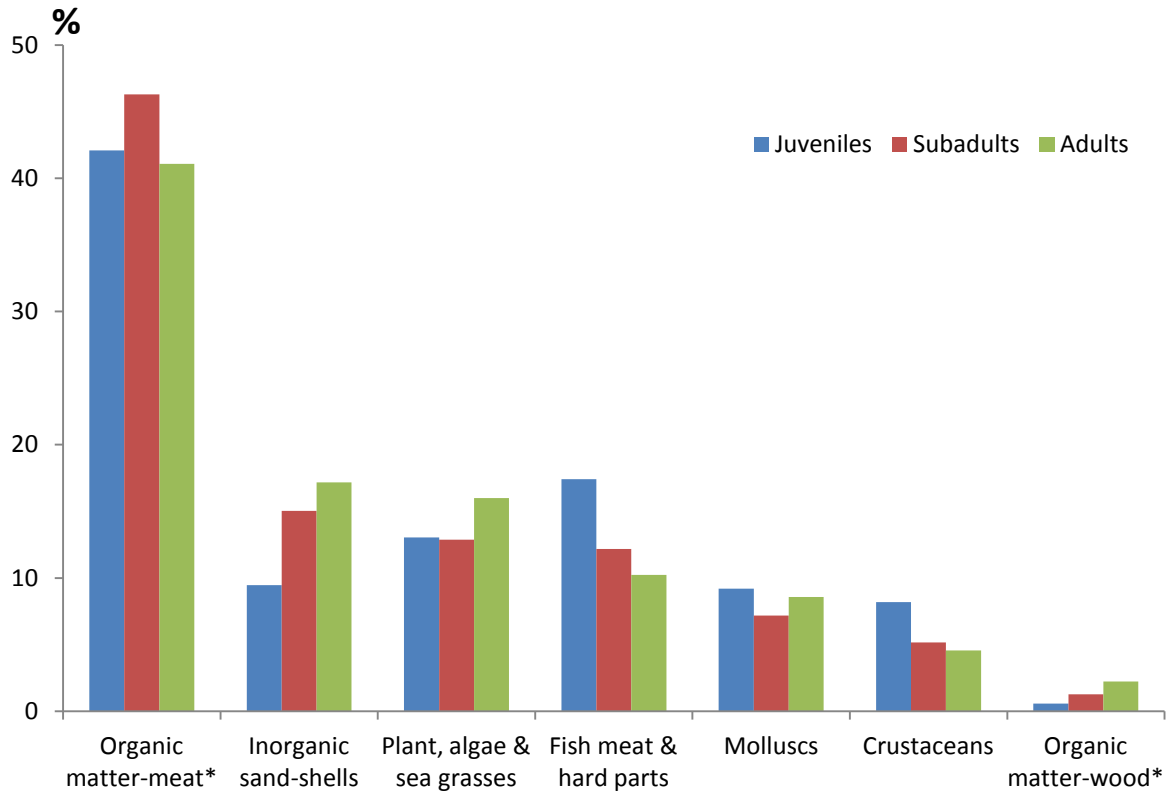

Figure 1.5. Composition of natural food of *S. serrata* through different growth stages in Indonesia (data source: La Sara et al., 2007; * refers to unidentified food).

Osmoregulation

Osmoregulation is an essential process for most aquatic crustaceans in order to maintain osmotic homeostasis under various salinity conditions. The organs involved in crustacean osmoregulation include gills, guts, and antennal glands (or excretory organs) (Mantel & Farmer, 1983). The gills have patches of special epithelial cells known as "ionocytes" which are most densely located in the posterior gills, thus, osmoregulation is more

efficiently regulated by the posterior gills (Mantel & Farmer, 1983; Péqueux, 1995). The digestive tract of brachyurans is divided into three regions including foregut, midgut and hindgut. The midgut is not lined with cuticle and the epithelial cells are in direct contact with the lumen. This makes the midgut more efficient in ion and water uptake (Icely & Nott, 1992).

The degree of osmoregulatory capacity of aquatic animals may be quantified by measuring the deviation between the haemolymph osmolality in relation to the environment as well as the associated enzymatic responses (McNamara & Faria, 2012; Romano & Zeng, 2012). Because the osmoregulatory capacity of crustaceans is often highly species-specific (Chen & Chia, 1997; Romano & Zeng, 2006), this greatly influences their ability to inhabit ecosystems that experience rapid salinity fluctuations. One of the most important osmoregulatory responses to cope with salinity changes is ion transport which is considerably fuelled by gill Na^+/K^+-ATPase activity which regulates the monovalent ions of Na^+, Cl^- and K^+ (Freire et al., 2008; Henry et al., 2012; McNamara & Faria, 2012). Typically, gill, Na^+/K^+-ATPase, increases when marine crustaceans are exposed to low salinities (Castilho et al., 2001; Palacios et al., 2004; Romano & Zeng, 2010) to allow for enhanced ion uptake.

Mud crabs are an euryhaline species which can inhabit both the open ocean and estuaries with its rapid salinity fluctuations (Keenan et al., 1998). Ruscoe et al. (2004) demonstrated that the survival or growth of crablet *S. serrata* remains unaffected by water salinity between 5 and 40 ‰, as *S. serrata* possesses strong abilities for hyper-osmoregulation and hypo-regulation of Na^+ (Chen & Chia, 1997). Juvenile *Scylla serrata* also appeared well adapted to low-salinity ranging from 4 to 28 ‰ (Romano et al., 2013). This was related to the significant increase of posterior gill, Na^+/K^+-ATPase, activity at both hypo- and hyper-osmotic conditions. However, lower growth and hepatosomatic indexes (HIS) were observed above 36 ‰. Similarly, studying the ability of osmoregulation of *Scylla paramamosain* also indicated that the osmotic pressure of the hemolymph changed immediately after the specimens were transferred from a salinity level of 25 ‰ to 5 ‰ and then 45 ‰ (Chung & Lin, 2006). Moreover, these authors found that the crabs did not seem to undergo any active regulation in concentrated sea water (45 ‰) since they acted almost as osmoconformers. Therefore, based on the ability of these physiological regulations, *S. paramamosain* can live in a wide range of salinity, and have more adaptive to low salinity habitats.

1.3 - MUD CRAB FARMING

1.3.1 - Production and market

Exploitation of the mud crab resources in the world has increased dramatically over the past 20 years, and has reached up to 45000 t in 2011 (Fig. 1.6). There is an unmet demand for mud crabs and this has led to the over-exploitation of wild populations in many areas, especially in Asian countries (www.shrimpnews.com). This has generated substantial interest in the mud crab aquaculture across many Southeast Asian countries. Crab production from farming has dramatically increased from 3788 t in1990 to reach 158309 t in 2011 (Fig. 1.6).

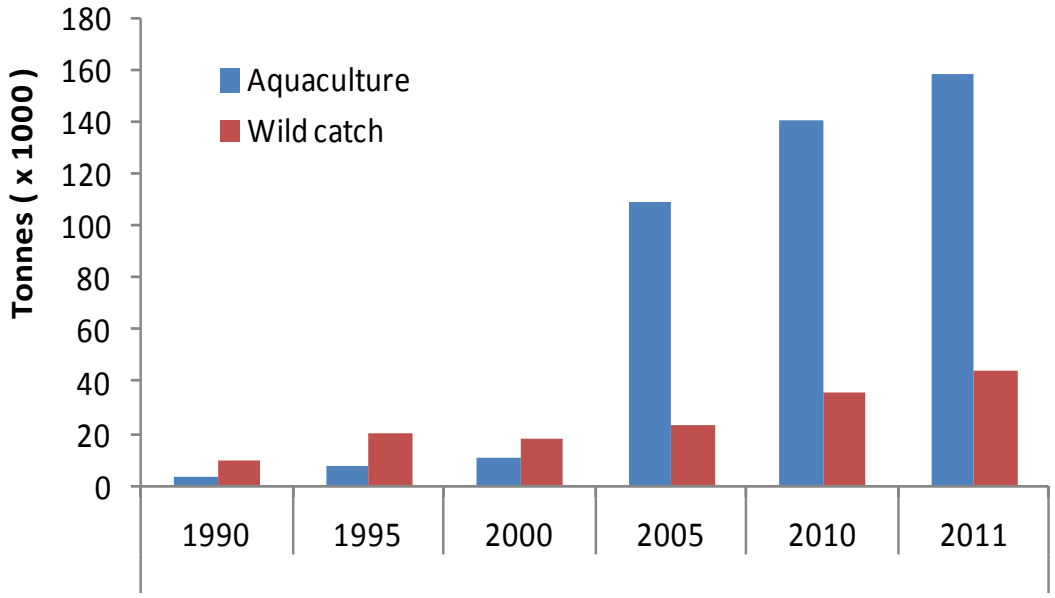

Figure 1.6. The production of wild catch and aquaculture of mud crab, *Scylla*; (data source: FAO FIGIS database; www.fao.org/ES/ess/figis.asp).

Annual aquaculture mud crab production in China is of a greater magnitude than that in Vietnam, the second highest mud crab producing country (Table 1.1). These FAO statistics are attributed to *S. serrata*, however, this data probably represents the production of all genus *Scylla* as the three other species of mud crab are also farmed in these countries (Shelley, 2008). Mud crabs are primarily marketed live, but on the other hand, they are also sold as a cooked product in Australia or frozen for the newly emerging soft-shell crab market around the world (www.shrimpnews.com). They are consumed in the producing country and also exported to Hong Kong, Singapore, Republic of Korea and Taiwan (Their price is dependent on the season,

size, sex, gonad maturity and country of origin (www.fao.org/fishery). Production of globally farmed mud crabs has reached over 140000 t and valued at nearly 400 million USD in 2010 (www.fao.org/figis).

Table 1.1. The production of aquaculture mud crab, *Scylla*, in tonnes in South East Asia.

	Year									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
China				83306	94935	97463	93363	101529	113852	115881
Indonesia	5126	3879	9039	7152	2243	4379	5516	6631	7642	7516
Malaysia	225	219	311	204	174	162	141	86	71	14
Philipines	4968	4608	4747	4809	6245	6861	7800	9308	11625	13730
Thailand	9	5	10	10	23	15		11	23	25
Vietnam*					7000		22082			30000

Data source: FAO FIGIS database; www.fao.org/ES/ess/figis.asp.

* Data source in Vietnam: 2004 from MoFI (2005); 2006 from pers. comm. (2007); 2009 from www. Shrimp news.com.

1.3.2 - Perspectives and issues

The unmet mud crab demand has generated a continuously increased production from aquaculture and over-exploitation in many areas (Fielder & Allan, 2003). Decreasing trends of catch size, yield and catch per unit effort has been reported in Australia (Mounsey, 1989), Sri Lanka (Jayamanne, 1992), Thailand (Tiensongrusmee & Pratoomchat, 1999), Philippines (Lebata et al., 2007) and other Southeast Asia (Overton & Macintosh, 1997). In Southeast Asia, large areas of mangroves have been lost to over fishing and deforestation for aquaculture and human settlements (Primavera, 2000). Such situations along with over exploitation of wild stocks have initiated the increased development of hatchery technology for mud crabs (Lindner, 2005; Thach, 2007). One difficult issue of mud crab aquaculture productivity is cannibalism, requiring low rearing densities (0.5-1.5 crab/m^2; Thach, 2009; FAO, 2011). In addition, the commercial formulated diets are not to be widely adopted by the mud crab farmers. Trash fish is still the principle feed used for crab farming (Petersen, 2013). The

substitution of fresh food for dry pellets in mud crab farming would reduce the risk of disease outbreaks and deterioration of the pond bottom (Thach, 2009; FAO, 2011).

1.3.3 - Hatchery

Studies on breeding of mud crab in captivity were successful in the Philippines, Japan, Vietnam, and Australia with survival rates of up to 15% from zoea to crablets (Nguyen, 2000; Fielder & Allan, 2003). Further developments include improvement of broodstock and larval nutrition (Alava et al., 2007; Nghia et al., 2007), and nursery production (Mann et al., 2007; Rodriguez et al., 2007). Nowadays, mud crab hatchery technology has been improved remarkably in Vietnam. Over 100 millions of crab seeds are produced by about 250 mud crab hatcheries every year (Thach, 2009).

Broodstock

The mature female crabs can be obtained from the wild or from crab farming (Millamena & Quinitio, 2000; FAO, 2011). Among these sources, wild broodstock have the best reproductive performance while the eggs from crab farming broodstock are occasionally heavily infested with parasites (Quinitio & Parado-Estepa, 2003). This result underlines the need for further research on broodstock nutrition for pond-reared broodstock, to equal the reproductive performance of wild broodstock (FAO, 2011). For example, in Vietnam the mud crab breeders are usually fed on a mix of fresh crustaceans, mollusks, fish and annelid worms to get better vitellogenesis, better larval quality and better metamorphosis. Supplements such as vitamins and minerals can also be used with natural diet to ensure its nutrient completeness. Formulated diets have been tested to feed mud crab broodstock. Best results on fecundity and spawning were obtained when formulated feeds were used as a supplement to fresh or frozen feeds (Millamena & Quinitio, 2000; Millamena & Bangcaya, 2001). However, formulated diets are currently not being used extensively enough.

Breeders, whatever their origins, should be scrubbed and treated with a formalin bath (50-100 $\mu l.L^{-1}$) for at least 1 hour (Millamena & Bangcaya, 2001). They can be held at densities of 1-5 $crabs.m^{-2}$, in aerated seawater of 30-35 ‰ and 25-32 ^{o}C (Thach, 2009; FAO 2011). Natural spawning of crabs occurs in the most hatcheries. However, eyestalk ablation can be used to shorten the pre-spawning period (Baylon & Failaman, 2001; Quinitio et al., 2001). There is little and conflicting information on the effect of eyestalk ablation on egg and

larval quality (Millamena & Quinitio, 2000). The authors reported lower fertilization and lower hatch rates of eggs obtained from ablated crabs while Mann et al. (1999a) recorded that ablated crabs produced larger eggs with better hatch rates.

As mud crabs are nocturnal and normally inhabit turbid estuaries (Barnes et al., 2002) they are maintained under low light conditions (Quinitio et al., 2001). In Vietnam, broodstock tanks are normally covered with a dark canvas to reduce light and increase feeding rate.

Spawning and incubation

Spawning may occur within 10-30 days after full ovarian maturation then hatching into zoea after 13-15 days of the incubation, depending on the temperature (Baylon & Failaman, 2001; Quinitio et al., 2001). Mud crabs require a substratum for spawning, they dig a depression in the substratum over which the abdominal flap is extended backward and into which the eggs are extruded (Rusdi et al., 1994). Absence of a substratum results in poor egg attachment and potential loss of the batch. Sand trays or sandy broodstock floors should be established on an artificial bottom plate, so that the sand can be aerated gently from below to prevent anaerobic conditions. An airlift system that draws water through the sand bed can ensure that aerobic conditions are maintained (FAO, 2011). Once the egg mass is extruded, the berried crabs are transferred into incubation or hatching tanks and their embryonic development is carefully monitored. The embryo development can be quickly assessed by the color change of the egg mass and observed under a microscope with low power magnification. During the incubation, berried crabs are not fed or lightly fed, in order to maintain water quality and to prevent eggs contamination, mild aeration is continuously provided and total water volume is changed daily (Thach, 2009).

Larval rearing

Zoea (Z) larvae are often hatched between 6:30 am and 8:00 am (Hai et al., 2001; Hamasaki, 2002; Thach, 2009). The larvae should be collected immediately after hatching because Z1 larvae are highly sensitive to fungal and bacterial infections from a variety of sources including the egg envelope (Hamasaki & Hatai, 1993a; Mann et al., 1999b). The success of larval rearing depends on a range of factors, such as: temperature stability, low level of organic matter in the water, low bacterial levels in rearing tanks, suitable salinity, keeping larvae in suspension and consistent high-quality feeds (FAO, 2011).

All equipment used in larval rearing should be cleaned and sterilized with chlorine at 100-200 ppm. The rearing water should be maintained between 28-30 ^{o}C, 29-31 ‰ for Z1-Z5 and 25-27 ‰ for megalopa to crablet, the pH level should be between 8.0 and 8.6, and NH_3-N & NO_2-N < 0.01 ppm (Thach, 2009). However, some other studies did not suggest reducing the water salinity during the larval nursing stage and the larvae are commonly reared from hatching through to the first crablet (C1) in full strength seawater (Cowan, 1984; Baylon et al., 2001b).

1.3.4 - Nursery pre-growing

Various nursery systems including tanks, ponds and hapa nets established in ponds have been developed for nursing early crablets. Crabs of less than 1.0 cm are nursed in net cages at 20-50 individual.m^{-2} until they reach 1.5-2.0 cm CW (phase 1) then they are transfered in ponds of 200-800 m^2 at 5-10 crabs.m^{-2} surrounding by nets or net fences until 3.0-4.0 cm CW (Phase 2) (www.fao.org/fishery/). A survival rate of 60-80% can be attained during the 15-20 days nursing period (Thach, 2009). Crabs are fed *ad libitum* once or twice daily on a combination of food items such as minced low-value fish, shellfish, chicken trash, chicken eggs, boiled corn, squid intestine or formulated feeds. Water renewal is carried out during spring tides, and the water level is maintained at 70-80 cm deep. Crablets are transported at 2-3 individuals.cm^{-2} in containers with 1.0-1.5 cm moist sand over the bottom. Survival rate can reach 90% when the transportation duration is less than 30 h. (Thach, 2009).

1.3.5 - Mud crab growing-out and nutrition

Until recently, mud crab aquaculture has been carried out in Southeast Asian countries, such as China, Vietnam and Philippines. Crabs are grown within various different production systems such as earthen ponds, mangrove pens, individual containers (for soft shell crab), or maintained in any possible unit during a short period of time for fattening. In each system, crabs can be reared in monoculture or polyculture with shrimp and/or fish, and seaweed (FAO, 2011). In Vietnam, culture techniques can be intensive with a stocking density of 0.5-5 crab. m^{-2} or extensive with a stocking density of 0.05-0.1 crabs.m^{-2} (Nguyen, 2003; Christensen et al., 2004). Depending on crab size at stocking, mud crabs attain 300-500 g within 4-6 months (Trino et al., 1999; Thach, 2009). Some culture systems such as pens in mangrove forests or extensive shallow mangrove silviculture ponds, do not require supplemental feeding (Lindner, 2005). Crab production is approximately 1 t.ha^{-1}.crop^{-1} for polyculture (Thach, 2009) and 1-2

$t.ha^{-1}.crop^{-1}$ for intensive pond culture system (Lindner, 2005). Mud crabs are still traditionally fed on diets including low valued fish, molluscs and small crustaceans. Although, until now, manufactured diets are available in some countries (e.g. China and the Philippines) the adoption is still poor and there is significant scope to improve formulations and to reduce their costs (Shelley, 2008). An investigation in Vietnam of Pertersen et al. (2013) indicated that if pellets are to be adopted widely by mud crab farmers, their perceptions regarding the poor adaptability of mud crab to pellets, relatively slow growth rates compared with trash fish need to be overcome. It means that further research on the cost-effective diets is still necessary.

Understanding the nutritional requirements and nutrient digestibility of the reared species are essential for the development of nutritionally balanced, cost-effective diets. Proteins and amino acids are essential nutrients required for maintenance, growth and reproduction in crustaceans as in other animals (Guillaume, 1997). The essential amino acid requirements for crabs and crustaceans include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Guillaume, 1997; Unnikrishnan & Paulraj, 2010). Tyrosine and cysteine potentially spare the requirement of phenylalanine and methionine, respectively, so they are considered semi-essential in the diet (Guillaume 1997). There is a significant correlation between the dietary amino acid requirements of a species and the pattern of amino acids in whole body tissue (Cowey & Tacon, 1983; Wilson & Poe, 1985). Consequently, dietary amino acid requirements of growing animals are often assumed to be similar to the amino acid composition of the tissue proteins formed during growth. However, determination of the exact amino acid requirements in crustaceans is difficult (Shiau, 1998). When using body composition as reference of requirement, one would overestimate the dietary requirement of essential amino acids and underestimate the need of other protein components (Saoud et al., 2012). This is the probably the reason for the difficulty to find a report on the specific amino acid requirements of mud crabs. While dietary protein is important for tissue synthesis, dietary lipids provide energy and essential fatty acids (EFAs), sterols, phospholipids and fat-soluble vitamins necessary for proper functioning of physiological processes and maintenance of biological structure and function of cell membranes (D'Abramo, 1997; Teshima, 1997). Lipid used as an energy source can also spare dietary proteins and reduce nitrogenous waste production (Lim & Sessa, 1995; Cho & Bureau, 2001). The ratio of energy from non-protein sources (lipids, carbohydradares) to protein levels must be supplied into the diets in sufficient amount to ensure that the dietary protein is used for tissue synthesis because among the major nutritional components of crustacean diets,

where protein is considered the most expensive (Cuzon & Guillaume et al., 1997; Cho et al., 2005). If this ratio is insufficient, the dietary protein may be catabolized and used as an energy source to satisfy maintenance before somatic growth. Conversely, high dietary lipid level and excess of the ratio of dietary energy and protein can cause significant reductions in the growth rate, feed consumption and also might reduce the utilization of other nutrients (D'Abramo, 1997). Along with dietary lipid, carbohydrates considering inessential nutrients generally provide an inexpensive source of energy for crustacean feeds (Shiau, 1997). They are mixed into feeds to reduce costs, for their binding activity during feed production and possibly for protein-sparing effects (Wouters et al., 2001; Pillay & Kutty, 2005). Additionally, carbohydrates play several roles in crustacean metabolism including glycogen storage, chitin synthesis and the formation of steroids and fatty acids (Dall et al., 1990; Ali, 1993; Sánchez-Paz et al., 2006). Along with macronutrients required by crustaceans, vitamins and minerals are essential micronutrients for their normal life processes. Insufficient vitamins and/or minerals lead to slower growth, negatively affecting reproduction and/or eventual mortality in crustaceans (Conklin, 1997).

Compared with shrimp, the information about the nutritional requirements of mud crabs have been still limited. Mud crabs *S. serrata* gained weight faster and had shorter molt period when feeding on high protein diets (up to 55%) and lipid (up to 15%) diets (FAO, 2011). However, the dietary requirement of mud crabs *Scylla* is not strict because they could have a good growth over a wide range of protein and lipid levels (Catacutan, 2002). The results of the study showed that a dietary protein level ranging from 32% to 40% with a 6-12% lipid level promoted the best growth performance. No significant difference in growth responses of mud crabs fed on formulated diets containing 35% and 40% crude protein had been recorded (Chin et al., 1992). Diets tested in the study of Anderson et al. (2004) showed the range of the nutritional requirements for mud crabs: 32-54% protein, 4.8-10.8% fat, 2.1-4.3% fiber and 0.6-22% ash. The study of Unnikrishnan & Paulraj (2010) on smaller size of *S.serrata* suggested that 45% - 47% dietary protein with a 8% lipid level was required for the best growth response in juvenile *S. serrata*.

Development of formulated feeds for mud crabs is becoming a major concern as trash fish and other wild resources used for aquaculture feed are under increasing pressure (FAO, 2011). Mud crabs are traditionally considered as carnivores and they prefer diets containing molluscs, fish and crustaceans (Hill, 1979). However, they can also digest many different food items, such as fiber and protein from plants (Catacutan et al., 2003; Tuan et al., 2006; Truong

et al., 2009). Among them, soybean is well known for its high protein content, well balanced amino acid profile, and stable market supply, as well as its reasonable cost (David & Arnold, 2000; Amaya et al. 2007a,b). Chen et al. (1994) reported that up to 33% fishmeal protein could be replaced by soybean cake for juvenile mitten crabs (*Eriocheir sinensis*) without reducing its growth rate. Recently, Luo et al. (2011) found that 30% inclusion of soybean meal and rapeseed meal mixture (1:1 ratio) could replace 40% fishmeal in diets for Chinese mitten crabs without impairing growth performance and feed utilization. However, the inclusion of the soy bean meal (SBM) in fish diets has been limited by relatively high levels of heat stable anti-nutritional and antigenic factors including protease inhibitors, oligosaccharides (e.g., stachyose, raffinose), saponins, isoflavones, phytate, and tannins (Francis et al., 2001). Although heat and enzyme treatments can neutralize some of these compounds, they are still a significant problem when including the soy bean meal in aquaculture feeds (Gatlin et al., 2007). While, soy protein concentrate (SPC), although more expensive, does not contain the alcohol-soluble fraction present in SBM and has a higher essential amino acid concentration. It also has greater nutrient digestibility compared with SBM and can be included at much higher concentrations in diets of piscivorous marine species (Bureau et al., 1998; USSEC 2008).

The biological, economic and environmental advantages of the use of pellets instead of trash fish diets are well documented (Petersen et al., 2013). There was preliminary evidence that while pelleted diets are more expensive than trash fish, growth rates using pellets are generally significantly higher and justify the extra expense (Petersen & Phuong 2011; Petersen & Glencross, 2012). However, until now it is still very difficult to find a report introducing the effectively exclusive use of formulated diets in mud crab farming although there are some commercial formulated diets being manufactured. For example, Vietnam is the second largest producer of mud crabs, but trash fish is still the major diet for crab farming. Consequently, if pellets are to be widely adopted by mud crab farmers, further research on crab nutritional requirements is necessary to formulate a cost-effective and balanced diet for mud crab grow-out farming. In addition, we have to consider the ability for alternative use of plant protein source to fishmeal in the diet formulation as trash fish and other wild resources for aquaculture feed are under increasing pressure (FAO, 2011).

Through the above global overview on biology and mud crab farming, hatchery technology is mainly being developed or studied for *S. serrata* and *S. paramamosain*. Despite indications of success at the laboratory scale, large-scale commercial hatchery development for *S. serrata* has not yet been developed. In Australia and Southeast Asia only the Northern

Territory Department of Primary Industries (NTDPI) hatchery has produced *S. serrata* with over 10% survival of zoea to crablet stage 1 (C1) on a consistent basis (Fielder & Allan, 2003). However, many problems are still inhibiting the expansion of commercial crab hatchery. Among these, the need to use live feeds makes the process expensive and subject to total collapse due to crashes of feed culture. Furthermore, live feed can also be a source of disease for crab larvae culture (Fielder & Allan, 2003). In this context, further study on the use of other effective diets to replace live food for mud crab larvae rearing is needed.

1.4 - PRIMARY RESULTS OF LARVAL, *SCYLLA SERRATA* PRODUCTION OBTAINED IN SAINT-VINCENT STATION, NEW CALEDONIA.

All the production trials were performed in the station of Lagoons, Ecosystems and Sustainable Aquaculture Department (LEAD/NC, IFREMER) located in Saint-Vincent station (21°51'50″ S, 166°3'0″ E) in the southwest of New Caledonia. The aim of these trials was to assess the ability to produce crablets, *Scylla serrata*, in New Caledonia and the possibility to produce the biological material needed for the nutritional research, which is the main objective of the present PhD.

1.4.1 - Experimental design

Two trials of larval production were designed:

1st trial: 5 mature female crabs without eyestalk ablation between April and July when air temperatures ranged between 20.3 ^{o}C and 24.3 ^{o}C.

2nd trial: 6 mature female crabs stimulated by eyestalk ablation between September and November when air temperatures ranged between 20.7 ^{o}C and 23.4 ^{o}C.

Larval production of crab, *S.serrata* was carried out following the steps:

i. Selection of broodstock: all the crabs used as broodstock were females from the wild, heavier than 600 g, larger than 14 cm carapace width (CW) and healthy with complete limbs. Females were identified as being sexually mature by their wide, dark, U-shaped abdomens fringed with setae (Warner, 1977; Robertson & Kruger, 1994; Ruppert & Barnes, 1994). Thus, the females were mated in the wild and carrying spermatophores (Robertson & Kruger, 1994).

ii. Maintenance of broodstock: crabs were stocked in 0.5-4 m^3 tanks with 15 cm sand trays located on the bottom (Fig. 1.7A,B), filled with sea water of 34-35 ‰ salinity and

temperature ranging 18.5-26 ^{o}C (1st trial), 27-29 ^{o}C (2nd trial). There was a heater system to adjust the water temperature in broodstock tanks of the 2nd trial. The water in the tanks was continuously aerated. Females were fed on fresh feed daily (shrimps, squids, snails and mussels) at the rate of 10-15% of their body weight. The amount of feed was adjusted depending on the consumption of previous feeding. 30% of the water volume was renewed daily. Uneaten feed was removed daily and the tank bottom was siphoned once a week.

iii. Triggering spawn: after one week of adaptation to culture conditions, both eyestalks of the crabs of the 2nd trial were ablated in order to stimulate spawning.

iv. Incubation: berried crabs were incubated in 60-150 L containers of seawater at a temperature of 28-29 ^{o}C and a salinity of 34-35 ‰. Aeration was provided during the entire incubation period. The water volume was completely renewed and crabs were fed on fresh food daily.

v. Larval rearing: the larvae were collected immediately after hatching and transferred to nursery tanks of 150-500 L cylinders with smooth inner surface (Fig. 1.7C,D). Larval nursing protocol of Thach (2009) was modified to suit our experimental conditions. For example, we did not feed zoea stages with rotifers, water temperature fluctuated naturally during trial 1 because the water heater system was not available, larval nursery tanks positioned in the open area.

vi. Quantification of larvae abundance: four 50 ml samples were scooped out of the tank at different depths. The larvae in each sample were counted and an average was calculated as the density of larvae per 50 ml, allowing for the estimation of the total number of larvae hatched.

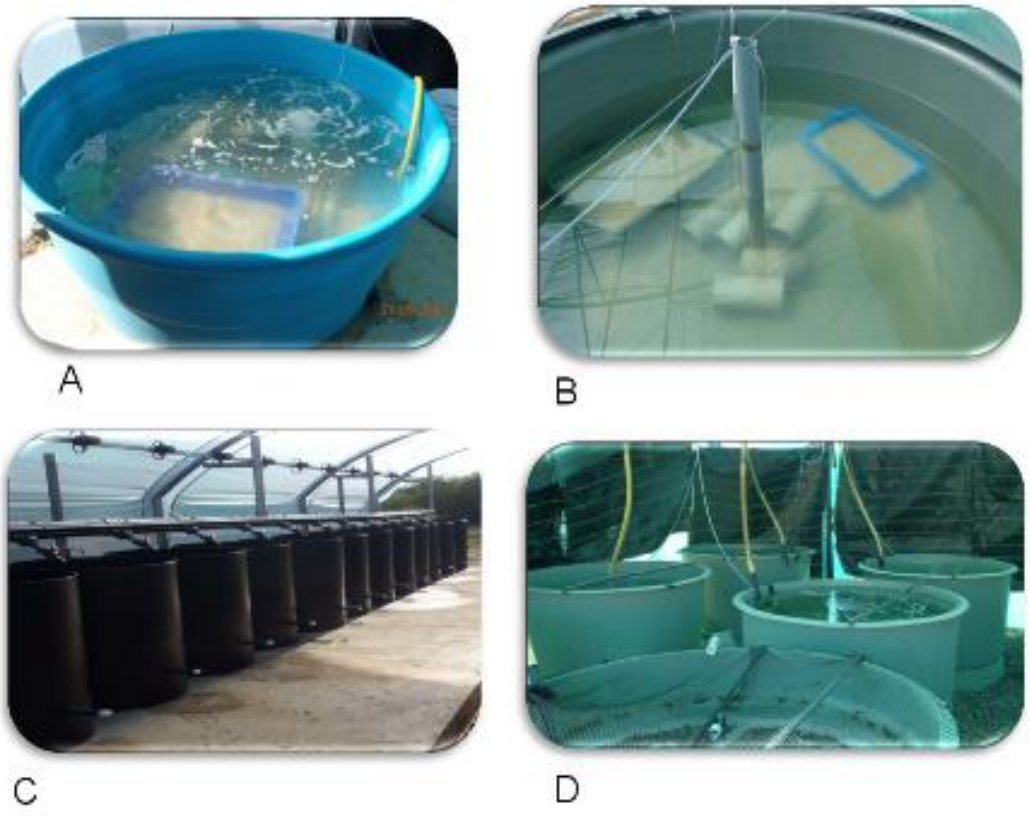

Figure 1.7. Broodstock tanks (A,B) and larval rearing tanks (C,D).

1.4.2 - Results and conclusions

Both the egg batch and larval quality produced from the females without eyestalk ablation were better than those from the ablated females.

1st trial (female crabs without eyestalk ablation): after 60-95 days at temperatures ranging 17.5-26 ^{o}C, 80% of the crabs spawned. The egg mass changed color from yellow to orange, brown and black after 10-13 days of incubation (Fig. 1.8). Then the eggs hatched into zoea (Z) and the larvae swam actively towards light (Fig. 1.9). The number of Z1 was 3.92 ± 1.00 million per egg batch. The hatch occurred within 1-2 hours. After 30 days of larval nursing, under a high fluctuating temperature of 16.5-26 ^{o}C, 38 larvae could metamorphose and reach megalopa but could not metamorphose into crablets

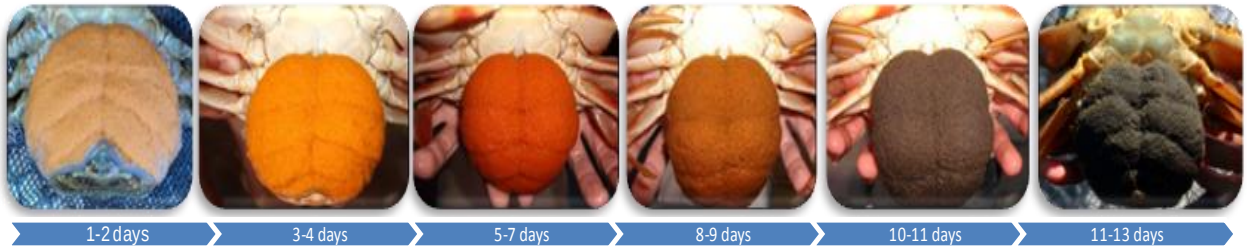

Figure 1.8. Changing color of egg mass from yellow to black during incubation according the incubation duration.

Figure 1.9. Newly hatched larvae swim towards light (in yellow cycle).

2nd trial (female crabs stimulated by eyestalk ablation): 100% female crabs spawned 8-12 days after eyestalk ablation. However, after spawning, eggs released from only 50% females could be attached to the hairs of the abdominal flap of the mother crab while those from the remaining could not. In addition, their egg masses were not solid and unclear grooves (Fig. 1.10). The egg mass could reach the brown stage but not the black stage as the eggs released from mother crabs without eyestalk ablation. Eggs hatched into weak, malformed larvae. The hatching duration lasted 2 days.

A B

Figure 1.10. Eggs released are not generated (A) and egg masse is not solid and unclear grooves (B).

The differences between the hatching durations and the mobility of larvae from females with or without eyestalk ablation could be due to the artificial triggering of the vitellogenese (accumulation of egg yolk). The crab females had not completely matured before their eyestalks were ablated. When the egg yolk cannot be fully constituted, nutritional deficiency and bad embryonic development can be observed. Consequently, after carrying out two production trials the results indicated that it was possible to produce berried crabs in New Caledonia under satisfactory culture conditions, even in the cold season. We assume that in New Caledonia we can get wild broodstocks of mud crab *S. serrata,* to produce eggs of high quality with high hatching rate, and produce healthy larvae. It is not suggested to produce berried crabs by eyestalk ablation. However, these results are only indicative because they had been obtained in inadequate rearing conditions. Water quality was not controlled, in a particular temperature, which could be stressful and impaired the reproductive performance of the broodstock and also the larvae development.

1.5 - PRIMARY RESULTS OF BIOFLOCS USE TO REPLACE LIVE FOOD (*ARTEMIA*) FOR REARING CRAB LARVAE

1.5.1 - Biofloc utilization in aquaculture

Microbial flocs (bioflocs) contain detritus and associated microorganisms and have been considered as a potential food source for aquaculture animals, such as fish or shrimps (Colman & Edwards, 1987; Moriarty, 1997; Avnimelech, 2007; Kuhn et al., 2009, 2010). The microorganism community, mainly protozoa grazers, rotifers, cyanobacteria, diatoms, provides a continuous natural food source (Emerenciano, et al., 2012). Bioflocs are similar in shape but different in density and size, ranging from 100-200 µm (Azim et al., 2008). Bioflocs contain high levels of crude protein (up to 49%) and potentially other nutrients such as essential fatty acids and amino acids (Azim & Little, 2008; Kuhn et al., 2009, 2010). Furthermore, bioflocs are considered as probiotics in aquaculture (Bairagi et al., 2002; Bairagi et al., 2004; Kesarcodi-Watson et al., 2008) and provide "extra components", such as the growth factor that could enhance the growth rate of aquaculture species (Emerenciano et al., 2011). A range of studies reported an increase in growth rates, general welfare and survival of shrimps reared in microbial floc based systems (Burford et al., 2004; Moss, 2000; Tacon et al., 2002; Wasielesky et al., 2006). Growth enhancement has been attributed to both bacterial and algae nutritional components. Up to 30% of conventional feeding ration can be lowered due to biofloc consumption by shrimp (Panjaitan, 2004). Burford et al. (2004) reported that more than 29% of the daily food consumed by *Litopenaeus vannamei* could be bioflocs.

Bioflocs used as an ingredient in aquaculture formulated diets have been investigated. Kuhn et al. (2009) reported that microbial flocs can be an alternative ingredient to fishmeal and soybean meal in shrimp diets, which will improve growth and potentially reduce feed cost as well. Kuhn et al. (2010) indicated that shrimps fed on diets including 10-30% bioflocs exhibited significantly higher survival rate and an average of 10% faster growth rate compared to a control diet. Recently, Bauer et al. (2012) investigated that fishmeals could be completely replaced with a mixture of soy protein concentrate (up to 67%) and microbial floc meal (up to 33%) in the formulated diet without adverse effects on *Litopenaeus vannamei* performance. However, regarding biofloc meal production, one bottleneck seems to be the large amount of wet biofloc biomass required to produce 1 kg of dry biofloc meal (Emerenciano et al., 2013). Avnimelech (2007) indicated that biofloc plug in 1 L settling cones contained only 1.4% of dry matter.

Regarding the option of using bioflocs as an alternative feed in crab aquaculture, the primary trials were carried out from January to February 2012 at Research Institute for Aquaculture No.3 (RIA 3), Vietnam. The aim of these trials was to assess the ability of using wet biofloc as an alternative to live food for crab larval stages.

1.5.2 - Experimental design

Biofloc production

Bioflocs were produced in two separated tanks of 2 and 10 m^3 with continuous aeration, T^oC = 24.5 ± 2.5, S ‰ = 27.5 ± 1.5 (Fig. 1.11). Shrimps *L. vannamei* were cultured in those tanks at a density of 50 animals.m^{-3} and fed on pellets twice daily to 3% of their body weight. Molasses were supplied in the tanks with 50% amount of pellet each day. After 20 days of culture, bioflocs started to be used for the experiment. Microbial flocs were collected by filtering through plankton net of 80-100 µm. Quantity of biofloc particles were counted by sedgewick-rafter cell S50.

Experiments

Total or partial fresh bioflocs were used to feed mud crab larvae from zoea (Z1, Z3, Z5) to megalopa stages through 4 trials (Table 1.2):

Trial 1 included 3 treatments. Z1 larvae were fed on: i) bioflocs; ii) 50% bioflocs and 50% *Artemia,* and iii) *Artemia* (control treatment).

Trial 2 included 2 treatments. Z3 larvae were fed on: i) bioflocs and ii) *Artemia* (control treatment).

Trial 3 included 2 treatments. Z5 larvae were fed on: i) bioflocs and ii) *Artemia* (control treatment).

Trial 4 included 2 treatments. Megalopa were fed on: i) bioflocs and ii) *Artemia* and process feed (control treatment).

S. parapamosain larvae (Z1, Z3, Z5 and megalopa) were obtained from the hatchery of RIA 3. Larvae were stocked into 100 L-conical tanks, 5 L-plastic vessels or 6 L-glass vessels depending on the quantity of larvae available (Fig. 1.12). Initial densities of crab larvae were 100 larvae.$L^{-1}$for Z trials and 50 larvae.$L^{-1}$for megalopa trial. Zoea were fed 4 times daily at 5:00-6:00 am, 11:00-12:00 am, 17:00-18:00 pm and 23:00-24:00 pm, with only *Artemia*

nauplii at the rate of 20-30 individuals.ml^{-1} or only bioflocs at the rate of 100-200 particles. ml^{-1} depending on the trial. The excess of biofloc located at the bottom of the tanks was siphoned the following day. Megalopa were fed 3 times daily at 5:00-6:00 am, 11:00-12:00 am, and 17:00-18:00 pm. 5 g.m^{-3} of processed feed sieved through 2 mm net were fed megalopa in the control treatment.

Figure 1.11. System of biofloc production for the experiment carried out in RIA 3, Vietnam.

Table 1.2. Experimental design for using total or partial bioflocs as alternative food to *Artemia* for mud crab larvae from zoea to megalopa.

TA	Stage	Duration (days)	Treatments			Repl	Larvae/L	EXP. U
1	Z1	7	flocs + *Artemia* *	100 % flocs	Control (*Artemia*)	3	100	5 L-plastic vessel
2	Z3	5		100% flocs	Control (*Artemia*)	1	100	100 L- tank
3	Z5	6		100% flocs	Control (*Artemia*)	1	100	100 L-tank
4	M	9		100% flocs	Control (*Artemia* + processed food)	3	50	6 L-glass vessel

Note: “TA” is trial; “Repl” is replicates; “M” is megalopa; “flocs” is fresh bioflocs; “flocs + *Artemia*” treatment: crabs were fed on bioflocs two times and *Artemia* nauplii two times per day; “EXP.U” is experimental Unit.

Figure 1.12. Trials of biofloc use as feed for zoea and megalopa.

1.5.3 - Results and conclusions

The survival rate of zoea fed on 100% fresh bioflocs was low for all treatments: from 0.5% (Z5-megalopa) to 2.2 ± 0.76% (Z1-Z2). The survival rate of larvae in the control treatments (larvae were fed on *Artemia*) was also low and ranged from 2.6% (Z5-megalopa) to 16.3 ± 4.04% (Z1-Z2).

During the 7 days of the experiment on Z1 (trial 1), water temperature varied between 24 °C and 27 ^{o}C, salinity was 29 ‰, pH ranged between 7.6 and 7.9. The survival rates, when larvae reached Z2 stage, were 16.3 ± 4.04%; 9.7 ± 2.08% and 2.2 ± 0.76% for the control; bioflocs and *Artemia* nauplii; and 100% bioflocs treatments, respectively.

During the 5 days of the experiment on Z3 (Trial 2), water temperature varied between 23 °C and 26 ^{o}C, salinity was 29 ‰, pH ranged between 7.6 and 7.9. The survival rates, when larvae reached Z4 stage were 7% for the control and only 2% for the 100% biofloc treatments.

During the 6 days of the experiment on Z5 (trial 3), water temperature varied between 23.5 and 27.5 ^{o}C; salinity was 27 ‰ and pH varied between 7.0 and 7.5. The survival rates when larvae reached megalopa were 2.6% for the control and only 0.5% for the 100% biofloc treatments.

During the 9 days of the experiment on megalopa (trial 4), water temperature varied from 23.5 to 25.5 ^{o}C, salinity was 26‰ and pH varied between 7.6 and 8.0. The first megalopa

became crablet 1 (C1) 7 days after starting the experiment. The survival rate when megalopa transferred into C1 was 92.3 ± 3.50% for the control and 93 ± 2.64% for the 100% biofloc treatment, 9 days after starting the experiment.

These results may indicate that zoea larval stages of mud crabs have low ability to use biofloc particles as food. The poor survival rates in the first trials with early larval stage may be explained by: i) the poor nutritional quality of biofloc particles used in these experiments, ii) biofloc particles stacked on antennules, mouth parts and spines of larvae which prevented larvae from swimming and feeding (Fig. 1.13), iii) unsuitable size of microbial flocs for zoeal larvae and iv) the poor quality of the larvae as survival rates of larvae fed on *Artemia* (control treatment) were also very low. Moreover, trials 2 and 3 were carried out without replicates which cannot allow us to test the differences. Consequently, the ability of alternative use of bioflocs to *Artemia* during zoeal stages could not be verified. These preliminary results need to be confirmed by other experiments to give a definitive conclusion.

Figure 1.13. Biolfoc articles stacked on antennules, mouth parts and spines of zoea.

The result of trial 4 with megalopa indicated that biofloc particles can replace *Artemia* nauplii as live feed. This result is of prime importance and allows us to consider the possibility to save cost as *Artemia* are very expensive and biofloc production can be cheaper.

Consequently, regarding the ability to produce crablets in New Caledonia and the possibility to use bioflocs instead of live feed (*Artemia*) during the larval stages, we can assume that:

i. We can get wild broodstocks of mud crabs *S. serrata* to produce eggs of high quality with high hatching rate and then produce healthy larvae. If the hatchery technology is adopted, there is capacity to produce crablets in New Caledonia;

ii. Biofloc particles can replace *Artemia* nauplii as live food for megalopa culture.

Nevertheless, in order to develop mud crab farming in New Caledonia, the development of an affordable practical feed is fundamental to provide sufficient nutrition for crab growth.

1.6 - AIMS OF THE THESIS STUDY

In New Caledonia, there is the strong political will to diversify aquaculture which is currently mainly based on shrimp farming. In this context, mud crabs have been considered as a potential species for aquaculture development. A research program has been started on this species as part of a scientific and technical cooperation between Research Institute for Aquaculture No. 3 (RIA No. 3) in Vietnam, the University of New Caledonia and IFREMER, and is supported by the Southern Province of New Caledonia. However, the possible development of crab farming in New Caledonia will require the development of an affordable practical feed that can be produced from selected and controlled raw materials available on the international market. Formulated feeds for aquaculture generally require large quantities of fishmeal as a source of protein and energy. Along with its sometimes limited availability, the cost of fishmeal has been constantly increasing over the years and this trend is expected to continue into the future. Hence for a sustainable mud crab aquaculture industry, it will be important to find a way to partially or completely replace fishmeal from feeds for mud crabs using alternative and more sustainable protein sources which can be used to formulate cost-effective feeds. Consequently, the objective of this thesis is to gain further information on the nutritional requirements of mud crabs, specifically information regarding the nutrient deposition for dynamic growth and energy budget in order to formulate a cost-effective and balanced feed for mud crab grow-out farming. Until now the main species of mud crab, genus *Scylla*, present in New Caledonia had not been adequately identified, though some preliminary information from a study of Keenan (1997) identified it as *S. serrata*. However, this study was based on only six specimens. Therefore, we had to first clarify how many species of mud crab are present in New Caledonia, and then having identified the most predominant commercial species, use this species in our nutritional study. The objectives of the study were to determine:

i. The nutrient deposition for dynamic growth and the energetic budget of juvenile mud crabs;

ii. The effect on tissue growth, feed efficiency and energy budget of the replacement of fishmeal with soy protein concentrate as the main protein source in the diet of mud crab juveniles;

iii. The optimum proportion of dietary of soy protein concentrate relative to dietary protein content for best crab growth.

2 - CHAPTER II: GENERAL MATERIALS AND METHODS

2.1 - IDENTIFICATION OF COMMERCIAL MUD CRABS SPECIES IN NEW CALEDONIA

Before starting on the specific research on the nutrition of mud crabs in New Caledonia, we had to establish if there were more than one species of mud crabs being exploited there. Commercial mud crab fishing occurs all along the coastal areas of the main island of New Caledonia. Therefore, in order to get sufficient numbers of specimens that would be representative of all the mud crab populations, fifty five mud crabs of genus *Scylla,* were obtained from the market where they were brought together from eight capture locations. These locations were representative of the main fishing areas in New Caledonia. Eight additional specimens were trapped without any commercial consideration in the upper estuary of the Nera River. Morphological keys and mitochondrial DNA (mtDNA) analyses were used in tandem to identify all the samples of mud crabs to the species level. Detailed description of this work is given in chapter III.

2.2 - EXPERIMENTAL STATION OF SAINT-VINCENT

All the nutrition experiments of this study were performed in the station of ADECAL (Agency for development of New-Caledonia) located in Saint-Vincent station (21°51’50″ S, 166°3’0″ E) in the southwest of New Caledonia (Fig. 2.1). This station created in 1970 has been in charge of aquaculture research carried out by IFREMER and has contributed to the development of shrimp farming in New Caledonia. The main research sectors are ecophysiology, water environment, pathogens, infections and epidemiology, technical and health monitoring, genetic, hatchery and growth-out farming. In 2013, the Technology Centre (CTA) of ADECAL was created to: i) transfer research results to the private sector, ii) monitor the shrimp industry, iii) improve at the competitiveness of aquaculture companies and iv) develop aquaculture diversification efforts (http://www .adecal.nc).

The infrastructures in Saint Vincent station include:

- An experimental building dedicated to pathology;
- Analytical laboratories including: i) a unit for microscopic observations and molecular biology studies (PCR, electrophoresis), ii) a unit for microbiology and chemical analyses and iii) a unit for feed processing;
- A "semi-wet" laboratory to process living specimens and samples with a dedicated room for water and sediment analyses;

- Animal experimental facilities including: i) outside experimental areas with various sizes of tanks (from 100 L to 4000 L) protected by shade canvas , ii) earthen ponds of various sizes (500-100000 m ²) and iii) an experimental hatchery unit.

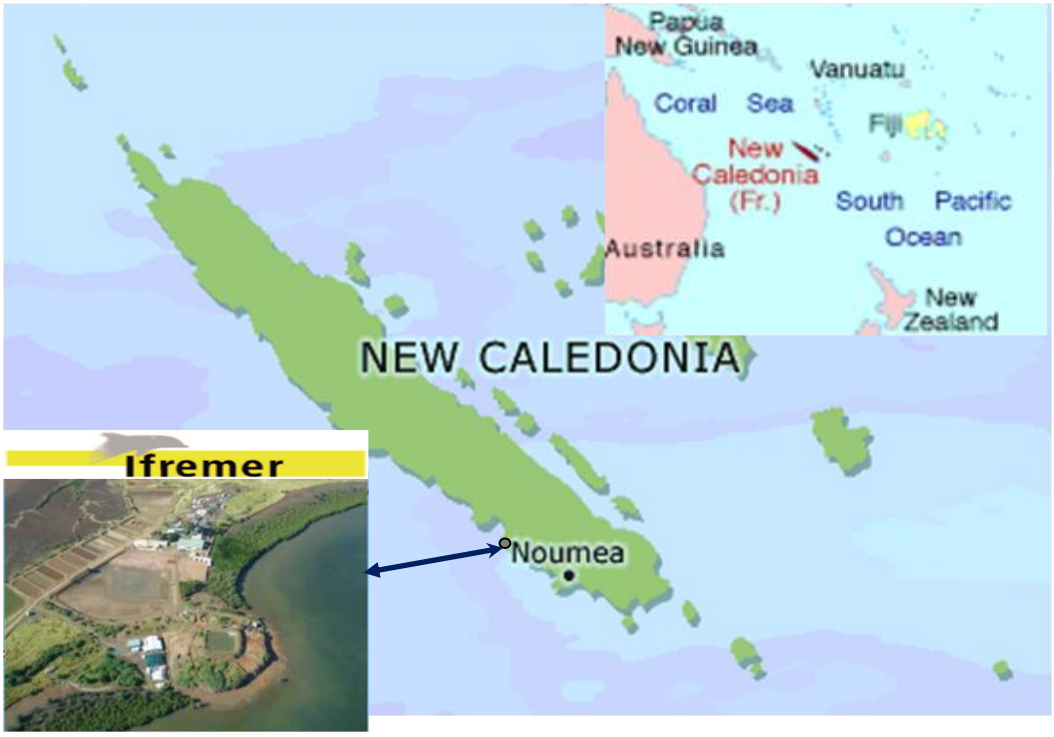

Figure 2.1. Location of New Caledonia ADECAL, Saint-Vincent station (21°51'50″ S, 166°3'0″ E). Source: Google earth.

2.3 - CRAB COLLECTION AND TRANSPORTATION

2.3.1 - Collection area

Crab Juveniles, *Scylla serrata,* were collected in the shallow intertidal zones (Fig. 2.2) associated to mangroves surrounding at Saint-Vincent station, during incoming tide. Most of the collection area is covered with heavily silted bottom. Water salinity (5-42 ‰) and temperature (14-35 °C) varies greatly during the year depending on the tide, the rainfall and the season. Crabs were collected by hand and a net racket**.**

Figure 2.2. Collection area of crab juveniles *Scylla serrata* in Saint-Vincent station.

2.3.2 - Crab transportation

Crab juveniles were kept in the containers of 0.2-0.3 m^2 with a density of 50 animals.m^{-2}. The bottom of the container was covered with a 20 cm-layer of moisture mangrove leaves or grasses. The survival rate was 100% after transport (4-6 hours).

2.4 - IDENTIFICATION OF CRAB MOLT STAGES

The juvenile crabs used in the study ranged from 10.43 to 30.10 g fresh body weight and from 4.12 to 5.76 cm carapace width.

Molt stages of crabs were identified using the criteria and methodology described by Drach & Tchernigovtzeff (1967), Freeman et al. (1987), Heasman (1980) and adapted to our study. The criteria used to identify each molt stage (Table 2.1) were based on observations of i) exocuticle, endocuticle and membranous layers of the cuticle at the edge of the paddle of the 5^{th} periopod and ii) the abdominal color of the crabs. The observation of the cuticle was carried out under a microscope with low magnification (x10-30). Because this part of the cuticle is transparent, this determination allowed us to work with live crabs. In our study, during one molt cycle, we considered: i) a postmolt period comprising A, B, C1, C2, C3 stages; ii) an intermolt period comprising C4, D1 stages and iii) a premolt period comprising of D2, D3, D4 stages.

Table 2.1. General criteria used for the identification of molt cycle stages of juveniles *S. serrata* (Heasman, 1980; Freeman et al., 1987).

Stage	Diagnostic characteristics
A – B	Soft, leathery, paper shell, flexible periopods. Consumption of exuvia usually begins before the end of this stage. Crabs are passive or have limited locomotion.
C1	Thoracic sternites translucent and of a bluish tinge. Exocuticle is thicker than endocuticle. Consumption of exuvia completed in early part of this stage. Almost full mobility.
C2	Thoracic sternites partially translucent and of a pale bluish tinge. Exocuticle is thinner than endocuticle. Full activity.
C3	Sternites not fully opaque and of a very faint bluish tinge. Membranous layer present. Full activity.
C4 - D1	Sternites fully opaque. Teeth of chelae usually display some degree of erosion. Integument visible through flattened natatory dactyl in most advanced individuals. Membranous layer present. Full activity.
D2 - D3	Separation of new pigmented epicuticle from old integument clearly evident through flattened natatory dactyl. Determination of ecdysial suture is evident. The suture becomes pale blue and fractures under slight thumb-nail pressure. Teeth of chelae usually highly eroded. Limb buds, when present, moderately to highly developed.
D4	Open ecdysial sutures.

2.5 - EXPERIMENTAL UNITS AND WATER RUNNING SYSTEM

2.5.1 - Experimental units

There were 3 majority experimental units used during this study:

i. An animal housing unit of 10 circular polyethylene tanks (diameter, 118.5 cm; height, 69 cm; surface area at the bottom, 92 cm²; capacity, 536 L) with an aeration supply and

continuous running water at the exchange rate of 200% per day. This unit was used to adapt crabs to the experimental conditions after their transfer from the wild.

ii. A reservoir unit of 4 plastic tanks of 1000 L, located at 2 m height and fitted with a heater to keep a constant experimental temperature (Fig. 2.3).

iii. A system of individual experimental tanks with an aeration supply for the experiment including: 60 rectangle polyethylene tanks of 18 L; 12 circular tanks of 150 L; 20 plastic aquariums of 10 L. The running water rate in this unit was 0.19 $L.min^{-1}$ (Fig. 2.3).

2.5.2 - Water running system

The seawater was pumped from the Saint-Vincent bay then stored in an earthen reservoir built at 8 meters above the sea level. From the earth reservoir, the water runs by gravity through a sand filter into: i) the animal housing unit; and ii) the experimental unit after passing through a net filter (25 μm) then a reservoir unit (Fig. 2.4).

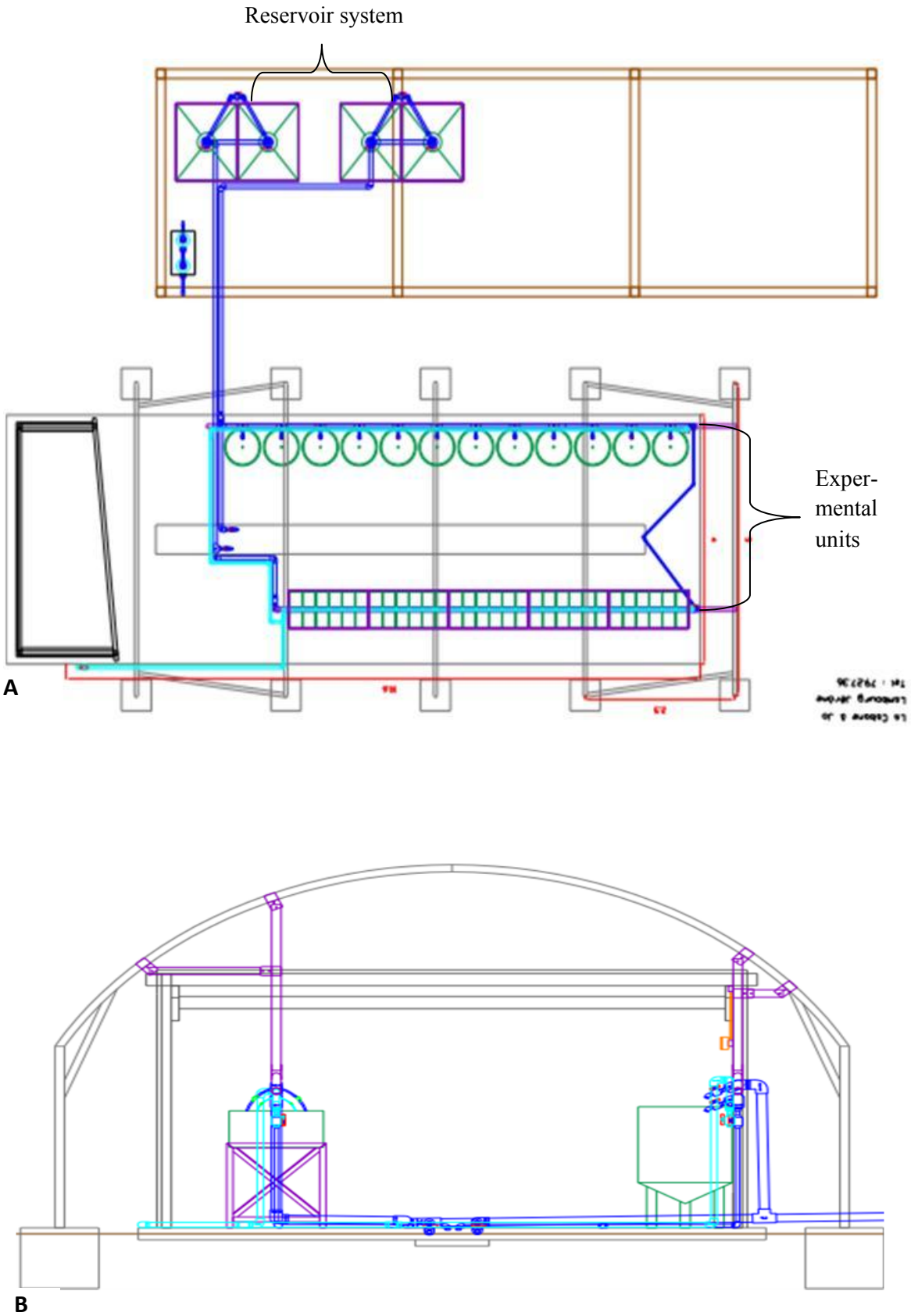

Figure 2.3. Design of the experimental units (A) and front view of the experimental zone (B).

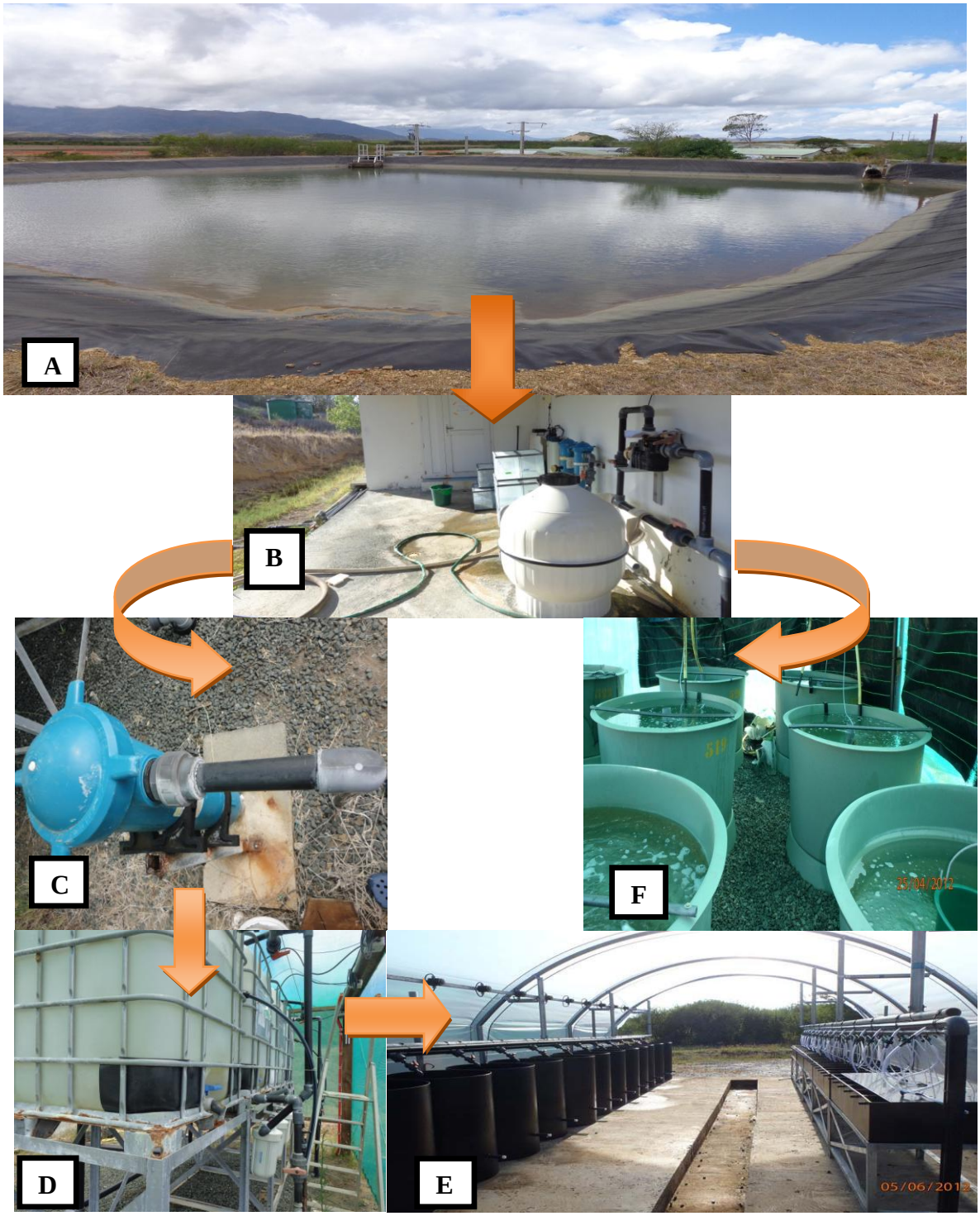

Figure 2.4. Water running system. Sea water is stored in the earthen pond (A) runs by gravity through a sand filter (B) into: the animal housing unit (F); and the experimental unit (E) after passing through a net filter (C) then a reservoir unit (D).

2.6 - FEED PROCESSING

2.6.1 - Feed ingredients

Feed ingredients used to produce the experimental the diets and their nutrient composition are given in Table 2.2 and Table 2.3. The main ingredients used to adjust nutritional composition of experimental diets were: fishmeal, soy protein concentrate (SPC) and wheat flour. Whatever the diet, the inclusion of 20% crab meal was applied to promote the palatability of the feed. Other ingredients included a binder, dicalcium phosphate, vitamin C, vitamin premix and trace elements, all of which were held at a constant inclusion rate across the diets for one experiment. The process and nutrients of main ingredients as follow:

Fishmeal

The fishmeal used in our experiments was Chilean low temperature fishmeal produced from anchovy and mackerel. The main steps of the fishmeal process (FAO, 1986) include: i) heating which coagulates the protein, breaks the fat depots and releases oil and physic-chemically bound water; ii) pressing which eliminates a large fraction of the liquids from the mass; iii) separation of the liquid into oil and water (stickwater); iv) evaporation of the stickwater into the concentrate (condensed fish solubles); iv) mixing the concentrate with the presscake (solid material), and drying in order to remove enough water from the wet material to form a stable meal and finally v) grinding the dried material to the desired particle size. The proximal composition and amino acid contents of the fishmeal are presented in Table 2.2 & Table 2.3.

Soy protein concentrate

The SPC used in our experiments was low-antigen SPC. It was obtained from the processing of mature, high quality soybeans (USSEC, 2008). The cleaned soybeans were dehulled and its oil was extracted. The residues consisted of defatted white flakes which were treated further with an aqueous alcohol extraction step to produce SPC. This extraction process removes the soluble carbohydrate and significantly lowers the levels of lectins, trypsin inhibitors, glycinin, β-conglycinin, saponins and oligosaccharides that are considered to be anti-nutritional factors (ANF's) in regular soybean meal. Finally, SPC is thermally processed to further reduce ANF's and produce low-antigen SPC. Its proximal composition and amino

acid contents are presented in Table 2.2 & Table 2.3. The amino acid composition of SPC was obtained from the literature (Sauvant et al. 2004).

Wheat flour

Wheat flour used in our experiments was produced by grinding the whole wheat grains used for animal feed. Its proximal nutrition content is indicated in Table 2.2.

Table 2.2. Diet ingredients and their proximal compositions.

Diet ingredients	Proximal analyses (dry matter basis)			
	Protein (%)	Lipid (%)	Ash (%)	Energy ($MJ.kg^{-1}$)
Fishmeal	73.73	11.18	12.40	22.47
Soy protein concentrate	64.02	2.47	7.00	23.53
Crab meal	42.23	1.90	46.47	11.47
Fish oil				39.27
Wheat flour	12.92	2.13	2.02	15.73
limestone				
Dicalcium phosphase				
Binder[1]				
Vitamin premix[2]				
Vitamin C[3]				
Trace elements[4]				

[1]Binder: gluten or Pegabind (R) synthetic resin from Bentoli Cie;

[2]Vitamin Premix for shrimp from BEC feed solutions PTY, LTD - ingredients: vitamin AD3 1000/200, vitamin B1 98% Thiamine, Mononitrate, vitamin B2 Riboflavin 80%, vitamin B3 99% Niacin, vitamin B5 98% D-Calpan, vitamine B6 98% Pyridoxine, Vitamin B9 97% Folic acid, vitamine D3 500, vitamin E 50 ADD, vitamin K3 43.7%;

[3]Vitamin C: Rovimix Stay-C 35 from DSM Cie;

[4]Trace elements from SICA Cie (Goodman Fielder).

Table 2.3. Amino acid compositions of the feed ingredients (%, on dry matter basis).

Amino acid (%)	Ingredients				
	Crab meal	Fish meal	SPC[(1)]	Wheat flour[(2)]	Gluten[(3)]
EAA					
Arginine	1.48	4.76	4.75	0.66	3.40
Histidine	1.24	2.02	1.70	0.33	1.60
Isoleucine	1.09	3.34	2.94	0.49	3.00
Leucine	1.46	5.58	4.71	0.89	5.60
Lysine	1.03	5.59	3.92	0.31	1.30
Methionine**	0.49	2.13	0.90	0.18	1.40
Phenylalanine	1.27	3.02	3.21	0.60	4.00
Threonine	1.16	3.15	2.48	0.35	2.10
Tryptophan***	0.37	0.87	0.83	0.14	0.90
Valine	1.52	3.92	3.09	0.53	3.30
NEAA					
Alanine	1.58	4.26	2.81	0.37	2.03
Aspartic Acid	2.68	6.95	7.28	0.41	2.41
Cysteine**	0.20	0.75	0.94	0.26	1.16
Glutamic Acid	3.36	9.52	11.43	3.40	27.4
Glycine	1.49	3.99	2.67	0.47	2.56
Proline	1.51	2.99	3.15	1.21	9.45
Serine	1.42	2.97	3.20	0.58	3.79
Taurine	ND	0.79	ND	ND	ND
Tyrosine	1.06	2.48	2.12	0.32	2.63

**from performic acid oxidation; ***from alkaline hydrolysis; ND: none detected;

EAA: essential amino acid; NEAA: nonessential amino acid; SPC: soy protein concentrate;

[(1)]: data from Sauvant et al. (2004); [(2)] & [(3)]: everaged value gathered from Hertrampf et al. (2000) and CSIRO analysis, personal communication, Smith, D.M., CSIRO Marine and Atmospheric Research.

2.6.2 - Experimental diet processing

The experimental diets were processed in the nutritional laboratory through a basic series of steps (Fig. 2.5): i) the ingredients were finely ground up in a grinder (Retsch®) with a 1 mm screen); ii) the meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca®) until the consistency was suitable for pelleting; iii) the mixture was then extruded through a 3 mm die in a meat grinder to produce pellets and finally iv) diets were steamed for 15 min and stored at -20°C before use.

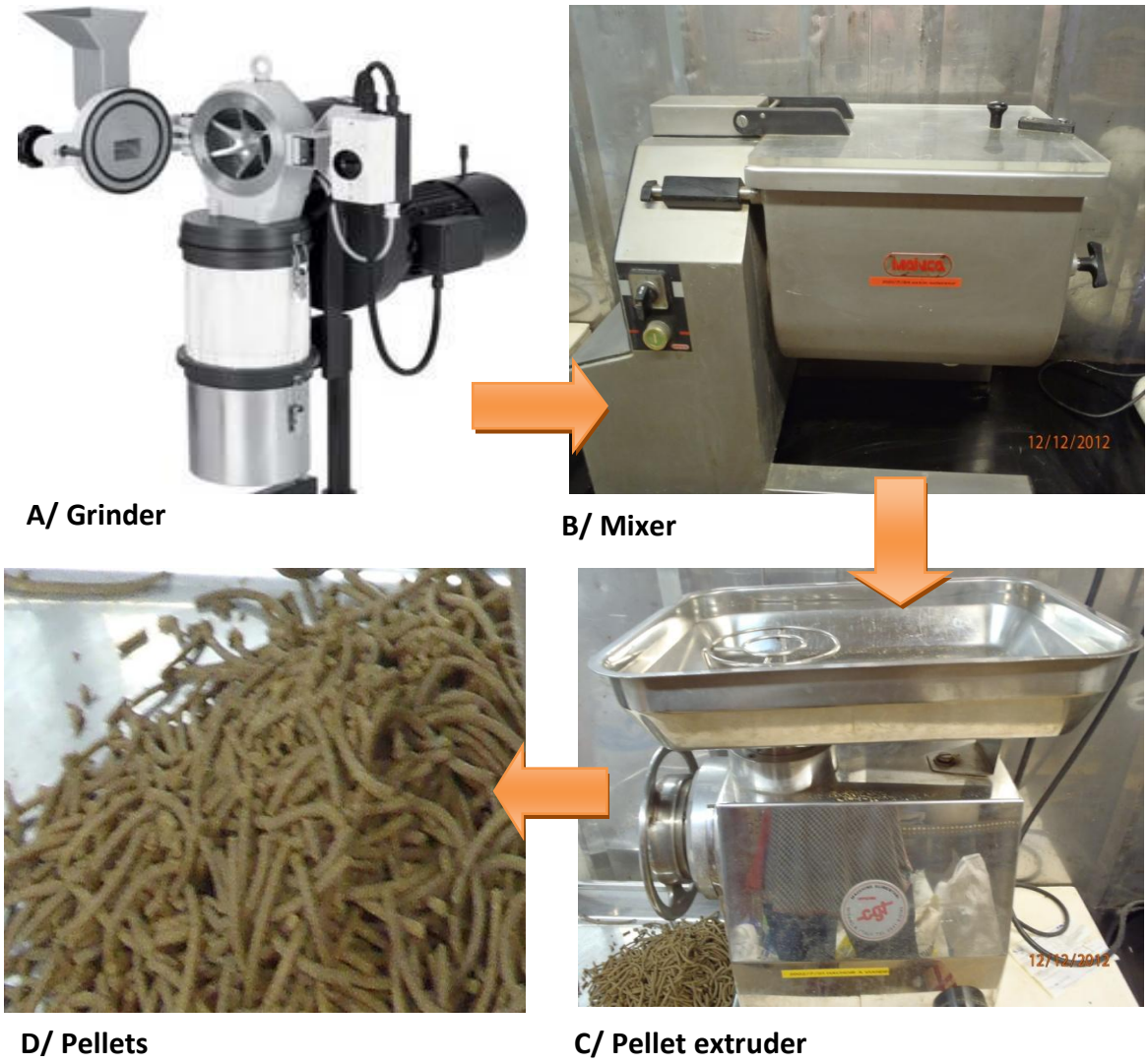

Figure 2.5. The chain of feed process following the direction of the arrows.

The protocol retained to produce the experimental diets consisted of:

i. Mixture A: the main ingredients including fishmeal, soy protein concentrate, crab meal, wheat flour, limestone and dicalcium phosphate were well mixed in a container.

ii. Mixture B: the micro-ingredients including vitamins and trace elements are mixed with a part of Mixture A. When this mixture was well blended, it was added to Mixture A and mixed again (Mixture AB) in a horizontal mixer.

iii. Checking the moisture content: A few grams of Mixture AB was taken and dried to determine its water content. In the mean time, Mixture AB was placed in the freezer at -20 °C until water content was known.

iv. Mixing binder, fish oil and water: the required amount of binder was well mixed with Mixture AB. Next, the required amount of fish oil was slowly added into Mixture AB while being mixed in a horizontal mixer. Then, water amount corresponding to 30% of a Mixture AB was added and well mixed until reach the suitable consistence for pelletting.

v. Pellet extrusion and storage: Pellets were extruded through a meat grinder and preserved in a freezer at -20 °C before use.

2.7 - BIOCHEMICAL ANALYSIS

The proximate composition of diets, feces, crabs were estimated according using AOAC (1995) method at Invivo laboratory, Binh Duong City, Vietnam. Energy contents of diets, fecal material, exuviae and crabs were determined using a calorimetric bomb (Fig. 2.6) (Parr® 6200, USA, calibrated by benzoic acid) at Ifremer laboratory, New Caledonia. Amino acids composition of diet was analyzed using Rutherfurd & Moughan (2000) and AOAC (1998) methods at the nutrition laboratory of Massey University, New Zealand. Usually, hydrochloric acid is used to determine the amino acid composition of proteins. However, not all amino acids can be accurately determined by acid hydrolysis as amino acids are highly diverse in their chemistry and have variable stabilities in acid. During the acid hydrolysis, tryptophan and cysteine are largely destroyed, methionine can be oxidised if oxygen is present, and asparagine and glutamine are converted to their acid derivatives, aspartic and glutamic acid respectively. Therefore, determination of aspartic acid and glutamic acid concentrations represent the sum of their acid and amide derivatives. More accurate estimates of cysteine, methionine and tryptophan can be achieved by oxidation and base hydrolysis procedures.

Figure 2.6. Calorimetric bomb used to determine energy content of samples.

2.8 - EXPERIMENTS

Two main experiments were designed for this study:

i. The effects of the partial or total replacement of fishmeal with soy protein concentrate (SPC) as the main protein source in the diet. The effects of this replacement on tissue growth, feed efficiency and energy budget during a molt cycle of the juvenile mud crab, *S. serrata,* were measured. In this experiment, four trials including growth response, digestibility, fecal production and nitrogenous excretion, were carried out. The detailed design of each trial is described in chapter IV.

ii. The feed intake, molt frequency, tissue growth, feed efficiency and energy budget during a molt cycle of mud crab juveniles, *Scylla serrata,* fed with graded levels of soy protein concentrate as main source of protein were tested. In this experiment, growth, digestibility, and ammonia excretion trials were conducted. The detailed design of each trial is described in chapter V.

3 - CHAPTER III: IDENTIFICATION OF THE MUD CRAB SPECIES OF GENUS *SCYLLA* TARGETED FOR AQUACULTURE IN NEW CALEDONIA

3.1 - INTRODUCTION

One species of mud crab, *S. serrata*, was identified in New Caledonia by Keenan et al. (1997) based on only 6 specimens collected and after the same author has revised the classification of mud crabs (Keenan et al, 1998). However, fishing area of commercial mud crabs are distributed all along the coastal areas of the main island of New-Caledonia which stretches from northwest to southeast nearly 400 km long and 50-70 km wide. Under these conditions the probability of the existence of several species, in addition to *S. serrata*, as *S. olivacea* could not be excluded with certainty. Indeed, these two species occur in south Pacific associated with mangrove forests and coastlines inundated with reduced salinity seawater. *S. olivacea* is distinguished from *S. serrata* by its smaller size, front teeth more rounded, one smooth granule on the cheliped carpus and pals of chela yellowish-orange (Poupin & Juncker, 2010). Therefore, we had to check if it exists one or more species of mud crabs exploited in New-Caledonia before we engage in specific research on nutrition. In case we would have several species it was possible to choose, based on the experience of other countries, the easiest to farm as a biological model for studies that we planned. Thus, in Vietnam where 4 species of mud crab coexist, all aspects of crab farming have focused on *S. paramamosain* as it is the most dominant species and considered easier to manage than *S. olivacea*, *S. tranquebarica* and *S. serrata*.

In this frame, the objective of the study presented below was to identify the species of mud crab present in New Caledonia using traditional morphological keys and genetic analyses (mitochondrial DNA). Our study was conducted with the majority crabs (55 individuals) sold in the market and from capture zones clearly identified. In addition, 8 specimens were trapped without any commercial consideration in the upper estuary of a River in blackish water.

3.2 - IDENTIFICATION OF THE COMMERCIAL MUD CRAB SPECIES OF GENUS *SCYLLA* DE HAAN, 1833 IN NEW CALEDONIA

By

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Key words: mud crab, mangrove crab, *Scylla serrata*, taxonomy, genetics, morphology

Abstract

In New Caledonia, the mud crab, genus *Scylla*, is a major resource for the local population living nearby mangroves. In some areas, the fishing effort has reached high levels, inducing a decline of the resource abundance and indicating a possible overexploitation. This evolution has raised concerns on the mud crab wild fishery management and also on the capacity of a possible aquaculture development in New Caledonia. However, the taxonomy of the commercial mud crab present in New Caledonia has never been confirmed yet. This information is fundamental before planning any study because this genus includes several species difficult to discriminate. The objective of this study was to identify the species of mud crab present in New Caledonia using traditional morphological keys and genetic analyses (mitochondrial DNA). In all, 63 crab samples from the west and northeast coastal mangroves of New Caledonia were gathered at Noumea market. Based on morphological traits, 55/63 crabs were identified as *S. serrata* and the identification of 8/63 was not confirmed. Genetic analyses confirmed that all 63 were crabs *S.serrata.* Thus, the only or at least the highly dominant commercial species present in New Caledonia is *S.serrata* (Forskål, 1775). If another species is present it should be very rare in the landings.

1. Introduction

The taxonomy of the mud crab genus, *Scylla* de Haan, 1833, has been unclear for a long time. Mud crab was considered as one species with different names (Keenan et al., 1998; Overton, 1999). Subsequently, the study of Estampador (1949) recognized three species: *Scylla serrata* (Forskål, 1775), *Scylla oceanica* (Dana, 1852), *Scylla tranquebarica* (Fabricus, 1798), and one new variety *Scylla serrata var. paramamosain*. These identifications were supported by Serène (1952) based on samples collected in Vietnam, who recognized four forms but applied only two species' names. However, the validity of the different species was called into question (Holthuis, 1978; Fuseya & Watanabe, 1996; Overton et al., 1997) and all taxa continued to be considered as one species, *S. serrata* (Stepheson & Campbell, 1960; Fortes, 1999). The confused taxonomy of the genus *Scylla* was a major constraint for the development of mud crab aquaculture as well as the management of wild fishery (Infofish, 1992; Brown, 1994). In the past, identification of mud crab genus, *Scylla* was based on traditional morphological keys built from relatively few samples collected in restricted areas (Overton, 1999). Recently the development of the genetic techniques such as: allozyme

electrophoresis and mitochondrial DNA (mtDNA) sequencing of cytochrome oxidase I, 12S and 16S rRNA genes, was applied to a large number of specimens collected in various areas of the globe to clarify the identification of species in the *Scylla* genus (Keenan et al., 1998; Imai et al., 2002). More recently, Imai et al. (2004) used the first internal transcribed spacer (ITS-1) and 16S rRNA and identified four *Scylla* species at early larval and juvenile stages. At the present state of knowledge, there are four valid *Scylla* species: *S. serrata* (Forskål, 1775), *S. olivacea* (Herbst, 1796), *S. tranquebarica* (Fabricius, 1798) and *S. paramamosain* (Estampador, 1949). The morphological key for the genus *Scylla* was also updated in the revision of Keenan et al. (1998).

In New Caledonia and other countries of the Indo-Pacific region, the mud crab, genus *Scylla*, is a major resource for many local populations located nearby mangroves (Angell, 1991; Brown, 1993). In some areas, fishing catch has reached high levels, inducing the decline of the stocks and a probable overexploitation (Rocklin, 2006). Identification of the species, information on its distribution and population structure is fundamental for the fishery management of wild stocks but also for potential aquaculture development (Keenan, 1997; Brown, 1994). One species, *S. serrata*, was identified in New Caledonia by Keenan (1997). However, only 6 specimens from New Caledonia were collected for this study while it is widely recognized that mud crabs of the Indo-Pacific region belong in more than one species of the genus *Scylla* (BOBP, 1992) and that these species can be sympatric (Keenan, 1997). Furthermore, commercial mud crabs in New Caledonia are distributed all along the coastal areas of the main island. Therefore, determining the species taxonomy must include enough specimens representative of all areas to accurately identifying the species present.

2. Materials and methods

2.1. Collection of samples

Fifty five specimens were collected at Noumea market where the crabs are all gathered from the west coast and northeast of New Caledonia: Poum, Arama, Kaala Gomen, Voh, Temala, Oundjo, Moindou, and la Foa. In addition, 8 specimens were trapped in the upper estuary of Nera River (Bourail) (Fig. 3.1).

Figure 3.1. Locations of New Caledonia (21° 30' 0" S, 165° 30' 0" E) and collection sites of the studied mud crabs of the genus *Scylla*. Source: Google earth.

The samples were collected weekly from 21 July to 12 August 2011. In all, 63 crabs were studied: mean carapace width was 14.8 ± 1.0 cm, minimum size was 12.6 cm and maximum size was 17.3 cm. This sample was representative of commercial mud crabs, *Scylla spp*., being fished in New Caledonia. Oshiro (1991) indicated that species differences in frontal spines are only recognizable in crabs greater than 8 cm carapace width. Nguyen (2009) also mentioned that this characteristic was difficult to distinguish for *Scylla* species when small. Our sample satisfied this condition.

2.2. Identification of species

Morphological keys and mitochondrial DNA (mtDNA) analyses were used in tandem to identify the species for all samples of mud crab, genus *Scylla* de Haan.

2.2.1. Morphological analysis

Morphological analysis was based on the key published by Keenan et al. (1998). This determination was done at the LIVE laboratory, New Caledonia University. The morphological identification steps were:

i/ Morphological diagnosis: characters listed in Table 3.1 and illustrated in Fig. 3.2, 3.3 and 3.4 were recorded for each sample. The data from carapace and cheliped of each sample were measured by the caliper to the nearest 0.1 mm. Carapace data include: carapace width (CW), internal carapace width (ICW) and frontal median spine height (FMSH). Cheliped data comprise inner carpus (ICS) and outer carpus spines (OCS).

ii/ Species identification: the set of characters recorded was compared with the morphological diagnosis for each species described in Keenan et al. (1998). Furthermore, a number of standard deviation units (Z-score) (Fowler et al., 1998) was used to compare values of ratios (ICS/OCS, FMSH/FW, FW/ICW) with those representative of each species.

Table 3.1. Morphological characters recorded to identify mud crab species (based on Keenan et al., 1998).

	Carpus spines	ICS/ OCS	FMSH/ FW	FW/ ICW	Frontal lope spines		Propodus spines
					Shape	Height	
Record	Both obvious; or inner absent, outer reduced ;	Value	Value	Value	Blunt point; blunted; triangular; or rounded	High; Mod; Mod high; or low	Obvious or reduced

CW: Carapace width;

ICS/OCS: Iner carpus spine/outer carpus spine;

FMSH/FW: Frontal median spine height / frontal width;

FW/ ICW: Carapace frontal width/Internal carapace width;

Mod: moderate.

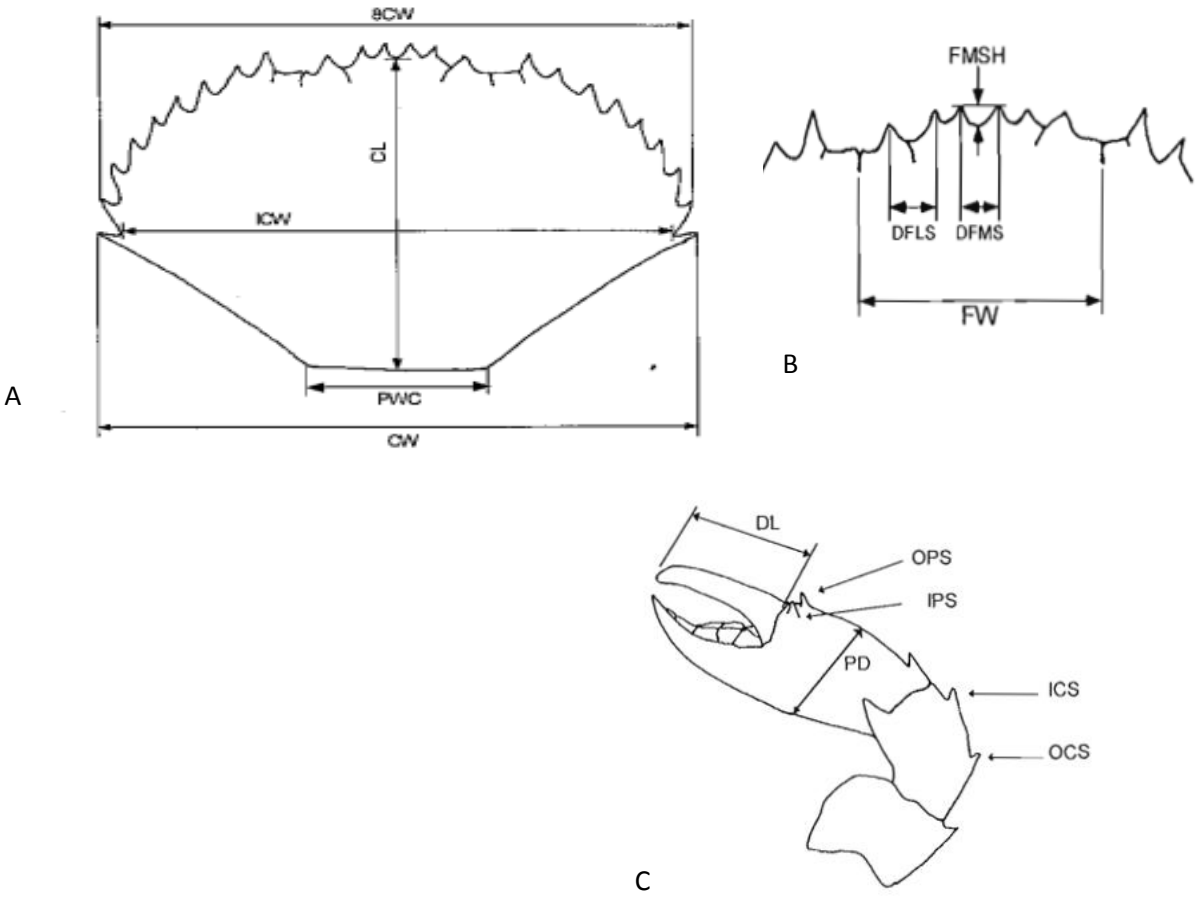


Figure 3.2 . Morphological characters measured on mud crab specimens taken from: A) carapace; B) frontal lobe and C) cheliped (Keenan et al., 1998).

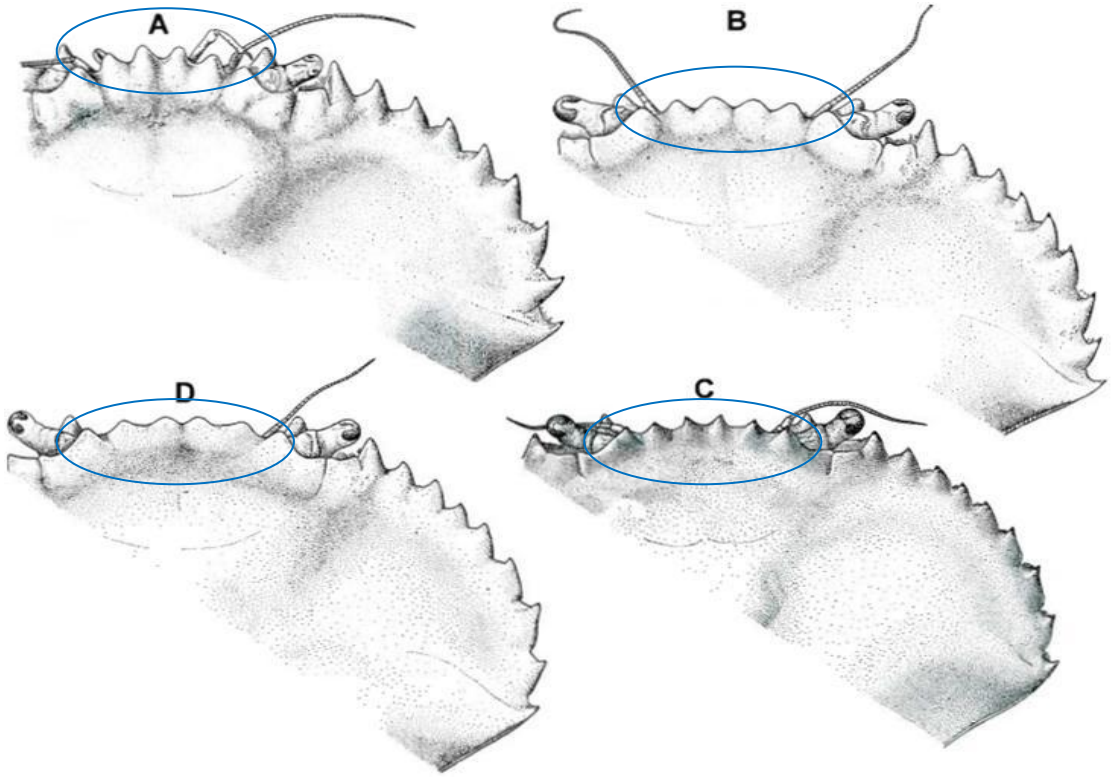


Figure 3.3. Different shapes of frontal slope spines (presented in cycles) of *Scylla* species: A) *S.serrata*; B) *S. tranquebarica*; C) *S. paramamosain*; D) *S.olivacea* (drawings of Keenan et al., 1998).

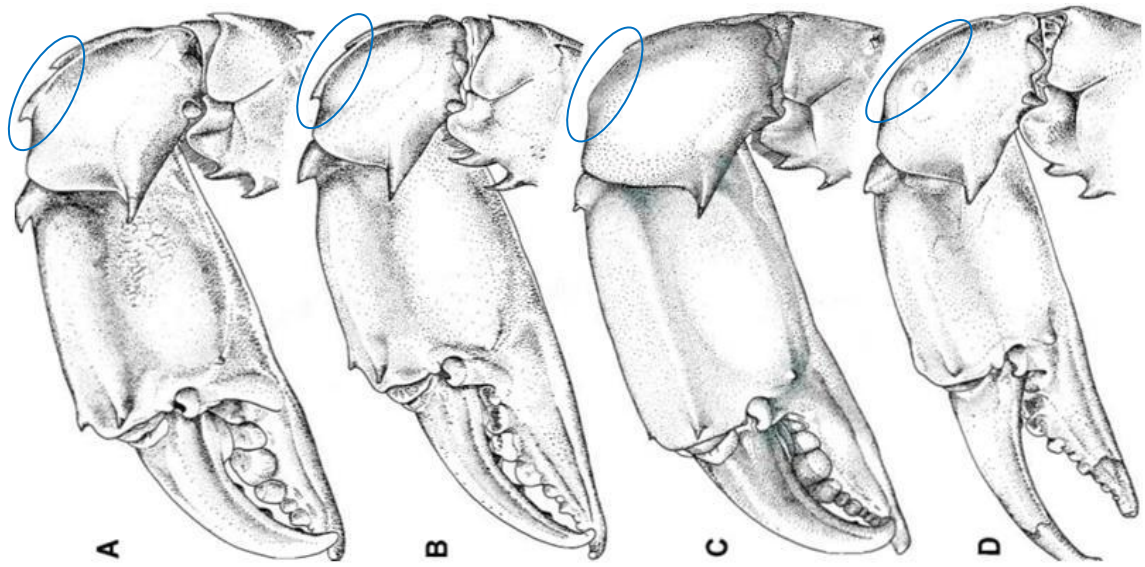


Figure 3.4. Different types of propodus spines *Scylla* species (indicated in the cycles): A) *S.serrata*; B*) S. tranquebarica*; C) *S. paramamosain*; D) *S.olivacea* (drawings of Keenan et al., 1998).

2.2.2. Mitochondrial DNA analyze

Tissue samples were taken from the fresh crab legs, then fixed in 70% ethanol and sent to the IRCP (Institut des Récifs Coralliens du Pacifique), and analyzed in the molecular genetic laboratory of Perpignan University (France) for DNA analysis:

i. DNA extraction: The analyses were done on tissue from the second or the third pair of limbs. A QIAxtractor robot was used for DNA extraction. 63 individual pieces of tissue (1-2 mm) were placed in an extraction plate with buffer and digestion enzyme, and incubated in a water bath at 55 °C overnight. The next day the DNA was extracted and trapped on a membrane with the QIAxtractor. DNAs were collected in an elution buffer. The success of the extraction was validated by a test on an agarose gel (TBE 0.5X, 0.5X agarose). Then DNAs were stored in a freezer at -20 °C.

ii. Amplification (PCR): A fragment of 597 base pairs (bp) of the gene coding for cytochrome oxidase I (COI, genebank numbers AF279309-32) was amplified by polymerase chain reaction (PCR) using two specific primers: 5'-T mtd10 TGA TTT TTT GGT CAT CCA GAA GT-3' and C/N 2769 5'-TT AAG AAA TAG TCC ATG TTG RGG GA-3' (Fratini & Vannini, 2002). The primers were provided by Eurofins MWG Operon®. For the PCR, dNTPs, Taq-polymeraza with its buffer, and $MgCl_2$ were provided by 5 PRIME®. The PCR mix used consisted of 17.3 µl pure water, 2.5 µl buffer, 1.25 µl $MgCl_2$, 3 µl dNTP, 0.4 µl of each primer, 0.15µl Taq Polymerase. The PCR amplification was performed in an Eppendorf Mastercycler Pro. It consisted of a 5 min denaturation phase at 94°C, followed by 40 cycles comprising: 1) a 30 s denaturation phase at 94°C, 2) a 30 s hybridization at 50°C, 3) an elongation phase during 1 min at 72°C, and 4) an extension phase during 10 min at 72°C. The PCR amplification was checked on agarose gel (TBE 0,5X, agarose 1X).

iii. Sequencing: Approximately 400-500 bases were routinely sequenced for each individual.

iv. Analysis: The results were analysed using BioEdit® software. The sequences were compared to the reference sequences of *Scylla serrata* already published and recorded in Genbank. A percentage of similarity between the nucleic acid sequences was calculated for each sample.

3. Results

3.1. Morphological analysis

The morphological characters recorded showed that among the 63 mud crabs collected, 55 were *S. serrata* (Table 3.2). These specimens had: high bluntly pointed frontal lobe spines with a tendency to concave margins and rounded interspaces, narrow anterolateral carapace spines with outer margin slightly concave, an obvious pair of large spines on carpus and propodus of chelipeds, and variable colors including purple, dark blue, blue or brownish. In addition, the ratios of ICS/OCS, FMSH/FW and FW/ICW ranged from 0.818 to 1.200, 0.035 to 0.074, and 0.345 to 0.407 respectively and were not significantly different (Z-score, $P > 0.05$) from the reference values given by Keenan et al. (1998) for *S. serrata*. Therefore, these 55 crabs were identified as *S. serrata*.

Seven other crabs were suspected of be *Scylla traquebarica* based on the frontal lobe spines which were considered as moderate height and blunt point. However, none of them displayed the whole set of morphology characters of *S. traquebarica* given by Keenan et al. (1998). Finally, one crab could not be identified because of the presence of 3 carpus spines.

Table 3.2. Morphological characters for the specimens of mud crab, genus *Scylla* collected in New Caledonia.

Specimen	ICS/OCS	FMSH/ FW	FW/ICW	Frontal lobe spines	Carpus spines	Species identified
1	1.200	0.056	0.360	High narrow, blunt point	Both spines prominent	*S. serrata*
2	1.000	0.051	0.358	High narrow, blunt point	Both spines prominent	*S. serrata*
3	0.923	0.067	0.370	High narrow, blunt point	Both spines prominent	*S. serrata*
4	1.000	0.057	0.386	High narrow, blunt point	Both spines prominent	*S. serrata*
5	1.000	0.066	0.369	High narrow, blunt point	3 spines prominent	???
6	0.917	0.057	0.381	High narrow, blunt point	Both spines prominent	*S. serrata*

7	0.909	0.056	0.353	High narrow, blunt point	Both spines prominent	*S. serrata*
8	0.667	0.060	0.397	High narrow, blunt point	Both spines prominent	*S. serrata*
9	1.071	0.040	0.382	Hight , blunt point	Both spines prominent	*S.serrata*
10	0.909	0.058	0.371	High , blunt point	Both spines prominent	*S.serrata*
11	1.111	0.036	0.379	High , blunt point	Both spines prominent	*S.serrata*
12	1.000	0.046	0.388	High, blunt point	Both spines prominent	*S.serrata*
13	0.909	0.074	0.375	High, blunt point	Both spines prominent	*S.serrata*
14	1.091	0.059	0.386	High, blunt point	Both spines prominent	*S.serrata*
15	1.050	0.042	0.380	High, blunt point	Both spines prominent	*S.serrata*
16	1.000	0.039	0.389	Moderate height, blunt point	Both spines prominent	*Suspect S. tranquebarica*
17	0.947	0.057	0.358	High, blunt point	Both spines prominent	*S.serrata*
18	0.955	0.577	0.361	High, blunt point	Both spines prominent	*S.serrata*
19	1.048	0.059	0.371	High, blunt point	Both spines prominent	*S.serrata*
20	0.850	0.065	0.368	High, blunt point	Both spines prominent	*S.serrata*
21	1.000	0.060	0.368	High, blunt point	Both spines prominent	*S.serrata*
22	1.333	0.060	0.407	High, blunt point	Both spines prominent	*S.serrata*
23	0.917	0.048	0.379	High, blunt point	Both spines prominent	*S.serrata*
24	1.100	0.062	0.380	High, blunt point	Both spines prominent	*S.serrata*
25	0.983	0.059	0.375	High, blunt point	Both spines prominent	*S.serrata*
26	1.200	0.069	0.404	High, blunt point	Both spines prominent	*S.serrata*
27	1.083	0.045	0.377	Moderate height, blunt	Both spines	*Suspect*

				point	prominent	*S.tranquebarica*
28	0.917	0.054	0.378	High, blunt point	Both spines prominent	*S.serrata*
29	1.091	0.048	0.397	Moderate height, blunt point	Both spines prominent	*Suspect S. tranquebarica*
30	0.947	0.074	0.371	High, blunt point	Both spines prominent	*S.serrata*
31	0.909	0.057	0.387	High High, blunt point	Both spines prominent	*S.serrata*
32	0.909	0.061	0.389	High, blunt point	Both spines prominent	*S.serrata*
33	1.200	0.053	0.358	High, blunt point	Both spines prominent	*S.serrata*
34	1.000	0.058	0.382	High, blunt point	Both spines prominent	*S.serrata*
35	0.900	0.079	0.392	High, blunt point	Both spines prominent	*S.serrata*
36	0.889	0.051	0.368	High, blunt point	Both spines prominent	*S.serrata*
37	0.889	0.059	0.389	High, blunt point	Both spines prominent	*S.serrata*
38	0.889	0.044	0.382	Moderate , shape point	Both spines prominent	*S.serrata*
39	1.000	0.036	0.386	Moderate , blunt point	Both spines prominent	*Suspect S. tranquebarica*
40	1.000	0.035	0.386	Moderate, blunt point	Both spines prominent	*Suspect S. tranquebarica*
41	1.000	0.039	0.371	High, blunt point	Both spines prominent	*S.serrata*
42	0.818	0.041	0.374	Moderate, blunt point	Both spines prominent	*Suspect S. tranquebarica*
43	1.000	0.062	0.399	High, blunt point	Both spines prominent	*S.serrata*
44	0.938	0.046	0.388	High , blunt point	Both spines prominent	*S.serrata*
45	1.000	0.048	0.373	Hight height, blunt point	Both spines prominent	*S.serrata*
46	0.900	0.058	0.398	Hight height, blunt point	Both spines prominent	*S.serrata*
47	0.938	0.063	0.352	Hight height, blunt point	Both spines prominent	*S.serrata*
48	0.900	0.054	0.381	High, blunt point	Both spines prominent	*S.serrata*

49	0.800	0.059	0.376	High, blunt point	Both spines prominent	*S.serrata*
50	0.867	0.049	0.349	High, blunt point	Both spines prominent	*S.serrata*
51	0.933	0.059	0.377	High, blunt point	Both spines prominent	*S.serrata*
42	0.923	0.074	0.378	High, blunt point	Both spines prominent	*S.serrata*
53	1.167	0.038	0.393	Moderate height, blunt point	Both spines prominent	*Suspect S. tranquebarica*
54	1.100	0.039	0.345	High , blunt point	Both spines prominent	*S.serrata*
55	0.923	0.051	0.388	High , blunt point	Both spines prominent	*S.serrata*
56	1.000	0.063	0.387	High, blunt point	Both spines prominent	*S.serrata*
57	0.833	0.061	0.386	High, blunt point	Both spines prominent	*S.serrata*
58	0.955	0.055	0.375	High, blunt point	Both spines prominent	*S.serrata*
59	1.000	0.068	0.366	High, blunt point	Both spines prominent	*S.serrata*
60	0.913	0.060	0.388	High, blunt point	Both spines prominent	*S.serrata*
61	0.892	0.060	0.408	High, blunt point	Both spines prominent	*S.serrata*
62	0.923	0.042	0.393	High, blunt point	Both spines prominent	*S.serrata*
63	0.917	0.051	0.388	High , blunt point	Both spines prominent	*S.serrata*

3.2. Mitochondrial (mt) DNA analysis

The genetic analysis of the 63 crabs showed blasts between 99.4% and 100% similarity with the reference sequence of *S. serrata* in the gene bank (Table 3.3). The standard for species identification is a blast superior to 99% similarity on the two nucleotide sequences with Genbank reference sequences. Therefore, it can be concluded that all 63 specimens collected were of the same species and can be identified as *Scylla serrata.* This result confirmed the results of the morphological analyze. Moreover, the samples suspected not to be *S. serrata* were *S. serrata.* It is therefore probable that only one species of mud crab presents

in New Caledonia or dominates largely, *S. serrata* (Fig. 3.5). The full classification is (Keenan, 1998; Thach, 2009):

Phylum: Arthropoda

Class: Crustacea

Subclass: Malacostraca

Order: Decapoda

Suborder: Reptantia

Family: Portunidea

Genus: Scylla

Species: *S. serrata* (Forskål, 1775)

Figure 3.5. *S. serrata* - commercial species in New Caledonia.

Table 3.3. Similarity of the blasts of the specimens of mud crab, genus *Scylla* studied with *S. serrata* reference sequences of the Genbank.

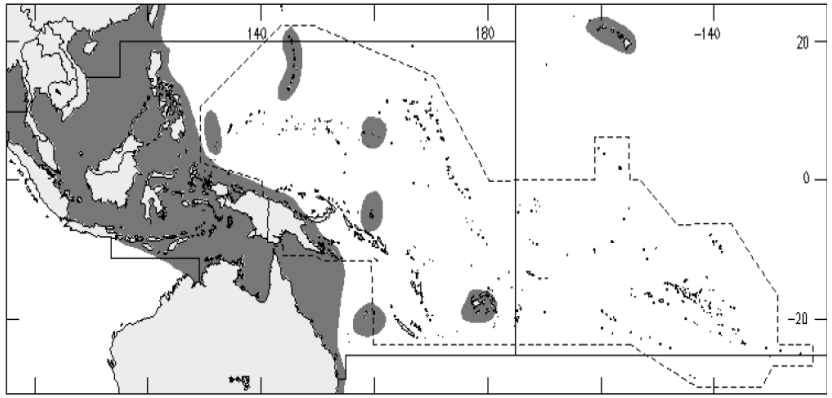
Specimen	Similarity in gen bank (%)	Species identification	Specimen	Similarity in gen bank (%)	Species identification	Specimen	Similarity in gen bank (%)	Species identification
1	99.6	*S. serrata*	22	99.6	*S. serrata*	43	99.4	*S. serrata*
2	99.6	*S. serrata*	23	99.6	*S. serrata*	44	99.6	*S. serrata*
3	99.6	*S. serrata*	24	99.6	*S. serrata*	45	100	*S. serrata*
4	99.6	*S. serrata*	25	99.6	*S. serrata*	46	99.6	*S. serrata*
5	100	*S. serrata*	26	99.8	*S. serrata*	47	99.4	*S. serrata*
6	99.6	*S. serrata*	27	99.6	*S. serrata*	48	99.6	*S. serrata*
7	99.4	*S. serrata*	28	99.4	*S. serrata*	49	99.6	*S. serrata*
8	98.9	*S. serrata*	29	99.4	*S. serrata*	50	99.4	*S. serrata*
9	99.6	*S. serrata*	30	99.4	*S. serrata*	51	100	*S. serrata*
10	99.9	*S. serrata*	31	99.6	*S. serrata*	52	99.6	*S. serrata*
11	100	*S. serrata*	32	100	*S. serrata*	53	99.4	*S. serrata*
12	99.6	*S. serrata*	33	99.6	*S. serrata*	54	99.6	*S. serrata*
13	99.6	*S. serrata*	34	100	*S. serrata*	55	99.4	*S. serrata*
14	100	*S. serrata*	35	99.4	*S. serrata*	56	100	*S. serrata*
15	99.4	*S. serrata*	36	99.6	*S. serrata*	57	99.8	*S. serrata*
16	100	*S. serrata*	37	99.4	*S. serrata*	58	99.6	*S. serrata*
17	100	*S. serrata*	38	99.6	*S. serrata*	59	99.6	*S. serrata*
18	99.4	*S. serrata*	39	99.8	*S. serrata*	60	99.4	*S. serrata*
19	99.6	*S. serrata*	40	99.6	*S. serrata*	61	99.4	*S. serrata*
20	99.4	*S. serrata*	41	100	*S. serrata*	62	99.4	*S. serrata*
21	99.4	*S. serrata*	42	100	*S. serrata*	63	99.6	*S. serrata*

4. Discussions and conclusions

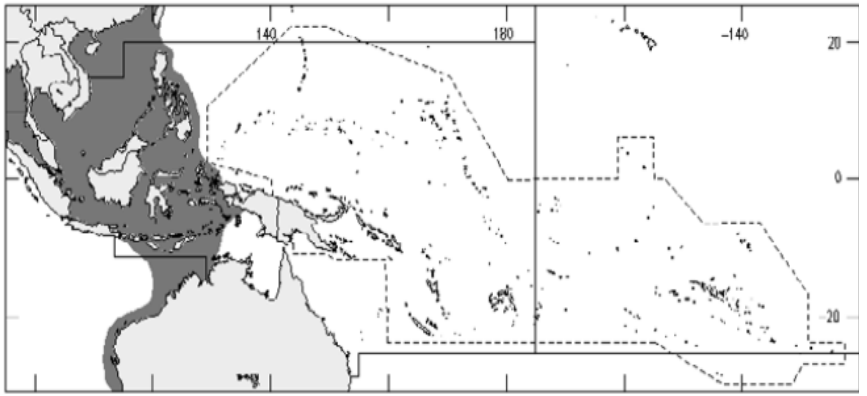
The distribution of 4 species of genus *Scylla* is presented in Fig. 3.6 (Carpenter & Niem, 1998). *Scylla serrata* is the most widely distributed ranging from the east African coast through Australia. This species is also reported in South Atlantic Ocean off Brazill (Melo, 1983). *S. serrata* and *S. olivacea* are sympatric in five areas: Gulf of Carpentaria, Panay, Taiwan and Kupang, and Western Australia (Taylor, 1984). In addition, three species *S. serrata*, *S. olivacea* and *S. tranquebarica* were found in Panay Island, Philippines. *S. olivacea* is the most abundant species in Philippines and Malaysia, where it is sympatric with *S. tranquebarica.* Both *S. olivacea* and *S. tranquebarica* have a distribution centered in the South China Sea, where *S. serrata* is almost completely absent (Keenan, 1997).

New Caledonia is located in the Indo-Pacific region and has the lagoon area of 24000 km^2 where is important habitat for fisheries and aquaculture, with 351 km^2 of mangrove forests, 88% along the west coast (Virly, 2008). Mangrove is the habitat of the mud crab *S. serrata* throughout the Indo-Pacific region and they usually support coastal fisheries (Lee, 1991; Brown, 1993; Bonine et al., 2008). Mangrove ecosystems with *S. serrata* have a high salinity, but this species can tolerate reduced salinity, mud bottoms of mangroves with fluctuating salinity being their preferred habitat (Keenan et al., 1998; Vay, 2001). Specimens analyzed in this study were collected from almost all the major wild crab fishery grounds and also where available nursery habitat is abundant (Dumas & Leopold, 2009). The salinity of these areas can be as high as 38 ppt (Marchand & Dumas, 2008), fluctuates because of the fresh water input from river estuaries such as La Foa, Voh, Temala and Nera, among others.

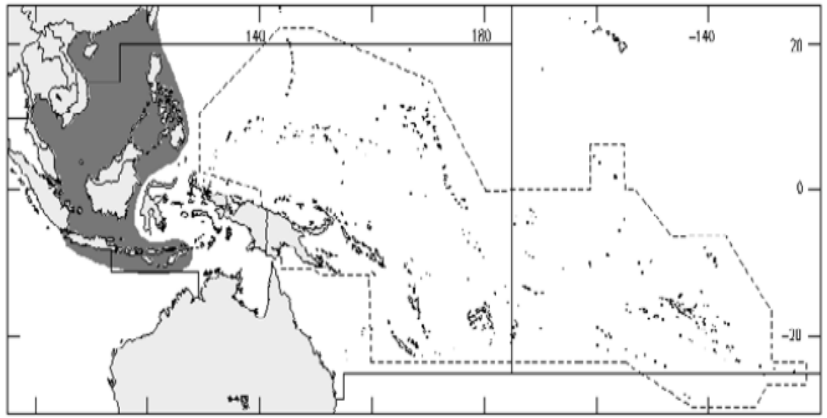
Using the morphological key provided by Keenan et al. (1998), we identified 57/63 crabs as *S. serrata*. It was not possible to identify the remaining 7/63 crabs without doubt. The reason was inaccurate measurements of the frontal median spine height (FMDH), the inner and outer carpus spines. These crabs were larger than 14 cm CW and these spines were easily worn out or broken. These characters must be considered with care. Furthermore, the morphological traits within a species can also be affected by its distribution and change between sites. For example, the colors of the specimens were very different, depending on the habitat and probably the diet. This observation is similar to Keenan et al. (1998). As indicated in study of Delathière (1990) on mud crab biology in New Caledonia, adult crabs had large ecological tolerance and thus exhibited wide distribution across mangrove, estuarine, and reef habitats.



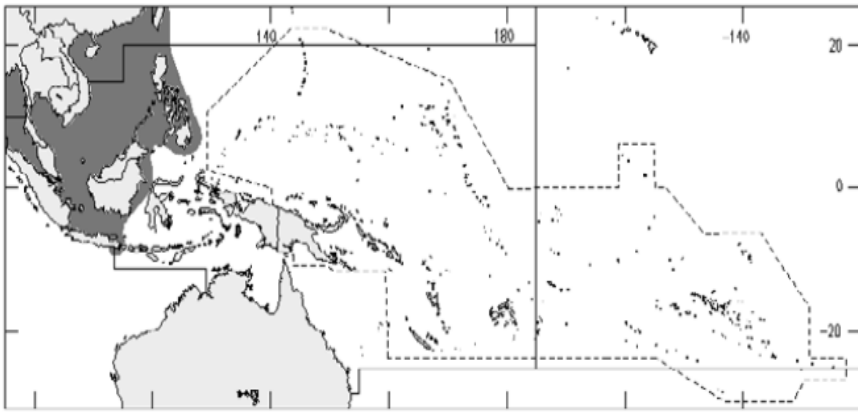
S. serrata



S.olivacea



S.paramamosain



S.tranquebarica

Figure 3.6. Distribution (indicated in dark areas) of 4 species of genus *Scylla*. Source: Carpenter & Niem (1998).

Based on genetic analysis, all 63 samples collected along the west and northeast coast of New Caledonia were identified as *S. serrata.* This result confirms the identification of Keenan (1997) who studied 6 specimens from New Caledonia.

In conclusion, we estimate that there is one commercial mud crab species in New Caledonia, *S. serrata.* If other species are present they should be very rare species. As considered above, *S. serrata* is widely spread throughout the Indo-Pacific region and can be sympatric with other species. Therefore, in New Caledonia in order to definitely confirm all mud crab species of the genus *Scylla*, more samples from each location along the coastline areas are needed.

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3.3 - CONCLUSION

Our study allows us to conclude that the mud crab fishery in New Calenonia concerns only one species: *Scylla serrata*. However, as in our material and method we focused mainly on crabs caught for marketing thus it is still possible that other species of mud crab exist in New Caledonian waters. Indeed, as considered above, *S. serrata* is widely spread throughout the Indo-Pacific region and can be sympatric with other species and notably *S. olivacea* in South Pacific (eastern Australia). However, if other species are present they should be very rare. Therefore, in New Caledonia in order to definitely confirm all mud crab species of the genus *Scylla*, more samples from each location along the coastline areas are needed and the study should not only target species of commercial interest.

Finally, the next step of our study will be done on the crab *S. serrata*. This species is probably best known as the point of view of its biology as that of his farming and this knowledge will be useful for the rest of our research in nutrition.

4 - CHAPTER IV: COMPARISON OF TWO PROTEIN SOURCES: FISHMEAL AND SOY PROTEIN CONCENTRATE ON THE FEED UTILIZATION AND GROWTH OF JUVENILE MUD CRAB, *SCYLLA SERRATA*

4.1 - INTRODUCTION

The presence and high percentage composition of plant fragments in the stomach of wild *S. serrata* at all growth stages suggests that this animal feeds on a wide variety of available food (La Sara et al., 2007). Hence, the trophic level of this animal may be categorized as an omnivore and its feeding behavior as a scavenger, opportunistic carnivores (Hutching & Saenger, 1987; Jayamane & Jinadasa, 1991). Regarding his diet in the wild, *S. serrata* should be adapted to the utilization of raw materials derived from plants. Experimental studies confirmed the capacity of *S. serrata* to digest fibers from all the plant feedstuffs (Catacutan et al., 2003). This may indicate that the crabs have some capacity to break down plant cellular structures to liberate the cell contents for better digestion (Anderson et al., 2004).

In Southeast Asia, mud crabs can be successfully raised on fresh diets including trash fish, fish wastes, slaughter wastes, kitchen leftovers, mussels, snails and shrimp heads (Shelley & Lovatelli, 2011). However, reliability of supply is frequently a problem and those natural food resources come under increasing pressure because of their use as feed for various types of aquaculture and for human consumption. Moreover, fresh diets require adequate refrigeration to be stored in good condition, which lead to additional costs. Furthermore, removal of large numbers of individual low value fish from the wild may impact negatively natural populations. Finally, uneaten fresh diet in the crab pond culture may rapidly cause a downturn in water quality and development of diseases.

In New Caledonia fresh diets regarding the low value fish, fish waste are not allowed to use as the direct feed for grow-out aquaculture even if this country gets annually about 750 tons of waste from fisheries, mainly tuna, which must be processed (ie hydrolysate) before being used as an ingredient for aquafeed (Bergé et al., 2011). Use of fresh fish waste to feed crab is problematic for two reasons: the first is logistic cost (transportation and refrigeration) and the second is biosecurity. Research on optimal crab nutrition and the development of suitable, cost-effective formulated feeds is therefore a prerequisite to develop crab aquaculture in New-Caledonia.

There has been some work done on the nutritional requirements of mud crabs, but much more needs to be done, particularly in the area of protein substitution and alternatives for fishmeal. Nevertheless, with the omnivorous nature of a crab's diet, it should not be too difficult to find suitable substitutes.

It is well known that protein is the most important and expensive dietary component and that fishmeal was the major dietary protein source of carnivorous fishes (Watanabe, 2002). Therefore, the dietary requirement of farmed fish can probably be met by sources other than fishmeal, including terrestrial animal products, single-cell, proteins and plant meals (Glencross et al, 2007). Besides ecological and ethical opposition to the use of aquatic resources as feed ingredient for high-value aquaculture species, there is real concern about the uncertain market availability and cost of fishmeal (Sookying et al., 2013). In the medium term, formulation cost of aquafeeds increases can only be avoided by a significant replacement of fishmeal by cheaper and reliable ingredients.

A range of terrestrial animal and plant-based ingredients can be efficiently digested by mud crabs, and have digestibility coefficients not significantly different from fishmeal (Catacutan et al., 2003). Processed animal protein ingredients such as blood meal, feather meal, meat and bone meal, and poultry byproduct meal are the most directly comparable substitute for fishmeal because their amino acid compositions are more similar to most plant products (Tacon, 1994; Allan et al., 2000; Edward et al., 2004). However the outbreak of bovine spongiform encephalophathy (BSE) led to ban on the use of animal products in aquaculture feeds in Europe and more specifically in New-Caledonia.

Various plant protein sources are already used in aquafeeds, including meals from oilseeds and legumes (soybean, rapeseed/canola, sunflower, cottonseed, peanut, peas, beans, and lupins), protein concentrates from grains (wheat and corn gluten). However, energy density from plant meal may be low because of its high content in carbohydrate (non-starch polysaccarides being the main issue) and some essential amino acids are generally deficient for fishes in plant protein sources (Gatlin et al., 2007). Among the potential protein sources, defatted soybean meal is available and cost effective, has a reasonably balanced amino acid profile compared with most other plant protein sources and it is well digested by mud crab *S. serrata* (Catacutan et al., 2003). However, concentrations of some essential amino acids (EAA) especially lysine, methionine and threonine are lower in SBM than in fishmeal (FM), and those EAA could be limiting in soy-based diets fed to aquatic animals (Gatlin et al., 2007). Nonetheless, concentrations of these EAA increase in soy protein concentrate (SPC) and approach or exceed those found in FM (Gatlin et al., 2007). In addition, the concentrations of anti-nutritional factors in SPC are lower than for soy bean meal (Bureau et al., 1998; Deng et al., 2006). All these advantages should not hide the high cost of SPC which is not always economically competitive with FM. However, the price of fishmeal will continue to increase

(Shepherd & Jackson, 2013), due to heightened demand on the international market mainly to feed aquaculture booming, especially in Asian countries and China in particular. Thus, SPC may ultimately represent a good alternative of protein source to fishmeal for aquafeed. A further concern in New Caledonia regarding plant proteins is the presence of genetically modified (GMO) products (Pusztai & Bardocz, 2006); precisely non-GMO SPC is still widely available on the international market. Consequently, these considerations highlight the interest to evaluate the SPC compared to the FM as protein sources on feed utilization and growth by the mud crab *S. serrata*.

Most of the previous studies on crabs have considered growth rate as gains in fresh weight. Nevertheless, crab fresh weight changes follow a basic pattern through the molt cycle, i.e. large and abrupt increases associated with rapid water uptake at ecdysis; further moderate gains associated with carapace mineralization and tissue growth during postmolt and relative stabilization in fresh weight during intermolt until the onset of the successive ecdysis (Heasmen, 1980). Increase in tissue mass, on the other hand, is a continuous process through the molt cycle (Freeman, 1990). The tissue growth can be measured as increase in dry weight, because tissue loses water in direct proportion to their gain in dry mass (Passano, 1960). Such growth goes along with definite changes in the ratio of dry and fresh weight of animals. Furthermore, Heasman (1980) showed rapid increases in organic matter as a proportion of fresh weight from molt stages B to C4 (postmolt stages) and lower increases from molt stage C4 to D2 (intermolt-premolt stages) in *S. serrata*. In a similar way, the actual growth of the muscle is thought to occur during the post-molt period when the carapace is already hardened, whereas immediately after the molt when the carapace is still soft the muscles appear to be watery and atrophied (Passano, 1960; Skinner, 1966). Based on this information, we studied the influence of the protein sources, FM versus SPC, on somatic growth of the juvenile crabs *S. serrata* during two periods of the molt cycle: post-molt and intermolt.

4.2 - EFFECT OF PROTEIN SOURCES, FISHMEAL *VERSUS* SOY PROTEIN CONCENTRATE, ON TISSUE GROWTH, FEED EFFICIENCY AND ENERGY BUDGET DURING A MOLT CYCLE OF JUVENILE MUD CRAB, *SCYLLA SERRATA*

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Key words: Juvenile crab, *Scylla serrata*, tissue growth, feed efficiency, protein requirement, fishmeal, soy protein concentrate, energy budget.

List of symbols and abbreviations

ACPD	Apparent Crude Protein Digestibility
ADMD	Apparent Dry Mater Digestibility
AGED	Apparent Gross Energy Digestibility
ERE	Energy Retention Efficiency
FCE	Feed Conversion Efficiency
FM	Fishmeal
PRE	Protein Retention Efficiency
SGR_d	Specific Growth Rate in dry weight (tissue growth)
SPC	Soy Protein Concentrate

Abstract

In this study, the nutritional value of soy protein concentrate (SPC) was evaluated as an alternative protein source to fishmeal for the juvenile crab *Scylla serrata* (average initial body weight = 22.1 ± 8.46 g and carapace width = 5.06 ± 0.70 cm). Three isoenergetic diets differing on their main protein sources: F100 (80% from fishmeal), F50S50 (40% from fish and 40% from soy protein concentrate meal) and S100 (80% from soy protein concentrate) were used to feed crab juveniles during 32 days in this study. A 3×5 factorial trial was conducted to assess the effects of feed intakes and different diets on tissue growth and energy budget of two crab groups: PMolt group with crabs whose tissue growth was studied during postmolt period (from ecdysis to C2-3 molt stages) and IMolt group was studied during intermolt period (from C3-4 to D1-3 molt stages).

The adjusted mean tissue growth, for a same amount of feed intake and the 3 diets combined, on dry matter basic (SGR_d, % dry iBW.d^{-1}) were 2.064 ± 0.324% and 0.492 ± 0.08% for PMolt and IMolt crabs respectively. The tissue growth of the PMolt crabs was not affected by the diets while IMolt crabs fed on diet S100 exhibited higher tissue growth. The apparent digestibility in dry mater (ADMD), crude protein (ACPD) and energy (AGED) was high (90-97.72%) regardless of the diet. Similarly, the efficiency in feed conversion (FCE, %), protein retention (PRE, %) and energy retention (ERE, %) were not affected by the different experimental diets, except for the IMolt crabs fed on diet S100 which exhibited higher values of the three parameters. However, values of FCE, PRE and ERE for PMolt crabs were 4 to 5 times higher than those for IMolt crabs. Similarly, feed intake in dry matter, energy and protein for optimal tissue growth of PMolt crabs were obviously higher than those for IMolt crabs. The energy budgets of juvenile crabs expressed as a % of energy intake were marginally affected by diet but were significantly different according the group of crabs: PMolt or IMolt. Maintenance energy was lower for PMolt (up to 49.84 ± 4.9%) than for IMolt crabs (up to 83.33 ± 2.45%). Excessive maintenance energy in IMolt crabs represents the energy portion set aside in anticipation of the next molt: shell energy content (4.97 ± 0.31%) and metabolism increase during ecdysis (± 28% of total energy intake). Conversely, recovery energy was significantly higher for PMolt (34.39 ± 0.99% of total energy intake) than IMolt crabs (8.33 ± 1.7% of total energy intake).

The results of this study showed that SPC is a good alternative dietary protein source, compared to fishmeal, for tissue growth during a molt cycle of crab juveniles *Scylla serrata*.

1. Introduction

The mud crab has been domesticated and farmed for many years in Asia, particularly in Vietnam where production is increasing quickly (www.shrimpnews.com). There is an unmet demand for mud crabs and this has led to over-exploitation of wild populations in many areas. Difficulties in obtaining wild caught juveniles for farming operations, and concerns about further over-exploitation have led to major investments in the development of new hatchery techniques (Allan & Fielder, 2003). For the past few years, techniques of captivity breeding and larval rearing have been developed allowing production to triple in 5 years (from 10000 tons in 2004 to 30000 in 2009) (John & Paul, 2012). However, the substantial crab farming operations which exist throughout Southeast Asia are mainly based on wild caught crablets (Allan & Fielder, 2003; Shelley, 2008) and animals in captivity have been fed with excessive trash fish which contaminates rearing water and leads to high mortality rates (Linder, 2005; FAO, 2011).

In New Caledonia, there is a strong political wish to diversify aquaculture, which until recently, has been based on blue shrimp (*Litopenaeus stylirostris*) farming. Among the different options available, the mud crab, *Scylla serrata,* is regarded as having great potential for aquaculture through its farming aptitudes and its high economical value. However, the possible development of crab farming in New Caledonia will necessarily involve the development of an artificial feed that can be produced from selected and controlled raw materials available on the international market.

Therefore, it is essential to assess the nutritional requirements of *S. serrata*. A few studies have been conducted on this subject. Sheen & Wu (1999) showed better lipid utilization by the crab than shrimp by evaluating several amounts of a mixture of cod liver oil and corn oil on the growth of juvenile crabs. In addition, the importance of a dietary source of cholesterol and polyunsaturated fatty acids (22:6 n-3, 20:4 n-6, 18:3 n-3) for the healthy growth of juvenile crabs (Sheen, 2000). Furthermore, a series of studies has been conducted to measure the digestibility of several potential ingredients for formulated feed for crabs (Catacutan et al., 2003; Truong et al., 2009). Results showed that crabs were able to digest many different ingredients, in particular fibre and protein from plants. Finally, the optimal protein inclusion level in the diet has been the subject of two studies (Catacutan, 2002; Unnikrishnan & Paulraj, 2010). Both studies concluded that the optimal protein concentration

(a mixture of fishmeal and soybean meal) in the feed for the best growth rate is between 30% and 47%.

Most of the previous studies have considered growth rate as gains in fresh weight. Fresh weight changes follow a basic pattern through the molt cycle, i.e. large and abrupt increases associated with rapid water uptake at ecdysis, further moderate gains associated with carapace mineralization and tissue growth during postmolt, and relative stabilization of fresh weight during intermolt until the onset of the successive ecdysis (Heasmen, 1980). Increase in tissue mass, on the other hand, is a continous process occurring throughout the molt cycle (Freeman, 1990). The tissue growth can be measured as an increase in dry weight because tissue loses water in direct proportion to their gain in dry mass (Passano, 1960).

This experimental study describes tissue growth, fecal production, nitrogenous excretion and energy budget of juvenile crabs during postmolt and intermolt periods, in relation to feed intake and sources of protein (fishmeal vs. soy protein concentrate). It provides a better understanding of the effect of protein sources on the tissue growth and bio-energetic balance of juvenile crabs during one molt cycle. This information is fundamental to adjust the feed formula and ration level for balance nutrition of captive aquaculture of juveniles *S. serrata.*

2. Materials and methods

2.1. Crabs and holding facilities

Juvenile crabs, *Scylla serrata,* were collected in the shallow intertidal zones associated to mangroves surrounding the experimental station (21°51’50″ S, 166°3’0″ E) in Boulouparis district, New Caledonia. Eighty crab juveniles were caught using a hand net, transferred to the laboratory and acclimated into 6 circular composite tanks (capacity 500 L) for two weeks prior to the beginning of the experiment. Next, sixty of these crabs were identified for their molt stages based on the criteria and methodology described by Drach & Tchernigovtzeff (1967) and Freman et al. (1987). These crabs were randomly individually assigned to sixty experimental rectangular polyethylene tanks (30 x 20 x 30 cm) covered with black lids. Two experimental crab groups, 30 individuals per each group, were considered: Postmolt (PMolt) and Intermolt (IMolt). At the beginning of the experiment, the PMolt group was constituted of crabs in premolt stage (from D1 to D3) which were about to molt and the IMolt group

included crabs which were in early intermolt stage (C3 to C4). During the trial, the PMolt crabs molted within the first two weeks and reached C2 or C3 stage at the end of the experiment. Conversely the IMolt crabs did not molt and they attained D2 or D3 stage. After being dried in soft paper, each crab was weighed to the nearest 0.01 g and its carapace width was measured to the nearest 0.01 mm.

Each experimental tank was continuously supplied with seawater running from a plastic reservoir (2000 liter capacity) at the rate of 0.19 $L.min^{-1}$. The water pumped from the lagoon was filtered through a 25 μm net bag into the reservoir. The temperature was automatically controlled using a heater. The temperature in the experimental tanks was measured every 3 hours using an automatic recording probe. During the experimental period, temperature, salinity, pH, and dissolved oxygen in the water were maintained at 28.5 ± 1.5 ^{o}C, 34.0 ± 1.5‰, 7.87 ± 0.09 and 4.46 ± 0.16 mg. L^{-1} respectively.

2.2. Diet preparation and composition

The crabs were fed on experimental diets produced in the laboratory: the ingredients were ground up in a grinder (Retsch®) with a 1 mm screen. Next, the meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca®) until the consistency was suitable for pelleting. The mixture was then extruded through a 3 mm die in a meat grinder. Pellets were then steamed for 15 min and stored at -20°C before use. The ingredient composition and proximate nutrient content of experimental diets are shown in Table 4.1A. The amino acid composition of each diet was estimated from the amino acid content of feed ingredients (indicated in Table 2.3 of chapter II) and is presented in Table 4.1B.

2.3. Experimental design

2.3.1. Growth trial

Table 4.2 shows the experimental design of this study. Sixty (60) tanks with individual crabs were assigned to two groups of 30 individuals each: PMolt and IMolt. As each crab was raised separately the experimental unit in this study was the individual. Each crab group was fed on three different experimental diets (F100; F50S50; and S100) distributed in 5 ration sizes (% of initial fresh body weight = % iBW): 0.5, 1, 1.5, 2 and *ad libitum*. Thus, 10 tanks of each crab group were randomly assigned to each diet (n = 10).

Table 4.1A. Proximal composition of ingredients and three experimental diets.

	Diets			Proximal analyze (dry matter basis)			
	F100	F50/S50	S100	Protein (%)	Lipid (%)	Ash (%)	Energy (MJ.kg^{-1})
Composition (%)							
Fish meal	53.28	26.64		73.73	11.18	12.40	22.47
Protein concentrate soy		28.70	57.39	64.02	2.47	7.00	23.53
Crab meal	19.59	19.59	19.59	42.23	1.90	46.47	11.47
Fish oil		1.96	3.92				39.27
Wheat	16.65	13.42	10.77	12.92	2.13	2.02	15.73
limestone	6.46	5.68	4.31				
Dicalcium phosphase	1.96	1.96	1.96				
Binder[1]	0.59	0.59	0.59				
Vitamin premix[2]	0.29	0.29	0.29				
Vitamin C[3]	0.88	0.88	0.88				
Trace elements	0.29	0.29	0.29				
Analysis (dry matter basis)							
Crude protein - P (%)	49.60	48.81	48.39				
Lipid (%)	4.98	4.69	4.19				
Ash (%)	23.82	21.73	16.22				
Gross energy - E (MJ.kg^{-1})	15.08	15.41	15.34				
P/E (g.Mj^{-1})	32.89	31.68	31.54				

[1]Binder: Pegabind (R) synthetic resin from Bentoli Cie;

[2]Vitamin Premix for shrimp from BEC feed solutions PTY, LTD - ingredients: vitamin AD3 1000/200, vitamin B1 98% Thiamine, Mononitrate, vitamin B2 Riboflavin 80%, vitamin B3 99% Niacin, vitamin B5 98% D-Calpan, vitamine B6 98% Pyridoxine, Vitamin B9 97% Folic acid, vitamine D3 500, vitamin E 50 ADD, vitamin K3 43.7%;

[3]Vitamin C: Rovimix Stay-C 35 from DSM Cie;

[4]Trace elements from SICA Cie (Goodman Fielder).

Table 4.1B. Amino acid composition (%, on dry matter basis) of three experimental diets.

	Experimental diets		
	F100	F50S50	S100
EAA			
Arginine	3.14	3.21	3.32
Histidine	3.12	3.05	3.01
IsoLeucine	2.22	2.15	2.11
Leucine	3.64	3.46	3.34
Lysine	3.45	3.04	2.66
Methionine	1.34	1.01	0.68
Phenylalanine	2.10	2.19	2.33
Threonine	2.10	1.95	1.82
Tryptophan	0.60	0.60	0.61
Valine	2.64	2.45	2.30
NEAA			
Alanine	2.82	2.45	2.11
AsparticAcid	4.59	4.81	5.07
Cystine	0.52	0.58	0.66
GlutamicAcid	6.75	7.40	8.29
Glycine	2.66	2.33	2.02
Proline	2.24	2.31	2.46
Serine	2.09	2.20	2.35
Tyrosine	1.69	1.62	1.57

EAA: essential amino acid; NEAA: nonessential amino acid.

Table 4.2. Experimental design of trials: growth, digestibility, fecal production, and nitrogenous excretion.

	Experimental diets														
	F100					F50S50					S100				
Growth trial															
Ration size (% iBW d^{-1})	0.5	1.0	1.5	2	*AL*	0.5	1.0	1.5	2	*AL*	0.5	1.0	1.5	2	*AL*
PMolt carbs (n)	10					10					10				
IMolt crabs (n)	10					10					10				
Feeding frequency	1/24h					1/24h					1/24h				
Fecal production trial															
Ration size (% iBW d^{-1})	0.5	1.0	1.5	2	AL	0.5	1.0	1.5	2	AL	0.5	1.0	1.5	2	AL
PMolt carbs (n)	10					10					10				
IMolt crabs (n)	10					10					10				
Feeding frequency	1/24h					1/24h					1/24h				
Digestibility trial															
Ration size (% iBW d^{-1})	*Ad libitum*					*Ad libitum*					*Ad libitum*				
Replicates (n)	5					5					5				
Feeding frequency	2/24h					2/24h					2/24h				
Nitrogenous excretion trial															
Ration size (% iBW d^{-1})	0.5	1.0	1.5	2	unfed	0.5	1.0	1.5	2	unfed	0.5	1.0	1.5	2	unfed
Replicates (n)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

AL: *Ad libitum*;
Pmolt crabs comprised crab juveniles of premolt stage (from D1 to D3) at the beginning of the experiment;
IMolt crabs included crab juveniles of early intermol stage (from C3 to C4) at the beginning of the experiment.

At the beginning of the experiment, the crabs were starved 48 hours and then weighed and measured. The initial average body weight and carapace size of crabs were 22.10 ± 8.46 g and 5.06 ± 0.70 cm respectively. The daily pre-weighed feed rations for each tank were delivered once a day at 6:00-7:00 am. Three hours after each meal the unconsumed feed was siphoned off, dried 24 hours at 60 °C and weighed. The leaching rate (dry matter loss of the pellet in the water) was determined by measuring remaining dry matter of the diet after 3 hours immersion. Then the amount of ingested feed (dry matter basis) was obtained following the equation:

$FI = dF – L – uF$

Where: FI = ingested feed; dF = distributed feed; L = leaching after 3 hours and uF = unconsumed feed.

Finally, the specific daily feed intake (ingested diet, % $iBW.d^{-1}$) of each crab was determined.

During the experimental period, the date of ecdysis of PMolt crab group, carapace width and body weight were recorded for each crab after molting. The exuviae was collected and weighed before and after drying at 80 °C for 24 h, then kept at -20 °C for energy analysis. The trial was conducted over 32 days. At the end of the experiment all crabs were left for 48 h starvation and then weighed, measured, dried at 80 °C for 48 h and kept at -20 °C before biochemical and energy analysis.

2.3.2. Digestibility trial

All diets contained 1% chromic oxide (Cr_2O_3) as an inert indicator to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED).

Feeding and fecal collection

For each experimental diet, five crabs were fed *ad libitum* twice daily until approximately 1.0 g of fecal material (dry weight) was collected (Table 4.2). Crab feces at the bottom of the tank were collected individually by Pasteur pipette and rinsed gently in distilled

water. Fecal matter from five crabs in each experiment diet was pooled. All samples were kept at -20 °C before freeze drying for analysis.

Chromium analysis and calculations

The chromium content of diets and fecal material used to calculate apparent digestibility values were determined using the method described by Furukawa & Tsukahara (1966). Apparent digestibilities of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) were calculated using the equation:

ADMD = 100 - 100 x (% Cr_2O_3 in feed / % Cr_2O_3 in feces)

The digestibility of crude protein (ACPD) or gross energy (AGED) was determined using the formula ACPD or AGED =100 - 100 x (% Cr_2O_3 in feed / % Cr_2O_3 in feces) x (% protein or $MJ.kg^{-1}$ energy in feces/ % protein or $MJ.kg^{-1}$ energy in feed).

2.3.3. Fecal production trial

During the experimental growth trial, fecal production was collected for each experimental unit (n = 60) three times a day during 10 days (Table 4.2). Then the feces of 2 crabs from each group, PMolt and IMolt, were pooled for each ration size of each diet. At the end of the trial, the collected fecal matter was freeze-dried, weighed and stored at -20 °C before biochemical and energy analysis.

2.3.4. Nitrogenous excretion trial

Three crabs were individually fed at five different ration sizes: unfed; 0.5; 1; 1.5; and 2% for each experimental diet (Table 4.2). Each crab was transferred into another individual aquarium with fixed water volume (30 L) immediately after the meal. Ammonia-N excretion (Weihraush et al., 2004) in each aquarium was measured twice according the method described in Grasshoff (1983): before and 24 h after transferring the crab. Then, the ammonia-N value was converted into energy by multiplying by 24.83 $kJ.g^{-1}$ (Elliot, 1976). The potential loss of nitrogenous compounds through bacterial action or diffusion was quantified by setting controls without crabs. The values of ammonia-N concentration of this control treament were used to adjust determined nitrogenous excretion.

2.4. Biochemical analysis

The proximate compositions of diets, feces, initial and final crabs were estimated according to the method of AOAC (1995) at Invivo laboratory, Vietnam. Moisture contents of crabs and feed were determined after oven-drying to a constant weight at 105 °C. Each sample was combusted at high temperature in pure oxygen then the nitrogen content was measured by thermal conductivity detection and converted to equivalent protein by an appropriate numerical factor, crude protein % = % N x 6.25. Crude lipid content was measured by solvent extraction with petroleum ether. Ash content was determined by a muffle furnace at 550 °C for 8-10 h. Energy contents of diets, fecal material, exuviae and crabs were determined by calorimetric bomb (Parr® 6200, USA, calibrated by benzoic acid) at Ifremer laboratory, New Caledonia.

2.5 Definition, calculation and statistics

The energy partitioning of the budget suggested by the National Research Council (NRC 2011) was adopted in this study with minor modification in order to comply with mud crab growth through molting. The energy budget of growing crabs is expressed in our study as:

$$IE = FE + UE + SE + RE + HE_m \quad (1)$$

Where IE is the energy intake which was obtained for each crab by calculating the difference between the feed distributed and the feed leached and uneaten;

FE is energy lost from the animal through the feces for each crab, FE was calculated from the model linking the feed intake (energy intake) and fecal production;

UE is energy lost from the animal through the total ammonia excretion which was calculated for each crab from the model linking the feed intake (energy intake) and nitrogenous excretion;

SE is surface energy losses which is exuvia lost at ecdysis for the PMolt crab group;

RE is the recovered energy which is channeled into growth. It is calculated for each crab through the dry weight gain converted into energy;

HE_m is the maintenance energy calculated for each crab from the equation (1):

$HE_m = IE – (FE + UE + SE + RE)$ (2)

Specific tissue growth rate (SGR_d, % of dry iBW.day^{-1}) was estimated through the equation:

$SGR_d = 100 \times (\text{dry fBW} - \text{dry iBW}) \times \text{dry iBW}^{-1} \times \text{day}^{-1}$

Where fBW and iBW are final and initial body weight, respectively.

Initial dry body weight (dry iBW) was estimated through the equation:

$Y = 0.3229 X + 0.4246$ ($R^2 = 0.96$, n = 30)

Where Y is dry body weight; X is fresh body weight. This equation was obtained from an extra sample assessment for crabs in intermolt and premolt stages.

Crabs of the PMolt group were selected in premolt stages (D1-3) at the beginning experiment. We showed in our extra sample assessment that dry body weight of individuals did not change significantly before and after ecdysis. Therefore in our study, for PMolt crabs we considered that "iBW after ecdysis" is equal to “dry BW” of the crabs at premolt stages. Consequently, growth period study lasted between ecdysis and the end of the experiment (18-22 days) for PMolt crab group, while the growth period was the same with the experimental duration (32 days) for IMolt crabs.

The efficiency in feed conversion (FCE), protein retention (PRE) and energy retention (ERE) is the ratio between weight gain in dry matter, protein and energy to the amount of feed (dry matter basic), protein or energy ingested, respectively (Brett & Groves., 1979; Sun & Chen., 2009; Talbot., 1993; NRC., 2011; Zhang et al., 2011).

Nitrogenous excretion (UE), fecal production (FE), recovered energy (RE), and maintenance energy (HE_m) were analyzed using Analysis of Covariance (ANCOVA) with feed intakes as the covariate. For these parameters, the adjusted individual data and mean were calculated for a same amount of ingested feed.

Optimum growth is defined as the best increase in dry body weight for the least feed intake (Brett, 1979). Therefore, the feed intake coresponding to the highest values of FCE was determined as the optimum intake, then converted into protein and energy required for optimal tissue growth of individual crab.

Differences among the various treatments were considered significant when $P < 0.05$. Shapiro-Wilk and Levene's test were used for checking normality and homogeneity of variances, respectively. Percentage data were transformed into arcsine to get normality. The Two way ANOVA fixed model was used to test the effects of diet and crab group (PMolt or IMolt) on body biochemical composition, energy content and each energy budget component (FE, UE, HE_m, RE). Analyses of Covariance (ANCOVA) were used to investigate the effect of different experimental diets on nitrogenous excretion, fecal production, and specific growth rate across ration size treatments. Data were Log transformed to get a linear relation between growth rate and feed intake for IMolt crabs. Digestibility coefficients of crabs fed on different experimental diets were compared by one way ANOVA as only one crab group was considered.

3. Results

3.1. Body chemical composition and energy content

The contents of protein, lipid, ash and energy in the body of PMolt and IMolt crabs juveniles fed on full meal for three different experimental diets are presented in Table 4.3. Whatever the crab group, PMolt or IMolt, no significant effect of the diet was shown (ANOVA, $P = 0.29$; 0.31; 0.74 and 0.13 for protein, lipid, ash and energy, respectively) on the biochemical composition and energy content of the animal.

The body lipid content was significantly higher for IMolt than for PMolt crabs (ANOVA, $P = 0.003$) for the 3 diets tested. On the contrary, whatever the diet considered, the average levels of protein (39.4-40.6%), ash (41.6-45.1%) and energy content (10.5-10.6 $kj.g^{-1}$ body.dry weight) of crabs in dry weight were not significantly different between the two crab groups (ANOVA, $P = 0.67$; 0.28; 0.98 for protein, ash and energy, respectively).

Table 4.3. Crab body proximal composition (%) and energy content (kj.g^{-1} of dry body weight) on dry weight basis of PMolt and IMolt crab groups fed *ad libitum*.

Crab's group	Diet	Protein content	Lipid content	Ash content	Energy content
PMolt	F100 (n = 3)	38.7 ± 1.1^{a}	1.5 ± 0.1^{a}	46.2 ± 2.0^{a}	10.4 ± 1.1^{a}
	F50S50 (n = 3)	37.6 ± 2.5^{a}	1.3 ± 0.8^{a}	46.3 ± 4.3^{a}	9.9 ± 1.1^{a}
	S100 (n = 3)	41.9 ± 4.2^{a}	1.9 ± 0.4^{a}	42.9 ± 3.4^{a}	11.2 ± 0.7^{a}
IMolt	F100 (n = 3)	39.9 ± 4.7^{a}	2.8 ± 0.8^{b}	42.2 ± 3.3^{a}	10.1 ± 0.1^{a}
	F50S50 (n = 3)	39.7 ± 1.3^{a}	2.4 ± 0.1^{b}	42.5 ± 0.9^{a}	10.0 ± 0.3^{a}
	S100 (n = 2)	42.3 ± 1.5^{a}	3.1 ± 0.4^{b}	40.0 ± 0.3^{a}	11.5 ± 1.1^{a}

Values are means ± SD (n = replicates per treatment), within the same column, means with different letters are significantly different ($P < 0.05$).

3.2. Diet digestibility

Apparent digestibility for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of the three experimental diets are given in Table 4.4. No significant difference in ADMD, ACPD and AGED was found between diets (ANOVA, P = 0.05; 0.43; 0.59 and 0.19, respectively). The averages of ADMD, ACPD and AGED were high for each diet: 90.34 ± 1.79%; 97.72 ± 0.44%; 90.42 ± 1.82% respectively.

3.3. Fecal production and nitrogenous excretion

The fecal production of juvenile crabs PMolt or IMolt increased significantly with feed intake (ANCOVA, $P < 0.01$) for all three experimental diets, whatever the crab group. The relationship between fecal production and feed intake was described as a linear function (Fig. 4.1). The diet composition did not affect the fecal production (ANCOVA, P = 0.46).

Nitrogenous excretion of juvenile crabs increased with feed intake. Exponential functions were used to describe this relationship for all three experimental diets (Fig. 4.2). Feed type and feed intake significantly affected nitrogenous excretion (ANCOVA, $P < 0.01$).

The later was significantly lower for F100, intermediate for S100 and higher for F50S50 (pairwise comparisions, $P < 0.01$).

Table 4.4. Apparent digestibilities (%) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of three experimental diets.

Diets	Digestibilities		
	ADMD	ACPD	AGED
F100	90.56 ± 2.38[a]	97.41 ± 0.65[a]	89.06 ± 2.76[a]
F50S50	91.03 ± 1.85[a]	97.86 ± 0.44[a]	92.27 ± 1.59[a]
S100	89.44 ± 1.15[a]	97.88 ± 0.23[a]	89.92 ± 1.1[a]

Values are means ± SD (n = 3 replicates per treatment), within the same column, means with the same letters are not significantly different ($P > 0.05$).

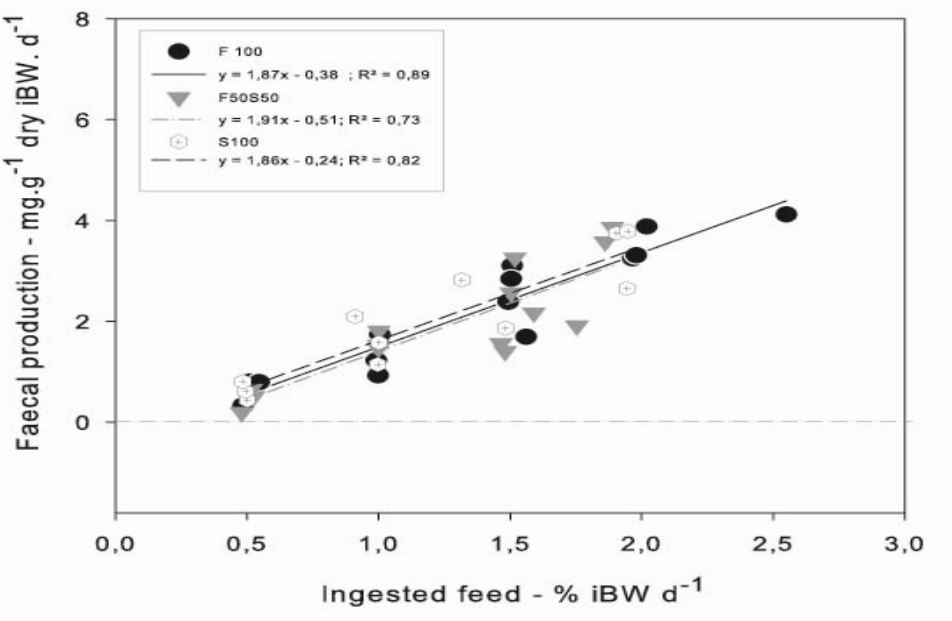

Figure 4.1. Relationships between fecal production of juvenile crabs and ingested feed for the three experimental diets. Amount of ingested feed is percentage of initial fresh body weight (iBW) per day.

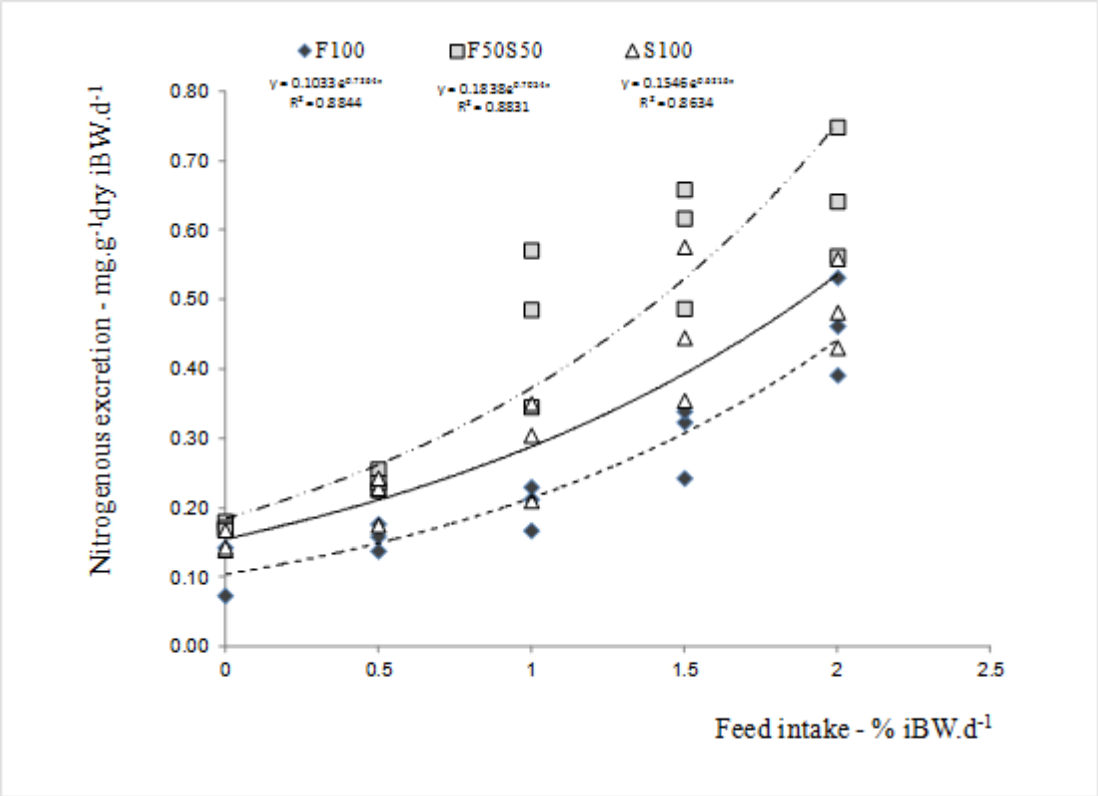

Figure 4.2. Relationships between nitrogenous excretion of juvenile crabs and feed intake for the 3 experimental diets. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

3.4. Growth and feed utilization

Crabs from PMolt group molted within the first two weeks of the experiment and no crabs molted in IMolt group. At the end of experiment, crabs were recorded in C2 to C3 and D2 to D3 molt stages for PMolt and IMolt groups, respectively.

The specific growth rate in dry weight or tissue growth (SGR_d) of juvenile crabs from the PMolt and IMolt groups increased with feed intake (ANCOVA, $P < 0.01$) whatever the experimental diet. Linear and logarithmic functions best fit the "tissue growth - feed intake" relationship of PMolt and IMolt crabs, respectively (Fig. 4.3 & Fig. 4.4).

ANCOVA analyze with feed intake as covariate (Fig. 4.5) allowed us to compare the adjusted means of tissue growth (SGR_d). For PMolt crab group, tissue growth was not significantly affected by diet type ($P = 0.43$). For IMolt crabs, it was significantly higher on diet S100 compared to F100 ($P = 0.02$) and F50S50 ($P = 0.04$); but no difference was observed between diet F100 and diet F50S50 ($P = 0.98$).

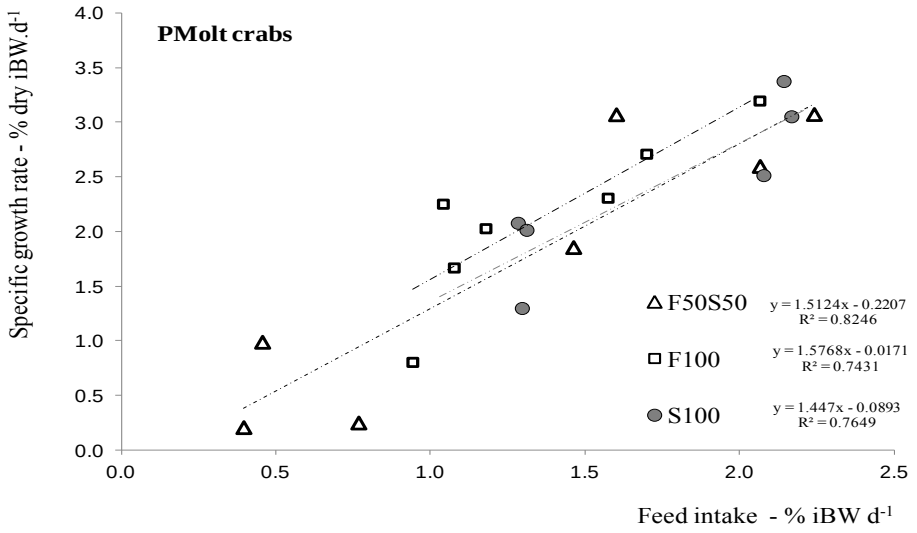

Figure 4.3. Relationship between specific growth rate (SGR_d) and feed intake of juvenile crabs for the 3 experimental diets for PMolt crabs. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

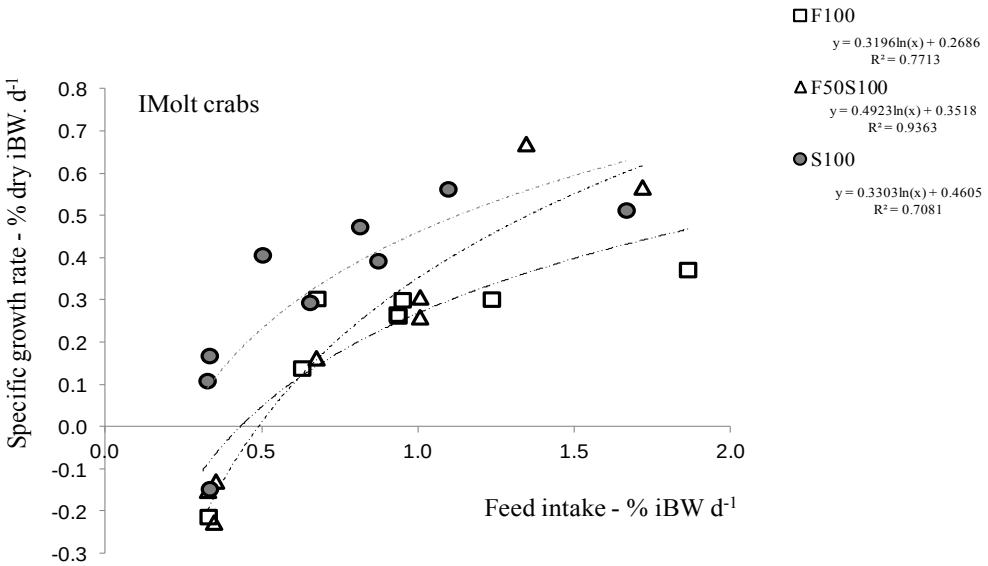

Figure 4.4. Relationship between specific growth rate (SGR_d) and feed intake of juvenile crabs for the 3 experimental diets at IMolt crabs. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

During a molt cycle, 80% of the tissue growth occurred during postmolt (PMolt crabs) and only 20% during intermolt (IMolt crabs) (Fig. 4.5).

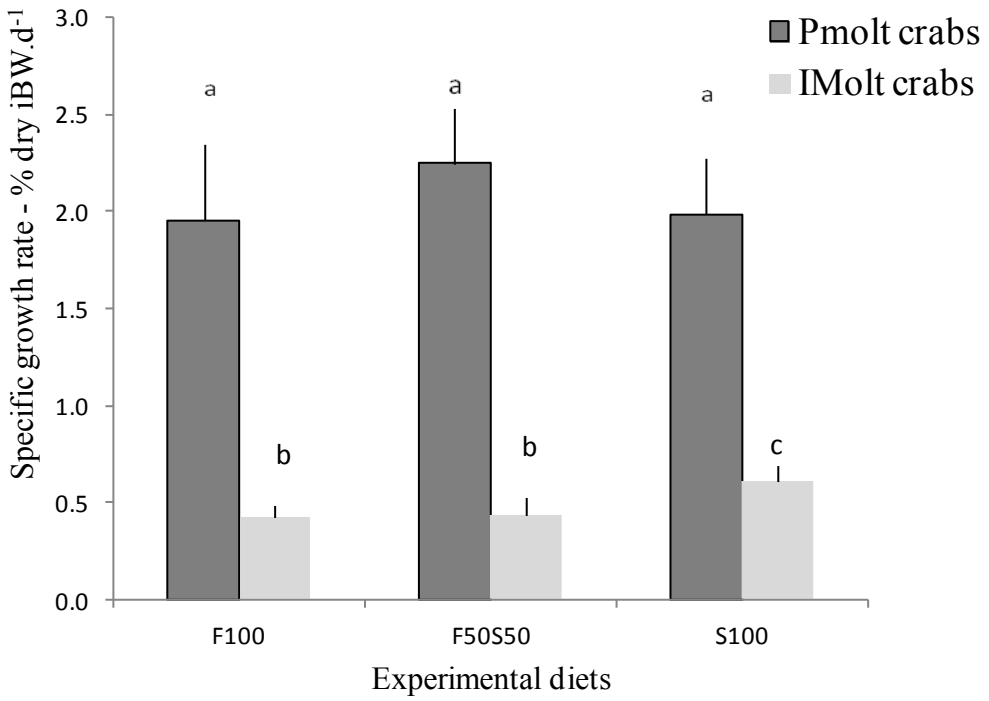

Figure 4.5. Adjusted mean specific growth rates (± SD) for average feed intakes of PMolt and IMolt crabs. Means with different superscript letters differ significantly (P < 0.05).

Feed intake values (in dry matter, energy and protein) for optimal tissue growth (the best growth rate for the minimum feed intake) of PMolt and IMolt crabs were calculated from the polynomial functions linking feed conversion efficiency (FCE) and feed intakes:

(i) For PMolt crabs, as FCE was not affected by the experimental diets, only one polynomial equation was defined by pooling the data (n = 16) from all 3 diets: $y = -36.296\ x^2 + 114.26\ x - 33.494$ ($R^2 = 0.65$)$^{(3)}$. The calculated feed intake for optimum growth is 4.57% of dry iBW.d^{-1} and the derived optimum values of energy and protein intakes are 0.70 kj.g^{-1} of dry iBW. d^{-1} and 0.022 g.g^{-1} of dry iBW. d^{-1}, respectively.

(ii) For IMolt crabs, as FCE was significantly higher in diet S100, two polynomial equations were defined: one for diet S100 treatment and one by pooling data (n = 14) for diets F100 and F50S50:

Diet S100 (n = 7): $y = -12.362\ x^2 + 22.008\ x + 8.4093\ (R^2 = 0.67)$ (4)

Diets [F100+F50S50] (n = 14): $y = -20.679\ x^2 + 54.56\ x - 22.728\ (R^2 = 0.76)$ (5)

Feed intakes for optimal growth are 2.72 and 4.24% of dry iBW.d^{-1} respectively for S100 and [F100+F50S50] diets. The corresponding values of energy and protein intakes are 0.42 kj. g^{-1} of dry iBW.d^{-1} and 0.013 g.g^{-1} of dry iBW.d^{-1} for diet S100 and 0.68 kj.g^{-1} of dry iBW.d^{-1} and 0.021 g. g^{-1} of dry iBW.d^{-1} for diet [F100+F50S50].

Estimated feed intake or ration size values for optimal growth reveal two trends: firstly, feed intake is obviously higher in the PMolt crab group, and secondly, consumption of diet S100 was lower than that of diets F100 and F50S50 in the IMolt crab group.

Adjusted mean values, for optimum feed intake, of feed conversion efficiency (FCE), protein retention efficiency (PRE) and energy retention efficiency (ERE) were calculated from equations (3); (4); and (5) for PMolt and IMolt crab groups fed on three different diets (Table 4.5). FCE, PRE and ERE values for PMolt crabs were respectively: 49.86 ± 7.01%, 39.29 ± 5.52%, 35.34 ± 4.27% and obviously 4-5 times higher than IMolt crabs with respective values of 11.37 ± 1.85%; 9.1 ± 1.48%; 7.84 ± 1.27%.

Within the PMolt crab group, experimental diets did not significantly influence FCE (ANOVA, P = 0.73), PRE (ANOVA, P = 0.72) and ERE (ANOVA, P = 0.19). In contrast, for the IMolt crab group FCE, PRE and ERE were significantly higher with diet S100 than with diet F100 and F50S50 (pairwise comparisions, $P < 0.01$).

Table 4.5. Adjusted mean values of efficiency in feed conversion (FCE), protein retention (PRE) and energy retention (ERE) at the optimal feed intake for crab groups, PMolt and IMolt, fed on three different diets.

Crab's groups	Experimental diet	Feed Efficiency (%)		
		[(i)]FCE	[(ii)]PRE	[(iii)]ERE
PMolt	F100 (n = 7)	47.57 ± 7.97[a]	37.49 ± 6.28[a]	34.29 ± 4.31[a]
	F50S50 (n = 7)	50.39 ± 5.29[a]	39.72 ± 4.17[a]	33.03 ± 3.41[a]
	S100 (n = 7)	51.61 ± 7.77[a]	40.68 ± 6.13[a]	38.70 ± 5.10[a]
IMolt	F100 (n = 5)	7.55 ± 0.87[b]	6.04 ± 0.70[b]	5.21 ± 0.60[b]
	F50S50 (n=5)	10.15 ± 3.38[b]	8.12 ± 2.70[b]	7.00 ± 2.33[b]
	S100 (n=6)	16.43 ± 1.29[c]	13.14 ± 1.03[c]	11.33 ± 0.89[c]

[(i)]FCE = 100 x (dry final body weight - dry initial body weight)/feed intake;

[(ii)]PRE = 100 x [(dry final body weight x final protein content) - (dry initial body weight x initial protein content)]/(feed intake x protein content);

[(iii)]ERE = 100 x [(dry final body weight x final energy content) - (dry initial body weight x initial energy content)]/(feed intake x energy content);

Values are means ± SD, within the same column, means with different letters are significantly different ($P < 0.05$).

3.5. Energy budget

Energy budgets were built from the adjusted means calculated for averaged feed intakes for each crab group (PMolt and IMolt) fed on the 3 diet treatments (Table 4.6). Average feed intakes were 0.65 $kJ.g^{-1}$of dry $iBW.d^{-1}$ and 0.45 $kJ.g^{-1}$of dry $iBW.d^{-1}$ for PMolt and IMolt crabs respectively. On average, the PMolt crab group consumed 30% more food than the IMolt group. The higher feed intake in the PMolt crab group led to obviously higher energy loss in feces (FE) and excretion (UE). Lost energy in ecdycis (SE) only concerned the PMolt crab group. Energy allocated for tissue growth (RE) in the PMolt group was 80 to 90% higher than for the IMolt crab group, even though feed intake was only 30% more for the PMolt group. Finally, maintenance energy (HE_m) was 16 to 21% lower in the PMolt than the IMolt crabs.

Whatever the crab group, the diet treatments had little effect on the energetic balance, except for FE which was significantly higher for treatment F100 (pairwise comparisions, $P < 0.01$), and for UE which was significantly higher for treatment F50S50 (pairwise comparisions, $P < 0.01$). Furthermore in the IMolt crab group, the energy allocated to tissue growth was significantly higher for animals fed on the diet S100 (pairwise comparisions, $P < 0.01$). The main differences in the adjusted energy budget calculated as % of energy intake between IMolt and PMolt crabs were observed for SE (Ecdysis), RE (Growth) and HE_m (Maintenance) (Table 4.7). Only the PMolt crab group was affected by SE which represents about 5% of total energy intake. Moreover, RE and HE_m were respectively 4 times higher and 1.7 times lower in the PMolt than the IMolt crab group. Low RE values of IMolt crabs reflect the slow tissue growth during this phase of molt cycle. On the other hand, the higher values of HE_m in IMolt crabs reflect energy storage in anticipation of the extra metabolic cost associated with the next molt which represents about 33% of the energy ingested by the IMolt crabs.

Diets had a significant effect on FE, EU, RE and HE_m. Energy lost in Feces (FE) and excretion (UE) were respectively higher for crabs fed on diets F100 and F50S50 (pairwise comparisions, $P < 0.01$). However, those diets marginally affected the global energy budget because FE and EU represent less than 15% of the total energy intake.

The percentage of energy allocated to growth (RE) was significantly higher (pairwise comparisions, $P < 0.01$) for crabs fed on S100 than F100 and F50S50 for both PMolt and IMolt crab groups (more than 5-7% of energy intake). In contrast, the percentage of energy allocated to maintenance (HE_m) of crabs fed on the S100 diet is lower than F100 and F50S50 diets for both PMolt (less than 4-6% of energy intake) and IMolt crabs (less 5-7% of energy intake). Finally, the experimental diets had no significant effect on energy lost in exuvia (SE).

Table 4.6. Adjusted mean values of energy budgets for PMolt and IMolt crabs fed on 3 different experimental diets.

Crab's groups	Experimental diets	Energy intake (IE)	Partitions of energy ($kj.g^{-1}$ of dry iBW. d^{-1})				
			Feces	Excretion	Ecdycis	Growth	Maintenance
		($kj.g^{-1}$ of dry iBW . d^{-1})	(FE)	(UE)	(SE)	(RE)	(HE_m)
PMolt crabs	F100 (n = 7)	0.65	0.075 ± 0.004^{a}	0.018 ± 0.003^{a}	0.031 ± 0.000^{a}	0.213 ± 0.044^{a}	0.308 ± 0.042^{a}
	F50S50 (n = 7)		0.068 ± 0.002^{b}	0.022 ± 0.003^{b}	0.032 ± 0.000^{a}	0.204 ± 0.035^{a}	0.320 ± 0.034^{a}
	S100 (n = 7)		0.067 ± 0.002^{b}	0.016 ± 0.001^{a}	0.032 ± 0.000^{a}	0.246 ± 0.052^{a}	0.282 ± 0.050^{a}
IMolt crabs	F100 (n = 5)	0.45	0.052 ± 0.004^{c}	0.009 ± 0.002^{c}		0.024 ± 0.004^{b}	0.367 ± 0.009^{b}
	F50S50 (n = 5)		0.043 ± 0.003^{d}	0.013 ± 0.002^{d}		0.033 ± 0.014^{b}	0.376 ± 0.011^{b}
	S100 (n = 6)		0.047 ± 0.003^{d}	0.010 ± 0.001^{c}		0.056 ± 0.006^{c}	0.345 ± 0.012^{c}

Values are means ± SD. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets within each crab group.

Table 4.7. Energy budgets in proportion of energy intake (IE): fecal production (FE), excretion (UE), ecdysis (SE), growth (RE) and maintenance (HEm) for PMolt and IMolt crab groups fed on three diet types: F100, F50S50, and S100.

Crab's groups	Experimental diets	% IE				
		FE	UE	SE	RE	HE_m
PMolt crabs	F100 (n = 7)	11.74 ± 0.52^{a}	2.83 ± 0.52^{a}	4.93 ± 0.44^{a}	33.17 ± 6.98^{a}	47.90 ± 6.58^{ae}
	F50S50 (n = 7)	10.59 ± 0.38^{b}	3.39 ± 0.45^{b}	5.01 ± 0.27^{a}	31.74 ± 5.65^{a}	49.84 ± 4.90^{a}
	S100 (n = 7)	10.44 ± 0.48^{b}	2.48 ± 0.13^{a}	4.98 ± 0.23^{a}	38.27 ± 7.57^{b}	44.16 ± 8.80^{be}
IMolt crabs	F100 (n = 5)	11.53 ± 0.79^{a}	2.03 ± 0.4^{ce}		5.31 ± 0.79^{c}	81.49 ± 2.02^{cf}
	F50S50 (n=5)	9.62 ± 0.58^{c}	2.78 ± 0.35^{d}		7.36 ± 3.05^{c}	83.33 ± 2.45^{c}
	S100 (n=6)	10.43 ± 0.70^{cb}	2.33 ± 0.33^{ae}		12.33 ± 1.27^{d}	76.45 ± 2.68^{df}

Values are means ± SD. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets which then being compared within and between crab groups.

4. Discussion and conclusion

Feed for aquaculture requires large quantities of ingredients from the sea and this sector is the largest consumer of fishmeal among the animal husbandry subsectors (Shepherd & Jackson, 2013). Indeed, fishmeal is the primary source of protein and energy in most aquafeeds and accounts for up to 75% of total feed formulation (Tacon, 1999). In 1989, fishmeal used in aquaculture accounted for only 10% (FAO, 2000), this had dramatically increased to 73% by 2010 (Shepherd & Jackson, 2013).

Production of carnivorous species, promoted in aquaculture, particularly marine crustaceans, marine finfish and salmonids, requires a lot of fishmeal (Rana et al., 2009). The cost of fishmeal is increasing over time as a result of the uncertainty of availability (Sookying et al., 2013). Hammerrsmith Marketing Ltd (2008) indicated that the price of fishmeal increased about 62.9% from August 2005 to June 2008. In such conditions, aquafeed can account for as much as 40-60% of the production cost (Hertrampf & Pie-dad-Pascual, 2000). In this framework, partially or completely shifting from fishmeal protein source to more sustainable protein sources in formulated diet is a priority for sustainable mud crab aquaculture development (Christense et al., 2004; Tuan et al., 2006).

In this study, tissue growth of the crabs was considered. This approach is fundamental as water content of crustaceans is high and varies dramatically through their molt cycles. Indeed, in crab *S. serrata*, free water content reached 88% in postmolt and decreased to 66 % in intermolt and premolt stage (Heasman,1980). Two tissue growth periods during a molt cycle of the juvenile crab *S. serrata* were identified in our experimental conditions: a postmolt period which lasted between 18 and 22 days and an intermolt period which lasted 32 days. Around 80% of tissue growth occurred during postmolt and the remaining 20% during intermolt. Thus, a fast tissue growth occurred after the molt until early intermolt stage. This fast tissue growth period was followed by a slow tissue growth which lasted over intermolt and premolt period. This result is consistent with findings of Heasman (1980) on growth study of mud crab juveniles (4.25-7.25 cm carapace width). The author indicated that the proportion of organic matter and soluble mineral salt rapidly increased around 80% during the postmolt stage (after ecdysis to C_3 stage), and the other 20% was gained during the remaining period covering molt stages C_4 to D_4.

In order to compare our results with other studies we had to transform the data of the later to express them in dry weight based on water content of crabs at relative stable stage. The Unnirishnan & Paulraj (2010) study on juvenile crabs *S.serrata* (iBW = 0.25 g) found that the optimum daily protein (0.065 g. $g^{-1}$of dry body.d^{-1}) and energy (2.351 kj. g^{-1} of dry body.d^{-1}) intakes were three times higher than that of the present study. These differences could be explained by the 10 times bigger crabs used in our study. Indeed, it is well admitted that protein and energy requirements decrease in bigger animals: for instance, 2 g and 10 g shrimps *L. vannamei* required daily respectively 0.014 and 0.007 $g.g^{-1}$ of digested protein and 0.558 and 0.298 $kj.g^{-1}.d^{-1}$ of digestible energy (Lupatsch et al., 2008).

Many plant-based ingredients are used as a source of proteins for aquafeed. Among them, soybean meal (SBM) is well known for its relatively high protein content, well balanced amino acid profile, and stable market supply, as well as its reasonable cost (David & Arnold, 2000; Amaya et al. 2007a,b). Chen et al. (1994) reported that up to 33% fishmeal protein could be replaced by soybean cake for juvenile mitten crab (*Eriocheir sinensis*) without reducing growth. Recently, Luo et al. (2011) found that 30% of soybean meal and rapeseed meal mixture (1:1 ratio) could replace 40% FM for Chinese mitten crab without impairing growth performance and feed utilization. However, the inclusion of SBM in fish diets has been limited by relatively high levels of heat stable antinutritional and antigenic factors including protease inhibitors, oligosaccharides (e.g., stachyose, raffinose), saponins, isoflavones, phytate, and tannins (Francis et al., 2001). Although heat and enzyme treatments can neutralize some of these compounds, they are still a significant problem when including SBM in aquaculture feeds (Gatlin et al., 2007). While, soy protein concentrate (SPC), although more expensive, does not contain the alcohol-soluble fraction present in SBM and has a higher essential amino acid concentration. It has also greater nutrient digestibility compared with SBM and can be included at much higher concentrations in diets of piscivorous marine species (Bureau et al., 1998, USSEC 2008).

In our study the juvenile mud crabs digested soybean protein concentrate as well as fishmeal. Moreover, the two ingredients exhibited high digestibility coefficients for dry matter, protein and energy. These results are consistent with previous studies on mud crabs reporting high soybean meal digestibility at an inclusion level of up to 45% with ADC values for ADMD, ACPD and AGED ranging from 80.4-95.7%, 91.7-97.1% and 88.6-97.9%, respectively (Catacutan *et al*., 2003; Tuan *et al*., 2006; Truong et al., 2008; Truong et al., 2009). Furthermore, Truong et al. (2008) showed that crude protein from soybean meal was

better digested by mud crabs than the other plant ingredients tested (canola, lupin and cotton seed meal).

Besides the high protein digestibility of SPC for the mud crab, our study showed that SPC can also totally replace fishmeal as a protein source in the diet without affecting the tissue growth of juvenile *S. serrata*. The SPC diet, without fishmeal, even led to a better tissue growth rate during intermolt (IMolt crab group) and higher efficiency values of feed conversion, protein retention and energy retention. This means that better utilization of SPC for mud crab juveniles during intermolt period. These results are supported by other authors who worked on crustaceans like shrimps. Suárez et al. (2009) showed that shifting from 80% fishmeal (FM) to soybean and canola meal did not affect the growth rate of *Litopenaeus vannamei*. Previously, Forster et al. (2002) replaced FM by SPC in diets for *L. vannamei* and suggested that SPC, with a methionine supplement, can substitute up to 75% of fishmeal. More recently, Bauer et al. (2012) showed that the replacement of all the fishmeal by alternative ingredients, soy protein concentrate and microbial floc, did not significantly affect growth, feed conversion ratio, specific growth rate, protein efficiency ratio or survival of the shrimp *L. vannamei*.

Opposite trends have also been observed. Lim and Dominy (1990) reported that a maximum of 40% of marine protein sources (fishmeal, shrimp head meal and squid meal) could be replaced by soybean meal in diets, but that higher FM replacement levels resulted in lower growth rates of the shrimp *L.vannamei*. Similarly, Paripatananont et al. (2001) showed that the maximum FM replacement levels by SPC without affecting the growth rate of the shrimp *Penaeus monodon* was 50%. These authors also reported that high concentrations of soy-based products in feed negatively affected the palatability of the diets for shrimps. However, in our study, no influence of SPC level was noted on feed intake. Indeed, all experimental diets contained the same amount (20%) of crustacean meal which is highly palatable for crabs.

The energy budgets of juvenile crabs determined in the present study are based on the average energy intake (IE) for each crab group (PMolt and IMolt). Adjusted values, for the corresponding average energy intake, were determined for energy lost in feces (FE), excretion (UE), exuveae (SE), and energy retained as growth (RE). All the data was obtained from the experiment except: (i) RE which was estimated considering the initial crab energy content calculated from the relationship defined between dry and fresh weight ($Y = 0.3229\ X +$

0.4246; R^2 = 0.96, n = 30); and (ii) energy for maintenance (HE_m) which was estimated indirectly from the equation HE_m = IE – (FE + UE +SE + RE); as theoretical energy budgets should be balanced. Consequently, these estimations include all errors associated with all directly calculated terms.

As the experimental unit in this study was the individual crab, it has been possible to establish the energy budget for each individual adjusted for an average amount of energy intake. In the energy budgets of both PMolt and IMolt crab groups, the proportions of energy intake lost in feces and ammonia excretion were small and similar for the three feed types tested. Generally, the level of nitrogenous excretion is of little importance in the bio-energetic flow for crustaceans (Villarreal, 1991). Furthermore, excretion contributed to no more than 3% of the energy budget of fish (Kaushik, 1998; Bureau et al., 2002) which is similar to the present study on crabs. The small proportion of FE and UE in the energy balance of the crabs in our study corresponds to those found by other authors for fishes and shrimps (Villarreal, 1991; Xie et al., 1997; Sun et al., 2006; Shun et al., 2007; Shun & Chen, 2009).

Based on NRC (2011), HE_m is defined as the amount of maintenance energy required for an animal to maintain zero energy for growth or equilibrium between IE and energy expenditure (FE, UE, SE, RE).

In the present study, we calculated HE_m in two ways: the first resolving the energy balance equation and the second from the model linking SGR and feed intake when growth is null. Resolving the energy balance equation, HE_m values were 0.304 and 0.358 kj. g^{-1} of dry iBW.d^{-1} for PMolt and IMolt crabs, respectively. These HE_m values included i) the heat increment of feeding (H_iE), ii) the formation and excretion of metabolic wastes; iii) the transformation and inter-conversion of the substrates and their retention in tissues; and iv) the molting process (NRC, 2011).

The HE_m value estimated from the model linking tissue growth, $SGR_{d,}$ and feed intake for zero growth (RE = 0), was 0.179 ± 0.003 kj.g^{-1} of dry iBW.d^{-1} (59.5 kj.$kg^{-1}$of wet iBW.d^{-1}) for PMolt and IMolt crabs which is about half the value of the HE_m estimated by the energy balance equation. When using this model, a lower level of feed intake (0.012 g.$g^{-1}$of dry iBW.d^{-1}) was considered compared to the estimate from the energy balance (0.042 g.$g^{-1}$of dry iBW.d^{-1} for PMolt crabs and 0.03 g.$g^{-1}$of dry iBW.d^{-1} for IMolt crabs) because meal size affects the heat increment of feeding (McGow & Curtis, 2013).

The maintenance energy value of the shrimp *Lipopenaeus vannamei* (345 kj.wet of $kg^{-1}.d^{-1}$) determined from the growth-meal size model (Lupatsch et al., 2008) was about 6 times higher than the HE_m value for crabs in our study. This difference could be explained by the fact that the mud crab is much less active than the shrimp, particularly in captivity.

Mud crab juvenile consumed a larger part of energy for maintenance than for growth, regardless of crab tissue growth period during the molt cycle and experimental diets. This result is consistent with other studies on fish: Nile tilapia, *Oreochromis niloticus* (Xie et al., 1997); Cobia, *Rachycentron canadum* (Sun et al., 2006, Shun & Chen., 2009); yellow grouper, *Epinephelus awoara* (Shun et al., 2007). However, other studies on crayfish showed a higher proportion of intake energy transferred to growth than to maintenance. Thus, *Cherax tenuimanus* and *C. destructor* could convert respectively 41.8-57.5% and 50% of ingested energy into growth (Villarreal, 1991; Woodland, 1969).

One striking result of this study on juvenile mud crabs is the estimated portion of energy intake chaneled to molt. Indeed, the energy for molting included energy lost in exoskeleton (SE) plus the energy required for molting which in our study was a portion of the maintenance energy (HE_m). Our study showed that crabs during intermolt exhibit a higher maintenance energy ratio (up to 83.33 ± 2.45%) than crabs during postmolt period (up to 49.84 ± 4.9%). The difference between the maintenance energy ratios of the crabs from the two growth periods showed that 33% of energy intake is stored and required for molting. This proportion included 5% lost with the exoskeleton and the remaining 28% for extra metabolism cost during ecdysis. This result is confirmed by the biochemical analysis indicating significantly higher lipid content in crabs during intermolt period. Lipid accumulation is done to anticipate ecdysis which requires energy, notably for osmoregulation during molting (GonzAlez-Gurriarhn et al., 1995; Tian et al., 2012). Similarly, another study on crab (*Eriocheir sinensis)* showed that lipids were accumulated in a digestive gland before molting, reaching a peak at late premolt and then decreasing during postmolt (Tian et al., 2012). A study on the shrimp *Penaeus semisulcatus* showed the same phenomenon, with lipid drops accumulating in hepatopancreatic R-cells from the intermolt to the premoltstage. Conversely only a few lipid drops were observed during the postmolt stage *(*Al-Mohanna & Nott, 1989).

The proportion of energy intake chaneled to tissue growth (RE) was lower during intermolt (12.32 ± 0.31%) than postmolt period (37.39 ± 6.73%). Thus, two interrelated but

antagonist physiological phenomena occurred: ecdysis is necessary for crab growth but it occurs at its expense.

Our study on the juvenile crab *S. serrata* led to three main conclusions:

- Soy protein concentrate can be used as a main source of dietary protein for crab juveniles in captivity.
- Soy protein concentrate sustained good tissue growth and good feed utilisation by the crab juveniles, in some cases better than fishmeal.
- When compared with fishmeal, soy protein concentrate did not affect the global energy balance of the crab juveniles.

Our results provide useful information for artificial feed formulation with SPC as main protein source and may also suggest the benefit to study nutritional requirement of mud crabs on the dry weight basis which is more accurate regarding the huge free water change during a molt cycle of this species. Finally, the methodology proposed will allow carrying out some specific nutritional experiments in shorter period without the necessity to wait for several molt cycles.

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4.3 - CONCLUSION

The present study allowed to better understanding of somatic growth during postmolt and intermolt phases and their corresponding energy balance of the juvenile crab *S. serrata*. To our knowledge, no equivalent results exist in the scientific literature. During postmolt and intermolt phases, somatic growth of juvenile crabs represented 80% and 20% respectively. Interestingly, energy is stored during intermolt period, mainly as fat, by anticipation of the next molt. Thus, the energy share associated with molting (loss of exuviae and energy lost mainly for osmoregulation) was estimated at 33% of total energy intake. This proportion is surprisingly extremely important and this result rises the question of the most suitable form of dietary energy to cover the energy expenditure for ecdysis? According to our results and those of several authors (Al-Mohanna & Nott, 1989; GonzAlez-Gurriarhn et al., 1995; Tian et al., 2012), it seems that lipids are used, first and foremost, as a source of energy for extra metabolism associated with ecdysis. Moreover, it was shown that juvenile crabs utilize higher dietary lipid levels more effectively than other crustaceans and shrimps in particular (Unnikrishnan et al., 2010).

The practical result of this study is a confirmation of the nutritional quality of the SPC as dietary proteins source for the juvenile mud crab. The SPC can, indeed, replace totally the fish meal as the main source of proteins, abstraction made by the other nutriments present in these two raw materials (lipids, carbohydrates, minerals).

These results lead us to encircle better the phenomenon of the somatic growth and show that the SPC is a good protein source for the mud crab. On the basis of the methodology used and the results gathered the next step is to determine the optimum dietary SPC and proteins incorporation in feed for the mud crab. This issue will be studied and discussed in the next chapter of this thesis.

5 - CHAPTER V: DIETARY SOY PROTEIN CONCENTRATE (SPC) UTILIZATION BY JUVENILE MUD CRAB, *SCYLLA SERRATA*

5.1 - INTRODUCTION

The objective of the previous study was to compare two sources of proteins on feed efficiency growth and energy budget of juvenile crab *S. serrata* (B.W. = 22.1 ± 8.46 g). Our results indicated that soy protein concentrate (SPC) can totally replace the fish meal (FM) as the main source of dietary proteins for juvenile crabs. Therefore, for the continuation of our study, we will focus only on the SPC as main source of dietary protein and assume that it is better for all the reasons evoked previously, to develop a practical diet without fish meal (FM).

In the diet S100 without FM (see Chapter IV), the dietary SPC proportion was of 57% which brought 75% of total dietary proteins. The remaining proteins were provided by crab meal (8%) and wheat (1.4%). Hence, the total dietary protein concentration of diet S100 was 48% which is higher than 40% determined as the optimum dietary proteins for juvenile crab (BW = 11.18 ± 0.66g) by Catacutan (2002).

Therefore, the aim of the present study is to find the optimum level of SPC, and thus dietary protein proportion in diet for best crab growth. For this purpose, we will evaluate the response of juvenile crabs to five isoenergetic diets with graduate levels of dietary SPC and respective dietary proteins, on feed intake and efficiency, growth (molt frequency and somatic growth) and energy budget.

The methodology used for this investigation will continue to study the growth considering two parameters: molt frequency and tissue gain measured as the increase in dry weight. We will also confirm that the free water content of crabs is extremely variable during the molt cycle and that it is fundamental to take into account this parameter when interested in tissue growth. Most of the previous studies have considered growth rate as gains in fresh weight which is very dependent on the water content and the molt stage of the animal.

5.2 - FEED INTAKE, MOLT FREQUENCY, TISSUE GROWTH, FEED EFFICIENCY AND ENERGY BUDGET DURING A MOLT CYCLE OF MUD CRAB JUVENILES, *SCYLLA SERRATA,* FED ON DIFFERENT PRACTICAL DIETS WITH GRADED LEVELS OF SOY PROTEIN CONCENTRATE AS MAIN SOURCE OF PROTEIN.

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Key words: Juvenile crab, *Scylla serrata*, tissue growth, feed efficiency, protein requirement, fishmeal, soy protein concentrate, energy budget.

List of symbols and abbreviations

ACPD	Apparent Crude Protein digestibility
ADMD	Apparent Dry Mater Digestibility
AGED	Apparent Gross Energy digestibility
FCR	Feed Conversion Ratio
PER	Protein Efficiency Ratio
SPC	Soy Protein Concentrate
VFI	Voluntary Feed Intake

Abstract

There has been growing interest in the development of mud crab aquaculture in New Caledonia. However, for this to become established at a commercial level, a cost-effective formulated feed based on internationally-available ingredients needs to be developed. We have evaluated the optimal dietary protein content for juvenile crabs, *Scylla serrata* using a series of diets with a protein content ranging from 27% to 49% and soy protein concentrate (SPC) as the main protein source. For this purpose, 54 individually housed crabs were allocated to five dietary treatments (n = 10 or 11). The crabs were fed *ad libitum*, for 81 days with the allocated diets. The apparent digestibilities of dry mater, crude protein and energy were high (96.2-97.3%), irrespective of the diet. The voluntary feed intake (VFI) of crabs widely varied from 46 to 220 $g.kg^{-1}$of fresh initial body weight per week ($iBW.week^{-1}$) whatever the diet. However, SPC intake and protein intake increased significantly with dietary protein content up to the diet with 40% crude protein, but did not increase further with diets containing 44% and 49% crude protein. The cumulative molts were strongly affected by the VFI levels or energy intake and also, to a lesser extent, by the levels of SPC or protein in diets. Two phases in tissue gain were observed after ecdysis: an initial deposition phase lasting around 30 days followed by a plateau which lasted until the next molt. The daily tissue growth was 16.5% of dry body weight (dry BW) one day after ecdysis and dramatically decreased to 3.6% of dry BW over the first 10 days, then decreased more slowly to the minimum value of 1.3% of dry BW over the next 70 days. During the course of experiment, the best growth (tissue growth and molt frequency) and the best feed efficiency (FCR, PER, retention of proteins and lipids) were obtained with crabs fed on the diet with 40% crude protein. This result was confirmed by a bioenergetic study which showed significantly higher allocation of the energy intake for growth (RE) of crabs fed on diet 40% crude protein. Finally, under our experimental conditions, 1 kg of juvenile crabs required 6.5 ± 1.1 g of protein per day. This level was obtained with the diet SPC-42 that contained 40% of protein of which almost three quarters were derived from SPC. Two hypotheses are proposed to explain the negative effect of high level of SPC or protein on growth and feed efficiency for crabs fed on in diets containing 52% and 60% SPC.

1. Introduction

The mud crab has been domesticated and farmed for many years in Asia, particularly in Vietnam where production is increasing quickly *(www.shrimpnews.com).* There is an unmet demand for mud crabs and this has led to over-exploitation of wild populations in many areas. Difficulties in obtaining wild caught juveniles for farming operations, and concerns about further over-exploitation have led to major investments in the development of new hatchery techniques (Allan & Fielder, 2003). For the past few years, techniques for breeding in captivity and larval rearing have been developed allowing production to triple in 5 years (from 10,000 tons in 2004 to 30,000 in 2009) (John & Paul, 2012). However, the substantial crab farming operations which now exist throughout Southeast Asia are still mainly based on wild caught crablets (Allan & Fielder, 2003) and animals in captivity are fed with excessive amounts of trash fish which contaminate the rearing water and lead to high mortality rates (Linder, 2005).

In New Caledonia, there is a strong political wish to diversify aquaculture, which until recently, has been based on blue shrimp (*Litopenaeus stylirostris*) farming. Among the different options available, the mud crab, *Scylla serrata,* is regarded as having great potential for aquaculture through its farming characteristics and its high economic value. However, the possible development of crab farming in New Caledonia will require the development of an artificial feed that can be produced from selected and controlled raw materials that are available on the international market.

Therefore, it is essential to assess the nutritional requirements of *S. serrata*. A few studies have been carried out on this subject. Sheen & Wu (1999) showed better utilization of lipids by the crab than by shrimp through a study in which they measured the growth response of juvenile crabs that has been fed diets with a range of inclusion levels of a mixture of cod liver oil and corn oil. Later, 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. Furthermore, a series of studies have been carried out to measure the digestibility of several potential ingredients for formulated feed for crabs (Catacutan et al., 2003; Truong et al., 2009). These results showed that crabs were able to digest many different ingredients, in particular fibre and protein from plants. Finally, the optimal protein inclusion level in the diet has been the subject of two studies (Catacutan, 2002; Unnikrishnan & Paulraj,

2010). Both studies concluded that the optimal protein concentration (a mixture of fish meal and soybean meal) in the feed for the best growth rate is between 30% and 47%.

Feed for aquaculture generally requires large quantities of ingredients from the sea and this sector is the largest consumer of fishmeal among the animal husbandry subsectors (Shepherd & Jackson, 2013). Indeed, fishmeal is the primary source of protein and energy in most aquafeeds and accounts for up to 75% of the mass of total aquaculture feed (Tacon, 1999). In 1989, fishmeal used in aquaculture accounted for only 10% of global production (FAO, 2000), this had dramatically increased to 73% by 2010 (Shepherd & Jackson, 2013).

Production of carnivorous species, promoted in aquaculture, particularly marine crustaceans, marine finfish and salmonids, requires a lot of fishmeal (Rana et al., 2009). The cost of fishmeal is increasing over time as a result of the increased competition for available supplies (Sookying et al., 2013). Hammersmith Marketing Ltd (2008) indicated that the price of fishmeal increased by about 62.9% from August 2005 to June 2008. In these conditions, feed can account for as much as 40-60% of the cost in aquaculture production (Hertrampf & Pie-dad-Pascual, 2000). In this framework, partially or completely shifting from a fishmeal protein source to more sustainable protein sources in formulated feeds is a priority for sustainable mud crab aquaculture development (Christense et al., 2004; Tuan et al., 2006).

Many plant-based ingredients are used as a source of proteins in aquafeeds. Among them, soybean meal (SBM) is well known for its relatively high protein content, well balanced amino acid profile, and stable market supply, as well as its reasonable cost (David & Arnold, 2000; Amaya et al., 2007a,b). Chen et al. (1994) reported that up to 33% fishmeal protein could be replaced by soybean cake for juvenile mitten crab (*Eriocheir sinensis*) without reducing growth. Recently, Luo et al. (2011) found that 30% inclusion of soybean meal and rapeseed meal mixture (1:1 ratio) could replace 40% fishmeal in diets for Chinese mitten crab without impairing growth performance and feed utilization. However, the inclusion of SBM in fish diets has been limited by relatively high levels of heat stable antinutritional and antigenic factors including protease inhibitors, oligosaccharides (e.g., stachyose, raffinose), saponins, isoflavones, phytate, and tannins (Francis et al., 2001). Although heat and enzyme treatments can neutralize some of these compounds, they are still a significant problem when including SBM in aquaculture feeds (Gatlin et al., 2007). Soy protein concentrate (SPC), although more expensive than SBM, does not contain the alcohol-soluble fraction present in SBM and has a higher essential amino acid concentration. It also has greater nutrient digestibility compared

with SBM and can be included at much higher concentrations in diets of piscivorous marine species (Bureau et al., 1998, USSEC 2008). Recently we showed that SPC is well digested and able to replace the fishmeal as the main source protein for crab juveniles *S.serrata* (Nguyen et al., submitted).

Most of the previous studies have considered growth rate as gains in fresh weight. Fresh weight changes follow a basic pattern through the molt cycle, i.e. large and abrupt increases associated with rapid water uptake at ecdysis; further moderate gains associated with carapace mineralization and tissue growth during postmolt and relative stabilization of fresh weight during intermolt until the onset of the successive ecdysis (Heasmen, 1980). Increase in tissue mass, on the other hand, is a continuous process occurring through the molt cycle (Freeman, 1990). The tissue growth can be measured as the increase in dry weight as tissues lose water in direct proportion to their gain in dry mass (Passano, 1960).

The present study is complementary to the previous study which looked for the ability of SPC to replace fishmeal as main protein source for *S. serrata.* In this paper we report on the response of juvenile crabs *S. serrata* to graded levels of soy protein concentrate and respective dietary proteins on growth (somatic growth and molt frequency), feed efficiency (FCR, FCE, PRE...) and energy budget.

2. Materials and methods

2.1. Crabs and holding facilities

Juvenile crabs, *S. serrata,* were collected from the mangrove habitat surrounding the experimental station (21°51’50″ S, 166°3’0″ E) in the Boulouparis district, New Caledonia. Seventy juvenile crabs were caught using a hand net and transferred to the laboratory and acclimated into 8 circular composite tanks (capacity 500 L) for one week prior to the beginning of the experiment. The crabs were examined to identify their molt stage using the criteria and methodology described by Drach & Tchernigovtzeff (1967), Freman et al. (1987) and Heasman (1980). Fifty four of the crabs were identified as being in intermolt stage and were selected for the experiment. These crabs were randomly and individually assigned to fifty four experimental rectangular polyethylene tanks (30 x 20 x 30 cm) covered with black lids. After being dried in soft paper and cotton cloth, each crab was weighed to the nearest 0.01 g and its carapace width was measured to the nearest 0.01 mm.

Each experimental tank was continuously supplied with seawater running from a polyethylene reservoir (2000 liter capacity) at the rate of 0.19 $L.min^{-1}$. The water pumped from the lagoon was filtered through a 25 µm net bag into the reservoir. The temperature was automatically controlled using a heater. The temperature in the experimental tanks was measured every 3 hours using an automatic recording probe. During the experimental period, water environmental parameters were maintained with: $T^oC = 21.5 \pm 2.5$, S ‰ = 34.0 ± 1.5, pH = 7.97 ± 0.09 and $O_2 = 4.48 \pm 0.16$ mg L^{-1}.

2.2. Diet preparation and composition

The crabs were fed on five different experimental diets (SPC-12; SPC-32; SPC-42; SPC-52; and SPC-60) formulated with graded levels of SPC as main source of protein (Table 5.1A). These diets comprised the experimental treatments. The diets were produced in the laboratory using the following procedure: the dry ingredients were ground up in a grinder (Retsch®) with a 1 mm screen. The meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca®) until the consistency was suitable for pelleting. The mixture was then extruded through a 3 mm die in a meat grinder. Pellets were steamed for 15 min and stored at -20 °C before use. The ingredient compositions and proximate nutrient contents of experimental diets are shown in Table 5.1A. The amino acid composition of each diet was computed from the amino acid content of the ingredient analysis (indicated in Table 2.3 in chapter II) and is presented in Table 5.1B.

Table 5.1A. Ingredients and proximal composition of five experimental diets.

	Experimental diets				
	SPC-12	SPC-32	SPC-42	SPC-52	SPC-60
Composition (%)					
Soy protein concentrate	12.00	32.00	42.00	52.00	60.00
Crab meal	20.00	20.00	20.00	20.00	20.00
Wheat Flour	53.50	35.00	25.50	16.00	8.00
Fish oil	4.00	4.00	4.00	4.00	4.00
Soy oil	2.50	1.00	0.50	0.00	0.00
Gluten	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00
vitamin C[1]	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50
Trace elements[3]	1.00	1.00	1.00	1.00	1.00
Analysis (dry matter basis)					
Crude protein (%)	26.87	35.52	39.69	44.15	49.17
Lipid (%)	6.30	4.80	4.62	4.36	4.43
Ash (%)	13.95	14.54	22.36	21.80	27.43
Gross energy (MJ. kg^{-1})	17.96	17.26	17.87	18.61	18.44
P/E ($g.Mj^{-1}$)	16.79	20.58	22.13	23.72	25.56

[1]Vitamin C: Rovimix Stay-C 35 from DSM Cie;

[2]Vitamin premix for shrimp from BEC feed solutions PTY, LTD – ingredients: vitamin AD3 1000/200, vitamin B1 98% Thiamine, Mononitrate, vitamin B2 Riboflavin 80%, vitamin B3 99% Niacin, vitamin B5 98% D-Calpan, vitamine B6 98% Pyridoxine, Vitamin B9 97% Folic acid, vitamin D3 500, vitamin E 50 ADD, vitamin K3 43.7%;

[3]Trace elements from SICA Cie (Goodman Fielder).

Table 5.1B. Amino acid composition (%, on dry matter basis) of five experimental diets.

	Experimental diets				
	SPC-12	SPC-32	SPC-42	SPC-52	SPC-60
EAA					
Arginine	1.39	2.22	2.63	3.04	3.37
Histidine	2.26	2.54	2.68	2.82	2.93
IsoLeucine	0.98	1.48	1.73	1.97	2.17
Leucine	1.61	2.39	2.78	3.16	3.47
Lysine	0.91	1.64	2.00	2.36	2.65
Methionine	0.37	0.52	0.59	0.66	0.72
Phenylalanine	1.16	1.69	1.96	2.22	2.43
Threonine	0.82	1.25	1.47	1.68	1.85
Tryptophan	0.29	0.43	0.50	0.57	0.63
Valine	1.12	1.64	1.90	2.16	2.37
NEAA					
Alanine	0.95	1.45	1.69	1.94	2.13
Aspartic Acid	1.75	3.13	3.82	4.51	5.06
Cystine	0.35	0.49	0.56	0.63	0.68
Glutamic Acid	5.23	6.89	7.71	8.53	9.17
Glycine	0.99	1.44	1.66	1.89	2.06
Proline	1.80	2.21	2.41	2.61	2.76
Serine	1.17	1.70	1.97	2.23	2.44
Tyrosine	0.77	1.13	1.32	1.50	1.64

EAA: essential amino acids; NEAA: nonessential amino acids.

2.3. Experimental design

2.3.1. Growth trial

At the beginning of the experiment, the crabs were starved for 48 hours and then weighed and measured. The initial average body weight and carapace size of the crabs was 20.43 ± 9.81 g and 4.92 ± 0.81 cm, respectively. As each crab was held separately, the experimental unit in this study was the individual. Fifty four tanks with individual crabs were

assigned to the five different dietary treatments. Thus, 11 tanks were randomly assigned to each treatment (n = 11), except for the treatment with diet SPC-12 (n = 10). The crabs were fed once each day *ad libitum*. The daily pre-weighed feed allocation for each tank was delivered at 6:00-7:00 am. Three hours after each meal, the uneaten feed was siphoned off, dried 24 hours at 60 °C and weighed.

The leaching rate (dry matter loss of the pellet in the water) was determined by measuring remaining dry matter in a weighed sample of each diet after 3 hours immersion. Then the amount of voluntary feed intake (VFI) for each crab was obtained following this equation:

$$VFI = dF - L - uF$$

Where: VFI = voluntary feed intake; dF = distributed Feed; L = leaching after 3 hours and uF = unconsumed Feed.

During the experimental period, the date of ecdysis, carapace width and live body weight were recorded for each crab after molting, and then they were transferred back to the tanks. The exuviae were collected and weighed before and after drying at 80 °C for 24 h, then kept at -20 °C for energy analysis. The trial was maintained for 81 days. At the end of the experiment, all of the crabs were left unfed for 48 h and then weighed, measured, dried at 80 °C for 48 h and kept at -20 °C before biochemical and energy analysis.

2.3.2. Digestibility trial

All diets contained 1% chromic oxide (Cr_2O_3) as an inert maker to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED). The chromium content of diets and fecal material used to calculate apparent digestibility values were determined using the method described by Furukawa & Tsukahara (1966). Apparent digestibilities of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) were calculated following the equations:

$$ADMD = 100 - 100 \times (\%Cr_2O_3 \text{ in feed} / \%Cr_2O_3 \text{ in feces})$$

The digestibility of crude protein (ACPD) or gross energy (AGED) was determined using the formula ACPD or AGED = 100 - 100 x (%Cr_2O_3 in feed / %Cr_2O_3 in feces) x (% protein or MJ. kg^{-1} energy in feces/% protein or MJ.kg^{-1} energy in feed).

2.3.3. Nitrogenous excretion trial

Three crabs from each treatment were individually fed *ad libitum* with their assigned experimental diet. Immediately after the meal, each crab was transferred into another individual aquarium containing a fixed volume of water (10 L). Ammonia-N excretion in each aquarium was measured twice: before and 24h after transferring the crab, using the method described in Grasshoff (1983). The ammonia-N value was converted into energy by multiplying by 24.83 $kJ.g^{-1}$ (Elliot, 1976). The potential loss of nitrogenous compounds through bacterial action or diffusion was quantified by setting controls without crabs. The values of ammonia-N concentration of this control treament were used to adjust determined nitrogenous excretion. During the experimental period, this excretion trial was repeated every 25 days to get the average value for ammonia-N excretion.

2.4. Biochemical analysis

The proximate compositions of diets, feces, initial and final crabs were determined at the Invivo laboratory, Vietnam according to the methods of AOAC (1995). Moisture contents of crabs and feed were determined after oven-drying to a constant weight at 105 °C. Each sample was combusted at high temperature in pure oxygen then the nitrogen content was measured by thermal conductivity detection and converted to equivalent protein content by an appropriate numerical factor, crude protein % = %N * 6.25. Crude lipid content was measured by solvent extraction with petroleum ether. Ash content was determined following combustion in a muffle furnace at 550 °C for 8-10 h. Energy contents of diets, fecal material, exuviae and crabs were determined by calorimetric bomb (Parr® 6200, USA, calibrated by benzoic acid) at Ifremer laboratory, New Caledonia.

2.5. Definitions, calculations and statistics

2.5.1. Monitoring the molting

Cumulative molting (CM in %) can be calculated using the following formula:

$$CM = (\sum_{i-0}^{n} Mi/Cr) \times 100$$

Where i = day i; Mi = number of molts on day i; Cr = number of crabs in each treatment.

2.5.2. Growth measurements

Growth parameters of crabs in this study were determined as: tissue growth, tissue gain and nutrient gains (protein, lipid and ash).

Tissue growth after ecdysis (% of dry BWa.e.d^{-1}) or during the course of experiment (% of dry iBW.d^{-1}) and tissue gain (% of dry BWa.e.) were measured on the basis of the increase in dry weight (Passano, 1960):

Tissue growth after ecdysis = 100 x ((fBW - BWa.e.) - ash gained) / (BWa.e. x days after ecdysis)

Tissue growth during the course of experiment = 100 x ((fBW - iBW) - ash gained) / (iBW x days of experiment)

Tissue gain = 100 x ((fBW - BWa.e.) - ash gained) / BWa.e.)

Protein, lipid or ash gain = 100 x (protein, lipid or ash retained/ BWa.e.),

where, iBW, BWa.e. are dry initial body weight and dry body weight after ecdysis, respectively; fBW is final dry body weight.

2.5.3. Feed efficiency

Feed conversion ratio (FCR) was calculated as:

FCR = dry voluntary feed intake/dry weight gain

Protein efficiency ratio (PER) was calculated as:

PER = dry weight gain/total protein ingested

Protein, lipid or energy retention was calculated as:

Protein, lipid or energy retention (%) = 100 x (protein, lipid or energy retained/total protein, lipid or energy intake).

2.5.4. Energy budget assessment

The partitions of the energy budget suggested by the National Research Council (NRC 2011) were adopted in this study with minor modification in order to comply with mud crab growth through ecdysis. The energy budget of growing crabs is expressed in our study as:

$IE = FE + UE + SE + RE + HE_m$ (1)

Where IE = the energy intake which was obtained for each crab by calculating the difference between the feed distributed and the feed leached and uneaten;

FE = energy lost from the animal through the feces for each crab, FE was calculated by the difference betwwen the voluntary feed intake and the digested feed estimated from its apparent digestibility;

UE = energy lost from the animal through the total ammonia excretion which was asseesed for each crab from the trial of nitrogenous excetion previously indicated;

SE = surface energy losses which is exuvia lost at ecdysis;

RE = recovered energy which is channeled into growth. It was calculated for each crab through the dry weight gain converted into energy;

HE_m = maintenance energy calculated for each crab from the equation (1):

$HE_m = IE – (FE + UE + SE + RE)$ (2)

2.5.5. Statistical analysis

Differences among the various treatments were considered significant when $P < 0.05$. Shapiro-Wilk and Levene's test were used for checking normality and homogeneity of variances, respectively. Percentage data were transformed to their arcsine values to get a normal distribution of the data. One-way ANOVA was applied to i) test the effect of dietary

treatments on VFI, and ii) compare the digestibility coefficient of each diet. ANCOVA was used with energy intake as a covariate to assess the effect of the different diets on the tissue growth, feed efficiency parameters (FCR, PER, retention of protein, lipid, and energy), and the partitioning of the energy budget.

3. Results

3.1. Feed digestibility and voluntary intake

Apparent digestibility for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of the five experimental diets are given in Table 5.2. No significant difference between diets (ANOVA, $P > 0.05$) in ADMD, ACPD and AGED was found; apparent digestibilities were high with average values of 96.02 ± 1.59% for ADMD; 97.26 ± 0.66% for ACPD and 97.02 ± 0.54% for AGED.

Table 5.2. Apparent digestibilities (%) for dry matter (ADMD), crude protein (ACPD), and gross energy (AGED) of five experimental diets.

Diets	Apparent digestibilities		
	ADMD	ACPD	AGED
CP25	95.96 ± 1.44[a]	96.85 ± 0.82[a]	96.84 ± 0.70[a]
CP35	95.80 ± 2.02[a]	96.99 ± 0.92[a]	96.47 ± 0.55[a]
CP40	96.29 ± 1.82[a]	97.65 ± 0.57[a]	97.09 ± 0.42[a]
CP45	96.03 ± 1.04[a]	97.12 ± 0.63[a]	97.04 ± 0.55[a]
CP50	96.00 ± 1.62[a]	97.68 ± 0.34[a]	97.67 ± 0.47[a]

Values are means ± SD (n = 3 replicates per treatment). Within the same column, means with the same letters are not significantly different ($P > 0.05$).

The averages of voluntary feed intake (VFI), SPC and protein intakes for each diet are presented in Table 5.3. There were no significant differences in VFI (ANOVA, $P = 0.78$) among the treatments. However, the variability of the data was high whatever the diet (CV = 36.7%, n = 50) with a minimum VFI of 45.7 and maximum of 220.2 $g.kg^{-1}$of fresh iBW.week^{-}

[1]. Soy and protein intakes significantly increased from the diet SPC-12 to the diet SPC-42 and then reached a plateau for the two following diets with higher inclusion levels of SPC.

Table 5.3. Voluntary feed intake, SPC (soy protein concentrate) intake and protein intake (g.kg^{-1}of fresh iBW.week^{-1}) for crabs fed on five different experimental diets.

	Experimental diets				
	SPC-12 (n = 10)	SPC-32 (n = 10)	SPC-42 (n = 10)	SPC-52 (n = 10)	SPC-60 (n = 10)
Voluntary feed intake (g.kg^{-1}of iBW.week^{-1})	96.7 ± 25.3^{a}	106.7 ± 18.6 ^{a}	114.0 ± 18.7^{a}	107.4 ± 31.0^{a}	102.7 ± 21.5^{a}
SPC intake (g.kg^{-1}of iBW.week^{-1})	11.6 ± 3.0^{a}	34.1 ± 5.8^{b}	47.9 ± 7.9^{bc}	55.9 ± 16.1^{bc}	61.6 ± 12.9^{c}
Protein intake (g.kg^{-1}of iBW.week^{-1})	26.0 ± 6.8^{a}	37.9 ± 6.5^{a}	45.3 ± 7.4^{b}	47.4 ± 13.7^{b}	50.5 ± 10.6^{b}

Values are means and standard deviation. Within the same row, means with different letters are significantly different ($P < 0.05$); iBW = fresh initial body weight.

As VFI was not affected by the diet composition, we pooled all the data to study the feed consumption during the course of the experiment (Fig. 5.1). During the first 14 days after ecdysis, crabs consumed about 200 g.kg^{-1} of fresh iBW.week^{-1} and then the VFI decreased until Day 35. From Day 49 until the Day 77, VFI remained at a baseline value of around 100 g.kg^{-1}fresh iBW.week^{-1}. Over the last 5 days before ecdysis, the VFI dropped dramatically to the value of 50 g.kg^{-1} of fresh iBW.week^{-1}.

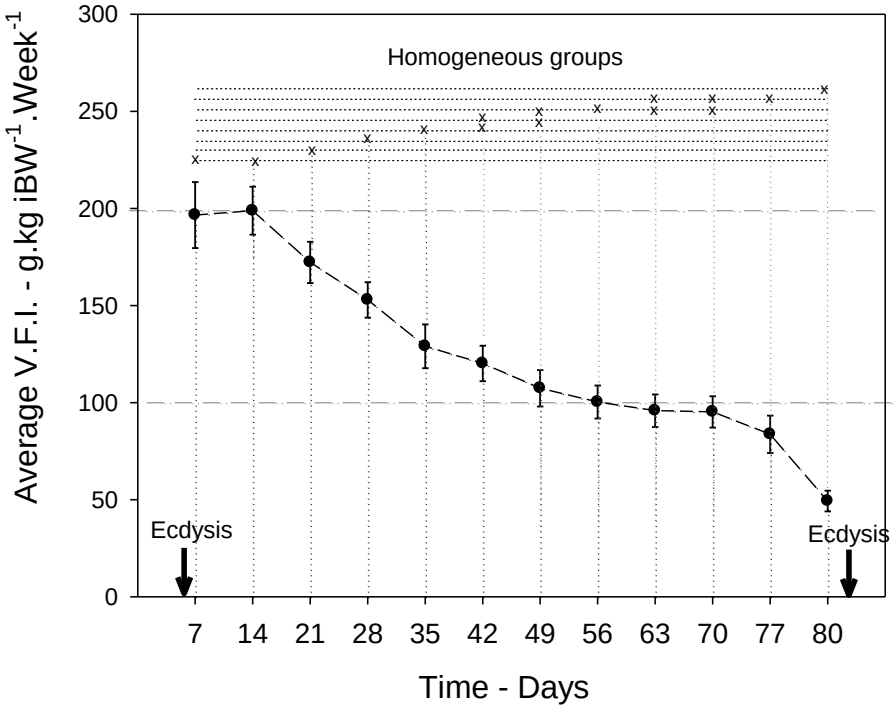

Figure 5.1. Voluntary feed intake (VFI, g.kg^{-1}of fresh iBW.week^{-1}) of crabs fed on whatever the diet, during a molt cycle (days). Homogenous groups determined from Tukey test are presented above the curve according time.

3.2. Molt cycle

The cumulative molts were clearly affected by the voluntary feed or energy intake levels, all diets combined (Fig. 5.2A). Two crab groups were identified; a low VFI group (1.05 ± 0.12% of iBW.d^{-1}) and a high VFI group (1.97 ± 0.12% iBW.d^{-1}). Cumulative molts, during the course of experiment were 50% and 100%, respectively in the low and high VFI groups.

The cumulated molts were also affected by the SPC or protein contents in the diets (Fig. 5.2B). After 80 days, the molting rates was 60% for crabs fed on the diets SPC-12, and 90% for crabs fed on the diets SPC-32, SPC-52 and SPC-60. Whereas, only 50 days after starting the experiment, 90% of the crabs fed on diet SPC-42 had molted.

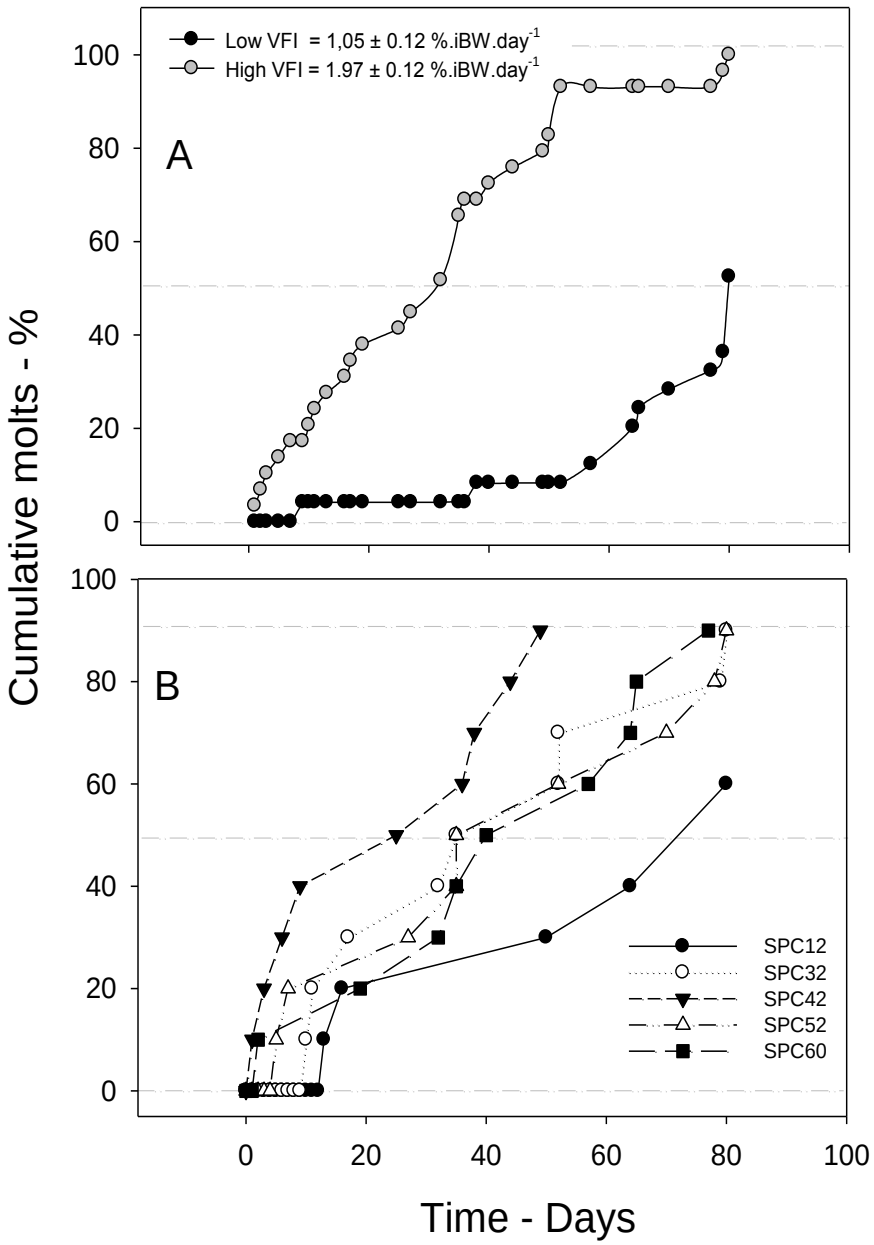

Figure 5.2. Cumulative molts of crabs (% of initial crab numbers) according to the voluntary feed intake (VFI) levels (A) and different diets (B). iBW = initial fresh body weight.

3.3. Growth

3.3.1. Body weight gain and carapace increment at molt

The fresh weight gain after ecdysis (molt stage B) averaged 89.5 ± 4.1%. It was not affected by the diet, except significantly higher (ANOVA, $P < 0.05$) for crabs fed on diet SPC-42 compared with SPC-52 (Fig. 5.3A). The average carapace increment was 23 ± 2.7% and was not affected by the diet consumed (Fig. 5.3B).

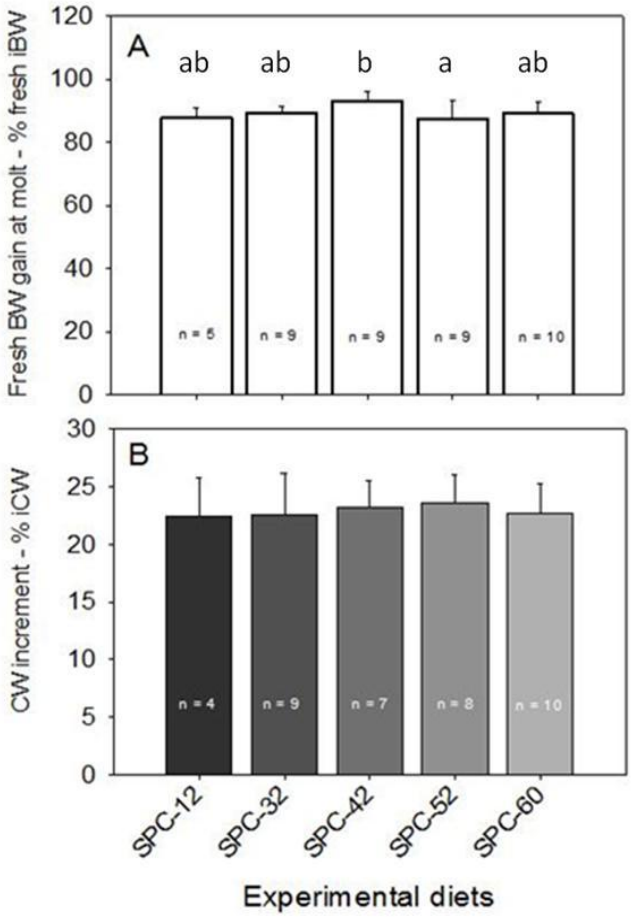

Figure 5.3. Fresh body weight gain after one day of ecdysis (A) and carapace increment after one day of ecdysis (B) for crabs fed on different experimental diets.

3.3.2. Tissue growth during molt cycle

The water content of postmolt crabs was 85% one day after ecdysis (Fig. 5.4) and then decreased over the next 23 days to reach a baseline of 62.4 ± 0.6% of water which then remained relatively constant until the next molt.

The tissue gain, measured as the increase in dry organic matter as proportion of dry body weight after ecdysis (BWa.e.), increased for about 30 days after ecdysis and then it remained relatively constant until the next molt (Fig. 5.5A).

The tissue growth rate was highest two days after ecdysis (16.5%) and dropped dramatically during the first 10 days to 3.6%. Then the decrease was slower to the minimum value of 1.3% just before the next ecdysis (Fig. 5.5B).

Crab body protein and lipid change during the intermolt cycle and showed the same trend as the tissue growth (Fig. 5.6A, 5.6B, respectively) with two distinct phases: an initial increase followed by a plateau. The initial increase of body protein content started about one week earlier than the increase of the lipids. Ash gain did not follow the trends of protein and lipid. It increased rapidly during the first five days after ecdysis and then remained stable during most of the intermolt period (Fig. 5.6C).

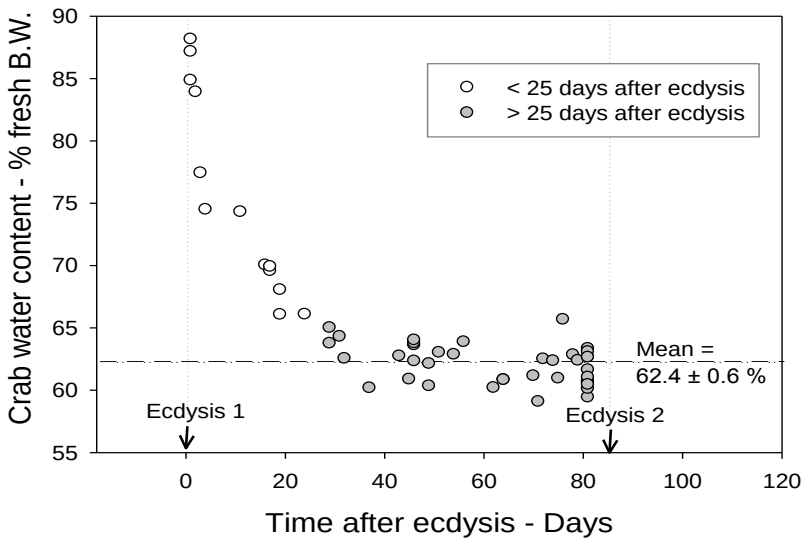

Figure 5.4. Crab water content during a molt cycle. Mean value of the data calculated for crabs after 25 days of ecdysis. The fast decline trend of water content during the first 25 days after ecdysis "blank cycles"while stable trend for crabs after 25 days of ecdysis "brown cycles ".

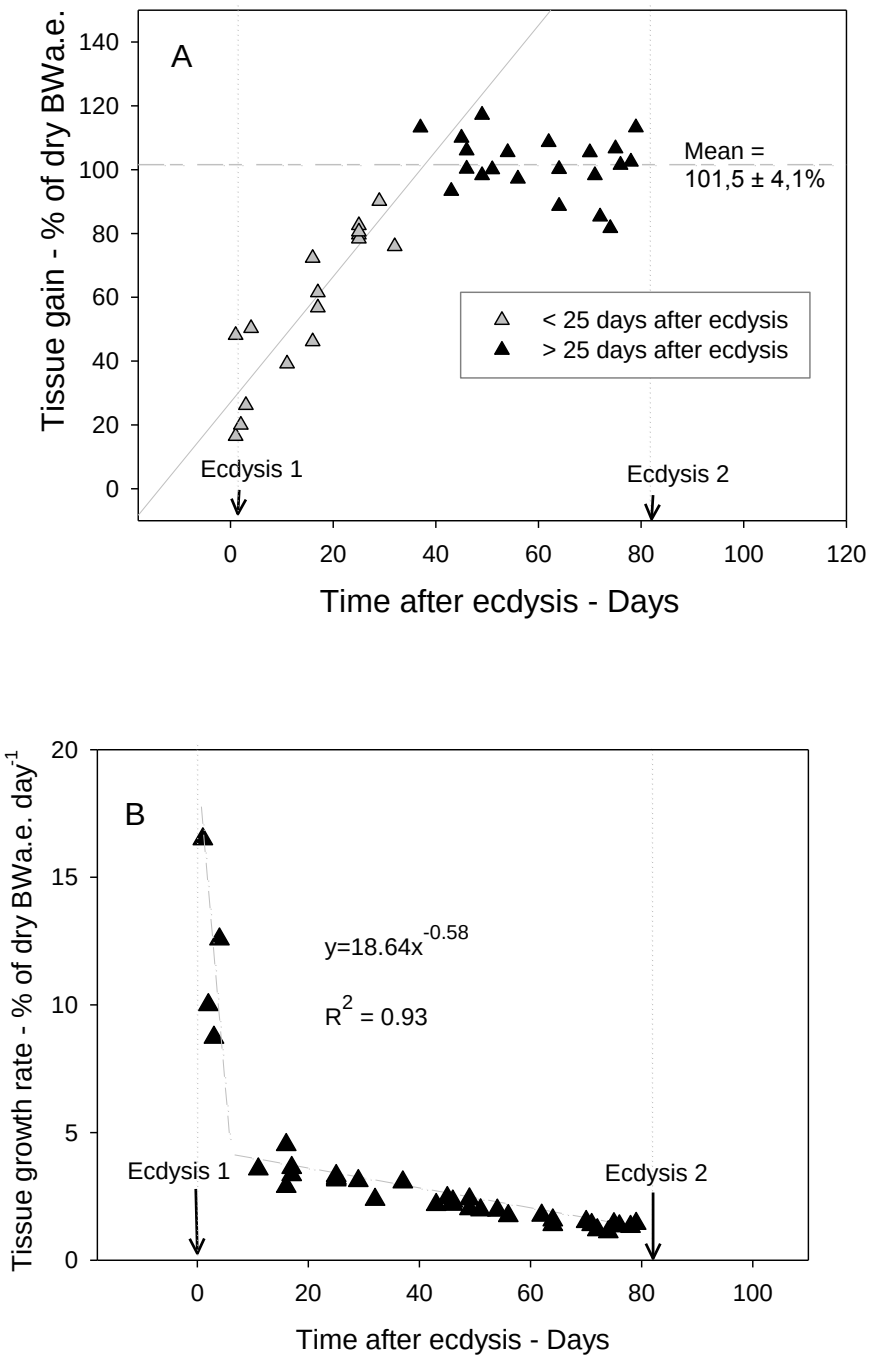

Figure 5.5. Growth of crabs during a molt cycle: (A) is tissue gain expressed in % of dry body weight after ecdysis - % of dry BWa.e.; mean value was determined for crabs after 25 days of ecdysis and (B) is tissue growth rate expressed in % of dry body weight after ecdysis per day - % of dry BWa.e.day^{-1}.

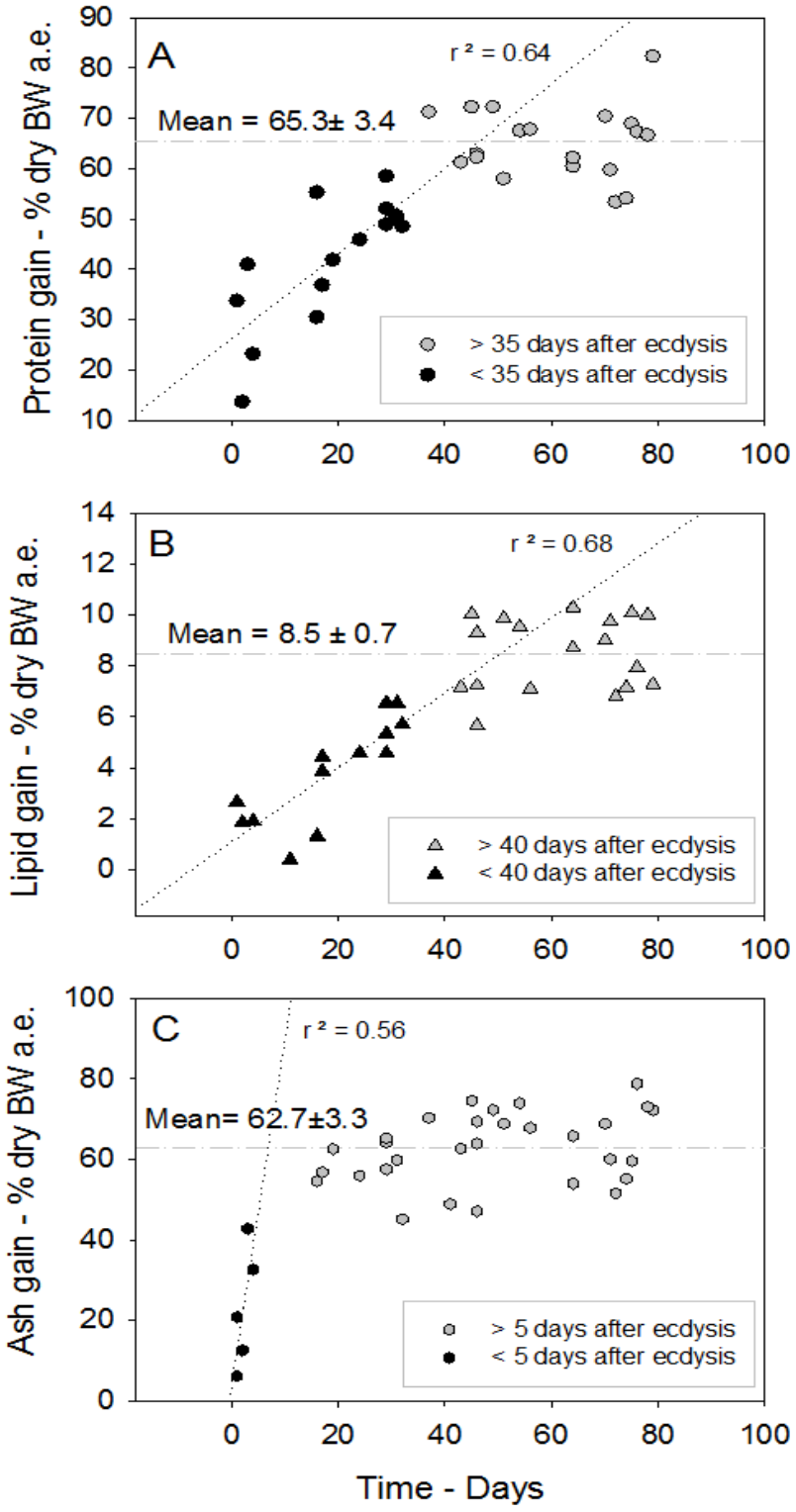

Figure 5.6. Nutrient gains of crabs expressed as % of dry body weight after ecdysis (% dry BWa.e.) during a molt cycle: (A) protein gain; (B) lipid gain and (C) ash gain.

3.3.3. Tissue growth and feed efficiency according to the dietary treatments

To compare the effect of the different diets on growth, feed efficiency and energy budget, only the crabs which had molted a minimum of 30 days before and had reached the plateau of their tissue growth were retained for the experiment. In the treatment SPC-12, only 2 crabs satisfied this condition, thus we have not considered this treatment any further.

As voluntary feed intake (VFI) was highly variable, regardless of dietary treatment, we have to consider the effect of its level on crab tissue growth. Indeed, there is a significant linear relation between energy intake and growth rate for each dietary treatment, with the exception of diet SPC-32 which however has linear relationship (Fig. 5.7). Thus, ANCOVA was applied for all the diet treatments with energy intake as a covariate in order to reduce error variance within each treatment allowing us to more accurately assess the effect of dietary treatment on dependent parameters (tissue growth, feed efficiency).

Dietary treatments did not significantly affect tissue growth rate (ANCOVA, $P = 0.054$), nor the FCR (ANCOVA, $P = 0.135$), PER (ANCOVA, $P = 0.127$) and protein retention (ANCOVA, $P = 0.112$), but they did affect lipid retention (ANCOVA, $P < 0.001$), and energy retention (ANCOVA, $P = 0.013$) (Table 5.4). For lipid retention, the highest values were found for the diet SPC-32 and SPC-42 and then it decreased with the diet SPC-52 and SPC-60. For energy retention there was no clear trend, with lowest value for diet SPC-32 and SPC-52 and highest values for diets SPC-42 and SPC-60.

3.3.4. Energy budget according the dietary treatments

To analyse the energy budgets for the different diets only crab juveniles which had molted a minimum of 30 days before were retained for the experiment.

The dietary treatments did not significantly affect the energy budget allocated in FE and SE (ANCOVA, $P > 0.05$). Conversely, diet affected UE (ANCOVA $P < 0.001$), RE (ANCOVA, $P = 0.005$), and HE_m (ANCOVA, $P = 0.013$). UE increased in the similar way to SPC or protein content in the diets with significant rise for SPC-52 and SPC-60 (Table 5.5).

For RE and $HE_{m,}$ there were no clear pattern but we noted significantly higher value for RE and lower for HEm with crabs fed on diet SPC-42. The average energy budget

accounted for RE was higher for crabs fed on diets SPC-32 and SPC-42 compared to other diets; however, this difference was not significant.

Under our experimental conditions, the main proportion of energy intake was allocated for maintenance (75%) and growth (21%). The remaining proportion of energy intake (4%) was shared among FE (2.5%), UE (0.6%) and SE (0.9%).

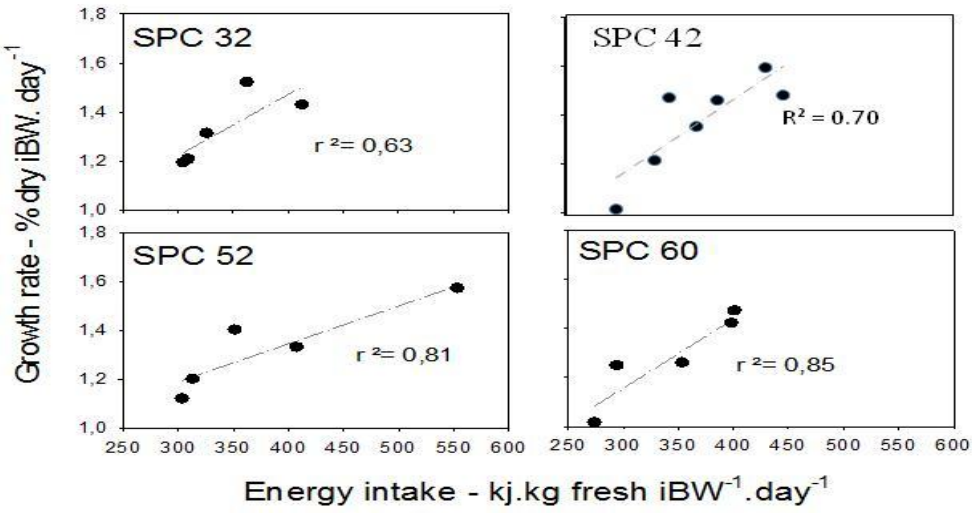

Figure 5.7. Linear relationships between growth rates (% of dry iBW.day^{-1}) and energy intakes of crabs fed on different experimental diets: SPC-32; SPC-42; SPC-52 and SPC-60; Significant r^2, P = 0.10; 0.02; 0.04; 0.03 for SPC-32; SPC-42; SPC-52; SPC-60, respectively.

Table 5.4. Feed efficiency in crab juveniles fed on different experimental diets.

Parameters	Experimental diets				
	SPC-12	SPC-32 (n = 5)	SPC-42 (n = 7)	SPC-52 (n = 5)	SPC-60 (n = 5)
Tissue growth rate (% of dry iBW.d^{-1})	ND	0.81 ± 0.05^{a}	0.86 ± 0.06^{a}	0.76 ± 0.04^{a}	0.78 ± 0.05^{a}
FCR	ND	3.88 ± 0.22^{a}	3.68 ± 0.26^{a}	4.07 ± 0.26^{a}	4.05 ± 0.28^{a}
PER	ND	0.62 ± 0.04^{a}	0.65 ± 0.04^{a}	0.59 ± 0.04^{a}	0.59 ± 0.03^{a}
Protein retention (%)	ND	24.95 ± 1.53^{a}	26.45 ± 1.75^{a}	23.80 ± 1.55^{a}	23.98 ± 1.56^{a}
Lipid retention (%)	ND	32.65 ± 2.32^{a}	35.27 ± 2.33^{a}	24.57 ± 1.21^{b}	25.20 ± 1.64^{b}
Energy retention (%)	ND	18.48 ± 1.13^{abc}	18.92 ± 1.25^{b}	16.35 ± 1.07^{c}	19.62 ± 1.08^{b}

Values are adjusted means ± confident intervals, with energy intake (EI = 0.93 ± 0.06 kj.g^{-1}of dry iBW.d^{-1}) as covariate. Within the same row, different letters indicate significant differences (P < 0.05) between the diets; ND is not determined.

Table 5.5. Energy budget of crabs fed on different experimental diets with the same energy intake.

Diets	Energy intake (IE) ($kj.g^{-1}$ of dry iBW . d^{-1})	Partitions of energy ($kj.g^{-1}$ of dry iBW. d^{-1})				
		Faeces (FE)	Excretion (UE)	Ecdycis (SE)	Growth (RE)	Maintenance (HE_m)
SPC-12	ND	ND	ND	ND	ND	ND
SPC-32 (n = 5)	0.93 ± 0.06	0.025 ± 0.002^{a}	$0.004 \pm < 0.001^{a}$	0.008 ± 0.001^{a}	0.195 ± 0.011^{abc}	0.690 ± 0.012^{abc}
SPC-42(n = 7)		0.023 ± 0.001^{a}	$0.004 \pm < 0.001^{a}$	$0.009 \pm < 0.001^{a}$	0.211 ± 0.014^{b}	0.682 ± 0.034^{b}
SPC-52 (n = 5)		0.023 ± 0.002^{a}	$0.006 \pm < 0.001^{b}$	0.008 ± 0.001^{a}	0.178 ± 0.008^{c}	0.713 ± 0.008^{c}
SPC-60 (n = 5)		0.024 ± 0.002^{a}	$0.009 \pm < 0.001^{c}$	$0.009 \pm < 0.001^{a}$	0.184 ± 0.016^{c}	0.703 ± 0.018^{c}

Values are means ± confident interval. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets; ND is not determined.

4. Discussions and conclusions

4.1. Feeds digestibility

In our study, digestibility of the experimental diets was not affected by the level of dietary soy protein concentrate (SPC). This result confirms the results of our previous study on crab *S. serrata* that showed high digestibility coefficients for dry matter, protein and energy of SPC, which were also similar to those of fishmeal (Nguyen et al., submitted). These results are also consistent with previous studies on mud crabs reporting high soybean meal digestibility at an inclusion level of up to 45%, with ADC values for ADMD, ACPD and AGED ranging from 80.4 to 95.7 %; 91.7 to 97.1% and 88.6 to 97.9, respectively (Catacutan et al., 2003; Tuan et al., 2006; Truong et al., 2008; Truong et al., 2009). Furthermore, Truong et al. (2008) showed that crude protein from soy bean meal was more completely digested by mud crabs than the other plant ingredients tested (canola, lupin and cotton seed meal). Although SPC is more expensive than SBM, it does not contain the alcohol-soluble fraction present in SBM and has a higher essential amino acid concentration. It also has greater nutrient digestibility compared with SBM and can be included at much higher concentrations in diets of piscivorous marine species (Bureau et al., 1998, USSEC 2008).

4.2. Voluntary feed intake (VFI)

In the present study, VFI varies greatly between individuals (global CV = 36.7%) with a general average of 105.5 $g.kg^{-1}$of $iBW.week^{-1}$. As the experimental diets were formulated to be isoenergetic, the variability of energy intake was similar to the VFI variability. Interestingly, the average VFI did not differ among diets tested, even the corresponding SPC intake increased significantly as its concentration in the diet increased. Jiang et al. (2013) obtained a similar result with the crab *Eriocheir sinensis* and Suarez et al. (2009) showed no difference in feed intake for the shrimp *Litopenaeus vannamei* when the replacement of fishmeal by soybean-canola meal reached 100%. Nevertheless, Luo et al. (2011) reported that feed intake of Chinese mitten crab (*Eriocheir sinensis*) was significantly reduced when a mixed source of dietary soybean meal and rapeseed meal exceeded 30%. As the VFI were very variable in our study, whatever the diet tested, the amount of protein or SPC ingested relied not only on the diet composition (dietary level of SPC) but also on the VFI. Thus, several crabs fed on the diets with higher protein contents, did in fact ingest less protein than some animals fed on diets with lower protein contents. However, the general trend showed that the protein intake increased significantly from the diet SPC-12 to the diet SPC-42 and then remained stable. Consequently, the surplus of SPC in the diets SPC-52 and SPC-60 did not result in higher protein consumption. We can then assume that the diet SPC-42 is the best compromise between dietary levels of SPC and availability of proteins for crabs.

4.3. Molt frequency

The duration of molt cycle stages in *S. serrata* (and all decapod crustaceans) are the net result of complex interactions between many endogenous (genetic) and exogenous factors (Heasman, 1980). Amongst these factors, temperature, nutrition, autotomy and regeneration, size and sex and sexual maturity are important (Heasman, 1980). Few studies have been devoted to the quantitative and still less to the qualitative influence of nutrition on the molting frequency of crustaceans. However, the general trend is that if food supply is reduced below the optimum level, there is invariably a reduction in growth rate (Hartnoll, 1982). This reduction is due to the combination of an extended intermolt period (lower molt frequency) and a reduced molt increment (carapace and body weight growth at molt). Our study confirmed this trend as the level of voluntary food intake (VFI) greatly influenced the molt frequency. We showed also that, for an equivalent VFI or energy intake, the dietary level of

SPC or protein influenced the molt frequency which was highest for the diet SPC-42. The molt frequency increased from diet SPC-12 to diet SPC-42 and then decreased for the diets SPC-52 and SPC-60. These results are novel and we could hardly find an equivalent study showing the influence of dietary protein on the molts frequency of crustaceans. The lower molt frequency of the crabs fed on diet SPC-12 could be explained by a deficiency in protein and essential amino acids required for tissue growth especially during posmolt period**.** Comparing with the previous studies (Millamena, et al., 1996; 1997; 1998; 1999), the optimal essential amino acid specifications for shrimp (*P. monodon*) found in the diet of 40% crude protein were higher than that in SPC-12, in which, particularly, only one third of methionine was presented. This can also be explained by the high carbohydrate content of the diet SPC-12 which can lead to glucose load resulting in persistent hyperglycemia and growth impairment. However, this second explanation is unlikely, because base on the reported findings for different fish and shrimp species, the maximum recommended levels of digestible starch inclusion in feed fall within 20-40% in shrimp and up to 50% in omnivorous species among which is the mud crab (La Sara et al., 2007; NRC, 2011). Another factor, mineral content, could also affect on the molt frequency of crabs because minerals are essential micronutrients necessary for normal file processes, the deficiency in mineral leads to longer intermolt period, then slower growth in crustaceans (David & Lawrance, 1997). In the present study, a high gradient content of ash followed the increase of SPC. Therefore, the lowest ash content in SPC-12 could result in the lowest molt frequency of crabs. Nervetheless, basing on the previous studies on mud crabs of Catacutan (2002), Anderson et al. (2004) and Unnikrishnan & Paulraj (2010), it appears ash content of 14% in diet SPC-12 could not be insufficient. Therefore, this explaination is also unlikely.

To explain the decline in molts frequency for the diets SPC-52 and SPC-60, two hypotheses can be put forward: the first relates to the toxicity of ammonia and the second to the antinutritional factors encountered in soy bean.

Regarding the first hypothesis, after feeding the majority of amino acids in excess of what is required for protein synthesis, are catabolized in the liver (Campbell, 1991), releasing ammonia and resulting in transient increases in plasma ammonia level and ammonia excretion rate in fish (Wicks & Randall, 2002). In crustaceans, ammonia, excreted by the gills as NH_4^+ ions, is the major form of nitrogen excreted, accounting for 86% of total nitrogen excretion for *Carcinus maenas* (Needham, 1957). During the molt cycle an increase in nitrogen metabolism is correlated with a simultaneous increase in general metabolism (Regnault, 1979). Thus a

good correlation exists between ammonia excreted and oxygen consumed. Both are highest immediately after ecdysis or after a meal (Regnault, 1979; Nguyen et al., submitted). For *S. serrata,* there is also a correlation between ammonia excreted and meal size as well as dietary proteins of the diets (Nguyen et al., submitted). Thus, a transient postprandial surge in blood ammonia concentration could occur in crabs as it has been described for fishes (Kaushik & Teles, 1985; Wicks & Randall, 2002) and lead to postprandial ammonia toxicity. In our study, crabs fed on diets SPC-52 and SPC-60, with the highest dietary protein content, could be confronted with ammonia toxicity after feeding, leading to lower molt frequency compared to animals fed SPC-42 diet.

The second hypothesis could explain the negative effect of high SPC inclusions on molt frequency of juvenile crabs *S. serrata*. Anti-nutritional factors (ANFs) are present in soy bean meal, such as protease inhibitors, tannins, lectins and non-starch polysaccharides (Francis et al., 2001). However, in our study SPC has been used and produced by aqueous alcohol extraction from defatted soybeans, which removes a majority of the phytate, lectins, saponins and oligosaccharides (Anderson & Wolf, 1995; Bureau et al., 1998; Deng et al., 2006; Refstie et al., 2001). Although SPC has a low content of ANFs, they could still be a significant problem when SPC is included at high levels in the diet. *S. serrata* may be particularly susceptible to these anti-nutritional factors.

4.4. Growth

Most growth studies on crabs only consider the increase in size and fresh weight with time. The highest increase in fresh weight (almost 90%) of the crab *S. serrata* occurs in the very brief period of rapid water uptake (Heasman, 1980). Fresh weight changes follow a basic pattern through the molt cycle in all species of crustaceans, i.e. large and abrupt increases associated with rapid water uptake at ecdysis. Further moderate gains are associated with mineralization of the integument and the accumulation of organic matter during late molt stages and a relative stabilization of fresh weight during intermolt until the onset of the successive ecdysis (Heasman, 1980). The water content (% of fresh body weight) of juvenile *S.serrata* had a clear and progressive decline from a maximum of 88% following ecdysis to an average baseline of 62.4 ± 0.6%. Our results are in close to those of Heasman (1980). The high percentage of free water levels after ecdysis largely reflect the relative amount of water absorbed during and immediately after ecdysis and hence the relative increase in volume of

the animal. Although the weight growth in crabs in connection with the molt has an interrupted character, the somatic growth and the accumulation of energy reserves in tissues are continuous processes. Consequently, such growth is accompanied by definite changes in the ratio of dry and fresh weight during the molt cycle. Thus the period of rapid water uptake associated with ecdysis in *S. serrata* is followed by an increase in dry matter content in the relative proportion of initial body dry weight, reaching a plateau 30 days after ecdysis. The most rapid growth rate occurred during the first days after molt and then decreased until the next molt. Our results are in similar to those of Heasman (1980) who showed that increases in organic matter, as a proportion of fresh weight, were higher from molt stages B to C3 (postmolt stages) than from molt stage C4 to D2 (intermolt-premolt stages) for *S. serrata*. According to Passano (1960) and Skinner (1966), the growth of the muscle is thought to occur during the post-molt period when the carapace is already hardened, whereas immediately after the molt when the carapace is still soft the muscles appear to be watery and atrophied.

Protein gain expressed as the relative proportion of initial body dry weight followed the tissue growth rate pattern. The proportion increased during the first 35 days after ecdysis. This increase reflected a high protein accretion during the postmolt period. El Haj & Houlihan (1987) also showed an increase of the protein synthesis rate in the extensor muscle during the post-ecdysial period of the crab *Carcinus maenas*.

The changes in the lipid gain were similar to those of tissue and protein. Lipids were accumulated by the crab during the first 40 days following ecdysis and then remained stable at 8.49 ± 0.67% of the body dry weight. In our previous study we showed that lipid level was higher in premolt crabs than in postmolt crabs (Nguyen et al., submitted). Furthermore, Copeman et al. (2012) found that triacylglycerol levels in “red king crab” (*Paralithodes camtschaticus*) rapidly accumulated during seven days following a molt. In *Eriocheir sinensis*, the accumulation of lipids prior to molting appeared to result from the increase in neutral lipids because the polar lipid content remained constant during the molt cycle. The neutral lipids, particularly triacylglycerides, are the predominant molecules of energy storage and, consequently, the major source of energy (Stoner et al., 2010). In our previous study (Nguyen et al. submitted), total lipids per unit of body dry weight in intermolt and premolt *S. serrata* (2.8 %) was lower than those reported for the crab *Rhithropanopeus harrisii* (5%) (Nates & McKenney, 2000), the rock lobster *Panulirus cygnus* (7%) (Liddy et al., 2005) and red king crab, *Paralithodes camtschaticus* (2 to 3.6%) (Stoner et al., 2013).

Conversely to tissue, protein and lipid gains, the proportion of mineral salts (in dry weight) increased rapidly during the 5 days following ecdysis and then reach a plateau until the next molt. Heasman (1980) already noted the rapid mineralization of the cuticle for newly molted *S. serrata*. Similarly, the crab *Eriocheir sinensis* actively incorporated minerals after ecdysis (Tian et al., 2012)

In our study we found a linear relationship between VFI (or energy intake) and growth and based on this correlation we calculated the adjusted means of growth for a same amount of energy intake. Tissue growth for a same amount of VFI was not significantly affected by the feed composition, although a higher value was observed with treatment SPC-42. This diet resulted in a better FCR, PER, and retention of protein, lipid and energy. The higher lipid and energy retention of crabs fed on SPC-42 diet must be related to the increase in their molt frequency. This result is consistent with Adelung (1971) who found that the principal determinant of molt frequency in crab *Carcinus maenas* was its rate of tissue growth. This author also stated that reserve of energy must be accumulated before a molt can occur. In our study, tissue growth and energy storage were faster with diet SPC-42 and the next molt occurred earlier compared with crabs fed on other diets. This would explain the higher molt frequency observed for the crabs fed on SPC-42.

This study has also shown that the quantity or composition of the diet greatly influenced somatic growth and molting frequency, but that they have no influence on the increase in size at ecdysis. According to Hartnoll (1982), the overall growth rate of crustaceans is a function of the molt cycle duration and the molt increment. The relative importance of these two processes varies between species, and within species between different experimental protocols. However, in most cases the most important factor is molt cycle duration. Our results on *S. serrata* are consistent with those in the literature (Hartnoll, 1982).

The best growth (tissue growth and molt frequency) and the best feed efficiency (FCR, PER, retention of energy, proteins and lipids) obtained with SPC-42 diet was confirmed by the bioenergetic study. A decreased of retained energy (RE) was observed when dietary protein content increased (Table 5.5). The best RE was observed for the diet SPC-42. Increased energy retention in lower dietary protein diets (SPC-42 and SPC-32) may be related to the efficiency of nutrient utilization. Excess dietary protein is diverted for catabolism, reducing protein retention (Guzman et al., 2001). Furthermore, it has been demonstrated in

young pigs that dietary protein is used less efficiently than carbohydrate or lipid for energy retention (Van Milgen et al., 2001). If a similar process is occurring for crabs in our study, it should result in increased heat production leading to higher energy allocated for maintenance (HEm) and increased urinary energy losses in diets SPC-52 and SPC-60.

The best growth (somatic growth and molt frequency) and the best feed efficiency were obtained, under our experimental conditions, when 1 kg crabs consumed daily 6.5 ± 1.1 g of protein (P/E = 22.13 $g.Mj^{-1}$). This ingestion rate was obtained with the diet SPC-42 that contained 40% protein of which almost three quarters was derived from SPC. Intake of protein with diets SPC-12 and SPC-32 was obviously insufficient for optimal growth, while the excess intake of protein (or SPC) with diets SPC-52 and SPC-60 adversely affected the growth of juvenile crabs. Our results are similar to those of Catacutan (2002) who has shown that the optimal dietary protein in diet was 40% for juvenile crabs. However, at a younger stage, crabs had much higher voluntary feed intake (21.8 $g.kg^{-1}$of $BW.d^{-1}$) and the optimum level of crude protein in the diet was 45% (Unnikrishnan & Paulraj, 2010). Finally our work demonstrated the advantage of following somatic growth and molt frequency when investigating nutritional requirements of the crab which is subject to very significant changes in water content during the molting cycle.

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5.3 - CONCLUSION

From the point of view of fundamental knowledge our study allowed to better understand somatic growth (SG) and dietary energy utilization during a molt cycle of juvenile crab *S. serrata*. The most rapid SG rate occurred during the first days after ecdysis and then decrease until the next molt. In an interesting way we observed that the voluntary feed or energy intake (VFI) evolved in the same way as the SG rate: the animal eats dramatically more during two weeks following the ecdysis to cover its high energy requirement during this period of intensified SG. Then, the VFI decreases in a parallel to the SG rate until a minimum value shortly before the next molt. Interestingly, the level of VFI or intake of energy greatly influenced the molt frequency, and even more interesting, for the same level of VFI the nature of the diets also had a significant influence on the molt frequency. In fact, nature of the diet, its energy utilization and molt cycle duration are linked as we showed that the diets which leaded to the better recovered energy for growth (RE) also allowed the shortest molt cycle. However, in this study, VFI and nature of the diet have no influence on the increase in size of the crab at ecdysis. This last result is in agreement with the scientific literature which shows that, in the majority of the cases, the molt cycle duration is more important factor than the molt increment on the overall growth rate of crustaceans (Hartnoll, 1982).

Overall, from the practical point of view, our results confirm those of Catacutan (2002) than that the crab *S. serrata*, grows well when fed diets containing 32%-40% dietary protein. With 40% of dietary crude protein mainly derived from SPC, in our experimental conditions, 1kg of crab ingested weekly 45.3 ± 7.4 g of proteins and this consumption leaded to the best tissue growth rate and the highest protein efficiency ratio (PER). Besides, our study confirms the high nutritional quality of the SPC as dietary proteins source for the juvenile *S. serrata*.

Finally the above highlighting about great changes of the VFI during the course of the molt cycle could lead to adaptations of the feeding strategy of mud crabs in commercial farm.

6 - CHAPTER VI: GENERAL CONCLUSIONS

Before we undertook the studies on feed and nutrient requirements of mud crabs we checked to see if we were dealing with one or several species of mud crabs. To do this, we carried out a study using traditional morphological keys and genetic analyses which allowed us to conclude that the mud crab fishery in New Caledonia involved only one species: *Scylla serrata*. This species is the most widely distributed of all the mud crab species, ranging from the east African coast to Australia. *S. serrata* and *S. olivacea* are sympatric in five areas: Gulf of Carpentaria, Panay, Taiwan, Kupang and Western Australia (Taylor, 1984). The habitat of *S. serrata* is associated with mangrove forests inundated with full salinity oceanic water for greater part of the year. This species can tolerate reduced salinity. However, it is also more abundant than the other three species in high salinity environments. Females migrate offshore, often as far as the continental shelf (Hill, 1994). *S. serrata* is also the largest of the four *Scylla* species (Vay, 2001) and exhibits the highest growth rate (Robertson, 1987). This last characteristic is of course very interesting for aquaculture.

Generally, each of the four species of *Scylla* has subtly different biology, which suggests that there will be variations in optimal aquaculture production techniques (FAO, 2011). For *S. serrata,* optimal salinity and temperature for growth appears to be in the range of 10-25‰ and 26-30°C respectively (FAO, 2011). The presence and high percentage composition of plant fragments in the stomach of wild *S. serrata* at all growth stages suggests that this animal feeds on a wide variety of food items (La Sara et al., 2007). Hence, this species is omnivorous, a scavenger and opportunistic carnivore (Hutching & Saenger, 1987; Jayamane & Jinadasa, 1991). Considering its diet in the wild, it appears that *S. serrata* should be well adapted to the utilization of raw materials derived from plants, which is promising in terms of aquaculture.

Within the genus *Scylla*, *S. serrata* is probably the best known species. Its biology has been studied but very little information is available on its farming. However, scientific literature on mud crabs is still relatively sparse compared to publications on *penaeid* shrimps. Therefore at the beginning of this thesis, we were facing a virtually unknown field where much remains to be discovered. The present work is one of the first steps in the understanding of crab nutrition in aquaculture and it opened up new areas of investigation and ideas that are presented below.

6.1 - ADVANCES IN PHYSIOLOGY AND NUTRITION OF THE CRAB *S. SERRATA*

Most of the previous studies on crabs have considered growth rate in terms of gain in fresh or live weight. Nevertheless, crab fresh weight changes follow a basic pattern through the molt cycle, i.e. large an abrupt increases in live weight (98%) associated with rapid water uptake at ecdysis; further moderate gains (11%) associated with carapace mineralization and tissue growth during postmolt stages and relative stabilization in live weight during intermolt stages until the onset of successive ecdysis (Heasman, 1980). Our studies with *S. serrata* have shown a maximum proportion of the body water of 88% after ecdysis, followed by a clear and progressive decline in water content to an average baseline of 62.4 ± 0.6%. The high percentage of free water levels after ecdysis largely reflects the relative amount of water absorbed during and immediately following ecdysis and hence the relative increase in volume of the animal.

Although live growth in crustaceans in connection with the molt has a discontinuous character, the tissue growth and the accumulation of energy reserves are continuous processes that occur throughout the molt cycle (Freeman, 1990). In our study with juvenile *S. serrata,* we confirmed the continuous process of tissue growth. However, we observed that the rate of this process changes over the molting cycle. We have identified two tissue growth phases. The first phase starts at the ecdysis, lasts 30% of the molting cycle and corresponds to postmolt stages. The second phase covers the remaining 70% of the molting cycle and corresponds to intermolt stages. We showed that 80% and 20% of the tissue growth occurs respectively during the postmolt and the intermolt stages of the molt cycle. From our data and those of Heasman (1980) we propose schematic growth models of juvenile crab *S. serrata* over two molt cycles where we can distinguish a discontinuous live weight gain at ecdysis and a continuous tissue growth separated in two successive phases for each molt cycle (Fig. 6.1). Protein and lipid gains were found to follow the same trend as tissue gain with high deposition rate after ecdysis, which then decrease from postmolt to intermolt stages. In contrast, we noted an extreme rapid increase of mineral salts corresponding to mineralization of the cuticle after ecdysis, which is disconnected from tissue growth.

Interestingly, we also observed that the feed consumption rate or voluntary feed intake (VFI) depends on the tissue growth rate. The VFI is maximum during two weeks following the ecdysis when tissue growth rate is high then decreases in a parallel to the tissue growth rate until a minimum value shortly before the next molt (Fig. 6.1). In these conditions, almost

50% of the food is consumed during the first third of the molt cycle when the tissue growth rate is the highest. To our knowledge, this phenomenon has never been described before. However, Regnault (1979) showed a good correlation between ammonia excreted and oxygen consumed for the shrimp *Crangon crangon*. Both were higher after ecdysis and during postmolt indicating a greater feed consumption during these molt stages.

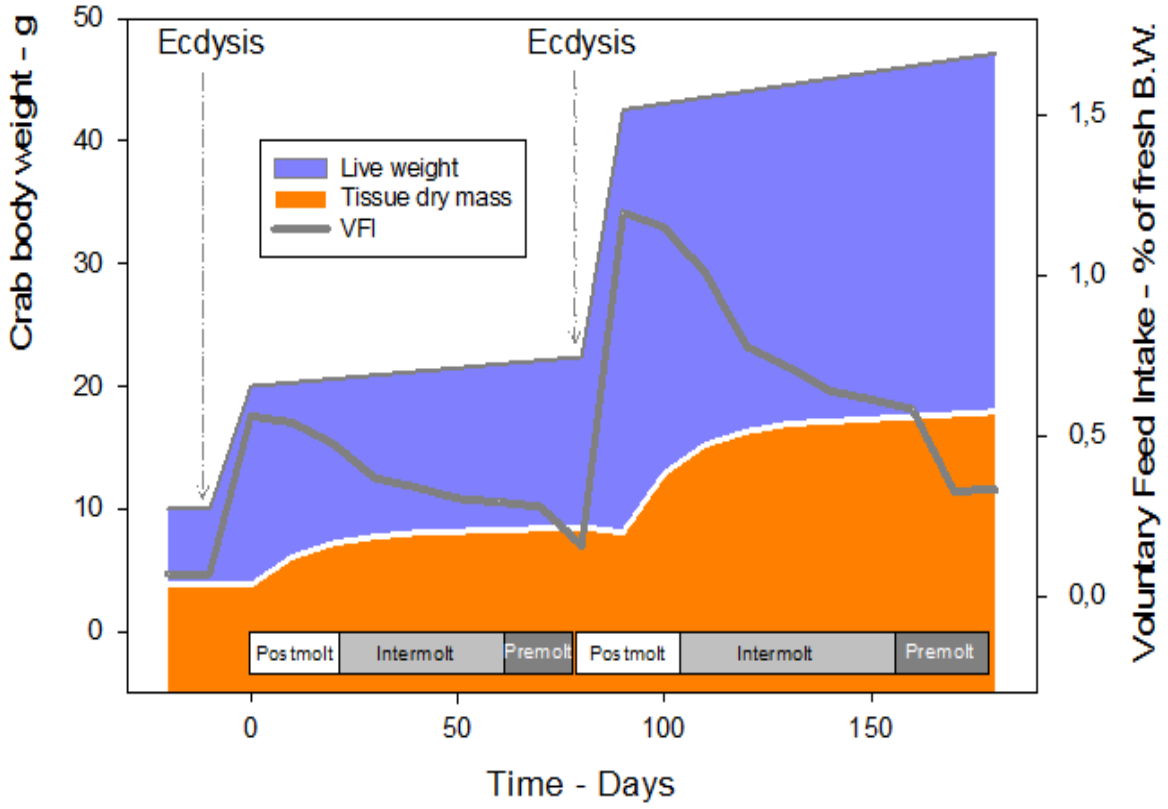

Figure 6.1. Schematic growth models and voluntary feed intake (VFI) of juvenile crab *S. serrata* over two molt cycles (MCD).

The duration of 2 molt cycles = MCD 1 + MCD 2, where: MCD 1 is our result, MCD 2 = MCD 1 + 24 % of MCD 1 (based on Heasman, 1980). After ecdysis, live weight gain is 90% of body weight immediately after ecdysis and then 11% until the next ecdysis. 80% of tissue growth is observed during the first 30% of MCD and the remaining 20% during the rest (70%) of MCD**.** VFI is dependent on tissue growth, dramatically increases following the fast tissue growth period and then decrease until the next ecdysis.

Another observation was made during our study with *S. serrata*: VFI or intake of energy has a strong influence on the molt frequency or intermolt cycle duration. A linear relationship was observed between VFI or energy intake and tissue growth. Moreover, when energy intake is reduced below the optimum level there is an extended molt cycle or a lower

molt frequency. However the growth in size or molt increment (live weight gain or carapace width gain) are not affected. Our study clearly demonstrates that the major effect of food intake on live body growth of juvenile *S. serrata* results is a change in the duration of the molting cycle and not on the size of the molt increment. There is currently insufficient knowledge on the factors initiating molting. However, it is highly probable that the major effect concerns the intermolt period because sufficient reserves of energy must be accumulated before a molt can occur (Adelung, 1971). Our results seem to confirm this assumption, as with *S. serrata* there is a close relationship between VFI and tissue growth, and between VFI and molting. When the feed intake is limited, tissue growth decreases and the molt cycle duration increases. One hypothesis on the mechanisms involved in triggering molting, when the threshold of energy becomes sufficient, is the implication of the hormonal control involving the molt inhibiting hormone (MIH) and the hyperglycaemic hormone (CHH) (Chang & Mykles, 2011). It is accepted that ecdysis is initiated when blood levels of the MIH fall, which could be triggered by accumulated energy level. The CHH, which has also a molt inhibiting activity, is produced under various stresses, including food reduction or starvation (Keller & Orth, 1990). Elevated CHH levels have been suggested as a possible factor in extending the intermolt period beyond its normal length with reduction in food supply (Oh & Hartnoll, 2000).

Energy intake is not the only factor that will impact the molt frequency of the crab *S. serrata*. Our studies have demonstrated that the duration of molt cycle could also be extended when the animals were fed a diet with a low concentration of dietary protein. This last nutrient plays an important role on the growth of muscle which is the greatest tissue mass of the crustacean. In the case of *S. serrata* protein gain reaches 70% of the dry tissue mass during one molt cycle.

The energy budgets of juvenile crabs, based on the energy intake, estimated dry body mass gain and maintenance energy, confirm the previous observations. Comparing the energy budgets for the two tissue growth phases shows two fundamental differences. The proportion of energy intake channeled to tissue growth or recovered energy (RE) was three times higher during postmolt period when tissue growth is high. Conversely, this maintenance energy (HEm) is twice as high during intermolt stages of the molt cycle. The difference between the maintenance energy proportions during the two growth periods gives an estimate of the energy stored for ecdysis. Thus, in our experimental conditions, approximately 33% of the intake energy is required for molting and stored during intermolt period (70% of the total

duration of the molt cycle). 5% of that energy is lost with the exoskeleton at ecdysis and around 28% is required to cover the extra metabolic cost associated with molting, especially for osmoregulation, as ecdysis implies large amount of water exchange. These results were confirmed by the biochemical analysis indicating higher lipid content in crabs during intermolt stages. Although, the phenomenon of lipid or energy accumulation in preparation for ecdysis is relatively well documented (Al-Mohanna & Nott, 1989; Tian et al., 2012), to our knowledge this is the first time that a crustacean bioenergetic study has been used to assess the energy share dedicated to molting. Thus, for ecdysis the crab requires a substantial proportion of ingested energy and this observation confirms that when the crab is underfed its somatic growth slows down and it is unable to store enough energy to go through the molt, which is therefore delayed.

Overall, as juvenile mud crabs require a large amount of energy for ecdysis, we observed a higher proportion of intake energy is used for maintenance (75%) than for growth (21%). In this work, HEm was calculated in two ways: resolving the energy balance equation and from the model linking growth rate and feed intake when growth is null. Maintenance energy (HEm) value estimated by the energy balance equation (116 kj.kg^{-1} of wet iBW.d^{-1}) was twice higher than the value obtained from the model linking tissue growth and feed intake (59.5 kj. kg^{-1} of wet iBW.d^{-1}). When using this last model, a lower level of feed intake (maintenance energy intake) was considered because the heat increment of feeding is less important (McGow & Curtis, 2013) and no energy is stored for molt. Furthermore, the difference between the values of HEm from the two models gives us another estimate of the energy dedicated to molt. The maintenance energy value (345 kj.wet kg^{-1}.d^{-1}) of the shrimp *Lipopenaeus vannamei* determined from the growth-meal size model (Lupatsch et al., 2008) was about 6 times higher than the HEm value of the crabs obtained in our study. This difference could be explained by the fact that the mud crab is much less active than the shrimp.

6.2 - ADVANCES IN APPLIED NUTRITION OF THE CRAB *S. SERRATA*

Based on the knowledge gained in the preliminary studies, the effect of different diets on growth (tissue growth and molt duration), energy balance and quantitative aspect of nutrient utilization (feed leaching rate, feed digestibility and voluntary feed intake) were analyzed. This approach is fundamental for the mud crabs whose water content varies dramatically through their molt stage. Following this principle two successive studies were undertaken.

In a first study, we showed that fishmeal (FM) could be totally replaced by soy protein concentrate (SPC) as the main source of protein. These two ingredients exhibited high digestibility coefficients for dry matter, protein and energy. When FM was totally replaced by SPC to an equivalent level of protein in the diet, crabs exhibited a tissue growth rate was not affected during postmolt. Furthermore, tissue growth rate was higher during intermolt stages leading to improve efficiency values of feed conversion, protein retention and energy retention. In the mean time the overall energy balance was not affected by the protein sources.

Based on these results, the second study showed that a practical diet with 40% of dietary proteins (P/E = 22.13 $g.Mj^{-1}$), of which almost three quarters was derived from SPC, resulted in a daily protein consumption of 6.5 ± 1.1 g per kg of live crab. This diet led to the best growth (tissue growth and molt frequency) and the best feed efficiency (FCR, PER, retention of proteins and lipids). This result was confirmed by a bioenergetic study which showed a significantly higher allocation of the energy intake for growth of crabs fed with this diet. We confirmed the results of Unnikrishnan & Paulraj (2010) and Catacutan (2002) who reported that higher dietary protein levels in diet (44 and 49%) reduced the growth rate (extended molt cycle in our study). To explain this phenomenon we put forward the hypothesis of the postprandial ammonia toxicity as it has been described for fishes (Wicks & Randall, 2002) without excluding, in our case, the toxicity of anti-nutritional factors still present at low concentrations in SPC.

6.3 - RECOMMENDATIONS AND CONCLUSIONS

Several recommendations can be put forward from the results of this thesis.

In terms of experimental methodology for nutrition studies of crab focusing on feed, nutrient utilization and growth, we strongly advise to take into account molting frequency and tissue growth (gain in dry body mass), the later being of utmost importance.

In terms of practical nutrition (i) we confirm the possibility of using the soy protein concentrate to partially or totally replace fishmeal. However, the decision will depend on the cost benefit ratio of such replacement knowing that SPC protein unit is still sometimes more expensive that its equivalent from fishmeal; (ii) a practical feed with 40% protein and a P/E ratio 22 $g.Mj^{-1}$ allows an optimum intake of energy and protein, feed efficiency and growth in young *S. serrata* crab.

We finally reached one of the objectives of this thesis by answering the question whether it is possible to raise the crab with a pellet made from ingredients available on the international market. This is not only possible, but also we can feed the crab on the formulated diets without fishmeal. Our results may contribute to a possible development of a sustainable mud crab aquaculture in New-Caledonia.

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Résumé - En Nouvelle-Calédonie il y a une forte volonté politique pour diversifier l'aquaculture qui repose encore aujourd'hui sur la crevetticulture. Dans ce contexte le crabe de palétuvier est considéré comme une espèce à fort potentiel. Un des principaux verrous au développement de la carcinoculture en Nouvelle-Calédonie est la disponibilité d'un aliment granulé commercial. Ainsi le principal objectif de cette thèse est d'améliorer notre connaissance des besoins nutritionnels du crabe de palétuvier afin d'être en mesure de formuler un aliment équilibré pour son élevage. Cependant avant d'aborder les études nutritionnelles nous avons vérifié le nombre d'espèces de crabes de palétuvier présentes en Nouvelle-Calédonie. Nos résultats d'études morphologiques et génétiques de 63 individus provenant de 9 sites des côtes Ouest et Nord-Est de la Nouvelle-Calédonie ont confirmé l'existence d'une unique espèce commercialisée: *Scylla serrata*. C'est donc sur cette espèce que nous avons travaillé en nutrition avec deux séries expérimentales ayant pour objectifs : i) d'évaluer le concentré protéique de soja (CPS) en comparaison avec la farine de poisson comme principale source en protéines et ii) de déterminer le taux optimum d'incorporation du CPS pour la mue et la croissance tissulaire des animaux. Nous avons ainsi observé deux phases de croissance tissulaire au cours d'un cycle de mue (CM): une phase rapide (CTR) qui démarre après la mue et dure jusqu'au début de l'intermue (elle représente 30% du CM) suivi d'une phase de croissante lente (CTL) sur toute la durée de l'intermue et jusqu'à la mue suivante (elle représente 70% du CM). L'accumulation des protéines et des lipides au cours du CM a suivi le même profil que la croissance tissulaire contrairement aux cendres qui ont augmenté de façon rapide durant les 5 jours suivant l'ecdysis pour atteindre un plateau jusqu'à la prochaine mue. Les deux phases de croissance étaient corrélées avec une prise de l'aliment par les animaux maximale pendant les deux premières semaines suivant la mue. Elle a diminué de moitié sur les 5 semaines suivantes et s'est maintenue ensuite à un niveau de base jusqu'à la prochaine mue. L'énergie ingérée était allouée principalement à la croissance et à la l'entretien respectivement durant les périodes CTR et CTL. Durant la phase de croissance lente, 28% de l'énergie ingérée étaient mis en réserve en prévision de la prochaine mue. Le remplacement de la farine de poisson par le CPS n'a pas modifié la croissance tissulaire, l'efficience de l'aliment et le bilan énergétique des animaux quelque soit la phase de croissance considérée. Le taux d'incorporation dans l'aliment de 42% de CPS a permis la meilleure croissance (fréquence de mue et croissance tissulaire), efficience de l'aliment et la rétention de l'énergie des protéines et des lipides. L'hypothèse d'une toxicité de l'ammonium issu de la dégradation des protéines en excès ou des facteurs antinutritionnels du soja est avancée pour expliquer les effets négatifs observés avec les aliments renfermant des taux d'incorporation élevés en CPS. En conclusion, nos travaux apportent des informations originales sur la croissance tissulaire et les dépenses énergétiques durant un cycle de mue et la capacité du crabe juvénile d'utiliser le CPS comme principale source de protéines. Sur ces bases nous sommes en mesure de préconiser des contraintes nutritionnelles permettant de formuler un aliment équilibré sans farine de poissons pour l'élevage du crabe de palétuvier *S. serrata*.

Abstract - In New Caledonia, there is the strong political will to diversify aquaculture which is mainly based on shrimp farming. In this context, mud crabs have been considered as a potential species for aquaculture development. One of the main constraints to develop crab farming is the availability of formulated feed. Thus, the main purpose of this thesis is to get information on the crab nutritional requirements in order to formulate a balanced diet. However, we had to clarify first how many species of mud crab were present in New-Caledonia. The result of our morphological and genetic investigations carried out on 63 specimens from 9 areas of the west and northeast coast of New-Caledonia confirmed that only one species, *Scylla serrata,* is commercialized in this country. Consequently, *S. serrata* was used in our nutritional study based on two experiments to: i) evaluate the soy protein concentrate (SPC) compared with the fish meal as the main protein source and ii) determine the optimum level of SPC in the diet for molting and tissue growth. We observed two tissue growth phases within one molt cycle (MC): a fast tissue growth (FTG) occurred after ecdysis until early intermolt stage (30% of MC) which is followed by a slow tissue growth (STG) period from intermolt to ecdysis (70% of MC). Protein and lipid deposition followed the same trend than tissue growth while ash level increased quickly during five days after molt and then remained stable until the next molt. The two growth phases were correlated with the voluntary feed intakes (VFI) which was maximum during 2 weeks after ecdysis and then decreased by 50% over the five following weeks to reach a baseline until the next molt. Intake energy was allocated mainly for growth during FTG period and for maintenance during STG period. During STG, 28% of the ingested energy was accumulated for the next ecdysis. Replacement of fishmeal by SPC as main protein source did not affect tissue growth, efficiency of feed utilization and energy budget of crabs whatever the tissue growth period considered. The dietary SPC inclusion of 42% in the diet promoted growth (molt frequency and tissue growth), feed efficiency and retention of energy, protein and lipid. Hypothesis related to ammonia toxicity from catabolism of proteins in excess or anti-nutritional factors from soybean could explain the negative effects of higher inclusion of SPC in the diet for juvenile crabs. In conclusion, our work brings novel information on tissue growth, energy budget during a molt cycle and the ability of juvenile crab to use SPC as a main source of protein. On this basis we suggest to formulate nutritionally balanced diet without fishmeal to farm juvenile mud crabs *S. serrata*.

Key words: Juvenile mud crab, *Scylla serrata*, tissue growth, molt cycle, molt frequency, ecdysis, voluntary feed intake, feed efficiency, fishmeal, soy protein concentrate, energy budget.