Effects of Pom-nang Seaweed, *Gracilaria fisheri* on Growth, Survival, Feed Efficiency and Catalase Production of Juvenile Mud Crab, *Scylla paramamosain*

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Fishery Science and Technology

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I hereby certify that this work has not been accepted in substance for any other degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ ผลของสาหร่ายผมนาง *Gracilaria fisheri* ต่ออัตราการเจริญเติบโต อัตราการรอดตาย ประสิทธิภาพการใช้อาหาร และการสร้างคะตาเลสของปูขาว *Scylla paramamosain* ระยะวัยรุ่น
ผู้เขียน นายวศินะ รุ่งเรือง
สาขาวิชา วิทยาศาสตร์และเทคโนโลยีประมง
ปีการศึกษา 2564

บทคัดย่อ

ผลของสาหร่ายผมนาง *Gracilaria fisheri* ต่ออัตราการเจริญเติบโต อัตราการรอดตาย ประสิทธิภาพการใช้อาหาร และการสร้างคะตาเลสของปูขาว *Scylla paramamosain* ระยะวัยรุ่น โดยการทดลองแบ่งเป็นสองส่วนคือ ส่วนที่หนึ่งเป็นการศึกษาผลของการเสริมสาหร่ายผมนางในอาหารที่ระดับต่างกันและการใส่สาหร่ายผมนางเป็นที่หลบซ่อน ส่วนที่สองเป็นการศึกษาความหนาแน่นของปูที่ระดับต่างกันและระดับความหนาแน่นของสาหร่ายผมนางที่เหมาะสม ในส่วนแรกได้ทำการศึกษาโดยใช้ปูขาวระยะวัยรุ่น น้ำหนัก 0.02 กรัม ซึ่งได้ออกแบบแผนการทดลองโดยมีสองปัจจัยได้แก่ ปัจจัยด้านอาหาร 5 ชุดการทดลอง (PSP0, PSP2, PSP4, PSP6 และชุดควบคุม) และปัจจัยทางด้านการใช้สาหร่ายผมนางเป็นที่หลบซ่อน 2 ชุดการทดลอง (มีและไม่มีที่หลบซ่อน) โดยให้อาหารวันละ 8 เปอร์เซ็นต์ของน้ำหนักตัว ทำการทดลองเป็นระยะเวลา 28 วัน จากการศึกษาพบว่าสูตรอาหารที่แตกต่างกันมีผลอย่างมีนัยสำคัญต่อน้ำหนักที่เพิ่มขึ้น อัตราการเจริญเติบโตจำเพาะของน้ำหนักตัว ประสิทธิภาพการใช้โปรตีน (P < 0.05) แต่ไม่มีผลต่ออัตราการแลกเนื้อ แอนไซม์คะตาเลส และอัตราการรอดตาย (P > 0.05) ของปูขาวระยะวัยรุ่น ผลจากการศึกษาปัจจัยของที่หลบซ่อน พบว่าปูขาวที่ใช้สาหร่ายผมนางเป็นที่หลบซ่อนมีประสิทธิภาพการใช้โปรตีน อัตราการแลกเนื้อ แอนไซม์คะตาเลส และอัตราการรอดตายแตกต่างอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับการไม่ใช้สาหร่ายเป็นที่หลบซ่อน (P <0.05) อย่างไรก็ตามจากการทดลองครั้งนี้พบอิทธิพลร่วมระหว่างสองปัจจัยต่อน้ำหนักที่เพิ่มขึ้น อัตราการเจริญเติบโตจำเพาะของน้ำหนักตัว ประสิทธิภาพการใช้โปรตีน (P <0.05) ผลจากการศึกษาครั้งนี้สามารถสรุปได้ว่าการใช้กุ้งเคย (ชุดควบคุม) เหมาะสมต่อการอนุบาลปูขาวระยะวัยรุ่นมากที่สุด อย่างไรก็ตามอาหารสำเร็จรูปเสริมด้วยสาหร่ายผมนางมีศักยภาพที่จะทดแทนกุ้งเคยได้ ถึงแม้ว่าประสิทธิภาพการเจริญเติบโตจะลดลงก็ตาม และการใช้สาหร่ายผมนางเป็นที่หลบซ่อนเป็นสิ่งจำเป็นสำหรับปูขาวระยะวัยรุ่นโดยมีแนวโน้มการเจริญเติบโต อัตราการรอดตาย และแอนไซม์คะตาเลสสูง นอกจากนี้ การใช้สาหร่ายเป็นที่หลบซ่อนและการใช้อาหารสำเร็จรูปเสริมด้วยสาหร่ายผมนางระดับ 4 เปอร์เซ็นต์ (PSP4) มีการเจริญเติบโตและอัตราการรอดสูงเมื่อเทียบอาหารสูตรอื่นๆ

สำหรับผลการทดลองความหนาแน่นของปูขาวและความหนาแน่นของสาหร่ายผมนางเพื่อเป็นที่หลบซ่อนต่ออัตราการเจริญเติบโตและอัตราการรอดตายของปูขาวระยะวัยรุ่น โดยออกแบบการทดลองโดยมีสองปัจจัยได้แก่ ความหนาแน่นของปูขาว (100, 200, 300 และ 400 ตัว/ตร.ม.) และความหนาแน่นของที่หลบซ่อน (100, 500, 1,000 กรัม/ตร.ม. และไม่มีที่หลบซ่อน) ให้อาหารด้วย PSP4 วันละ 8 เปอร์เซ็นต์ของน้ำหนักตัวเป็นระยะเวลา 28 วัน ผลการศึกษาพบว่า ความหนาแน่นของปูขาวที่เลี้ยงในระดับที่ต่างกันมีผลต่อค่าประสิทธิภาพการใช้โปรตีนและอัตราการแลกเนื้ออย่างมีนัยสำคัญ (P <0.05) ความหนาแน่นของสาหร่ายผมนางเป็นที่หลบซ่อนมีผลอย่างมีนัยสำคัญต่อ ประสิทธิภาพการใช้โปรตีน อัตราการแลกเนื้อ และอัตราการรอดชีวิต (P <0.05) จากการทดลองสามารถสรุปว่าการเลี้ยงปูขาวที่ความหนาแน่น 400 ตัว/ตร.ม. และการใส่สาหร่ายผมนางที่ความหนาแน่น 1,000 กรัม/ตร.ม. เป็นแนวทางที่เหมาะสมสำหรับอนุบาลปูขาวระยะวัยรุ่น ผลการศึกษาครั้งนี้จะช่วยพัฒนาเทคนิคและวิธีใหม่สำหรับการพัฒนาแนวทางการอนุบาลลูกปูขาวระยะวัยรุ่นต่อไป

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ABSTRACT

The study on the effects of Pom-nang seaweed, *Gracilaria fisheri* on growth, survival, feed efficiency and catalase production of juvenile mud crab, *Scylla paramamosain* was conducted. The main objectives of this study are to investigate (1) effect of dietary diets with different Pom-nang seaweed supplementation's levels on growth rate, survival rate, feed efficiency and catalase activity of juvenile mud crab and (2) influence of Pom-nang seaweed density as a shelter and stocking density on growth, feed efficiency, and survival rates of juvenile mud crab. Two experiments were conducted. The first experiment, juvenile mud crabs were individually stocked in plastic containers with an initial body weight of 0.02 g. Two factors including five dietary treatments (Pom-nang seaweed powder supplemented at the level of 0%, 2%, 4% and 6% and control; mysid shrimp) and two shelter treatments (with Pom-nang seaweed as shelter and without shelter) were designed. Juvenile mud crabs were fed at 8% body weight for 28 days. It was found that different diets had significant effects on weight gain (WG), specific growth rate on body weight (SGRw), protein efficiency ratio (PER) but not for feed conversion ratio (FCR), catalase activity (CAT) and survival rate of the crabs. The crabs reared with seaweeds as shelter had significantly different PER, FCR, CAT and survival rate values compared to those without seaweeds as shelter ($P < 0.05$). There were significant differences on the interactions of the combined factor on WG, SGR and PER ($P < 0.05$). It was found that mysid shrimp was the most appropriate food for nursing juvenile mud crab. However, formulated diets supplemented with seaweeds had a potential to replace mysid shrimp. The combination of seaweed as shelter and the formulated diets with 4% seaweed (PSP4) had the highest values of all growth performances and survival rate compared to the others. The second experiment, effect of stocking density and density of Pom-nang seaweed as shelter on growth and survival rates of juvenile mud crab were conducted using juvenile mud crab with an initial body weight of 0.02 g. Two factors including four stocking density treatments (100, 200, 300 and 400 crab/m^2) and four densities of Pom-nang seaweed as shelter (100 g/m^2, 500 g/m^2, 1,000 g/m^2 and without shelter) were tested. Juvenile mud crabs were fed with PSP4 at 8% body weight for 28 days. It was found that different stocking density had significant effects on PER and FCR ($P < 0.05$), and different density of Pom-nang seaweed as a shelter had significant effects on PER, FCR and survival rate ($P < 0.05$). The stocking density at 400 crab/m^2 and high density of Pom-nang seaweed as a shelter at 1,000 g/m^2 was the optimal rate for nursing juvenile mud crab as indicated by growth performances and survival rate. There was no impact of interaction effect of the combined factor. This study can support a new technique and method for the development of nursing mud crab in the future.

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CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Mud crab is commercially important species in many countries including Thailand. It is highly accepted as one of the main sources of food for human consumption (Kornthong *et al.*, 2019). Several provinces in Thailand such as Chantaburi, Trat, Samutsongkhram, Ranong, and Trang have been cultured mud crab for more than two decades (Areekijseree, Chuen-Im, and Panyarachun, 2010). With high demand both in local and international markets, they are one of the main target species required for aquaculture. However, in most of the culturing practice, the juvenile mud crabs were currently collected from wild. Production from hatchery cannot sustain the demand from farming industry.

The stage of nursing juvenile mud crabs, *Scylla paramamosain* is very crucial for the success of mud crab culture. However, there is a paucity of knowledge and information on the juvenile mud crabs rearing especially in Thailand. Factors that promoting their optimum growth and survival are essential, but the information is very limited on especially appropriate food for nursing juvenile mud crabs. Naturally, mud crabs consume marine detritus, mangrove plants, seagrass, mollusks, crustaceans and fish (Food and Agriculture Organization of the United Nations [FAO], 2011).

Macroalgae has been used to develop low-cost feed for aquatic animal and supplements in aquaculture due to their nutritional value and to replace animal ingredients (Niu *et al.*, 2015). It has been used as a source of nutrient food materials, water quality and habitat for aquatic animal including mud crab. Trino *et al.* (1999) used seaweeds (such as Gracilariopsis) as shelters for mud crab *S. serrata* in grow-out ponds as to provide substrates or shelters. This could lead to reduce cannibalism of mud crabs (Ut *et al.*, 2007). The used of Pom-nang seaweed as shelter help to improve survival rate of *S. paramamosain* during juvenile stages (Van *et al.*, 2021). *Gracillaria* spp. or Pom-nang seaweed has long been cultured in Thailand and used to promote the success of shrimp and crab culture (Ruangchuay *et al.*, 2010). Normally, the main components of Pom-nang seaweed including carbohydrates, protein, and polysaccharides have played important role in growth of crustaceans (Holme *et al.*, 2009). Seaweed supplementation help to improve feed efficiency and survival of *S. olivacea* fattening in ponds (Lestari *et al.*, 2020). In shrimp, many studies indicated that seaweed supplementation contributed to an improvement of growth, feed utilization, carcass quality and immune response of shrimp cultured. Seaweed supplemented in diet has been used to boost the immune response of *S. serrata* juveniles (Traifalgar, 2017). However, total replacement of fish meal by seaweeds showed negative impact on growth and feed efficiency of shrimp (Yu *et al.*, 2016). It is postulated that partial supplementation of seaweed to crab diet may promote growth rate, survival rate of mud crab, especially during juvenile stages.

Therefore, study on diets with different Pom-nang seaweed level supplemented, and Pom-nang seaweed used as shelter to determine growth rate, survival rate and immune response of juvenile mud crab is crucial and has potential to indirectly develop mud crab industry. The knowledge obtained from this study will be an important contribution to an advancement of nursing technique for *S. paramamosain* and lead to commercial production of the juvenile mud crab.

1.2 Related Literature

1.2.1 Mud crab, *Scylla* biology

1) Taxonomy of *Scylla*

Scylla serrata has frontal lobe spines of high, bluntly pointed with rounded interspaces. Outer carpus spine straight or slightly concave. Carpus inner spine and propodus spine were pairs of large spines obvious. Polygonal patterning clearly present on all appendages. (Figure 1.1A)

Scylla paramamosain has frontal lobe spines of moderately high, pointed with angular interspaces. Outer carpus spine reduced. Carpus inner spine absent and propodus spine were pairs of large spines obvious. Polygonal patterning present on last two pairs of legs, weak or absent on other appendages. (Figure 1.1B)

Scylla olivacea has frontal lobe spines of low, rounded with shallow interspaces. Outer carpus spine reduced. Carpus inner spine absent and propodus spine were reduced spines obvious. Polygonal patterning absents from all appendages. (Figure 1.1C)

Scylla tranquebarica has frontal lobe spines of moderate, blunted with rounded interspaces. Outer carpus spine convex. Carpus inner spine and propodus spine were pairs of large spines obvious. Polygonal patterning present on last two pairs of legs, weak or absent on other appendages. (Figure 1.1D)

Figure 1.1 *Scylla serrata* (A), *Scylla paramamosain* (B), *Scylla olivacea* (C), *Scylla tranquebarica* (D)

Source: Keenan *et al*. (1998)

2) Distribution

Scylla spp. inhabits mangrove areas across the Pacific and the Indian Oceans. Most *S. serrata* is spreading in South Africa, Japan and Australia (FAO, 2011). Keenan *et al*. (1998) was reported that *S. tranquebarica* and *S. olivacea* have narrow distribution along and *S. olivacea* were commonly found at lower salinities of mangrove forest and coastal areas (Keenan *et al*., 1998). *S. paramamosain* is commonly. (Figure 1.2)

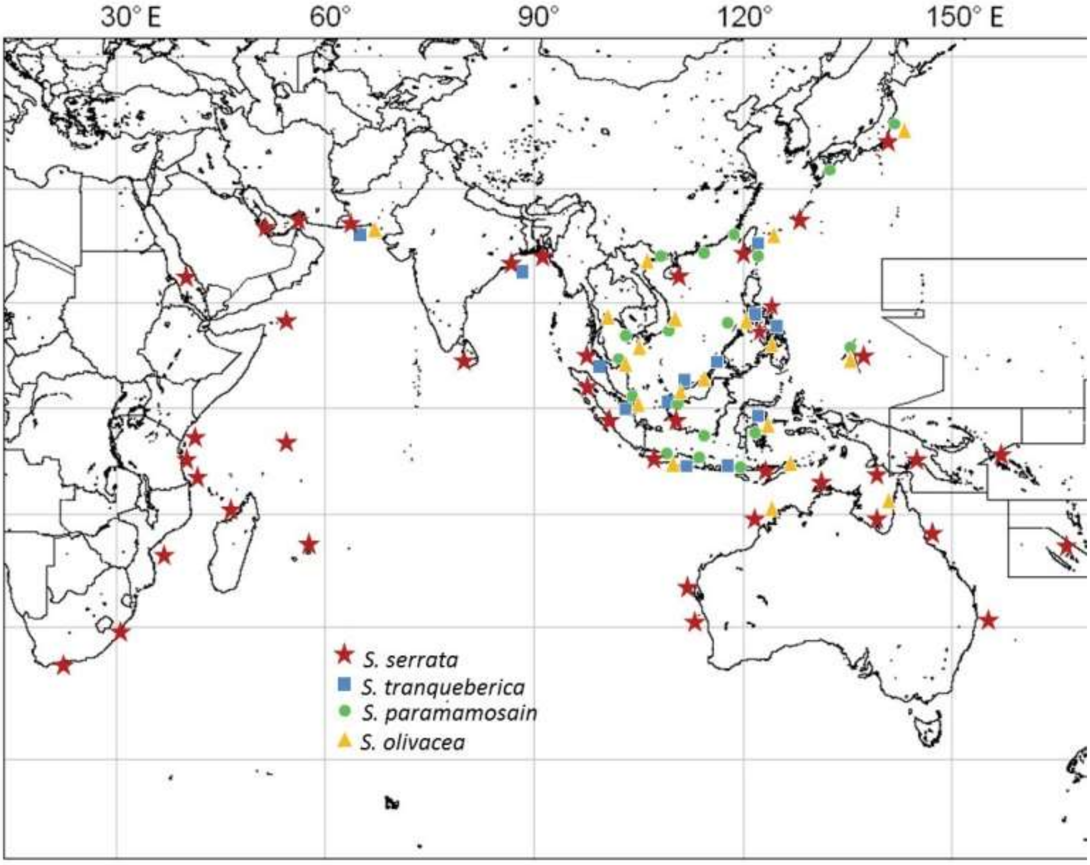

Figure 1.2 Distribution of four *Scylla* species

Source: Alberts-Hubatsch (2015)

3) Life cycle

Mud crabs are euryhaline species (Figure 1.3), which spend their adulthood in brackish water area and later migrate offshore for spawning (Srinivasagam *et al*., 2000). Adult males and females can be recognized by the shape of their abdominal flap, which is triangular for males and semi-circular for females (FAO, 2011).

Temperature affects the time it takes for eggs to hatch and larvae to be released once they have been spawned, with a shorter time at higher temperatures and longer time at lower temperatures (Zeng, 2007).

At hatching, this zoea stage is made of five sub-stages. The final zoea stage changes into a megalopa, then through another molt, these megalopa larvae metamorphose into juvenile crabs. It takes 25 to 31 days for the zoea larvae to develop into young crabs at water temperature ranged from 24.5 to 29.1°C, while salinity was kept at 28 to 33 psu (Motoh *et al*., 1977). Temperature has a negative affect with the duration of each larval stage, and their survival rate is influenced by both temperature and salinity (Cui1 *et al.*, 2021).

Once the crabs molt, the carapace width (CW) and body weight (BW) were increasing. However, as the crab ages, the frequency of molts decreases, and the rate of increase in body weight is greater than the rate of increase in carapace width. As a result, mud crab sizes increased, with the carapace width gradually slowing down

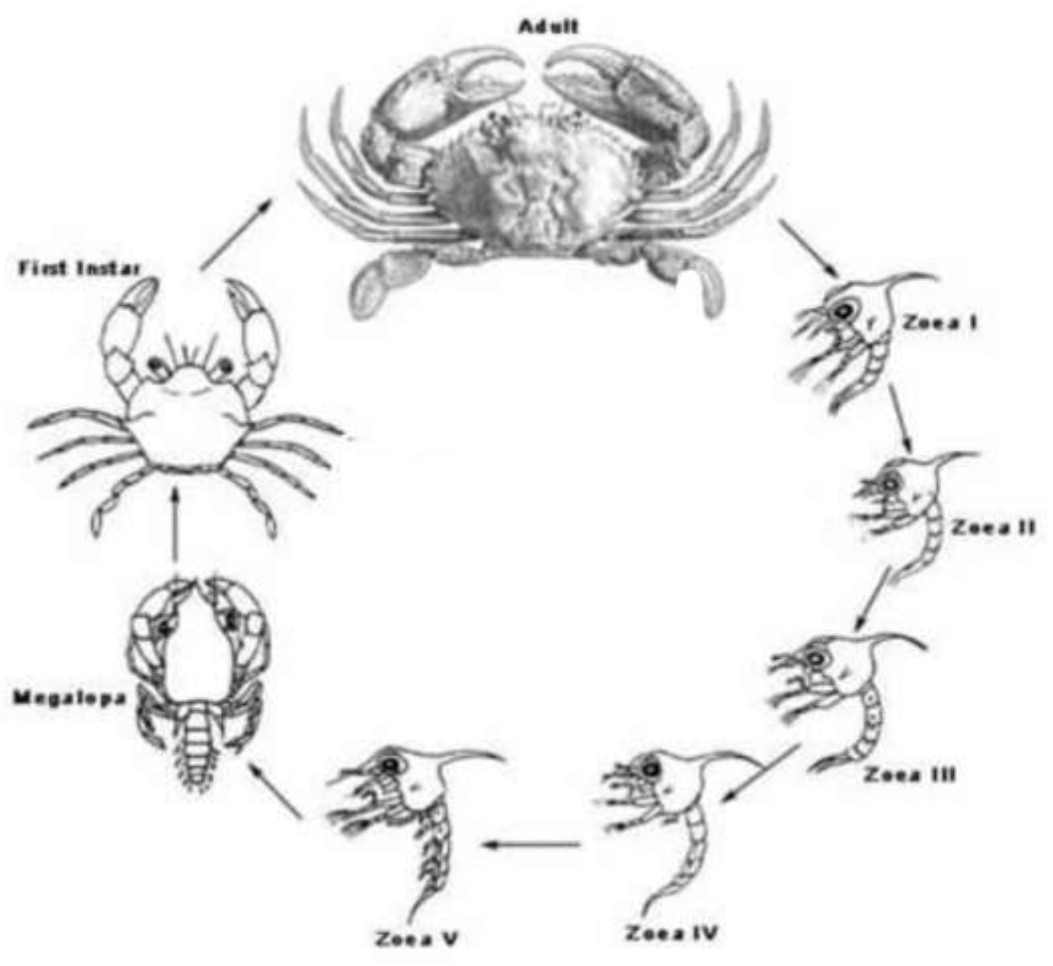

Figure 1.3 Life cycle of *Scylla* spp**.**

Source: Balasubramanian and Gopal (2014)

1.2.2 Hatchery operation of mud crab

1) Broodstock condition

Broodstock condition is essential in the provision of mud crab larvae. Broodstock can be sourced from the wild or are generally already mated and fertilized in wild or reared pond. Male broodstock usually no need to hold in broodstock tanks (Quinitio, 2001). 26 – 29 ℃ (Hamasaki, 2003). When the temperature rises, the egg's decrease development (Hamasaki, 2003). Mud crabs should be fed a high-quality, mixed fresh diet as shrimp, mussle, polychaete squid and artificial diet. Diet can lead to improvement in larval production and quality. Females can be feed with fresh mussel meat at 5 – 30 % body weight and provide over two feeds per day (Waiho *et al*., 2018). Uneaten feed should be get rid to preventing bacterial and fungal infestation (Hamasaki, 2003). Broodstock's tank should be using sand tray for crabs to be spawned. After spawning, ovigerous females (Quinitio, 2001). Ovigerous females will be not fed during the egg incubation period. The eggs hatched to zoea 1 after 12 – 15 days at temperature 25 – 32 °C (Ut *et al*., 2007).

2) Larvae nursing

The newly hatched larvae can be reared in rectangular tank or cylindrical tank in several 1,000 l at 50 – 120 larvae l^{-1} with mild aeration. The culture water was treated with 1–3 ppm streptomycin sulfate (Gong *et al*., 2015). The rearing salinity was 25 – 35 psu and the temperature range was 26 – 32 °C (2 °C difference in water temperature can lead to problems on growth) and 15 – 50% water was exchanged daily. The larvae were fed with *Tetraselmis* sp. and the rotifer *(Brachionus* sp.) at the stage of zoea 1, and the newly hatched *Artemia* nauplii was given to zoea 1 and continued up to zoea 5 stage at a density of 5 – 10 ml^{-1} until become to megalopa stage were observed. At megalopa stage, on-grown *Artemia* were added at the same density. *Nannochloropsis* sp. microalgae was provided as food to the rotifer (Baylon, 2010). The newly hatched larvae to first crab instar (C1) after 27 days (Ut *et al*., 2007).

3) Feeding and nutrition of larvae

The newly hatched larvae (first stage of zoea 1 (Z1) to zoea 2 (Z2)) can feed with *Tetraselmis* sp., rotifer and newly hatched *Artemia* naupli. Larvae mud crab will continue the feed with newly hatched *Artemia* at Zoea 3 (Z3). On-grown *Artemia* and minced mussel meat can feed from megalopa to first crab instar (Waiho *et al*., 2018). Microalgae can be used in mud crab larvae rearing, while microalgae are using to feed rotifers (Baylon, 2009) and also add to mud crab larval rearing tanks at densities of 0.5–5.0 × 10^4 cells/ml. Mud crab larvae may consume them but the nutritional value will be low compared with the rotifers or *Artemia*. In some study show that the use of *Nannochloropsis* has relatively high levels of EPA for larval mud crab development as low mortality, morphogenesis of larvae and abnormal molting in *S. serrata* and *S. paramamosain* (Syafaat *et al.,* 2019). Because of their small size and high nutritional value, rotifers can help boost the survival rate of mud crab larvae in the initial stage. Rotifers was feeding at the early-stage of mud crab in zoea 1 to zoea 2 stage in density at 20-40 individual /ml, when the mud crab larvae become zoea 3 and zoea 4 was feeding at density 10 individual /ml (Syafaat *et al.,* 2019). However, Nurdiani and Zeng (2007) mentioned that mud crab larvae can be feeding with rotifers (40 individual /ml) and *Artemia* nauplii (4 individual /ml) from zoea 1 until zoea 5. *Artemia* is the main feed for larvae mud crab. *Artemia* usually used at densities of 0.5- 1 individual/ml from zoea 2 or zoea 3 with newly hatched *Artemia* and on-grown *Artemia* at densities of 3-5 individual/ml for megalopa stage to first crab instar (Syafaat *et al.*, 2019). Artemia at umbrella stage can be used to feed newly hatched mud crab larvae for *S. serrata* larval culture. Feeding density of *Artemia* are increasing when larval stage increased (Baylon, 2009). The density of *Artemia* was increased around 1–2 individual /ml, when larvae reached zoea 2 and continued up to zoea 5 stage. Megalopa can feed on adult-sized *Artemia* at the same densities.

1.2.3 Early juvenile mud crab nursing technique

1) Nursery operations

Megalopa, 3-5 days-old in nursery tanks are stocked either into tanks at a density of the surface area around 2,000-4,000/m^3 (1,000-2,000/ton) of water available for megalopa to settle. It is recommended that 10–133 megalopa/m^2 be stocked into net cages in ponds at salinity of 15–45 psu and temperature of 20-32 ℃ (Syafaat *et al.*, 2021).

2) Stocking density

Juvenile mud crabs (*S. paramamosain*) with carapace width less than 1 cm were nursed at density 110-230 individual/m^2 until carapace width increase to 1.5-2.0 cm for 15 days, survival rates of 71%, 62% and 58% were reported, respectively (Ut *et al*., 2007). After that, the juvenile mud crabs were moved to a pond until reach carapace width 3.0-4.0 cm at density 30 individual/m^2 (Syafaat *et al.*, 2021). For longer nursery period of *S. paramamosain* at density 70 individual/m^2 had a survival rate of 52-66% in 30 days (Ut *et al*., 2007). Juvenile mud crabs (*S. olivacea*) reared at a density of 50 individual/m^2 showed lower survival rate of between 21 and 37% for 21-day trial (Syafaat *et al.*, 2017). Daly *et al*. (2009) found the survival of juvenile red king crab at low stocking density was significantly higher than high stocking density. Mud crab (*Scylla*) culture with low stocking density was the most efficient on feed conversion ratio but no for growth rate. High stocking density of crab was effect on appetite suppression, feeding inhibition, Sublethal injuries such as limb loss, or cannibalism with other juvenile crab (Trino *et al*., 1999).

2) Feeding of juvenile mud crab

The *Artemia* (5 days old) at 1-5 individual/ml and shrimp meat feeds at 2 g/m^3 are provided to the megalopa stage mud crab, prior to settlement. Once settled, combination of food items such as *Artemia*, shrimp meat and formulated feeds can be used to feed juvenile mud crabs once or twice daily. On-grown *Artemia*, up to adult size, can also be fed throughout the megalopa and early juvenile mud crab stages (Syafaat *et al.*, 2019). *Artemia* is often used as the main feed during the maintenance of early juvenile mud crab stages (Ly Van *et al*., 2021). The use of formulated feeds, with added spirulina powder together with *Artemia* nauplii has high survival rate in juvenile mud crabs *(S. paramamosain)* than treatment being fed only formulated feeds (without spirulina powder) and *Artemia* nauplii (Permadi and Juwana, 2015). Formulated diets of 45–55 percent crude protein and 9–15 percent lipid appear to support optimal growth in juvenile mud crab (Sheen and Wu, 1999).

3. Water Quality

Temperature and salinity are the two most important parameters to consider during the larvae rearing and nursery phases of juvenile mud crabs (Syafaat *et al.*, 2019). Salinity levels ranging from 5 to 40 psu have no effect on the survival of instar 2 juvenile mud crabs (*S. serrata*); the recommended salinity range for growth is between 10 and 20 psu. The temperature of 25-35 ℃ is suitable for growth and survival of juvenile mud crabs (Ruscoe *et al*., 2004).

4. Shelter

To reduce cannibalism during early juvenile mud crab nursery, black nylon, bunched netting or seaweed may be placed at the bottom of rearing tanks, as shelters (Syafaat *et al.*, 2019), Shelter to increases the surface area available for settled crabs. (Ly Van *et al*., 2021). Structured habitats, such as netting, leaf fronds, straw, PVC off-cuts or artificial sea grass provide significant shelter from predation and cannibalism in crabs (FAO, 2011). The density of seaweed as shelter at 1,000 g/m^2 and 2,000 g/m^2 significantly higher than 500 g/m^2 and without shelter in 28-day trial (Ly Van *et al.,* 2021).

1.2.4 Pom-nang seaweed, *Gracilaria fisheri,* biology

1) Taxonomy

A general description of *Gracilaria* has a variable morphology. It is a. Blades are usually red, but can be brownish, green, or almost black depending on light and nutrient conditions (Critchley, 1997).

Figure 1.4 *Gracilaria fisheri*

Source: Ruangchuay *et al.* (2007)

Table 1.1 Nutrient composition of *Gracilaria* spp. (% dry weight)

Species	Place	Moisture	Protein	Lipid	Ash	Reference
G. fisheri	Wild	8.1	7.5	3.9	17.5	Ruangchuay *et al.* (2006)
G. fisheri	Cultured	8.0	6.3	2.9	28.0	Ruangchuay *et al.* (2006)
G. arcuata	Wild	4.0	13.5	7.0	31.9	Younis *et al*. (2016)
G. changii	Wild	-	6.9	3.3	22.7	Yuan *et al.* (2008)

Table 1.2 Mineral composition of *G. fisheri* (mg.100 g^{-1} DW)

Mineral	Wild	Cultured
Calcium (Ca)	182.0	82.0
Phosphorus (P)	245.0	237.0
Sodium (Na)	438.0	165.0
Potassium (K)	4,389.0	10,794.0
Magnesium (Mg)	303.0	485.0
Iron (Fe)	61.0	90.0
Copper (Cu)	0.3	0.2
Zinc (Zn)	2.2	0.7
Chlorine (Cl)	1.9	1.9

Source: Ruangchuay *et al.* (2006)

The supplemented of dietary *G. lemaneiformis* at the level of 2%–3% showed high growth rate and survival rate than supplemented at 0, 1, 4 and 5% on shrimp culture in 56-day trial with an initial body weight about 0.27±0.00 g. The Pom-nang Seaweed powder (Yu *et al.,* 2016). The shrimp *Penaeus monodon* fed with diet supplement with wakame (*Undaria pinnatifida*) at 2% inclusion level has higher growth and survival rate than supplemented at 0,1,3,4,5 and 6% in 56-day trial with an initial body weight about 0.68±0.01 g. The mud crab *S.olivacea* with a initial body weight of 130-180 g, fed with diet supplement with *Kappaphycus alvarezi* seaweed at 30% showed high growth rate, feed efficiency and survival rate than seaweed supplemented at 0, 10 and 20% for 35-day trial in pond. (Yu *et al.*, 2016).

4) Pom-nang seaweed as shelter

Shelters increase the Shelters can reduce the frequency of intraspecific aggression in aquatic organisms and then improve the survival rate (He *et al*., 2017). The spider crab juveniles *Maja squinado* at density 2 individual/cylinder reared with natural seaweed as shelter showed higher survival rate than without shelter (Gil *et al*., 2019). Ut *et al.* (2007) who found survival rate of juvenile mud crab with shelter was higher than without shelter (sand substrate alone) reared for 15 days from first crab instar (*S. paramamosain*). Seaweeds provide as habitats can improve growth and survival of juvenile swimming crab *Portunus trituberculatus* with presence shelter was significantly higher than without shelter (He *et al*., 2017). Daly *et al.* (2009) also found that the survival of juvenile red king crab with shelter was significantly higher than without shelter. Ly Van *et al*. (2021) also found juvenile mud crab (*S. paramamosain*) reared at density 300 individual/m^2 with density of *G. tenuistipitata* seaweed as shelter at density 1,000 g/m^2 and 2,000 g/m^2 significantly higher than 500 g/m^2 and without shelter has the lowest survival rate in 28-day trial.

1.2.5 Nutritional requirement

Table 1.3 Dietary protein levels typically used in formulated diets for crustacean juveniles

Species	Dietary protein	Reference
Megalopa		
Scylla serrata	79.4%	Genodepa *et al.* (2004)
S. *serrata*	55%	Holme *et al.* (2006)
Juvenile		
Eriocheir sinensis	39–42.5%	Mu *et al.* (1998)
S. *serrata*	50%	Sheen and Wu (1999)
S. *serrata*	50%	Sheen (2000)
S. *serrata*	34.2–51.8%	Catacutan (2002)

Table 1.4 Dietary lipid levels used in previous nutritional studies with crustacean juvenile

Species	Lipid levels	Reference
Penauus vannamei	8.78–9.99%	Gong *et al.* (2000a)
Macrobrachium rosenbergii	2–10%	Sheen and D'Abramo (1991)
Scylla serrata	1.7–13.8%	Sheen and Wu (1999)
S. *serrata*	8–9.5%	Sheen (2000)
S. *serrata*	6 and 12%	Catacutan (2002)

Table 1.5 Cholesterol levels previously used in nutritional with crustacean juveniles

Species	Cholesterol level	Reference
Megalopa		
Scylla serrata	0.14–1.0%	Holme *et al.* (2006a)
S. serrata	1%	Genodepa *et al.* (2004)
Juvenile		
Penauus monodon	0.2–0.8%	Sheen *et al.* (1994)
P. vannamei	0.23–0.42%	Duerr and Walsh (1996)
P. vannamei	0–0.5%	Gong *et al.* (2000b)
S. serrata	1%	Sheen and Wu (1999)
S. *serrata*	0.51%	Sheen (2000)

4) Carbohydrates

Carbohydrates are an energy sources and as essential structural components in organisms. Studies on juveniles *P. monodon* fed with diets containing varied levels of carbohydrates (3-35%) reported the diets was not affected by starch content (Catacutan, 1991). For mud crab, (Holme *et al*., 2009). show in Table 1.7

Table 1.6 Dietary carbohydrate levels previously used in experimental formulated diets for crustacean juveniles

Species	Dietary level	Reference
Penauus monodon	3–35%	Catacutan (1991)
Scylla serrata	13.5-27%	Sheen (2000)

1.3 Objectives

1. To evaluate effect of dietary diets with different Pom-nang seaweed (*G. fisheri*) supplementation's levels on growth rate, survival rate, feed utilization and catalase activity of juvenile mud crab

2. To assess influence of Pom-nang seaweed density (*G. fisheri*) as a shelter and stocking density on growth, feed utilization, and survival rates of juvenile mud crab.

1.4 Expected advantages

As a limited supply of juvenile mud crab collected from the wild and less production from hatchery has serious impact on supply chain of mud crab industry. This is the main factor controlling the advancement of this industry besides very high demand from grow-out farm and consumers. This study will help to create new knowledge in developing low-cost feeds for optimum growth of juvenile mud crab, using dietary diets with different Pom-nang seaweed supplementation, and applying Pom-nang seaweed as shelter to improve nursing technique for juvenile mud crab. This will contribute to the advancement of nursing practice of *S. paramamosain* and lead to the commercial production of juvenile mud crab.

CHAPTER 2
METHODOLOGY

2.1 Acclimatization of animals

Crab megalopa (*S. paramamosain*) was purchased from Pakpanang Basin Royal Fisheries Development Center was acclimated into 8 tanks (2×3×0.2 m^3) at density of 100 megalopae/m^2 (salinity 25 psu and temperature 27 °C). They were fed with on-grown *Artemia* and formulated diet (PSP0), size 500-750 µm, until reaching juvenile mud crab at the stage of instar II (C2). They were harvested and fully intact and active instar II crabs were used in the experiment. The measurement of the juvenile mud crabs was recorded. The juvenile mud crab was gently dried using tissue paper and were individually weighed for an initial body weight (IBW) by electronic balance (0.0001 g). The carapace width was measured by vernier caliper (0.1 mm) (Ruscoe *et al.*, 2004).

2.2 Diet preparation

Pom-nang seaweed (*G. fisheri*) was obtained from Muang Pattani district, Pattani Province. The seaweed was washed and dried by sunlight before finely grounded by using a laboratory mill (Fig 2.1A). Feed formulation consisted of fish meal, squid meal, soybean meal, shrimp head meal, wheat flour, rice bran, fish oil, vitamin, mineral, and varying concentration of Pom-nang seaweed powder. The Pom-nang seaweed powder (PSP) was supplemented at the level of 0%, 2%, 4% and 6% (corresponding to PSP0, PSP2, PSP4, and PSP6). The ingredients were then mixed, and 1 mm feed pellets were produced using a pelletizer (Fig 2.1B). Proximate analysis was measured for all four diet formulas (Fig 2.1C) (Appendix 1).

Table 2.1 Composition of the formulated diets with graded levels of Pom-nang seaweed powder

Ingredient (%)	**PSP0**	**PSP2**	**PSP4**	**PSP6**
Fish meal	35	35	35	35
PSP	0	2	4	6
Squid meal	15	15	15	15
Soybean meal	12	11.5	11	10.5
Shrimp head meal	10	10	10	10
Wheat flour	19.9	18.4	16.9	15.4
Rice bran	1.1	1.1	1.1	1.1
Fish oil	6	6	6	6
Mineral	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5
Total	100	100	100	100

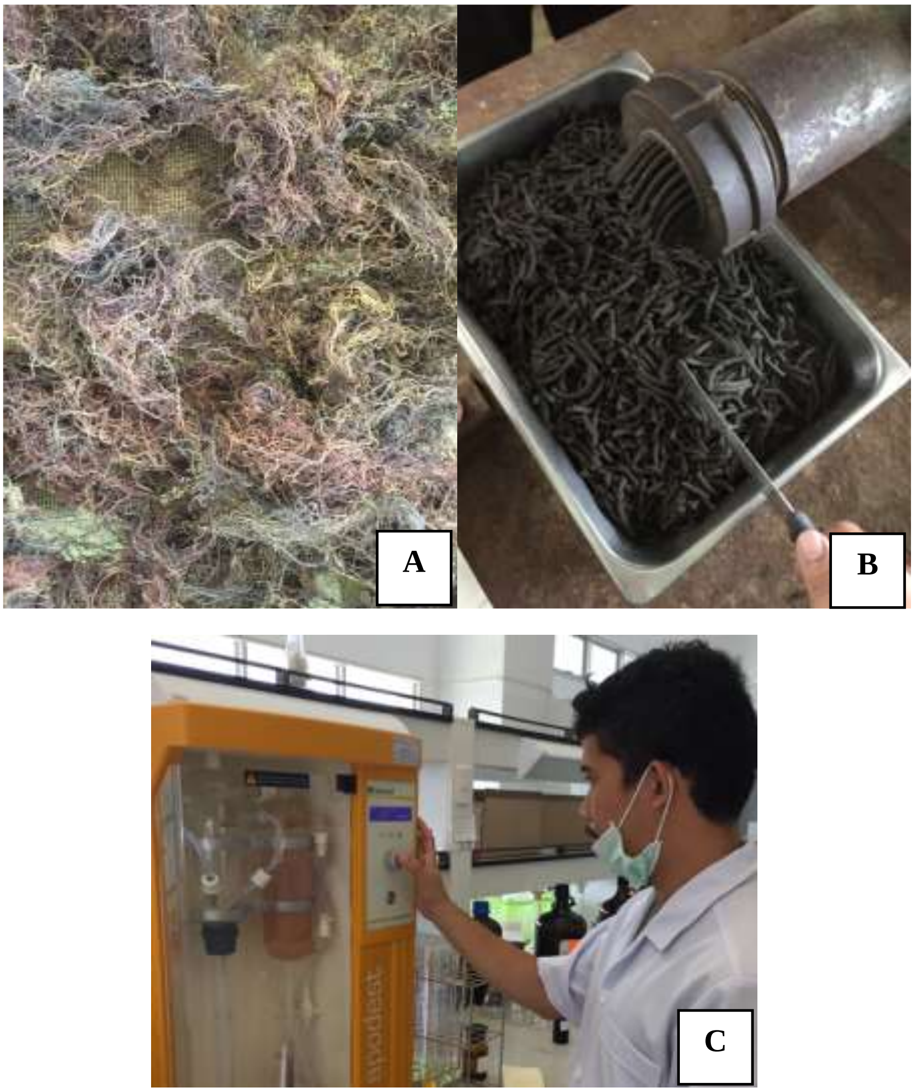

Figure 2.1 Dried Pom-nang seaweed (A), Producing pellet (B), Proximate analysis (C).

2.3 Experiment 1: Effect of dietary PSP diets and Pom-nang seaweed as a shelter

Juveniles *S. paramamosain* (C2) were individually stocked in 950 ml plastic container (116 mm diameter × 146 mm deep). The experimental condition was controlled at the salinity of 25 psu and temperature of 27 °C (Fig 2.2). Juvenile mud crabs with an initial body weight of 0.02 g were distributed randomly into plastic containers. Sand (particle size > 1 mm) was washed and dried by sunlight before using as substate in the containers. All containers were covered with a 20 mm deep layer of sand (Ut *et al.,* 2007).

Figure 2.2 Juvenile of *S. paramamosain* in plastic containers

A two-factor factorial design was used in the experiment (dietary diets × Pom-nang seaweed as a shelter) with five dietary treatments (PSP0, PSP2, PSP4, PSP6, and control; mysid shrimp) and two sheltered treatments (with seaweed as a shelter at density 100 g/m^2 and without seaweed as a shelter (Gil *et al.*, 2019)). Three replicates for each treatment were conducted. Ten juvenile mud crabs were used per replicate (Fig 2.3A). Altogether 300 individual crabs were used in this study (Fig 2.3B).

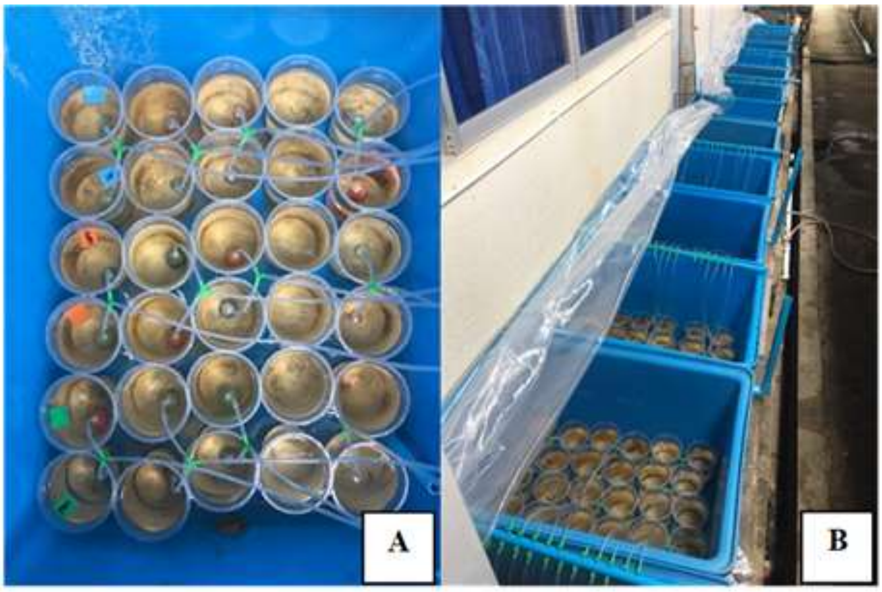

Figure 2.3 The experimental condition (A), 300 individual crabs were used in this study (B).

Juvenile mud crabs were fed twice a day (08:00 and 16:00 hour) with the experimental diets at 8% body weight for each treatment followed the methodology of

Zheng *et al.* (2018). Three hours after each meal, the uneaten feed was siphoned out (Unnikrishnan & Paulraj, 2010). They were grown for 28 days before all crabs were measured individually for carapace width and weighed.

2.3.1 Water quality

Approximately, ten percent of water were exchanged daily (Syafaat *et al.*, 2019*)*. Daily water parameters including temperature, alkalinity and pH were recorded at 09:00. Salinity was measured with a refractometer. Dissolved oxygen, temperature and pH were measured using dissolved oxygen meter (YSI PRO 2030). Alkalinity was measured every three days in each tank by titration method (Fig 2.4). Ammonia was measured every three days by phenol hypochlorite method (Ruscoe *et al.,* 2004) (Appendix 1).

Figure 2.4 The measurements of dissolved oxygen, temperature and pH.

2.3.2 Growth experimental

An initial average body weight and carapace size of the crabs were measured at the beginning of the experiment. A weekly monitoring of average body weight (BW), carapace width (CW) and survival rate of the crabs were recorded (Fig 2.5). Several attributes were calculated as follows (based on the methodology done by Yu et al., 2016).

-Specific growth rate (SGR %)

$$SGR\ on\ carapace\ width = 100 \times \frac{ln\ \ Final\ CW\ (mm) - ln\ Initial\ CW\ (mm)}{Experimental\ duration\ in\ days}$$

$$SGR\ on\ body\ weight = 100 \times \frac{ln\ \ Final\ BW\ (g) - ln\ Initial\ BW\ (g)}{Experimental\ duration\ in\ days}$$

-Percent weight gain (WG %)

$$WG = 100 \times \frac{Final\ BW\ (g) - Initial\ BW\ (g)}{Initial\ body\ weight\ (g)}$$

-Feed conversion ratio (FCR)

$$Voluntary\ feed\ intake = Distributed\ feed - unconsumed\ feed$$

$$FCR = \frac{Dry\ voluntary\ feed\ intake}{Total\ final\ BW - Total\ initial\ BW}$$

-Protein efficiency ratio (PER)

$$PER = 100 \times \frac{Total\ final\ BW - Total\ initial\ BW}{Total\ amount\ of\ the\ feed \times Protein\ content\ in\ the\ feed}$$

-Survival rate

$$Survival\ (\%) = 100 \times \frac{Final\ amount\ of\ crabs}{Initial\ amount\ of\ crabs}$$

Figure 2.5 Monitoring of average body weight (BW), carapace width (CW) and survival rate of the crab.

2.3.3 Catalase activity

Three juvenile mud crabs from each treatment were randomly selected to measure immunity after 24 h of feeding trial sampling. Immune enhancement level due to seaweed were measured by catalase activity (CAT) by the method described by Claiborne (1985) and Iqbal *et al*. (1998). The assay mixture consisted of 0.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml of crab homogenate supernatant (10% w/v) in a total volume of 2.0 ml. The change in absorbance was recorded at 240 nm at the end of experiment (Fig 2.6). The enzyme activity was calculated as nmol H_2O_2 consumed/min/mg protein usi ng a molar extinction coefficient of 6.4×10^3 $M^{-1}cm^{-1}$.

Figure 2. 6 The changes of absorbance in catalase activity were observed by using spectrophotometer.

2.3.4 Statistical analysis

To determine the effects of both factors on WG, FCR, SGR, PER, CAT and survival, a two-way analysis of variance (ANOVA) were used. Once the significant difference was found, Duncan' s multiple range test was applied to identify the difference of each treatment (Appendix 2).

2.4 Experiment 2: Effect of stocking density and shelter density

Juveniles *S. paramamosain* (C2) were stocked in 30 l plastic containers (0.34 m width × 0.48 m length, 0.15 m^2 of surface area). The controlled environment was at 25 psu of salinity and 27 °C of temperature (Fig 2.7A). Juvenile mud crabs with an initial body weight of 0.02 g were placed randomly into plastic containers (Fig 2.7B). Sand (particle size > 1 mm) was washed and dried by sunlight before using as substate. All containers were covered with a 20 mm deep layer of sand (Ut *et al.*, 2007).

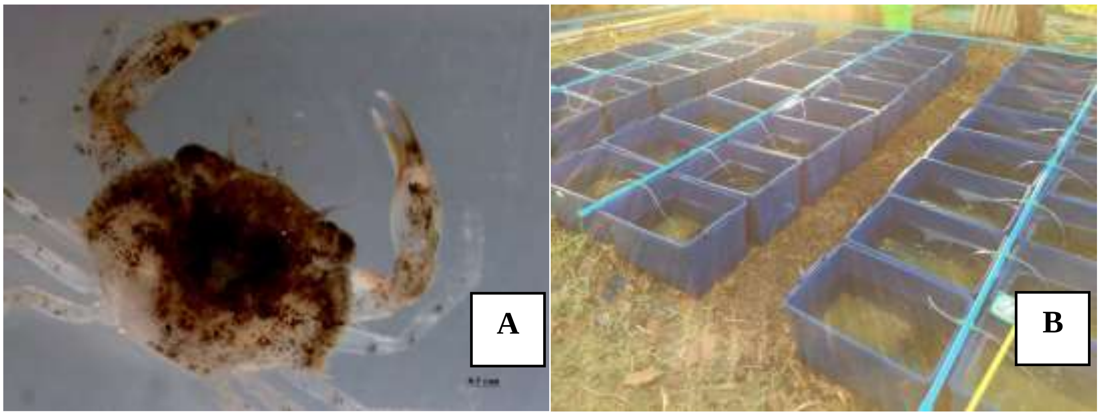

Figure 2. 7 *S. paramamosain* juvenile at second crab instar (A). Tank arrangement (B)

A two-factor factorial design was used (stocking density × shelter density) with four stocking density treatments (100, 200, 300 and 400 crab/m^2) and four densities of Pom-nang seaweed as a shelter 100 g/m^2, 500 g/m^2, 1,000 g/m^2 and without shelter (Fig 2.8). Three replicates for each treatment were conducted.

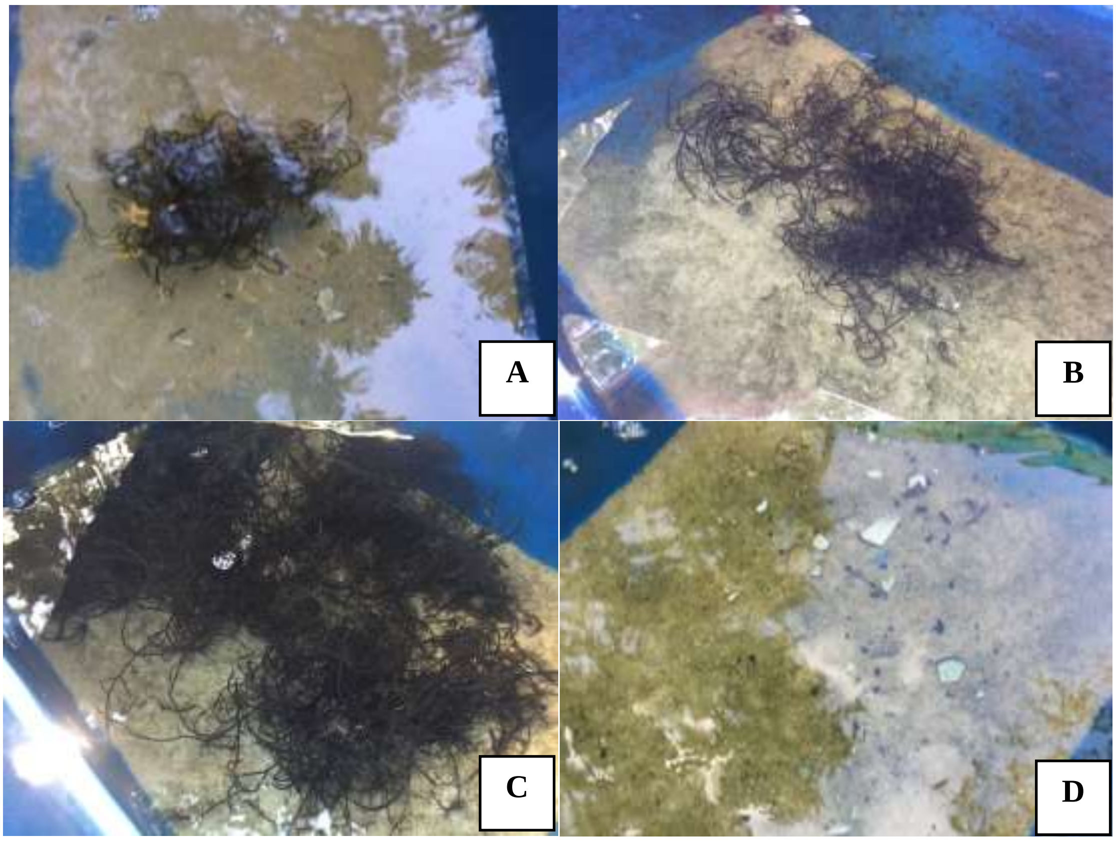

Figure 2.8 Four densities of Pom-nang seaweed as a shelter 100 g/m^2(A), 500 g/m^2(B), 1,000 g/m^2 (C) and without shelter(D).

Juvenile mud crabs were fed with the best formulated diet as found in the experiment 1 (PSP4) at 8% body weight for each treatment (Zheng *et al.,* 2018), twice a day (08:00 and 16:00 hour) (Fig 2.9). Three hours after each meal, the uneaten feed was siphoned out (Unnikrishnan & Paulraj, 2010). They were grown for 28 days before all crabs were measured individually for carapace width and weighed for final body weight.

Figure 2.9 Fed with Pom-nang seaweed powder at the level of 4% (PSP4) at 8% body weight for juvenile crabs

2.4.1 Water quality

Approximately, fifty percent of water were exchanged twice a week (Ly Van *et al.*, 2021). Daily water parameters including temperature, alkalinity and pH were recorded at 09:00 in several containers. Salinity was measured with a refractometer. Dissolved oxygen, temperature and pH were measured using dissolved oxygen meter (YSI PRO 2030). Alkalinity was measured every three days using titration method. Ammonia was measured every three days using phenol hypochlorite method (Ruscoe *et al.*, 2004).

2.4.2 Growth experimental

An initial average body weight and carapace size of the crabs were measured at the beginning of the experiment. A weekly monitoring of average body weight and survival rate of the crabs were recorded. Several attributes as WG, FCR, SGR, PER and survival rate were calculated as follows (Yu *et al.*, 2016).

2.4.3 Statistical analysis

To determine the effects of both factors on WG, FCR, SGR, PER and survival, a two-way analysis of variance (ANOVA) were used. Once the significance was found, Duncan' s multiple range test was applied to identify the difference of each treatment.

CHAPTER 3
RESULTS

3.1 Effect of dietary PSP diets and Pom-nang seaweed as a shelter on crab growth rate, survival rate, feed efficiency and catalase activity

The mean initial body weight and carapace width (±S.D.) of the juvenile mud crab was 0.022 ± 0.003g and 4.8±0.1 mm, respectively. In this experiment, the feed was made by supplementing the diet with PSP with an average of 45% protein concentration. It was found that there was no difference of protein concentration for all dietary formulas ($P > 0.05$). However, the control diet; mysid shrimp had significantly higher protein concentration, 65%, compared to the dietary diets ($P < 0.05$) (Table 3.1). Water parameter during experimental period including temperature, salinity, pH, ammonia nitrogen, and dissolved oxygen were 27 to 29 °C, 25 to 26 psu, 7.78 to 8.16, <0.05 mg/l, and > 4.7 mg/l, respectively.

Table 3.1 Values of proximate analysis of nutritional values (%) of experimental diets with different levels of Pom-nang seaweed powder (PSP) inclusion in feed. PSP0; PSP2, PSP4 and PSP6. Values with different superscript letters within a same column are significantly different ($P < 0.05$).

Proximate analysis	PSP0	PSP2	PSP4	PSP6	Control diet
Crude protein	44.06±3.41[a]	45.46±1.83[a]	46.50±0.97[a]	44.92±0.79[a]	64.82±2.10[b]
Crude fat	9.48±0.29[a]	9.53±0.38[a]	9.53±0.24[a]	9.58±0.34[a]	2.33±0.11[b]
Ash	11.51±0.04[a]	10.70±0.19[a]	11.02±0.01[a]	11.30±0.11[a]	11.25±0.24[a]

It is found that juvenile mud crab living with Pom-nang seaweed as shelter combination with control feed; mysid shrimp demonstrated the highest values on growth as percent weight gain (2,168.1±80.8%), specific growth rate on body weight (11.15±0.13%), specific growth rate on carapace width (3.9±0.2%) and survival rate (86.7±23.1%) when compared to other diets. Crabs nursing with Pom-nang seaweed as shelter combination with Pom-nang seaweed powder supplemented at the level of 4% in diet had the highest value of PER (2.30 ± 0.21) and the lowest value of FCR (0.94±0.21). The average values (Mean ± S.D) for growth performance, catalase activity and survival rate of juvenile mud crab nursing with different diets and shelter were observed and the results were shown in Table 3.2 and Table 3.3.

Table 3.2 Initial carapace width (ICW), final carapace width (FCW), initial body weight (IBW), final body weight (FBW) and specific growth rate on carapace width (SGRc) of juvenile mud crab nursing with different diets and shelter.

Diet	Shelter	ICW (mm)	FCW (mm)	IBW (g)	FBW (g)	SGRc (%/d)
PSP0	with	5.0±0.2	12.2±0.3	0.021±0.002	0.297±0.108	3.2±0.2
	without	4.8±0.0	11.9±0.7	0.023±0.011	0.212±0.088	3.2±0.2
PSP2	with	4.8±0.0	10.3±0.5	0.022±0.002	0.203±0.066	2.7±0.2
	without	4.8±0.0	11.0±1.0	0.021±0.004	0.231±0.088	3.0±0.3
PSP4	with	4.9±0.2	13.0±0.8	0.023±0.002	0.417±0.205	3.5±0.1
	without	4.9±0.2	10.1±1.3	0.025±0.007	0.193±0.091	2.6±0.5
PSP6	with	4.7±0.0	11.2±0.3	0.024±0.010	0.233±0.098	3.1±0.1
	without	4.8±0.1	10.7±0.7	0.023±0.006	0.179±0.043	2.9±0.3
control	with	4.8±0.1	14.4±0.8	0.022±0.003	0.506±0.139	3.9±0.2
	without	4.7±0.1	13.0±0.3	0.021±0.002	0.438±0.171	3.6±0.1

Table 3. 3 Percent weight gain (%), specific growth rate on body weight (%/d), protein efficiency ratio, feed conversion ratio, catalase activity (nmol H_2O_2 consumed/min/mg protein) and survival rate (%) of juvenile mud crab nursing with different diets and shelter.

Diet	shelter	WG	SGRw	PER	FCR	SR	CAT
PSP0	with	1,345.4±290.4	9.49±0.77	2.09±0.07	1.19±0.05	80.0±17.3	0.079±0.002
	without	851.6±270.3	7.96±0.95	1.53±0.41	1.69±0.51	83.3±15.3	0.076±0.004
PSP2	with	810.9±163.8	7.85±0.62	1.68±0.18	1.76±0.41	76.7±15.3	0.083±0.003
	without	991.9±273.0	8.46±0.92	1.29±0.31	2.11±0.71	66.7±5.8	0.075±0.004
PSP4	with	1,755.1±535.2	10.33±1.07	2.30±0.21	0.94±0.21	73.3±5.8	0.078±0.007
	without	674.2±237.0	7.18±1.19	1.38±0.22	2.02±0.47	63.3±5.8	0.076±0.003
PSP6	with	894.3±232.5	8.14±0.83	1.76±0.15	1.44±0.17	83.3±11.5	0.082±0.006
	without	680.7±161.3	7.28±0.79	1.72±0.26	2.45±0.53	70.0±10.0	0.075±0.001
control	with	2,168.1±80.8	11.15±0.13	1.04±0.27	1.62±0.53	86.7±23.1	0.080±0.009
	without	1,963.1±154.9	10.80±0.26	0.84±0.13	2.48±0.25	63.3±5.8	0.078±0.006

Statistically, the different diets (PSP0, PSP2, PSP4, PSP6 and control, shrimp) significantly affected WG, SGRw, PER ($P < 0.05$) but not on FCR, CAT and survival rate of the crabs ($P > 0.05$). However, crabs nursing under Pom-nang seaweeds as shelter had significantly different WG, SGRw, PER, FCR and survival rate compared to those without Pom-nang seaweeds as shelter ($P < 0.05$). There were the impacts of interaction effect on the combined factor on WG, SGRw and PER (Table 3.4).

Table 3.4 Two-way ANOVA table of percent weight gain (WG), specific growth rate on body weight (SGRw), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR) and catalase activity (CAT) of juvenile mud crab nursing with different diets and shelter.

Source	**SS**	**df**	**MS**	***F***	**Sig.**
WG					
Diet	6109266.11	4	1527316.53	21.455	<0.001
Shelter	985522.59	1	985522.589	13.844	0.001
Diet * Shelter	1313491.70	4	328372.924	4.613	0.008
SGRw					
Diet	37.91	4	9.479	14.137	<0.001
Shelter	8.31	1	8.309	12.393	0.002
Diet * Shelter	11.82	4	2.955	4.408	0.010
PER					
Diet	3.38	4	0.844	14.736	<0.001
Shelter	1.33	1	1.327	23.166	<0.001
Diet * Shelter	0.70	4	0.176	3.070	0.040
FCR					
Diet	1.98	4	0.494	2.675	0.062
Shelter	4.30	1	4.304	23.296	<0.001
Diet * Shelter	0.62	4	0.155	0.840	0.516
SR					
Diet	613.33	4	153.333	0.920	0.472
Shelter	853.33	1	853.333	5.120	0.035
Diet * Shelter	546.67	4	136.667	0.820	0.528
CAT					
Diet	0.00	4	0.000	0.143	0.964
Shelter	0.00	1	0.000	4.955	0.038
Diet * Shelter	0.00	4	0.000	0.491	0.743

SS=sun of squares; MS= mean squares

Results from Duncan' s multiple range test on the comparison among different dietary treatments found that the WG of the control feed (mysid shrimp = 2,065.6±157.6%) was significantly higher than PSP0 (1,098.5±368.9%), PSP2 (901.4±224.4%), PSP4 (1,214.6±698.2%) and PSP6 (787.5±213.8%). While there was no difference among other dietary diets (Figure 3.1). Similar result was found for SGRw in which the control feed (mysid shrimp = 10.98±0.26%) was significantly higher than PSP0 (8.72±1.14%), PSP2 (8.16±0.78%), PSP4 (8.76±2.00%) and PSP6 (7.71±0.86%) but no difference among dietary diets ($P > 0.05$) (Figure 3.2). For PER, the formulated diets as PSP0 (1.81±0.40), PSP2 (1.49±0.31), PSP4 (1.84±0.54) and

PSP6 (1.74±0.19) was significantly higher than control feed (mysid shrimp = 0.94±0.22) as shown in Figure 3.3.

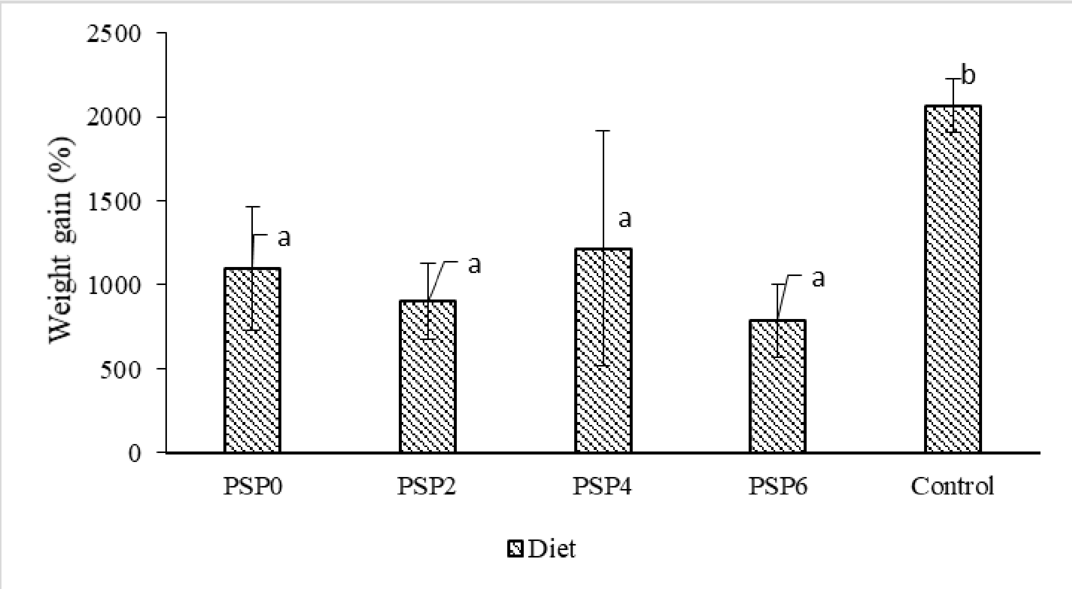

Figure 3.1 Comparison among different dietary treatments on weight gain (%) of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different (P < 0.05).

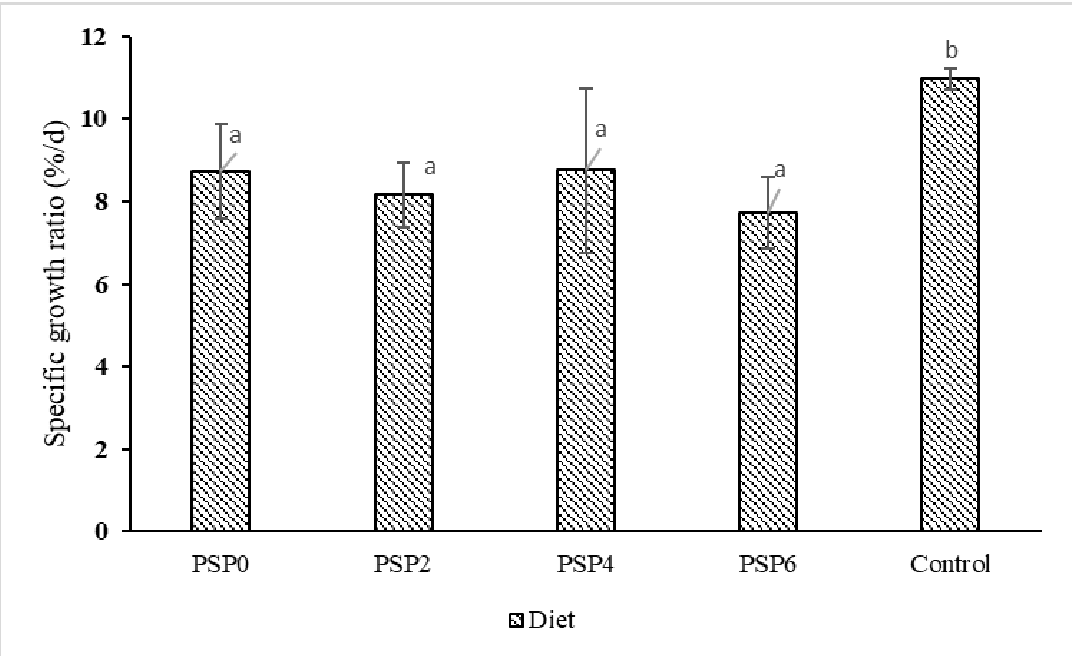

Figure 3.2 Comparison among different dietary treatment on specific growth rate on body weight (SGRw, %/day) of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different (P < 0.05).

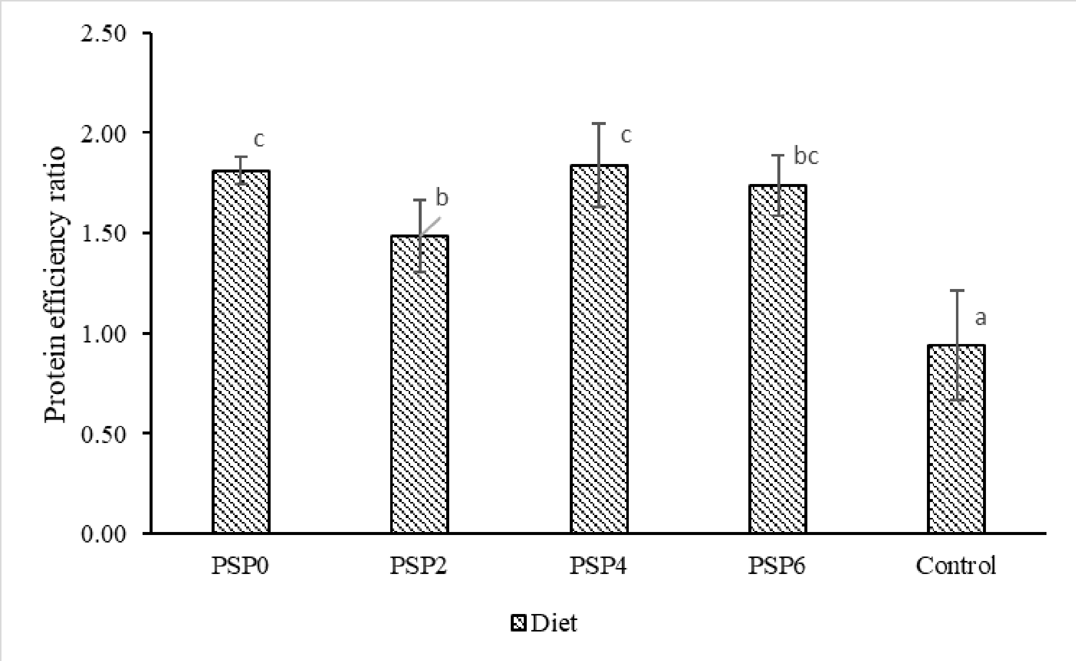

Figure 3.3 Comparison among different dietary treatment on protein efficiency ratio of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P < 0.05$).

Results of compared values including WG%, SGRw, PER, CAT and survival rate between crabs nursing with and without seaweeds as shelter are shown in Table 3.5.

Table 3.5 Effect of shelter on weight gain (WG), specific growth rate on body weight (SGRw) and specific growth rate on carapace width (SGRc), protein efficiency ratio (PER), feed conversion ratio (FCR), catalase activity (CAT) and survival of juvenile mud crab, *Scylla paramamosain*. Values with different superscript letters within a same column are significantly different ($P < 0.05$).

Shelter	With seaweeds as shelter	Without seaweeds as shelter
WG/%(g)	$1,394.8\pm590.5^{b}$	$1,032.3\pm532.2^{a}$
SGRw%(g/day)	9.39 ± 1.45^{b}	8.34 ± 1.55^{a}
Survival%	80.0 ± 14.1^{b}	69.3 ± 11.0^{a}
FCR	1.39 ± 0.41^{a}	2.15 ± 0.53^{b}
PER	1.77 ± 0.47^{b}	1.35 ± 0.39^{a}
CAT	0.082 ± 0.005^{b}	0.076 ± 0.003^{a}

The impacts of interaction effect of five dietary treatments and two sheltered treatments were observed on WG, SGRw and PER ($P < 0.05$). The details are shown in Figures 3.4, 3.5 and 3.6.

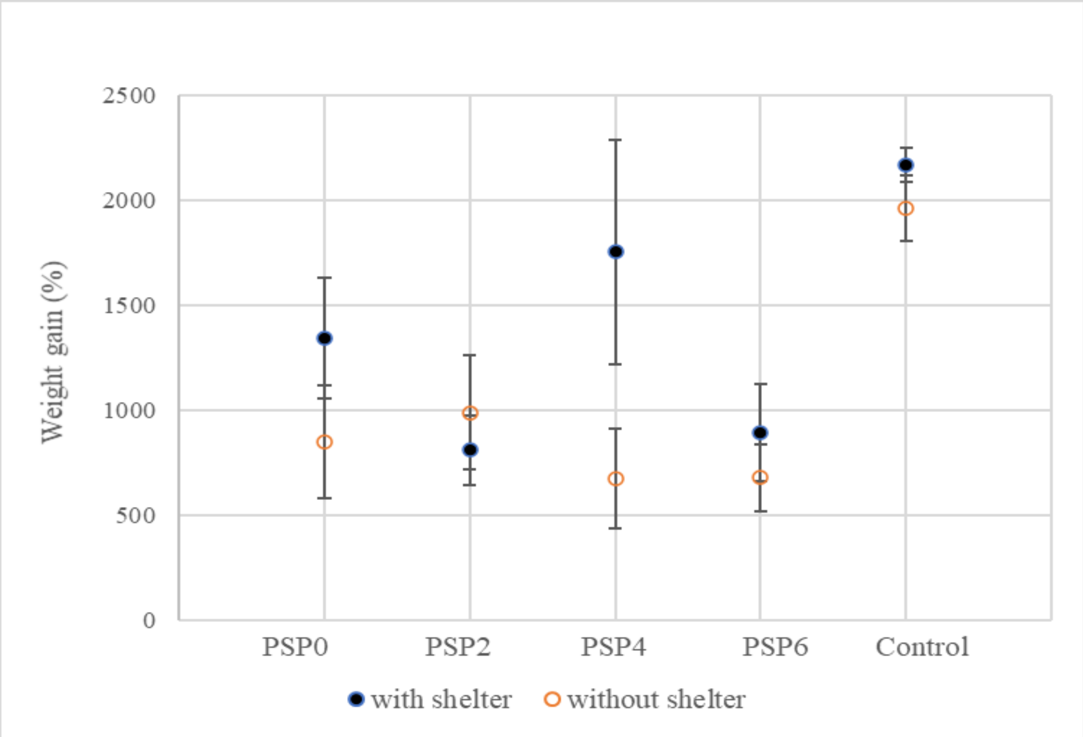

Figure 3.4 Weight gain (%) of juvenile mud crab, *S. paramamosain* in five dietary diet treatments (PSP0, PSP2, PSP4, PSP6, and control) and two sheltered treatments (with and without Pom-nang seaweed as a shelter)

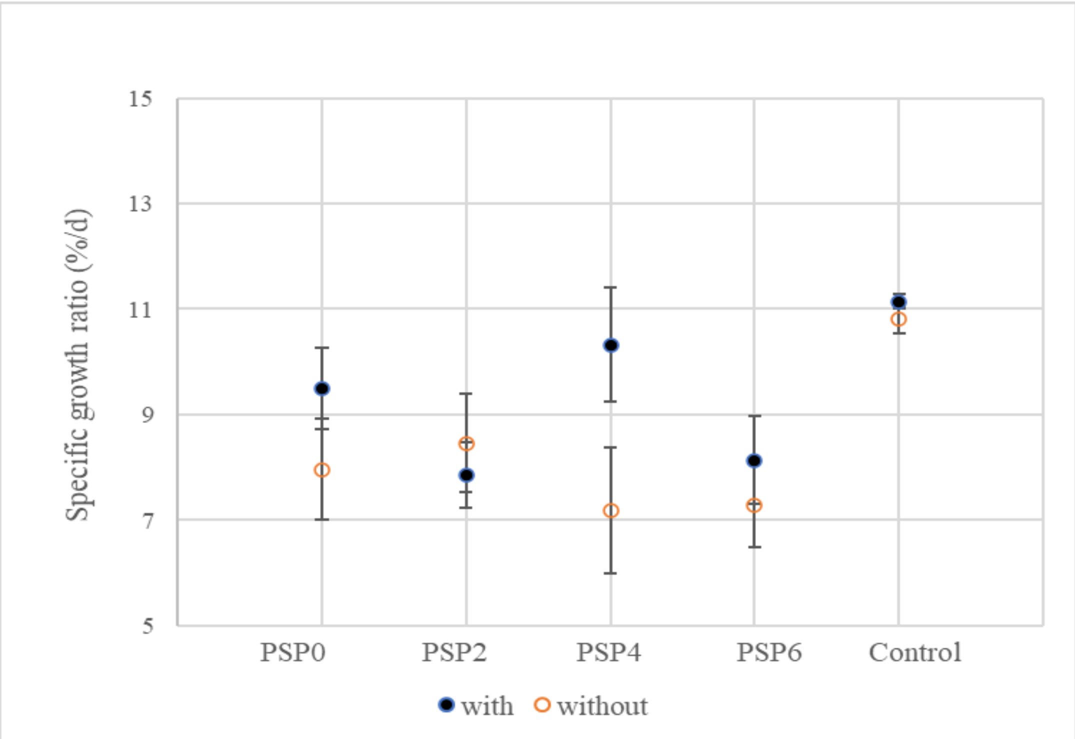

Figure 3.5 Specific growth rate on body weight (%/d) of juvenile mud crab, *S. paramamosain* in five dietary diet treatments (PSP0, PSP2, PSP4, PSP6, and control) and two sheltered treatments (with and without Pom-nang seaweed as a shelter)

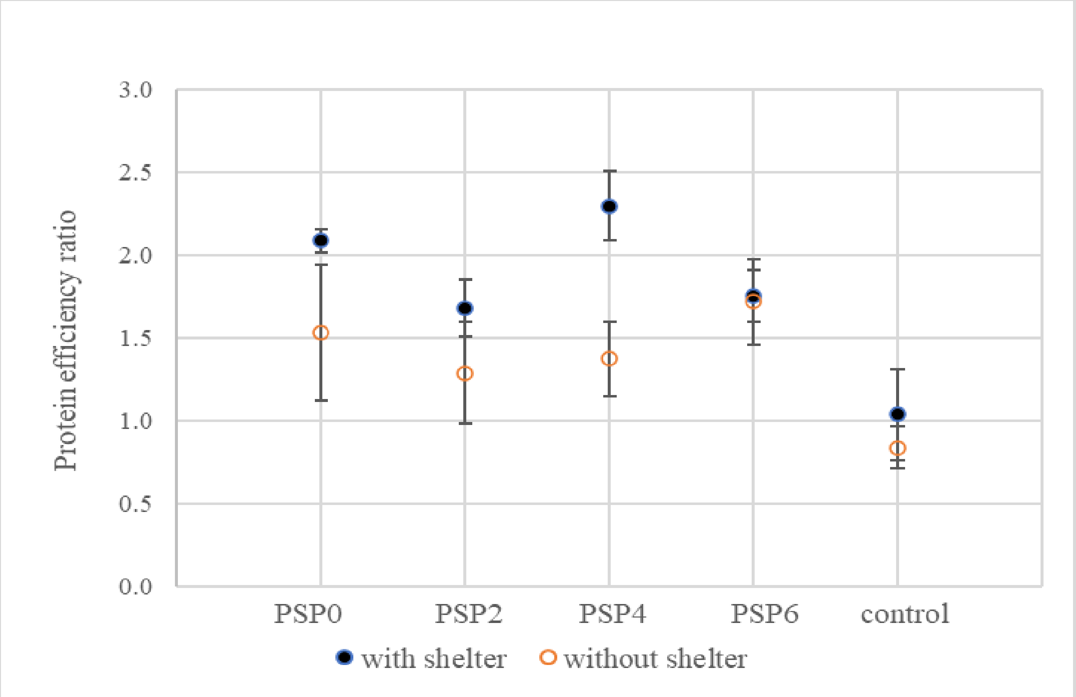

Figure 3.6 Protein efficiency ratio of juvenile mud crab, *S. paramamosain* in five dietary diet treatments (PSP0, PSP2, PSP4, PSP6, and control) and two sheltered treatments (with and without Pom-nang seaweed as a shelter)

3.2 Effect of stocking density and shelter density on growth rate, survival rate and feed efficiency

The mean initial body weight and carapace width (±S.D.) of the juvenile mud crab was 0.023 ± 0.003 g and 4.8±0.1 mm respectively. Water parameters during experimental period including temperature, salinity, pH, alkalinity level, ammonia nitrogen, and dissolved oxygen were 27 to 29 °C, 25 to 26 psu, 7.4 to 8.7, 77 – 150 mg/l, 0.01-0.22 mg/l, and > 3.8 mg/l, respectively.

It was found that growth of juvenile mud crab nursing with low density at 100 crab/m^2 combined with Pom-nang seaweed shelter at 500 g/m^2 had the highest values of weight gain (3,131.1±916.5%) and protein efficiency ratio (1.95±0.78). The density at 100 crab/m^2 and 200 crab/m^2 Pom-nang seaweed shelter at 500 g/m^2 had the highest values on specific growth rate on body weight (12.32±0.95% and 13.32±0.40%, respectively). However, crab nursing with the density of 100 crab/m^2 combined with seaweed shelter density at 1,000 g/m^2 had the highest value on survival rate (61.7±5.8%). For FCR, stocking density at 100 crab/m^2 combined with seaweed shelter at 1,000 g/m^2 had the lowest value compared to other treatments. Results of average values (Mean ± S.D) for percent weight gain (Figure 3.6), specific growth rate (Figure 3.8), protein efficiency ratio (Figure 3.9), feed conversion ratio (Figure 3.10) and survival rate (Figure 3.11) of juvenile mud crab nursing with different stocking density and seaweed shelter density were shown in Table 3.6 and Table 3.7

Table 3.6 Initial carapace width (ICW), final carapace width (FCW), initial body weight (IBW), final body weight (FBW) and specific growth rate on carapace width (SGRc) of juvenile mud crab nursing with different stocking density and shelter density.

Stocking density (crab/m^2)	**Shelter density (g/m^2)**	**ICW (mm)**	**FCW (mm)**	**IBW (g)**	**FBW (g)**	**SGRc (%/d)**
100	100	4.8±0.1	13.5±1.3	0.024±0.003	0.665±0.192	3.7±0.4
	500	4.7±0.1	13.6±1.0	0.021±0.001	0.662±0.144	3.8±0.3
	1,000	4.7±0.0	12.5±1.7	0.024±0.003	0.412±0.150	3.4±0.5
	without	4.8±0.2	14.3±0.7	0.022±0.001	0.578±0.178	3.9±0.2
200	100	4.8±0.1	12.3±0.3	0.022±0.001	0.572±0.213	3.4±0.1
	500	4.8±0.1	13.1±0.6	0.021±0.002	0.659±0.084	3.6±0.2
	1,000	4.7±0.1	11.6±0.5	0.023±0.004	0.456±0.085	3.2±0.2
	without	4.9±0.2	12.3±1.9	0.021±0.001	0.615±0.127	3.3±0.7
300	100	4.8±0.1	13.6±0.5	0.025±0.002	0.664±0.013	3.8±0.2
	500	4.8±0.2	12.7±1.0	0.023±0.004	0.668±0.200	3.5±0.3
	1,000	4.8±0.2	13.2±0.9	0.021±0.001	0.538±0.208	3.6±0.2
	without	4.8±0.1	14.1±1.1	0.023±0.001	0.624±0.062	3.8±0.3
400	100	4.8±0.0	14.1±1.2	0.026±0.003	0.774±0.183	3.8±0.3
	500	4.8±0.1	14.0±1.8	0.021±0.002	0.593±0.185	3.8±0.4
	1,000	4.7±0.1	14.3±0.1	0.024±0.005	0.715±0.244	3.9±0.1
	without	4.8±0.0	12.9±1.3	0.021±0.002	0.388±0.104	3.5±0.4

Table 3. 7 Percent weight gain (WG%), specific growth rate on body weight (SGRw), protein efficiency ratio (PER), feed conversion ratio (FCR),) and survival rate (SR) of juvenile mud crab nursing with different stocking density and shelter density.

Stocking density (crab/m^2)	Shelter density (g/m^2)	WG (%)	SGRw (%/d)	SR (%)	FCR	PER
100	100	2,757.4±905.4	11.86±1.06	40.0±15.0	1.36±0.43	1.71±0.62
	500	3,131.1±916.5	12.32±0.95	40.0±18.0	1.28±0.67	1.95±0.78
	1,000	1,697.4±851.2	10.07±1.58	61.7±5.8	1.16±0.25	1.91±0.38
	without	2,496.9±774.9	11.52±1.11	20.0±8.7	1.99±1.09	1.27±0.55
200	100	2,425.9±810.4	11.41±1.14	34.2±14.2	1.46±0.52	1.59±0.47
	500	3,066.4±352.4	12.32±0.40	39.2±10.4	1.45±0.26	1.51±0.25
	1,000	1,971.0±797.1	10.66±1.28	50.0±2.5	1.69±0.12	1.27±0.09
	without	2,807.3±440.9	12.01±0.52	19.2±6.3	2.24±0.30	0.97±0.12
300	100	2,576.3±198.3	11.73±0.26	20.0±6.7	2.50±0.31	0.87±0.12
	500	2,763.2±795.9	11.88±1.07	42.8±10.8	1.76±0.21	1.23±0.14
	1,000	2,412.1±898.0	11.35±1.38	52.8±5.1	1.48±0.14	1.46±0.13
	without	2,662.9±140.4	11.85±0.18	20.6±5.4	2.28±0.80	1.03±0.37
400	100	2,950.9±729.8	12.13±0.93	14.2±4.4	2.35±0.17	0.92±0.07
	500	2,721.8±833.8	11.81±1.18	31.3±5.7	1.81±0.35	1.22±0.24
	1,000	2,819.2±609.7	11.99±0.81	47.5±17.6	1.87±0.23	1.16±0.14
	without	1,752.5±595.1	10.30±1.18	23.8±5.8	2.92±1.09	0.80±0.25

It was found that different stocking density (100, 200, 300 and 400 crab/m^2) had significant effects on FCR and PER ($P < 0.05$) but not for WG, SGR and survival rate of the crabs ($P > 0.05$) (Table 3.8). However, different density of Pom-nang seaweed as shelter (100 g/m^2, 500 g/m^2, 1,000 g/m^2 and without shelter) had significant effects on FCR, PER and survival rate ($P < 0.05$) but not for WG and SGR of the crabs ($P > 0.05$). There was no interaction effect of the combined factor between stocking density and shelter density on WG, FCR, SGR, PER and survival rate ($P > 0.05$).

Table 3. 8 Two-way ANOVA table of percent weight gain (WG), specific growth rate on body weight (SGRw), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR) and catalase activity (CAT) of juvenile mud crab nursing with different stocking density and shelter density.

Source	**SS**	**df**	**MS**	**F**	**Sig.**
WG					
Stocking density	41548.40	3	13849.47	0.028	0.99
Shelter density	3276929.24	3	1092309.75	2.172	0.11
Stocking density * Shelter density	4985304.34	9	553922.70	1.101	0.39
SGRw					
Stocking density	0.41	3	0.14	0.132	0.94
Shelterdensity	7.64	3	2.55	2.455	0.08
Stocking density * Shelter density	12.71	9	1.41	1.361	0.25
SR					
Stocking density	771.37	3	257.12	2.528	0.08
Shelterdensity	7158.12	3	2386.04	23.462	0.00
Stocking density * Shelter density	1125.89	9	125.10	1.23	0.31
FCR					
Stocking density	4.27	3	1.42	5.077	0.01
Shelterdensity	5.11	3	1.70	6.078	0.00
Stocking density * Shelter density	1.71	9	0.19	0.678	0.72
PER					
Stocking density	3.25	3	1.08	8.351	0.00
Shelter density	1.61	3	0.54	4.151	0.01
Stocking density * Shelter density	0.89	9	0.10	0.76	0.65

SS=sun of squares; MS= mean squares

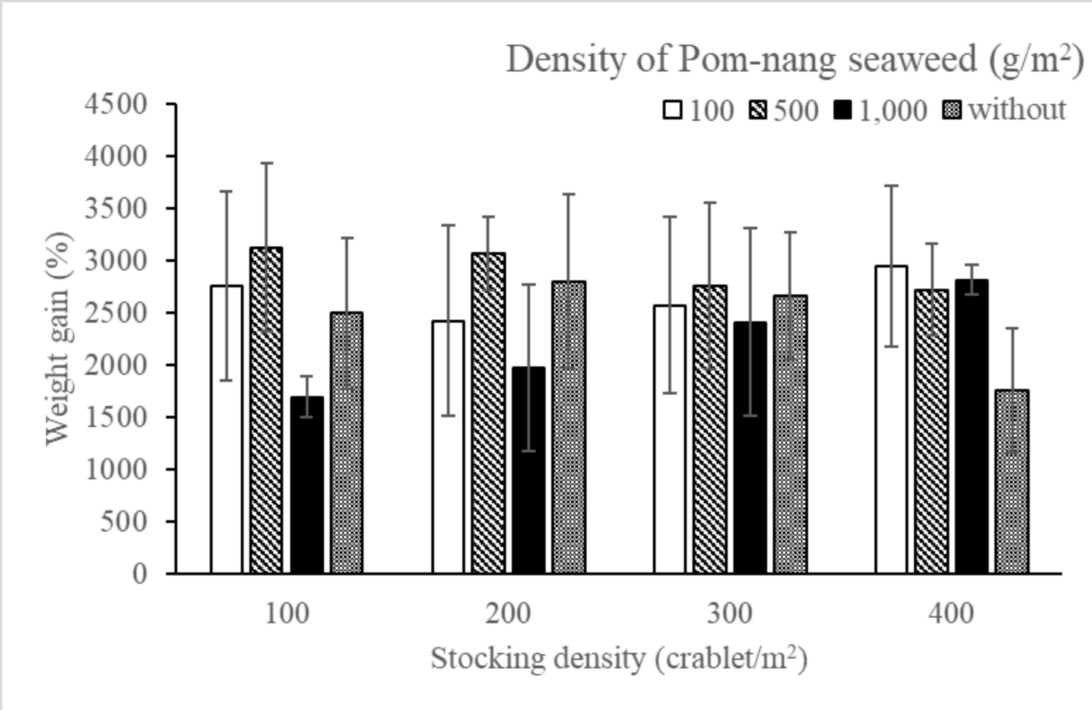

Figure 3.7 Weight gain (%) of juvenile mud crab, *S. paramamosain* nursing with different stocking density (100, 200, 300 and 400 crab/m^2) and shelter density (100, 500, 1,000 g/m^2 and without shelter

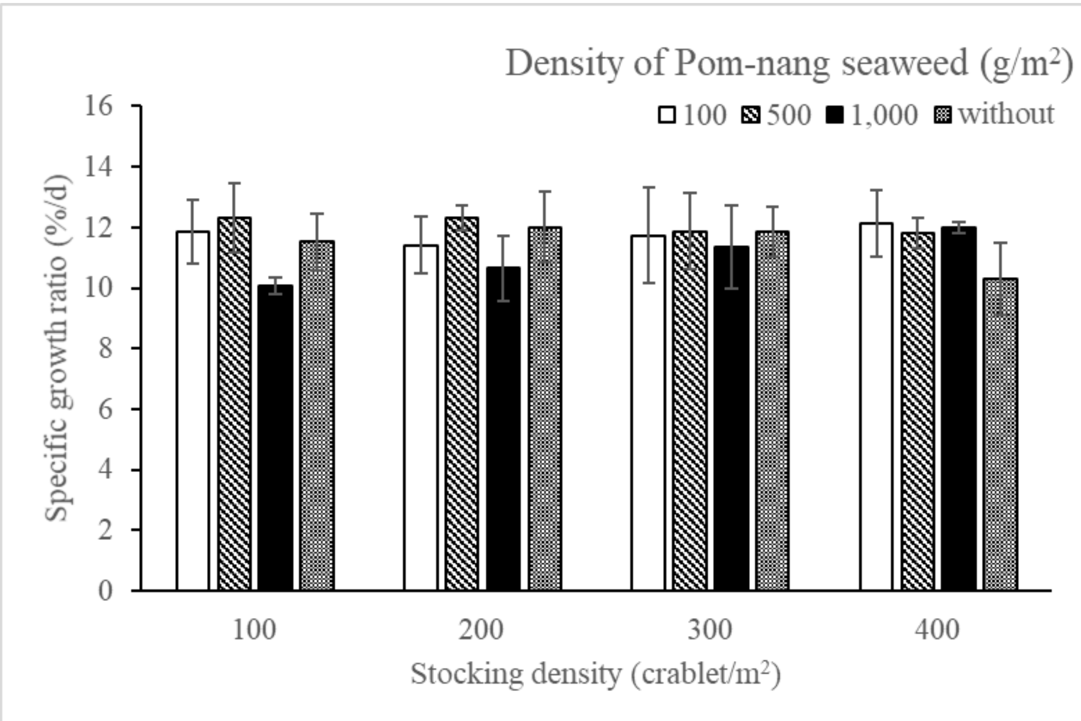

Figure 3.8 specific growth rate on body weight (%/d) of juvenile mud crab, *S. paramamosain* nursing with different stocking density (100, 200, 300 and 400 crab/m^2) and shelter density (100, 500, 1,000 g/m^2 and without shelter)

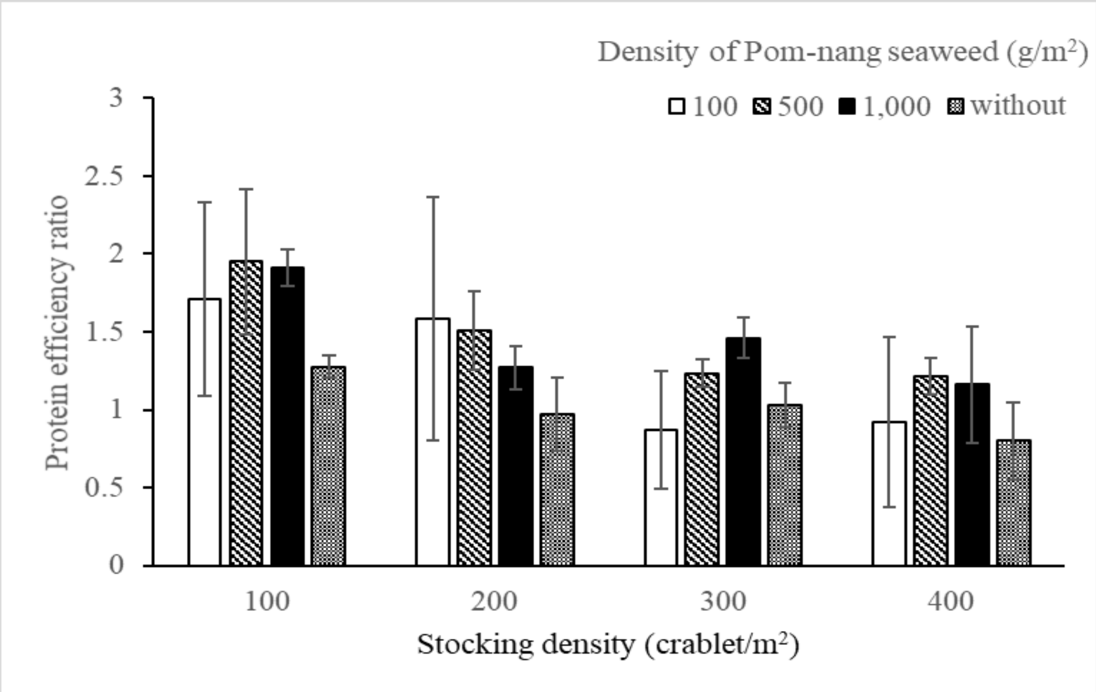

Figure 3.9 Protein efficiency ratio of juvenile mud crab, *S. paramamosain* nursing with different stocking density (100, 200, 300 and 400 crab/m^2) and shelter density (100, 500, 1,000 g/m^2 and without shelter)

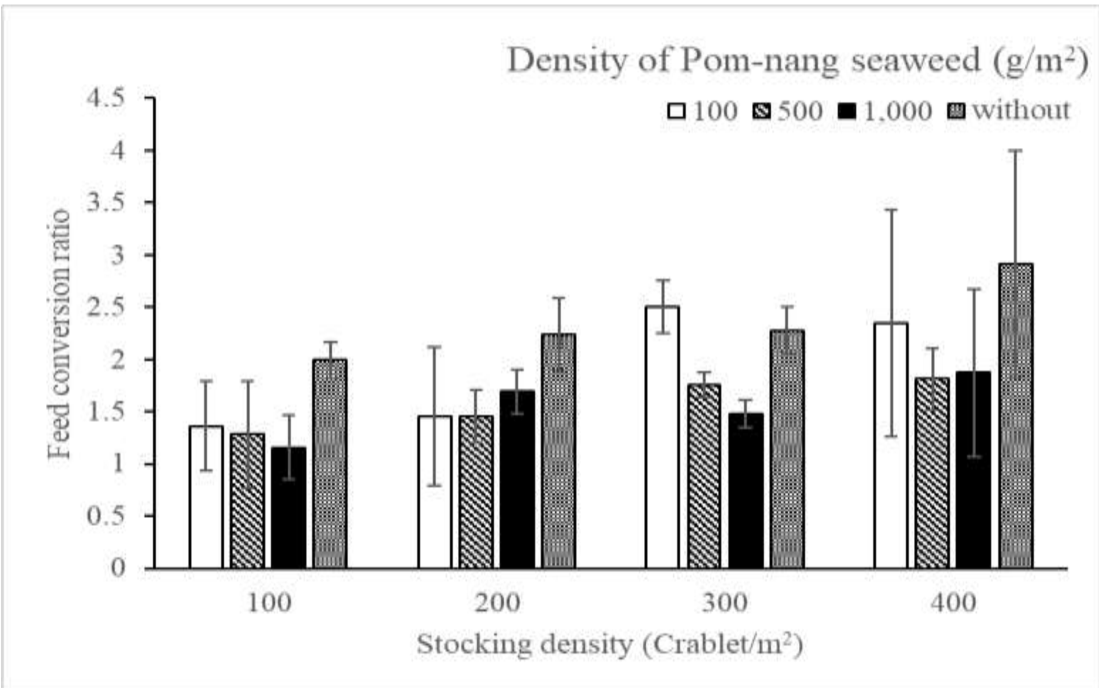

Figure 3.10 Feed conversion ratio of juvenile mud crab, *S. paramamosain* nursing with different stocking density (100, 200, 300 and 400 crab/m^2) and shelter density (100, 500, 1,000 g/m^2 and without shelter)

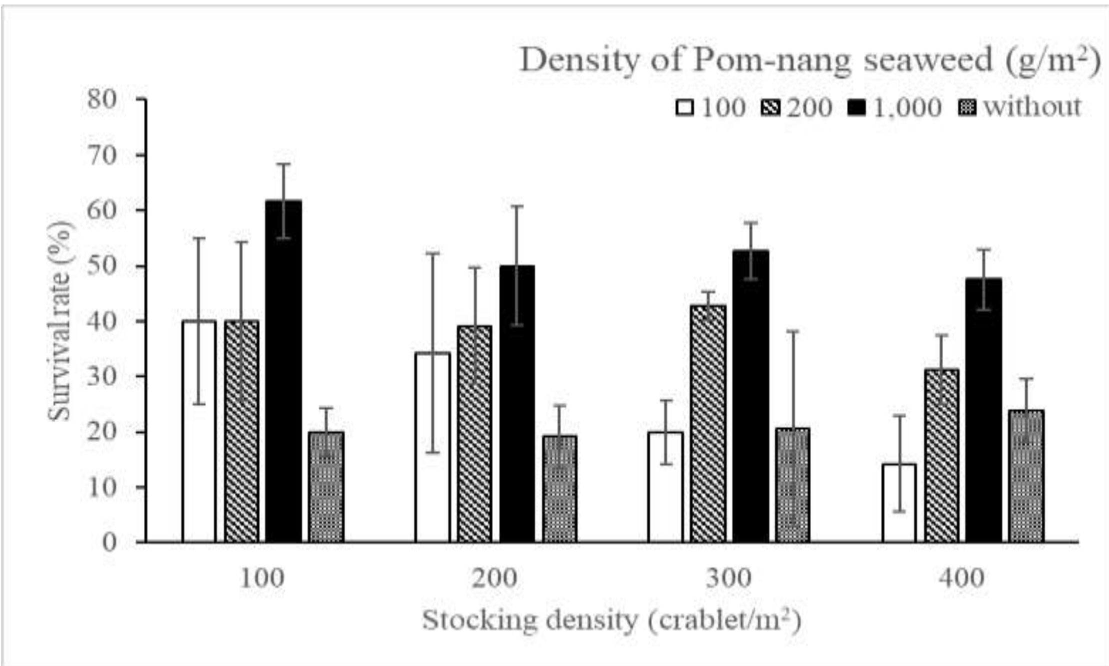

Figure 3.11 Survival rate (%) of juvenile mud crab, *S. paramamosain* nursing with different stocking density (100, 200, 300 and 400 crab/m^2) and shelter density (100, 500, 1,000 g/m^2 and without shelter)

Results from Duncan' s multiple range test on the comparison among different stocking density treatments found that the FCR for the density of 100 crab/m^2 (1.45±0.67) was significantly lower than those 300 crab/m^2 (2.01±0.57) and 400 crab/m^2 (2.24±0.68). For PER, the stocking density of 100 crab/m^2 (1.71±0.58) was significantly higher than those of 200 crab/m^2 (1.34±0.34), 300 crab/m^2 (1.15±0.30) and 400 crab/m^2 (1.02±0.24). For survival rate, it was found that difference density was no significant effect on survival rate ($P > 0.05$) (Figure 3.14).

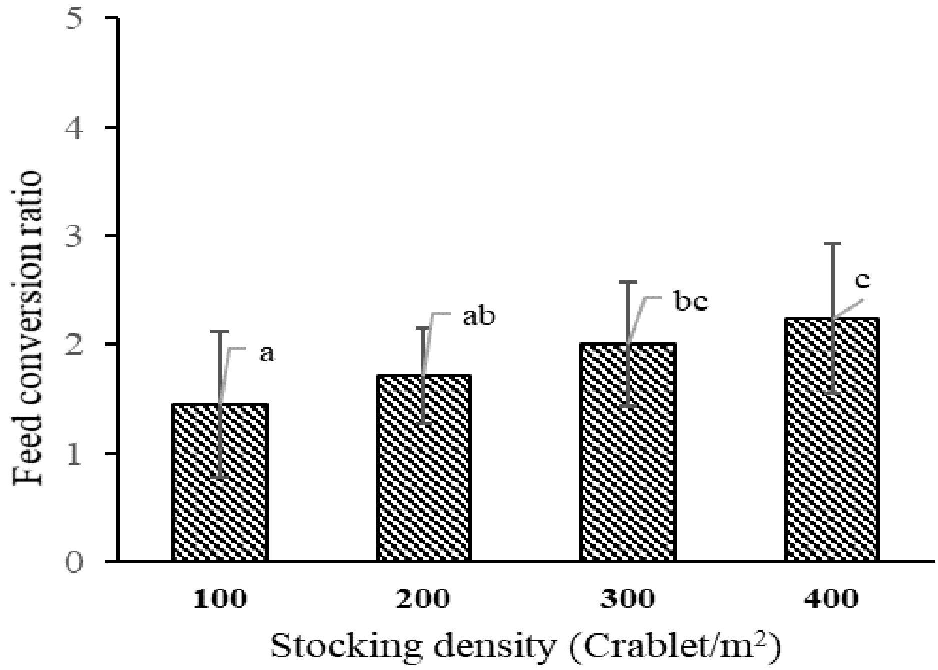

Figure 3.12 Effects of stocking density on feed conversion ratio of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P > 0.05$).

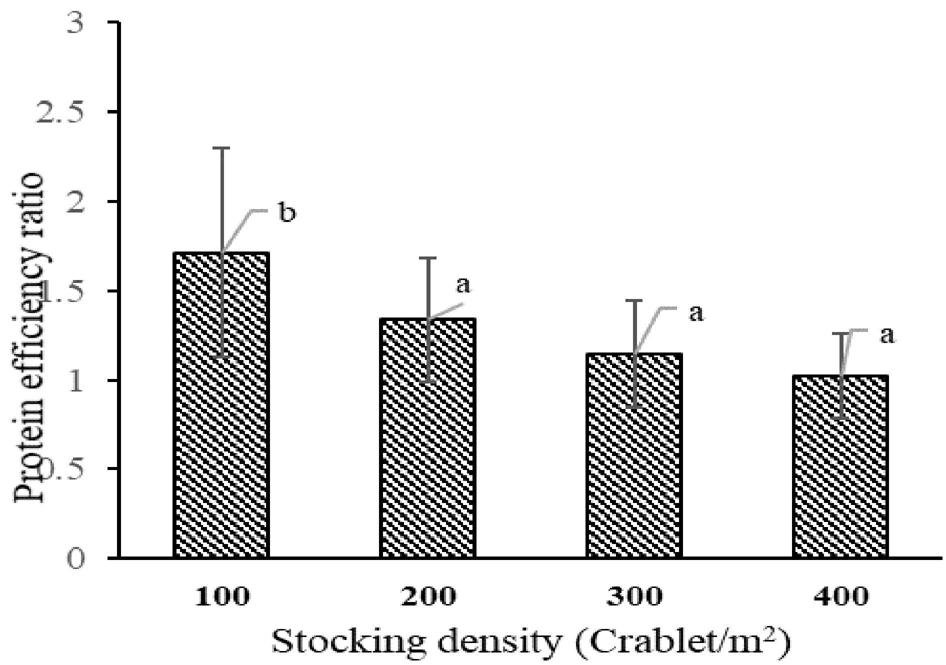

Figure 3.13 Effects of stocking density on protein efficiency ratio of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P > 0.05$).

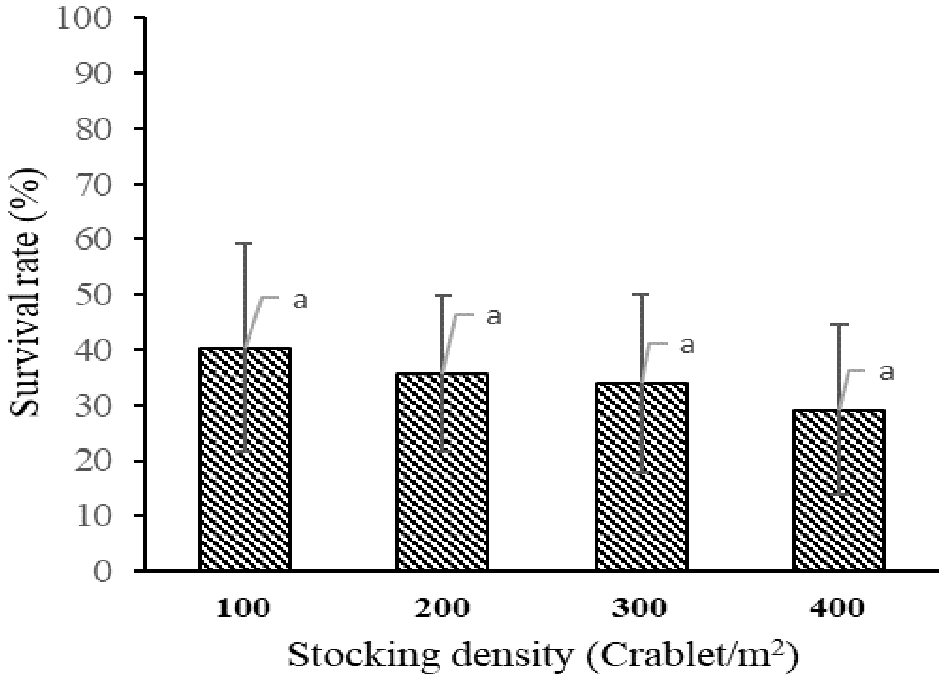

Figure 3.14 Effects of stocking density on survival rate of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different (P > 0.05).

The comparison among different density of Pom-nang seaweed as shelter found that the FCR of Pom-nang seaweed density at 500 g/m^2 (1.58±0.42) and 1,000 g/m^2 (1.55±0.32) was significantly lower than those without Pom-nang seaweed as a shelter (2.36±0.83) (Figure 3.14). However, the Pom-nang seaweed density at 100 g/m^2 (1.92±0.63) was no difference among other Pom-nang seaweed density (P > 0.05). Similar result was also found for PER, density of Pom-nang seaweed at 500 g/m^2 (1.48±0.48) and 1,000 g/m^2 (1.45±0.35) was significantly higher than those of without Pom-nang seaweed as a shelter (1.02±0.35). However, the Pom-nang seaweed density at 100 g/m^2 (1.27±0.52) did not differ with other Pom-nang seaweed densities (P > 0.05) (Figure 3.15). For survival rate, it was found high density of Pom-nang seaweed at 1,000 g/m^2 (53.0±10.0%) showed significantly higher survival rate than 500 g/m^2 (38.3±11.2) The lowest survival rate was found in 100 g/m^2 (27.1±14.4%) and without shelter (20.9±5.9%). The different effects of survival rate from different density of Pom-nang seaweed as shelter are shown in Figure 3.16.

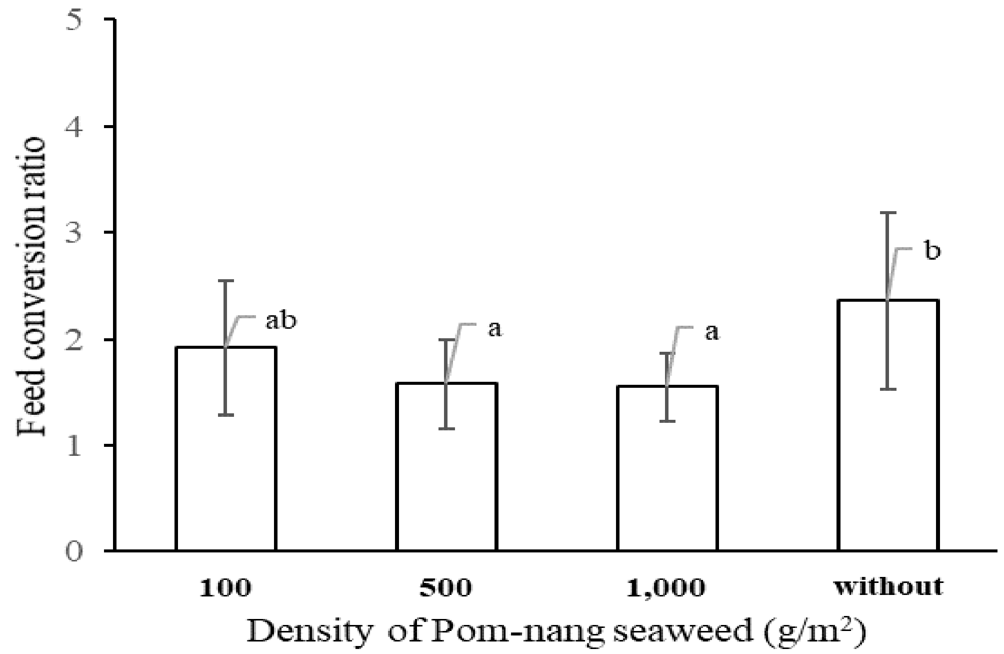

Figure 3.15 Effects of different density Pom-nang seaweed as shelter on feed conversion ratio of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P > 0.05$).

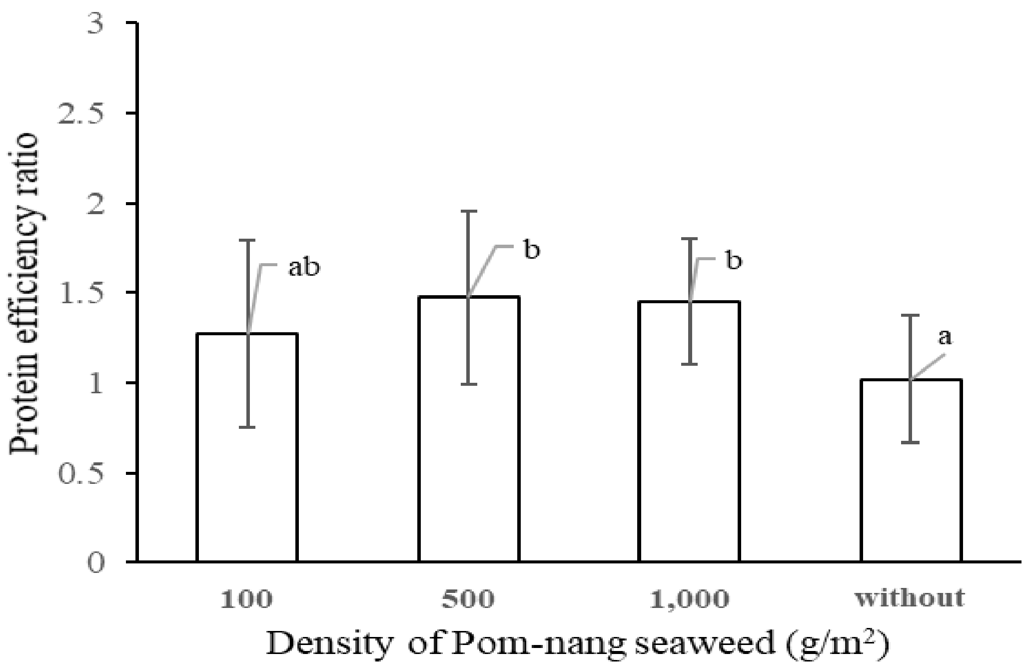

Figure 3.16 Effects of density Pom-nang seaweed as shelter on protein efficiency ratio of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P > 0.05$).

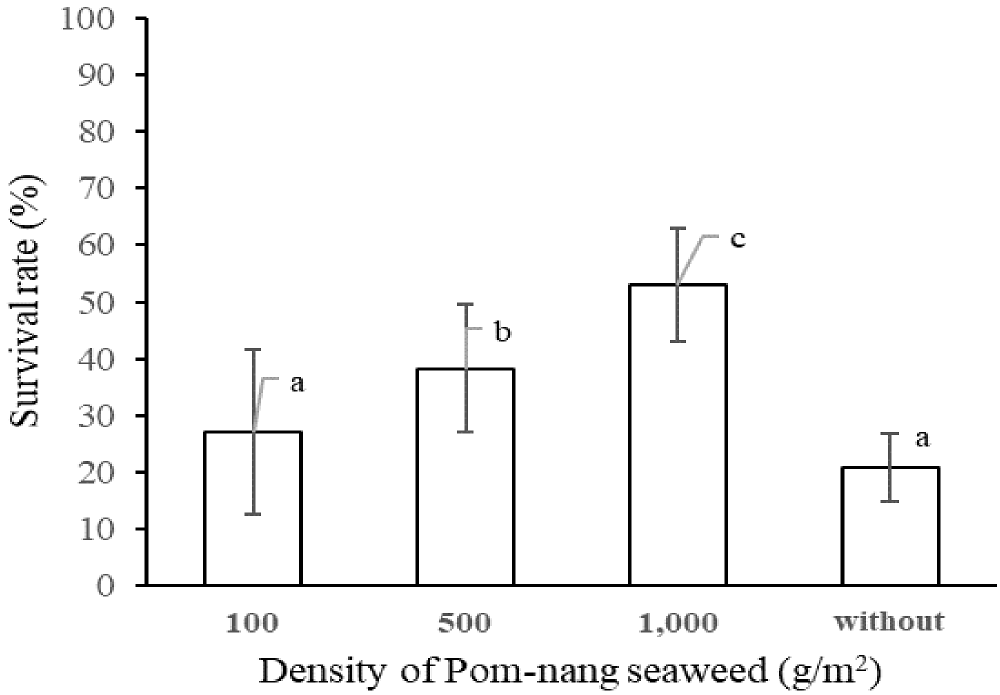

Figure 3.17 Effects of different density Pom-nang seaweed as shelter on survival rate of juvenile mud crab, *S. paramamosain*. Values with different letters on the tops of bars are significantly different ($P > 0.05$).

CHAPTER 4
DISCUSSION

4.1 Suitable food and effect of dietary PSP diets

It was confirmed that fresh diet, mysid shrimp, was the most effective diets for nursing juvenile mud crab compared to the formulated diets based on WG, SGRw and SGRc. Formulated diets have been found to reduce growth and survival rate in several species (Alaminos and Domingues,2008; Syafaat *et al*., 2019; Gil *et al*., 2019). Compared to other studies on mud crab, Holme at al. (2006) found that juvenile *S. serrata* molted from megalopa fed with live feed (*Artemia*) showed significant higher carapace width, final dry weight and better development time than formulated diets. Alaminos and Domingues (2008) found juvenile spider crabs *M. brachydactyla* (average age 2 months) fed with fresh diet (mussel meat) showed significant higher growth rate than artificial feed (crustacea pellet). However, the use of formulated diets can provide nutritional requirements for the growth, protein efficiency and immunity of crabs, and are easy to use, transport and store because they are prepared to be directly administered (Alaminos and Domingues, 2008). In this study, juvenile mud crab fed with formulated diets (45% protein concentration) showed significant higher PER than Fresh diet (65% protein concentration). Nguyen *et al*. (2014) found juvenile mud crab fed with 42 percent protein concentration showed higher PER than 52 and 60 percent protein concentration in formulated diets. Dietary protein intake was increased, there was a decrease in retained energy. Excess dietary protein is diverted for catabolism creating an imbalance in the nutrient profile of the diets, reducing protein retention. (Nguyen *et al*., 2014). Furthermore, in this study, fresh diet (mysid shrimp) is no difference in terms of FCR, CAT and survival rate between fresh diet and other formulated feed. A similar survival rate with Syafaat *et al*. (2019) found mud crab larvae rearing from Zoea 1 to juvenile mud crab using live feed show better survival rate but there was no significant difference ($P > 0.05$) on rearing with artificial feed of juvenile mud crab, *S. tranquebarica*. Gil *et al*. (2019) found spider crab juveniles *M. squinado* feed with artificial feed was significant higher survival than fresh diet (mussel meat) in 70-day trial an initial carapace length (CL) of 3.320 ± 0.059 mm. It is therefore suggested that using of formulated feed is also highly possible in nursing juvenile mud crab for mud crab industry.

The use of seaweed as feed supplement had been applied in many shrimp studies. Yu *et al*. (2016) suggested that juvenile *Litopenaeus vannamei* had the highest values of final mean weight, WG, SGRw, PER and FCR when shrimp fed with diet supplemented with *G. lemaneiformis* 3% in 56-day trial with an initial body weight about 0.27±0.00 g. Niu *et al*. (2015) revealed that the highest final mean weight, WG and SGRw when shrimp *Penaeus monodon* fed with diet supplement with wakame (*Undaria pinnatifida*) at 2% inclusion level in 56-day trial with an initial body weight about 0.68±0.01 g. The suitable supplemented of seaweed *G. fisheri* enhances the growth performance of the juvenile mud crab. The growth of crab did not increase with increasing Pom-nang seaweed supplemented in diet. The diets supplemented with Pom-nang seaweed at level of 2% and 4% was high growth rate than 6%. Most of studies, Yu *et al*., 2016 and Niu *et al*., 2015 found that high level of Pom-nang seaweed supplemented (4-6%) in diet has poor growth rate on shrimp. It was also found in this study that among formulated diets, the juvenile mud crab fed with the diets supplemented with 4% of Pom-nang seaweed (PSP4) combined with Pom-nang seaweed as shelter had the highest final mean weight, WG SGRc, SGRw and PER than those of juvenile mud crab fed with PSP0, PSP2 and PSP6. This value indicates that the optimum supplement rate of Pom-nang seaweed (*G. fisheri*) in the diet for juvenile mud crab shall be 4% for 28-day trial with the crab juvenile of an initial body weight about 0.02±0.00 g.

Immune respond is important for defense system of aquatic animals. This study found that feed supplemented with Pom-nang seaweed did not compromise the crab immune system. There wa no significant difference on CAT value between mysid shrimp as feed and other formulated supplemented with Pom-nang seaweed. It is coincident with the study reported by Niu *et al*. (2015) who revealed that different levels of wakame supplemented at 0, 1, 2, 3, 4 and 5% was no significant on hepatopancreas immune indices (superoxide dismutase) of shrimp. Liu *et al*. (2020) found banana shrimp *Fenneropenaeus merguiensis* feed with *Enteromorpha* polysaccharides supplemented in diet at level 0.1, 0.2 and 0.3% was no significant on hemolymph immune as SOD, ACP, PO and lysozyme activities. However, CAT was significantly higher in Pom-nang seaweed as a shelter compared to those without shelter. The use of seaweeds as shelter resulted in the seaweeds absorbing nutrients generated from crab waste, improved water quality in the culture medium, and a conducive habitat for crab development (Ly Van *et al*., 2021), thus, resulting in increased growth, feed efficiency and immune of juvenile mud crab. Mud crabs are omnivores, whose food includes crustaceans, fishes, mollusks, detritus, and plants; the seaweed add as a shelter could consumed by crab directly. However, at the end of experiment, final weight gain of Pom-nang seaweed its increased. The use of seaweed as feed supplement could improve growth and immunity in many studies. This observation was supported by Wei *et al*. (2015) who found that dietary containing *Enteromorpha* polysaccharides improved the immune enzyme as CAT, phosphatase (ACP) and superoxide dismutase (SOD) activities of coelomocytes in sea cucumbers. Zhou *et al*. (2020) also suggested that *Enteromorpha* polysaccharides supplementation increased the serum SOD, CAT, ACP and lysozyme activities in carp *Carassius auratus*.

4.2 Influence of stocking density and Pom-nang seaweed density as a shelter

The growth and survival of juvenile mud crab are essential in the nursery phase. One of the major factors influencing survival of crab is cannibalism. Several studies demonstrated that low stocking density significantly reduces cannibalism and increases the survival of juvenile mud crab (Trino *et al*., 1999; Ut *et al*.,2007; Daly *et al*., 2009). In the present study, lower stocking density provided the better results for feeding activity as FC R and PER than high stocking density of juvenile mud crab. It is suggested that the low stocking density reduces search and capture efficiencies, resulting in a higher protein efficiency ratio (Moksnes *et al*., 1997; Gil *et al*., 2019). However, stocking density was no significantly on growth and survival of juvenile mud crab such as WG, SGRw and survival rate.

The survival rates of juvenile mud crab(C2) nursing with different stocking density range of 29-40% for 28-day trial. These figures are similar with those reported by Ut *et al*. (2007) who found stocking density at 100, 175 and 230 crab/m^2 show not significant on survival rate for 15-day trial of juvenile mud crab, *S. paramamosain*. Moreover, Trino *et al*. (1999) found mud crab (*Scylla*) culture with low stocking density resulted in the most efficient FCR and highest survival rate compared with high stocking density. However, the different stocking density was no significant different on growth rate. Daly *et al*. (2009) found the survival of juvenile red king crab at low stocking density was significantly higher than high stocking density but no impact on growth rate. This can explain that the high stocking density affecting on appetite suppression, feeding inhibition, Sub-lethal injuries such as limb loss, or cannibalism with other juvenile crab (Trino *et al*., 1999). In nursery phase, low stocking density may promote high survival rate of juvenile mud crab. However, to find optimal stocking density, a tradeoff between survival rate and end production exists (Daly *et al*., 2009). In this study, it is evident that the high production was found at the high stocking density (400 crab/m^2). This indicates the optimal stocking density in nursery phase of juvenile mud crab might be 400 crab/m^2 with the presence of high density of seaweed as shelter.

Cannibalism was observed during a moulting process of juvenile mud crab. As the carapace is not completely hardened, the congeners can attack vulnerable crab (Gil *et al*., 2019). It is observed in this study that the cannibalism increased by day of culture and size of juvenile mud crab. Numerous studies demonstrated that the presence of shelter can significantly reduce cannibalism and increase the survival rate of juvenile mud crab (Daly *et al*., 2009; Ut *et al*., 2007, Gil *et al*., 2019). Consistently, in this study, a significantly higher survival rate was observed with the presence of shelter. For optimal density of shelter, it is suggested that nursing juvenile mud crab with high density of Pom-nang seaweeds as shelter (1,000 g/m^2) helps to improve nursing performance. This is based on values of several parameters including PER, FCR and survival rate. The study on growth rate, on the other hand, revealed an inverse relationship between survival and growth rates. It showed that growth of crabs in terms of WG and SGRw is related to survival rate in the stocking densities of 100 and 200 crab/m^2. A similar result was reported by Ly Van *et al*. (2021) who found that growth of juvenile mud crab, *S. paramamosain* in high density of seaweeds as shelter (2,000 g/m^2) showed lower body weight and SGRw than low density of seaweeds as shelter (500 g/m^2). The communal culture's reduced crab survival rate was presumably due to a high incidence of cannibalism during molting. As a result, survivors not only ate the experimental diet, but also ate newly molted crabs from the nursing tank, resulting in good growth. (Ly Van *et al*., 2021). Different density of Pom-nang seaweed helps to improve survival rate. The survival rate increased when density of Pom-nang seaweed as shelter increased. This may because of the high density of shelters can help to reduce the search and capture efficiencies of congeners crab from vulnerable crab (Moksnes *et al*., 1997; Gil *et al*., 2019). The assumption is that high density of Pom-nang seaweed as shelter will help in reducing cannibalism and self-defending mechanism leading to more effective feeding activity such as reducing feed conversion ratio and improve protein efficiency ratio. Therefore, the use of Pom-nang seaweed is therefore essential in nursing juvenile mud crab. However, this study found that providing Pom-nang seaweeds as shelter or without shelter was no effect on growth rate such as WG and SGRw. It is in agreement with Ut *et al*. (2007) who found survival rate of juvenile mud crab with shelter was higher than without shelter (sand substrate alone) reared for 15 days from first crab instar, but not significant on growth of juvenile mud crab. The high growth and survival rates of crab in the presence of shelter found in this study supported the earlier work by He *et al.* (2017), Daly *et al.* (2009) and Gil *et al.* (2019). He *et al*. (2017) found that WG, SGRw and survival of juvenile swimming crab, *Portunus trituberculatus*, with the presence of shelter was significantly higher than without shelter. Daly *et al*. (2009) also found that the survival rate of juvenile red king crab with shelter was significantly higher than without shelter. In addition, Gil *et al*. (2019) found the survival and growth rates of spider crab juveniles *M. squinado* with shelter was significantly higher than without shelter.

Thus, nursing juvenile mud crab with Pom-nang seaweed as shelter helps to improve nursing performance based on higher values of several parameters including growth performance, survival rate and catalase activity (CAT). Therefore, the use of Pom-nang seaweed as shelter is essential in nursing juvenile mud crab. The assumption is that Pom-nang seaweed will help in reducing stress from external factors such as light and self-defending mechanism leading to more effective feeding activity. This finding is in agreement with Ut *et al*. (2007) who found survival rate of juvenile mud crab with shelter was higher than without shelter (sand substrate alone) reared for 15 days from first crab instar. A similar with Gil *et al*. (2019), the spider crab juveniles *Maja squinado* reared with natural seaweed as shelter showed higher survival rate than without shelter.

CHAPTER 5
CONCLUSION AND SUGGESTION

5.1 Effect of dietary PSP diets and Pom-nang seaweed as a shelter

It is concluded that mysid shrimp is the most appropriated food for nursing juvenile mud crab, *S. paramamosain*, and formulated diets supplemented with seaweeds has a potential to replace mysid shrimp. The use of *G. fisheri* as shelter is essential for nursing juvenile mud crabs as indicated by all growth performances. Additionally, the combination of seaweed as shelter and the formulated diets with 4% seaweed had the highest values of growth performances as weight gain, specific growth rate on body weight and specific growth rate on carapace width and protein efficiency ratio compared to others. However, Pom-nang seaweed as a shelter played an important role on feed conversion ratio, survival rate and catalase activity for nursing juvenile mud crab.

5.2 Effect of stocking density and shelter density on growth rate and survival rate

The stocking density of juvenile mud crab and density of Pom-nang seaweed as a shelter are important for feed conversion ratio, protein efficiency ratio and survival rates of juvenile mud crabs. Low stocking density is the most suitable on feed conversion and protein efficiency ratios. The important factor on survival rate for nursing juvenile mud crab is seaweed shelter density. The optimal density of Pom-nang seaweed for the best survival rate is 1,000 g/m^2. However, this study suggests that the optimum stocking density is 400 crab/m^2 combined with high density of Pom-nang seaweeds as shelter at 1,000 g/m^2 as this combined factor provided high survival rate and high-end production for 28-day trial.

5.3 Suggestion

5.3.1 Pom-nang seaweed used as shelter may consume by crab directly in the form of fresh or detritus (decayed seaweed), hence promoting growth performances of juvenile mud crab. The use of Pom-nang seaweed is highly recommended for crab nursing process.

5.3.2 The further study on the formulated diets supplemented with Pom-nang seaweeds should be investigated in another culture phase including broodstock phase, growth-out phase, and fattening phase or soft-shell rearing of mud crab, *Scylla paramamosain.*

5.3.3 More study on the effect of Pom-nang seaweed on growth and survival rates of juvenile mud crab should be conducted with other factors such as water quality, predator and others.

5.3.4 To discover the best nursing condition, further investigation should be conducted with other densities of crab, density of shelter or level of seaweeds supplemented in diet.

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APPENDICES

APPENDIX 1

METHODOLOGY FOR CHEMICAL ANALYSIS

1.1 Materials and instruments

1.1.1 Materials

- Plastic containers
- Air Pumps
- Air Stones
- Dip-net
- Tong
- Crucible
- Flasks (50, 250 and 500 mL) (Schott Duran, Germany)
- Volume metric (50, 100, 500 and 1,000 mL) (Witeg, Germany)
- Desiccator
- Cylinder (LabFocus, Thailand)
- Vernier calipers

1.1.2 Instruments

- Thermometer
- pH meter (Metrohm, Harisau Switzerland)
- Salinity Refractometer (Atago, Japan)
- Electric balance (Denver, Thailand)
- Hot air oven
- Muffle furnace
- Spectrophotometer (Genesys, China)
- Centrifuge (Hettich Universal, Tuttlingen)
- Dissolved oxygen meter (YSI PRO 2030)

1.2) Water analysis

1.2.1 Alkalinity analysis (Titrimetric method)

The water sample used of 50 mL and was contained in the 50 mL flasks. Three drops of Phenolphthalein were added into these flasks. Water sample in the flasks were then titrated by 0.02 N H_2SO_4. After titrating, they were added three drops of Methyl red and titrated again by 0.02 N H_2SO_4. The total alkalinity in water sample was calculated by using the formula,

Total alkalinity (mg/L) = [(A+B) * 0.02*50000]/V

Where A = the endpoint of the first titration (mL)

B = the endpoint of the second titration (mL)

V = volume of water sample (mL)

1.2.2 Ammonia analysis (phenol hypochlorite method)

The water sample used of 10 mL was contained in the 50 mL test tube. After that 0.5 ml of phenol solution and 0.5 ml of oxidizing agent were added into these test tube. The changes in absorbance were recorded at 640 nm.

1.3 Proximate analysis

1.3.1 Determination of protein content (Kjeldahl method; AOAC,2000)

The sample used of 0.252 g was contained in the Kjeldalh flask. Mixed catalyst: $CuSO_4$ of 0.1 g, $NaSO_4$ 2 g and conc.H_2SO_4 25 g were added into these flasks. Prepare a tube containing the above chemical except sample as blank. Boil at 400 ^oC until solution clears. Cool and add 15 ml of Distilled water and 40% NaOH 50 ml in Kjeldalh flask. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 3-4 indicator in receiver. Rotate to mix content thoroughly; then until all NH_4 is distilled. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution. The total protein in sample was calculated by using the formula,

Protein (%) = ((A-B) x N x 1.4007 x 6.25)/W

Where A = volume (ml) of 0.2 N HCL used sample titration

B = volume (ml) of 0.2 N HCL used in blank titration

N = Normality o HCl

W = weight (g) of sample

14.007 = atomocof nitrogen

6.25 = the protein nitrogen conversation factor

1.3.2 Determination of ash content

Place the crucible and lid in the furnace at 550 ^{o}C to ensure that impurities on the surface of crucible are burned off. Cool the crucible in the desiccator (30 min). Weigh the crucible and lid with 3 decimal places. The sample used of 5 g into the crucible. Heat over low Bunsen Flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace. Heat at 550 ^{o}C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator. Weigh the ash with crucible and lid. The total ash content in sample was calculated by using the formula,

Ash (%) = (Weight of ash / Weight of sample) x 100

1.3.3 Determination of fat content

Place the bottle and lid in the incubator at 105 oC overnight. The sample used of 5 g to paper filter and wrap and take into extraction thimble and transfer into Soxhlet. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle. Ture on the water cool and heating mantle in Soxhlet apparatus. Evaporate the solvent by using the vacuum condenser. Incubate the bottle at 80-90 oC until solvent is completely evaporate and bottle is completely dry. Transfer with partially covered lid to the desiccator until cool. The total fat content in sample was calculated by using the formula,

Fat (%) = (Weight of fat / Weight of sample) x 100

APPENDIX 2

ANALYSIS OF VARIANCE (ANOVA)

Table 1. ANOVA of weight gain of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.408E6a	9	934253.377	13.124	0
Intercept	44180000	1	44180000	620.595	0
Diet	6109266.11	4	1527316.53	21.455	0
Shelter	985522.589	1	985522.589	13.844	0.001
Diet * Shelter	1313491.7	4	328372.924	4.613	0.008
Error	1423753.72	20	71187.686		
Total	54010000	30			
Corrected Total	9832034.12	29			

Table 2. ANOVA of specific growth rate on body weight of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	58.044a	9	6.449	9.619	0
Intercept	2357.078	1	2357.078	3515.596	0
Diet	37.914	4	9.479	14.137	0
Shelter	8.309	1	8.309	12.393	0.002
Diet * Shelter	11.821	4	2.955	4.408	0.01
Error	13.409	20	0.67		
Total	2428.531	30			
Corrected Total	71.453	29			

Table 3. ANOVA of specific growth rate on carapace width of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.878a	9	0.542	8.956	0
Intercept	301.34	1	301.34	4979	0
Diet	3.213	4	0.803	13.271	0
Shelter	0.442	1	0.442	7.298	0.014
Diet * Shelter	1.224	4	0.306	5.056	0.006
Error	1.21	20	0.061		
Total	307.429	30			
Corrected Total	6.089	29			

Table 4. ANOVA of protein efficiency ratio of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.408a	9	0.601	10.488	0
Intercept	73.302	1	73.302	1279	0
Diet	3.377	4	0.844	14.736	0
Shelter	1.327	1	1.327	23.166	0
Diet * Shelter	0.704	4	0.176	3.07	0.04
Error	1.146	20	0.057		
Total	79.855	30			
Corrected Total	6.553	29			

Table 5. ANOVA of feed conversion ratio of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.902a	9	0.767	4.15	0.004

Intercept	93.958	1	93.958	508.542	0
Diet	1.977	4	0.494	2.675	0.062
Shelter	4.304	1	4.304	23.296	0
Diet * Shelter	0.621	4	0.155	0.84	0.516
Error	3.695	20	0.185		
Total	104.555	30			
Corrected Total	10.597	29			

Table 6. ANOVA of survival rate of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2013.333a	9	223.704	1.342	0.278
Intercept	167253.333	1	167253.333	1004	0
Diet	613.333	4	153.333	0.92	0.472
Shelter	853.333	1	853.333	5.12	0.035
Diet * Shelter	546.667	4	136.667	0.82	0.528
Error	3333.333	20	166.667		
Total	172600	30			
Corrected Total	5346.667	29			

Table 7. ANOVA of catalase activity of juvenile mud crab under different dietary diets and Pom-nang seaweed as a shelter.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.000a	9	0.00002083	0.832	0.595
Intercept	0.183	1	0.183	7322	0
Diet	0.00001433	4	0.000003583	0.143	0.964

Shelter	0	1	0	4.955	0.038
Diet * Shelter	0.00004913	4	0.00001228	0.491	0.743
Error	0.001	20	0.00002503		
Total	0.184	30			
Corrected Total	0.001	29			

Table 8. ANOVA of weight gain of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.304E6a	15	553585.465	1.101	0.394
Intercept	315400000	1	315400000	626.987	0
Stocking density	41548.395	3	13849.465	0.028	0.994
Shelter density	3276929.24	3	1092309.75	2.172	0.111
Stocking * Shelter	4985304.34	9	553922.704	1.101	0.39
Error	16100000	32	503003.032		
Total	339800000	48			
Corrected Total	24400000	47			

Table 9. ANOVA of specific growth rate on body weight of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.764a	15	1.384	1.334	0.239
Intercept	6432.228	1	6432.228	6200	0
Stocking density	0.412	3	0.137	0.132	0.94
Shelter density	7.641	3	2.547	2.455	0.081
Stocking * Shelter	12.711	9	1.412	1.361	0.246

Error	33.2	32	1.037		
Total	6486.191	48			
Corrected Total	53.963	47			

Table 10. ANOVA of specific growth rate on carapace width of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.312a	15	0.154	1.457	0.181
Intercept	630.315	1	630.315	5957	0
Stocking density	1.24	3	0.413	3.906	0.017
Shelter density	0.127	3	0.042	0.399	0.754
Stocking * Shelter	0.946	9	0.105	0.993	0.465
Error	3.386	32	0.106		
Total	636.013	48			
Corrected Total	5.698	47			

Table 11. ANOVA of survival rate of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9055.373a	15	603.692	5.936	0
Intercept	58178.65	1	58178.65	572.06	0
Stocking density	771.369	3	257.123	2.528	0.075
Shelter density	7158.119	3	2386.04	23.462	0
Stocking * Shelter	1125.885	9	125.098	1.23	0.312
Error	3254.407	32	101.7		
Total	70488.43	48			

Corrected Total	12309.78	47			

Table 12. ANOVA of feed conversion ratio of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.089a	15	0.739	2.638	0.01
Intercept	164.476	1	164.476	586.955	0
Stocking density	4.268	3	1.423	5.077	0.005
Shelter density	5.11	3	1.703	6.078	0.002
Stocking * Shelter	1.711	9	0.19	0.678	0.722
Error	8.967	32	0.28		
Total	184.532	48			
Corrected Total	20.056	47			

Table 13. ANOVA of protein efficiency ratio of juvenile mud crab under different stocking density and shelter density**.**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.744a	15	0.383	2.956	0.005
Intercept	81.651	1	81.651	630.429	0
Stocking density	3.245	3	1.082	8.351	0
Shelter density	1.613	3	0.538	4.151	0.014
Stocking * Shelter	0.886	9	0.098	0.76	0.653
Error	4.145	32	0.13		
Total	91.54	48			
Corrected Total	9.888	47			

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