INDUCED MOULTING OF MUD CRAB BY SPINACH EXTRACT FOR PRODUCING SOFT SHELL

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THESIS

Submitted to the

Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli

in practical fulfilment of the requirements for the degree of
MASTER OF FISHERIES SCIENCE

IN

FISH BIOTECHNOLOGY

BY

AJAY NEMICHAND SHAHARE
B. F. Sc.

Under the guidance of

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I certify that:

1. He has carried out the research work under my direct supervision and guidance in academic year 2016-18 and that the manuscript of the dissertation has been scrutinized by me.
2. The entire thesis comprises the candidate's own work and it is his own achievement. It has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of recognition.
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5. The final typed copy of the thesis, which is being submitted to the University office, has been carefully read by me for its material and languages and it is to my entire satisfaction.

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I hereby declare that this thesis or part thereof has not been submitted by me or other person to any University or Institute for a Degree or Diploma.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

III

LIST OF PLATES

ABSTRACT

Abstract

Moulting stimulant made was prepared by from spinach extract. The objective of this study was to standardize the optimum dose of spinach extract for inducing the moulting of crab and to study the required moulting period of crab. The research was carried out in indoor culture system in polypropylene boxes (18 x 12 x 8 inch). In this system, the water parameters were maintained, temperature-27-32^{0}C, salinity-16-30ppt, dissolved oxygen-≥ 5 ppm and pH-7.5-8.5. The experiment was carried out as per Completely Randomized Design (CRD) with four treatments and five replicates to evaluate the effect of growth and moulting period in mud crab. There were three doses of spinach extract doses tested, without injection (control). 0.015 mg/g of crab, 0.02 mg/g of crab and 0.025 mg/g of crab. The result showed that the dose of spinach extract injection had great influence on the growth parameter (weight, length, width) and moulting period of mud crab. Spinach extract dose of 0.025 mg/g of crab showed the higher growth percentage i.e. weight gain (70-72%), length gain (29-34 %), width gain (25-27 %) and moulting period required was 30-32 days. However, in term of productivity, dose of 0.025 mg/g of crab showed the better result for soft shell production of mud crab.

सारांश

खेकड्याचे कवच कमी कालावधीत टाकण्याकरीता पालक भाजीच्या अर्कांपासून उत्तेजक बनवले आहे. या अभ्यासाचा उद्देश पालक भाजीच्या अर्कांपासून तयार केलेल्या उत्तेजकाची मात्रा ठरविणे आणि खेकड्याचे कवच कमीत कमी कालावधीत काढणे हा होता. हे प्रयोग पॉलीप्रोपायलीन बॉक्समध्ये (१८ इंच x १२ इंच x ८ इंच) बंदिस्त खोलीत (इनडोअर) पुनर्वापर पद्धतीने करण्यात आले. या प्रयोगांती पाण्याच्या घटकाचे प्रमाण उदाहरणार्थ, तापमान २७-३२^0 सेल्सिअस, क्षारता १६-३० पीपीटी, पाण्यातील विरघळलेले प्राणवायू ≥५ पीपीएम आणि पाण्याचे सामू प्रमाण ७.५-८.५ राखण्यात आले. संपूर्ण प्रयोग हा (सी आर डी), चार उपचार आणि प्रती पाच प्रतिकृतीसह करण्यात आला. खेकड्याची वाढ आणि कवच टाकण्याच्या कालावधीचा अभ्यास करण्यासाठी हा प्रयोग करण्यात आला. पालक भाजीच्या अर्कांपासून तयार केलेल्या तीन मात्रांची चाचणी केली गेली त्यामध्ये इंजेकशन शिवाय (नियंत्रित), ०.०१५ मिलिग्रॅम/ग्राम खेकडा, ०.०२ मिलिग्रॅम/ग्राम खेकडा आणि ०.०२५ मिलिग्रॅम/ग्राम खेकडा असे प्रमाण घेण्यात आले. प्रयोगांती असे दिसून आले कि, उतेजकाचा खेकड्याची वाढ (वजन, लांबी, रुंदी) आणि खेकड्याचे कवच टाकण्यावर प्रभाव पडला. या तीन मात्रामध्ये, ०.०२५ मिलिग्रॅम/ग्राम खेकडा या मात्रेचा प्रभाव जास्तीतजास्त आढळून आला त्यामुळे वजनात (७०-७२%), लांबीत (२९-३४%) आणि रुंदीत (२५-२७%) वाढआढळून आली तसेच खेकड्याचे कवच टाकण्याचा कालावधी कमी म्हणजेच ३०-३२ दिवस आढळून आला तसेच या प्रयोगांती ०.०२५ मिलिग्रॅम/ग्राम खेकड्याच्या मात्रेमुळे मऊ खेकड्याची उत्पादकता सर्वाधिक मिळते हे दिसून आले .

INTRODUCTION

1. Introduction

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

Production of mud crabs in 2015 was around 226,390 metric tons (FAO, 2015) with a farm-gate value of US$1.06 billion. Commercial markets were primarily driven by live or frozen soft-shell crab sales (Quinitio and Lwin, 2009; Quinitio *et al.*, 2015). Mud crab aquaculture has been practiced for many years in Asia and especially the Southeast Asia region, but is based primarily on captured animals and fattening prior to sale.

There are four species of mangrove crabs used for commercial aquaculture, *Scylla serrata, S. tranquebarica, S. paramamosain,* and *S. olivacea* (Keenan and Blackshaw, 1999; Fushimi and Watanabe, 1999; Fratini and Vannini, 2002; Allan and Fielder, 2003). These four different species can be found together in many countries, for example at in the Philippines and Indonesia (Keenan and Blackshaw, 1999). Mud crabs live along

tropical, sub-tropical and warm temperate coastlines from Eastern Africa to the southern tip of Japan and northeast Australia (Allan and Fielder, 2003; Sara *et al.,* 2014).

The life cycle of the mangrove crabs starts with courtship and mating, which occur in brackish water then the fertilized female migrates offshore to spawn (Maheswarudu *et al.,* 2007; Shelley, 2008; Hubatsch *et al.,* 2015; Waiho *et al.,* 2018). Depending on species, a mature female can produce 1 to 6 million eggs. After mating, females retain sperm and without further intervention of a male, 2 or 3 egg masses can be produced (Shelley, 2008; Shelley and Lovatelli, 2011). Spawned eggs remain attachd to the pleopod hairs of the abdominal flap (berried female) and eggs hatch into zoea 1 stage. Mud crab have a complex life cycle, where larvae go through five zoea stages (3–5 days each) and one megalopa stage (7–10 days). The zoea and megalopa feed on zooplankton. Crab in-star and juveniles undergo several more molts until full maturity. Small crabs are found in estuaries, tidal flats and mangroves where they burrow in mud or sand, or hide under fallen leaves and shaded areas during the day (Shelley and Lovatelli, 2011). Mud crab can reach sexual maturity within 1 year (Lucas and Southgate, 2012).

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

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

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

Commercial exploration of swimming crab is rapidly increasing worldwide. During the year 2015-16 total production of crabs (fisheries plus aquaculture) reached almost 1300 thousand tons. One of the most valuable marketing forms is called soft-shell crab. The marketing of soft-shell crab, with prices starting at US$3.5 a unit, but going up to US$8.00–10.00, depending on the size and presentation form of the product (live,

cooled, frozen, or processed). In luxury restaurants, a dish containing soft shell crab may cost well over US$75.00. Increasingly, this market requires exporting companies to present some kind of quality certification of the product or process (mainly certifications related to food safety). Almost all soft-shell crab production is based on crabs caught in the wild, by either trawling or trapping. Crab populations are disturbed due to anthropogenic factor associated with human activity, so that obtaining the raw material (crabs in pre-molt stage) has become the biggest challenge for companies that market soft-shell swimming crab nowadays.

Presently in soft shell crab production unit, the crabs are kept in the boxes for moulting for period of 4-10 weeks. Therefore, crab stockiest or exporters require to wait for the moulted crab for about more than 10 weeks. Scientist tried to give moultig stimulant for getting early moulting and growth of mud crab (Aslamyah and Fujaya, 2010). Therefore considering the need of soft-shell crabs in market the present study was undertaken on following objectives.

Objectives

- To standardize the optimum dose of spinach extract for inducing the moulting of crab.
- To study the required moulting period.

REVIEW OF LITERATURE

2. Review of Literature

2.1 General features of mud crab, *Scylla* spp.

Taxonomical identification of the mud crab, *Scylla* spp. was reviewed and updated by referring the recent classification of *Scylla* spp. on the basis of nuclear and mitochondrial DNA analysis/marker, (Fushimi and Watanabe, 1999; Shelley, 2008; Shelley and Lovatelli, 2011; Sarower *et al.*, 2016).

2.1.1 Taxonomic classification of mud crab

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Portunidae

Genus: *Scylla*

Species: *S. serrata*

S. tranquebarica

S. olivacea

S. paramamosain

2.1.2 Biological features

Mud crabs commonly known as mangrove crabs, are marine, or estuarine Portunidae crabs that are mainly distributed in the warmer regions of the Indo-Pacific (Keenan and Blackshaw, 1999). The preferred habitats of these four crabs, of the genus *Scylla*, are intertidal mud flat river mouths generally covered by mangroves (Pavasovic *et al.*, 2004).

Generally mangrove crabs have a flattened, broad body covered by a fan-shaped carapace. Along the frontal margin of the carapace have six spines between the eyes and nine spines on either side of anterial lateral margine of carapace. Each has one pair of chelipeds and three pairs of walking legs. The fourth pair of legs are flattened and used for swimming. In males, the walking legs are used for clasping a female during copulation; females use these walking legs for scratching the eggs off just prior to hatching (Quinitio and Parado-Estepa, 2008). The chelipeds consist of enlarged segments (merus, carpus, propodus, dactylus and pollex) and are used for crushing shells and holding and bringing food to the mouth. The mouthparts are responsible for collection and processing food (Barker and Gibson, 1978). The eyes, antennules, antennae, dactylus and maxilipeds are used to sensory perception.

Mud crabs have separate sexes, immature females have a triangular-shaped abdomen or abdominal flap and mature females have a broader, semi-circular abdomen. Males have a T-shaped abdomen. Mature males have larger chelipeds than females of similar carapace size (Pavasovic *et al.*, 2004).

2.2 Moulting

Moulting stages of crustaceans are first described by Drach and Tchernigovtzeff (1969). Mangrove crab to grow in larger crabs must periodically shed or molt through a process known as moulting or ecdysis (Quinitio and Parado-Estepa, 2008). The exoskeleton is soft immediately after moulting. The crab expands its body and limbs by taking in water before the new shell hardens and usually increases in size by 30-50%. The moulting frequency is more in smaller crabs while less in bigger size crab. At the first zoeal stage, the animal is just over 1 mm. long but increases in size by moulting four times over the next 12 to 15 days (Parado-Estepa and Quinitio, 2011). During the fifth moult, the larval crab transforms into a megalopa with large (relative to its size), functional claws (Nguyen *et al.,* 2014). The megalopa later moults into a stage one crab. The newly moulted crab is especially vulnerable to cannibalism. The number of days between moults increases with size. Daily increase in carapace of mud crab stops but the daily weight gain continuously increases, noticeably for adult crabs. Daily average weight gain can be 3.4g in the grow-out phase (Nguyen *et al.,* 2014).

Crabs with an initial body weight of about 0.7 g grow to marketable size of above 300g after 4-6 months (Catacutan, 2002). With each moult, the size increase 25 to 50% (Huong *et al.,* 2010). A majority of crabs moult during night time and for crabs in the size range of 80g-160g, the next moult would normally occur within 25 days (Ganesh *et al.,* 2015). It is thought that wild mud crabs reach 100-mm carapace width in about a year and sexual maturity in about two years. Maturation time varies according to water temperature, with higher temperatures accelerating the growth and decreasing the time taken to reach maturity (Azra and Ikhwanuddin, 2015). The typical life span of a mud

crab is thought to be three to four years. Factors that trigger moulting are both environmental such as light, temperature, salinity, nutrients, microorganisms and water quality and within the body such as metabolism, hormone and enzyme systems (Keenan, 2004).

2.2.1 The major stages of an individual moult cycle

A) Pre-moult or pre-ecdysis stage

Moulting hormones are released to start ecdysis. The outer hard layer of the shell separates from the membranous layer and causes an evident mottled appearance of the old shed carapace (Stevens, 2002). Activity is reduced and feeding stops as the crab loses it's muscle insertion. Prior to moulting, a crab reabsorbs some of the calcium carbonate from the old exoskeleton, and then secretes enzymes to separate the old shell from the underlying skin (or epidermis). The epidermis secretes a new soft, paper-like shell beneath the old one. When this new shell has fully formed, the crab will be ready to moult (Stevens, 2002; Stevens, 2012; Stevens and Jewett, 2014).

The following Table 1 shows the signs that indicate the stage between moults of the crabs held for moulting to become soft-shell. (Keenan and Blackshaw, 1999; Chamchuen *et al.*, 2014).

Table 1: Signs during moulting stage

Sign	Description
Normal hard shell crabs	14-50 days prior to moult, depend on crab size and hard shell
White sign	Two weeks prior to moult. Also known as snots or greens. The white sign is simply the first faint outline of the second exoskeleton or new skin forming underneath the old as moulting approaches. It is very hard to distinguish.
Pink sign	One week prior to moult. A pink mark that appears on the crabs back fin. This marks the appearance of the new shell underneath its present hard shell.
Red sign	Two days prior to moult. It appears as a small reddish outline, no larger than a fingernail clipping.
Rank peeler	Hard crab which will moult in a matter of hours. Squeeze (or pinch) the end of a rank peelers swimming fin, the new soft shell underneath will be displaced.
Buster	The process of shedding its old shell. The crabs suck water to expand its body and split the old shell open. Then it starts wiggling out of the shell a process that can take up to three hours.
Soft-shell	Immediately following moult. The crab fully exits its old shell. But it leaves behind its esophagus, stomach lining and part of its intestine in the old shell.

B) Ecdysis

A day before moulting, the crab starts to absorb seawater, begins to swell and stops eating. This helps to expand the old shell and causes it to come apart at a special seam that runs around the body. The carapace then opens up like a lid and the crab backs out of the old shell and takes up water rapidly. The crab extracts itself from its old shell by pushing and compressing all of its appendages repeatedly. First he backs out, then pulls out their hind legs and front legs at the end crabs comes out completely from their old shell. This process takes 5-20 minutes, depending on the health of the crab, and is the most stressful part of the moult cycle (Stevens, 2002; Stevens, 2012; Stevens and Jewett, 2014).

C) Post moult

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

D) Inter moult

At the early part of the inter-moult stage, the carapace is almost hard but the walking legs are still flexible depending on the crabs size. The shell hardens completely after several days. The inter-moult stage is the longest among the moult stages. Before moulting occurs, crabs begin to accumulate food for energy and nutrient storage.

2.3 Soft shell production of mud crab (*Scylla* spp.)

Aslamyah and Fujaya (2010) studied on moulting stimulant and growth of mud crab (*Scylla* spp.), used of artificial feed that contain spinach extract. Four artificial diets with different protein levels (P) and carbohydrates (K) used in this study were feed A (P: 46.84%; K: 33.33%), B (P: 41.57%; K: 38.29%), C (P: 35.62%; K: 44.32%) and D (P: 30.62%; K: 49.13%), and as control is feed derived from non-waste materials. During the test, crab was cultured individually in cages placed in ponds. The results showed that the feed D with 30.62% of protein and 49.13% of carbohydrates and enriched with spinach extract (700 ng/g crab), gives the best results in inducing molting of mud crabs. In conclusion, artificial feed should consist of a mixture of various raw materials, so that their nutrients can be balanced and complementary.

Fujaya (2011) worked on growth and moulting of mud crab administrated by different doses of vitomolt. Optimization the dose of vitomolt injection on the growth and molting of mud crab (*Scylla* spp.). Three doses of vitomolt were tested, i.e. 9 μg/g crab, 15 μg/g, and 21 μg/g crab. The results showed that the dose of vitomolt injection had great influence on the growth and moulting of mud crab. Higher dose of vitomolt gave higher growth but its moulting percentage was different. Vitomolt dose of 15 μg/g crab

was the optimal dose to induce moulting of mud crab while the dose of 21 μg/g crab gave the highest growth which reached 53.6%. However, in terms of productivity, dose of 15 μg/g crab gave the highest production of soft crab.

Aslamyah and Fujaya (2011) studied on effectiveness of artificial diet enriched by spinach extract on moulting stimulation to produce soft shell crab. Utilization of the artificial feed is related to its expensive cost, with a very high protein concentration since it's mainly produced from fish based materials, so need to be studied artificial feed formulation with substitution of vegetable material in stimulating moulting and growth of mud crabs. Four artificial feed enriched in this study were feed A (fish, crab shells, and cassava), feed B (fish, silage, shell crab, and cassava), feed C (fish, silage, shell crab, soy flour, and cassava), and feed D (fish, silage, shell crab, soy flour, corn starch, and pollard), trash fish and feed A without EB as control. During the test, mud crab intermoult phase was culture individually in crab box placed in pond. The results showed that the percentage of moulting and weight growth in their respective in the feed A (44% and 41.96%); feed B (56% and 31.57%); feed C (74% and 23.20%); feed D (50% and 39.15%); trash feed control (24% and 50.66%); and feed A without EB (28% and 35.11%). An opposite phenomenon, where the feed C with the highest percentage of moulting but with the lowest growth rate, the opposite occurs in the control of trash feed. This is apparently the effect of spinach extract as a stimulant moulting, where performance can be optimized with a complete and balance nutrient composition.

Aslamyah and Fujaya (2013) studied on gastric evacuation rate, chemical body composition, liver and muscle glycogen, moulting and growth of mud crabs feeding on different percentages in the soft shell crab cultivation. Determine the exact percentage of

artificial feeding in the soft shell crabs cultivation based on the rate of gastric evacuation, chemical body composition, liver and muscle glycogen levels, and the percentage of moulting and growth of mud crabs. Research design using a completely randomized design with three treatments percentage feeding (2, 4, and 6% of body weight per day) feed is given to the composition of 30.86% protein, 7.2% fat, NFE (Nitrogen Free Extract) 48.89%, crude fiber 5.7% enriched with vitomolt 0.10415 mg / g of feed to the standard dose vitomolt and 0.4166 mg / g of feed for high-dose or vitomolt equivalent to 700 ng / g crab. Tests crabs *Scylla* sp. reared in individual box and placed in the pond. Based on the rate of gastric evacuation parameters, the chemical body composition, including protein, fat, fiber content, NFE, ash, and energy, liver and muscle glycogen levels, the percentage of moulting, and growth feeding in soft shell crabs cultivation can be done with the percentage of 2-4% of body weight per day.

Fujaya *et al.*, (2014) studied on the use of mulberry (*Morus alba*) extract in the mass production of blue swimming Crab (*Portunus pelagicus*) larvae to overcome the mortality rate due to moulting syndrome. Standardize doses of Mulberry extract (ME) on the survival rate of crab larvae which are managed to metamorphose to the next stage and on the rate of larval development, as well as identifying various factors causing mortality in the mass cultured larvae, mainly mortality caused by moulting syndrome. There were 4 treatments of different doses of mulberry extract being tested which are: 0 mg/100 g (control), 1 mg/100 g, 2 mg/100 g, and 4 mg/100 g. Mulberry extract was given through feeding since day-8 of the stocking, which is the time when larvae enter zoea 3. The rearing process was done over 19 days in a concrete tank with a volume of one ton with the initial number of zoea at ± 350.0. The findings showed that mulberry extract has a

significant influence on the survival rate, stage growth, and mortality rate of blue swimming crab larvae due to moulting syndrome. The higher the dose of ME in the artificial food, the higher was the survival rate and the lower the mortality rate due to moulting syndrome. The treatment with 4 mg of mulberry extract/100 mg was the only treatment which successfully enters megalopa and crab stage. Control treatment and the dose of 1 mg/100 g can only reach zoea 3 while the dose of 2 mg/100 g can only reach zoea 4. This study revealed that the total mortality rate was high, but it was found that the main cause was not molting syndrome. Mortality rate due to moulting syndrome in the treatment of the dose of 4 mg of mulberry extract was only ± 15.61% of the total larval mortality. The unidentified factors dominate the cause of mortality (± 57.47%).

Aslamyah and Fujaya (2014) studied on four feeding frequency (1 time per day, 1 time per 2 days, 1 time per 3 days, and 1 time per 4 days) tested. Feed with nutrient composition of 30.86% protein, 7.2% fat, nitrogen free extract (NFE) 48.89%, crude fiber 5.7% enriched with vitomolt 0.1041 5 mg/g of feed to the standard dose and 0.4166 mg vitomolt/g feed to high doses; or equal to 700 ng vitomolt/g crab. Test crabs of *Scylla* sp. reared in crabs box and placed in the pond. The results showed the frequency of feeding 1 time per 2 days resulted in the highest percentage of moulting (66.67%) and the lowest percentage of moulting was found at 1 per 4 times a day of feeding (36.67%). Growth parameters such as weight, carapace width, and feed efficiency did not differ among all treatments. Thus, feeding in the soft shell crab for the maximum production can be done with a frequency of 1 time per 2 days.

Tahya *et al.*, (2016) studied role of mandibular organ in moulting, growth, and survival of mud crab *Scylla olivacea.* Healthy crabs and ± 100 gram of body weight

(BW) were selected as test crabs. The crabs were maintained individual in crab box and fed fresh fish 10% of crabs body weight per day. The crabs were divided into 2 groups, control and injected of Mandibular organ (MO). The injection of mandibular organ increased the molting percentage and was found to have an effect of simultaneously moulting. The injection of MO had higher moulting percentage i.e. 80, 80, and 90%, than control crabs i.e. 20, 10, and 50%. In control crabs the time required for molting between 20 to 41 days to reach of 26.67%, while in injected crabs required shorter time i.e. 26 to 34 days to reach the percentage of 83.33%. Mandibular organ (MO) is one of many organs which play role in moulting and reproduction. The influence of exogenous MO in the progress of molt, growth, and mortality in commercial mud crab. Injection of MO into intermoult crabs increased molting percentages, moulting simultaneity, growth acceleration, and adaptability. The results confirm that the MO is involved in the control of moulting in *Scylla olivacea*.

MATERIAL AND METHODS

3. Materials and Methods

In the present work, *S. serrata* species was selected for experiment. The crab species were identified by the morphological characters such as number of spines, body shape, chelipeds (Keenan and Blackshaw, 1999). But of the 714 species of portunidae only a few species of surviving crabs are regularly marketed as soft shell crab in the world. Among the portunidae family, species such as *Scylla serrata*, *Portunus pelagicus* are the most cultivated and popular due to its cultivation simplicity and more demand for consumption. Therefore, in the present work *Scylla serrata* species was chosen for the study.

An experiment was undertaken to evaluate moulting and moulting period of mud crab (*Scylla serrata*) by administration of three different doses of spinach extract through injection @ 0 mg/g control (without injection), 0.015 mg/g crab, 0.02 mg/g crab and 0.025 mg/g crab.

Experimental location:

The experiment was conducted in the series of polypropylene crab box unit in a indoor system Shirgaon, near college of fisheries, Ratnagiri, owned by local entrepreneur Mr. Shetye.

3.1 Materials

3.1.1 Experimental animal:

The collected mud crab spp. *Scylla serrata* were in the range of 40-80 g (size range). Total 60 numbers of specimen were collected from local fish market Ratnagiri

(Plate 1). Experiments were designed by keeping the mud crab individually into total 20 boxes (with 5 sets 4 series). Four series arranged on the basis of different treatment. Before stocking the crabs were measured for their initial length, width, weight and dates of stocking were recorded.

3.1.2 Experimental box:

Rectangular polypropylene boxes (18" x 12" x 8") were used for keeping the crabs for carrying out the experiment in the indoor system setup at Shirgaon, Ratnagiri (Plate 2).

3.1.3 Feed:

Feeding management is an important aspect in soft shell crab production. Feeding was done daily in the early morning. The crabs were fed trash fishes which was 10% of the biomass using the formula:

Total weight of crabs x 10 ÷ 100

3.1.4 Maintenance of water quality:

Maintenance of water quality is an important aspect in soft shell production. This water system was properly setup in the indoor system. Filtered water was used by filtration system and then re-circulated. In this system the water parameters were maintained in optimum range for better survival. (Table 2)

3.1.5 Spinach leaves:

Fresh spinach leaves were used for preparation of doses of spinach extract.

Table 2: Maintenance of water parameters in polypropylene boxes.

Parameter	Value
Temperature	27-32^{0}C
Salinity	16-30 ppt
Dissolved oxygen	≥ 5 ppm
pH	7.5-8.5

Plate 1: Hard shell mud crab (*Scylla serrata*)

Plate 2: Experimental setup in the polypropylene boxes

3.2 Methodology

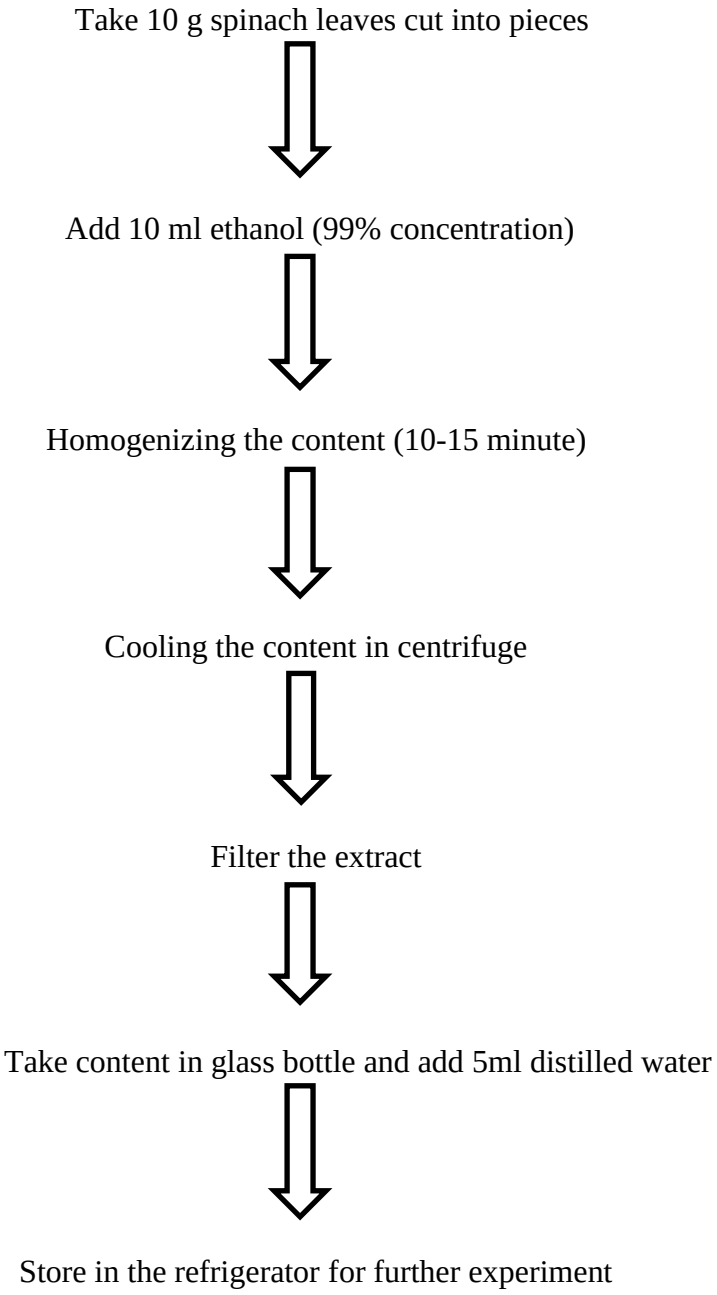
3.2.1 Experimental design:

The experiment was carried out as per Completely Randomized Design (CRD) with four treatments and five replicates to evaluate the effect of growth and moulting period in mud crab. Five crabs each were reared for the treatments as 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2), and 0.025 mg/g (T_3).

3.2.2 Spinach extraction:

Spinach extracts contain phytoecdysteroid, a substance which is well known to stimulate moulting in crabs. The extraction method as per Fujaya, (2011) was followed for the experiment. Initially 10g fresh spinach leaves were taken in mortar and pestles. 10 ml ethanol (99% concentration) was added to it and homogenized for 10-15 minutes. Then the cooled extract was centrifuged at 4500 rpm for 15 minute at temperature 20^0C. After the centrifuge process, the extract was filtered further by using a what-man filter paper. Collected filtrate was taken in the test tube and held in boiling water. The extract was boiled for 5-10 minutes. After boiling the extract was cooled and taken in glass bottle and 5ml distilled water was added in it. The extract was then stored in refrigerator for further experiment (Fig. 1 and Plate 3 & 4).

Fig. 1: Method for spinach extraction



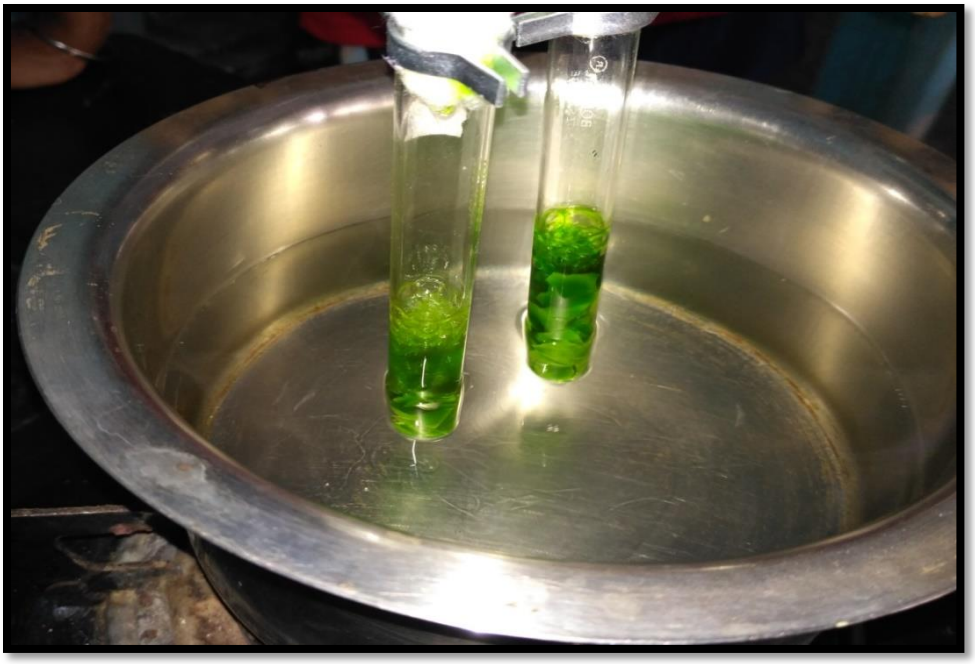


Plate 3: Spinach extracts process

Plate 4: Spinach extract

3.2.3 Injecting the crab:

The doses of spinach extract injection (T_1) 0.015 mg/g crab, (T_2) 0.02 mg/g crab and (T_3) 0.025 mg/g crab were prepared and injected to five mud crab of each set. The injection was administered at the base of last leg (coxa part) of mud crab. Before injection, area was swabbed with acetone. After the injection, the cotton dipped in acetone was again used for swabbing the injected area (Plate 5).

3.2.4 Inspection of crab:

During the experiment the biological parameters such as final weight, carapace length and carapace width of all the animals were recorded after moulting. The final weight of all animals were measured in gram using electrical balance (Plate 6). The total length and width of crab was measured in centimeter using a metallic scale with 0.5 cm accuracy (Plate 7). Moulting period of crab was also recorded (Plate 8).

The growth parameters were calculated as follows:

$$\text{Weight gain (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$ Soundarapandian *et al.*, 2013

$$\text{Length gain (\%)} = \frac{(\text{Final length} - \text{Initial length})}{\text{Initial length}} \times 100$$ Soundarapandian *et al.*, 2013

$$\text{Width gain (\%)} = \frac{(\text{Final width} - \text{Initial width})}{\text{Initial width}} \times 100$$ Soundarapandian *et al.*, 2013

3.3 Statistical Analysis:

All data of growth (weight, weight gain, length, length gain, width, width gain) were analyzed by One-way Analysis of Variance (ANOVA). If difference was found significant, the means were compared by tukey multiple comparison test. The statistical analysis performed by using SPSS 16.0.

Plate 5: Injection administrated to the mud crab coxal part

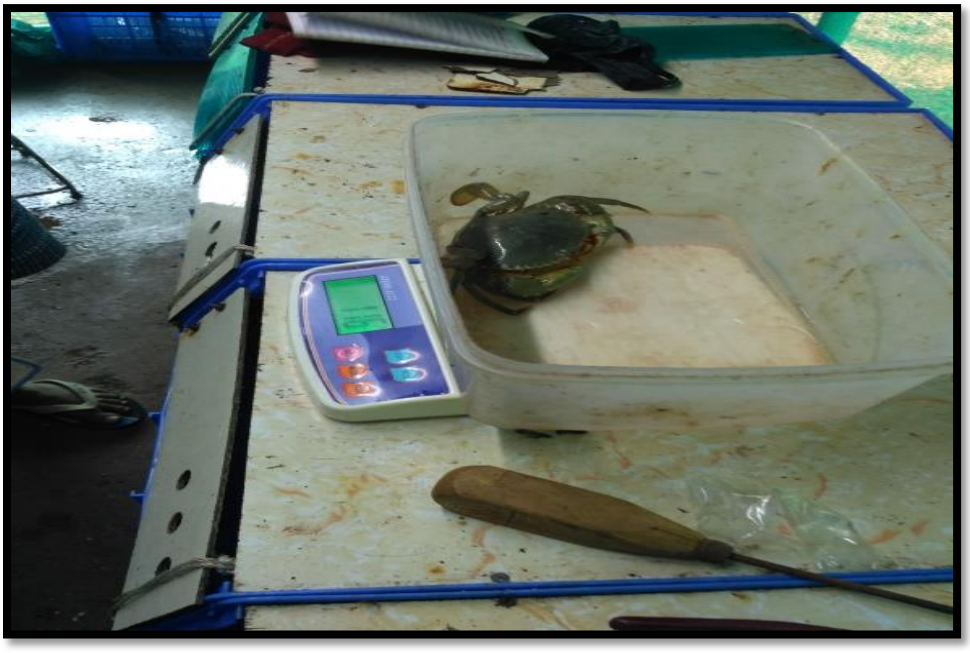


Plate 6: Weight gain observation in soft shell crab

Plate 7: Carapace length, carapace width measurement of soft shell crab

Plate 8: Moulted crab (soft shell) observed in polypropylene box

RESULTS

4. Results

The experiment was conducted to find out the effect of different doses of spinach extract on growth and moulting period of mud crab, *Scylla serrata* in polypropylene boxes. Results of the experiment conducted are presented as follows:

4.1 Growth Parameter

4.1.1 Weight (g)

The average final weight of *S. serrata* in three trials reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is given in Table 3 and Fig. 2. At the time of moulting of the crab, the final average weight of T_3 was significantly higher than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average final weight which was significantly greater than that of T_1 and T_2, whereas the lowest average final weight was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in final weight between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 4).

Thus, at the end of experiment it reveals that 0.025mg/g dose of spinach extract given the higher weight than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 3: Average final weight (g) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average final weight (g)			
	T_0	T_1	T_2	T_3
Initial	59.66 ± 4.66^{c}	59.26 ± 4.94^{d}	60.66 ± 4.69^{a}	60.33 ± 3.91^{b}
1st Trial	84.80 ± 5.35^{d}	90.20 ± 6.60^{c}	97.00 ± 5.67^{b}	103.00 ± 5.66^{a}
2nd Trial	83.20 ± 4.92^{d}	92.00 ± 5.40^{c}	96.40 ± 5.45^{b}	103.60 ± 4.71^{a}
3rd Trial	82.60 ± 4.89^{d}	90.80 ± 6.35^{c}	98.60 ± 5.97^{b}	103.40 ± 5.39^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups

Table 4: ANOVA for final weight (g) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	944.150	3	314.717	1.835	.181
	Within Groups	2743.600	16	171.475		
	Total	3687.750	19			
Trial 2	**Between Groups**	1092.000	3	364.000	2.761	.076
	Within Groups	2109.200	16	131.825		
	Total	3201.200	19			
Trial 3	**Between Groups**	1248.150	3	416.050	2.580	.090
	Within Groups	2580.400	16	161.275		
	Total	3828.550	19			

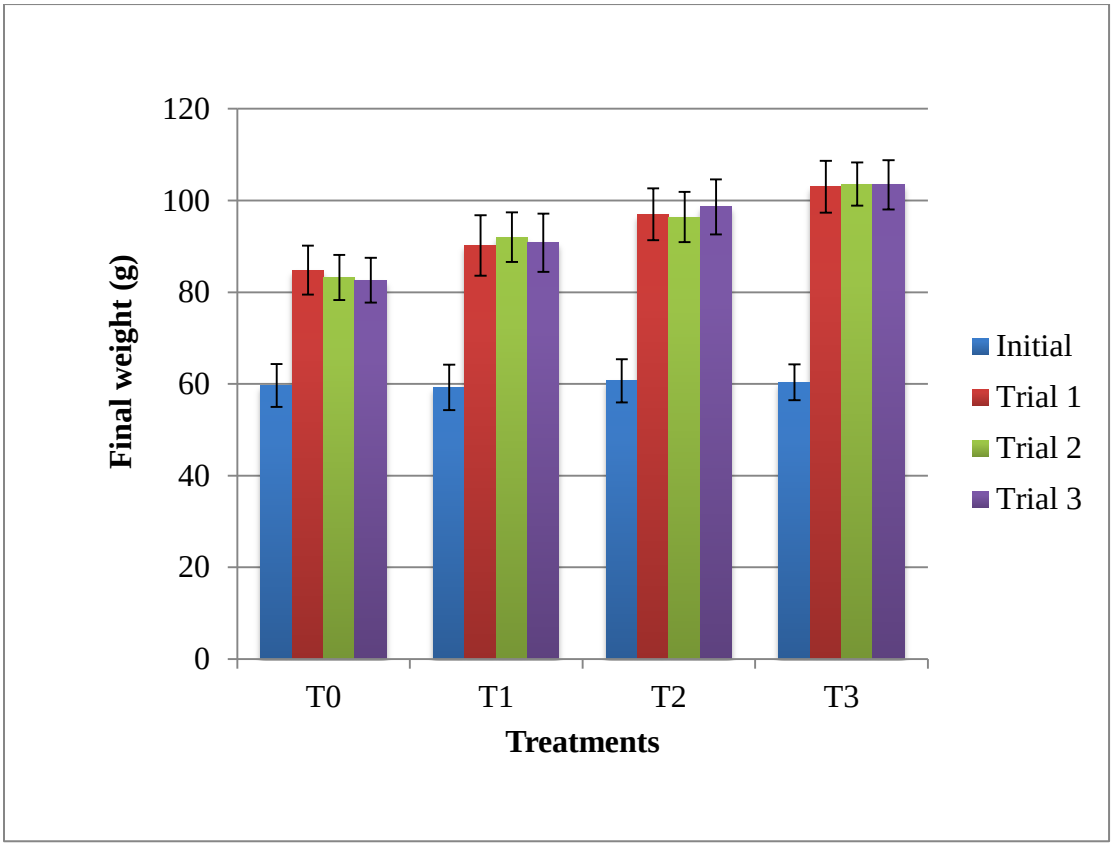


Fig 2: Final weight (g) of mud crab, *Scylla serrata*

4.1.2 Weight gain (%)

The average weight gain (%) of *S. serrata* in three trials of four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is shown in Table 5 and Fig. 3. At the time of moulting of the crab, the average weight gain (%) of T_3 was significantly higher ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average weight gain (%) which was significantly greater ($P<0.05$) than that of T_1 and T_2, whereas the lowest average weight gain (%) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in weight gain % between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 6).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract given the maximum weight gain percentage (70-72%) than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 5: Average weight gain (%) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average weight gain (%)			
	T_0	T_1	T_2	T_3
1st Trial	41.49 ± 2.38^{d}	54.79 ± 2.46^{c}	61.42 ± 3.29^{b}	72.41 ± 2.66^{a}
2nd Trial	41.23 ± 3.07^{d}	54.21 ± 2.59^{c}	62.22 ± 3.45^{b}	72.50 ± 2.12^{a}
3rd Trial	40.50 ± 3.09^{d}	53.49 ± 2.77^{c}	61.00 ± 3.17^{b}	70.79 ± 2.76^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 6: ANOVA for weight gain (%) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	2507.439	3	835.813	22.503	.000
	Within Groups	594.286	16	37.143		
	Total	3101.725	19			
Trial 2	**Between Groups**	2615.323	3	871.774	21.369	.000
	Within Groups	652.727	16	40.795		
	Total	3268.050	19			
Trial 3	**Between Groups**	2448.618	3	816.206	18.634	.000
	Within Groups	700.819	16	43.801		
	Total	3149.437	19			

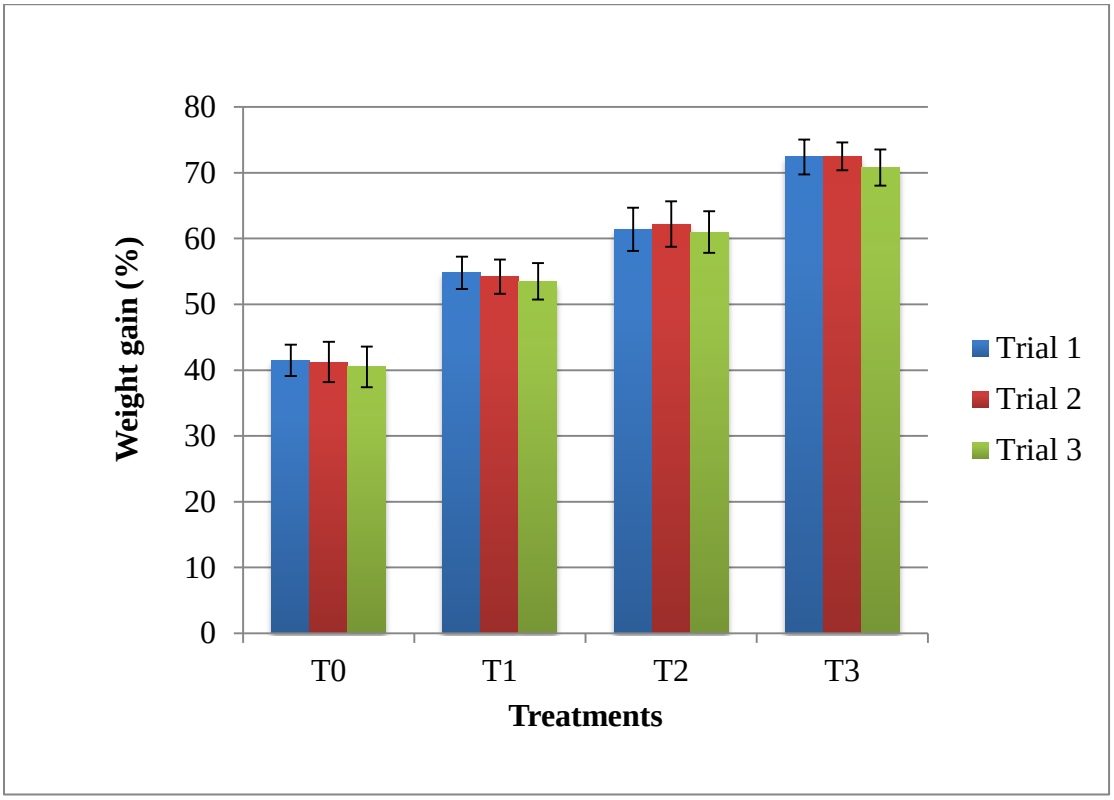


Fig 3: Weight gain (%) of mud crab, *Scylla serrata*

4.1.3 Final carapace length (cm)

The average final carapace length (cm) of *S. serrata* in three trials reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is showed in Table 7 and Fig. 4. At the time of moulting of the crab, the average final carapace length of T_3 was significantly higher ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average final carapace length (cm) which was significantly greater ($P<0.05$) than that of T_1 and T_2, whereas the lowest average final carapace length (cm) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in final carapace length between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 8).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract given the higher gain in carapace length than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 7: Average final carapace length (cm) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average final length (cm)			
	T_0	T_1	T_2	T_3
Initial	4.80 ± 0.12^{c}	4.78 ± 0.12^{d}	4.82 ± 0.12^{a}	4.81 ± 0.09^{b}
1st Trial	5.64 ± 0.21^{d}	5.80 ± 0.25^{c}	6.08 ± 0.18^{b}	6.30 ± 0.15^{a}
2nd Trial	5.58 ± 0.20^{d}	5.84 ± 0.20^{c}	6.10 ± 0.17^{b}	6.38 ± 0.10^{a}
3rd Trial	5.56 ± 0.20^{d}	5.76 ± 0.23^{c}	6.14 ± 0.22^{b}	6.32 ± 0.18^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 8: ANOVA for final carapace length (cm) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	1.289	3	.430	2.059	.146
	Within Groups	3.340	16	.209		
	Total	4.629	19			
Trial 2	**Between Groups**	1.769	3	.590	3.704	.034
	Within Groups	2.548	16	.159		
	Total	4.317	19			
Trial 3	**Between Groups**	1.805	3	.602	2.687	.081
	Within Groups	3.584	16	.224		
	Total	5.389	19			

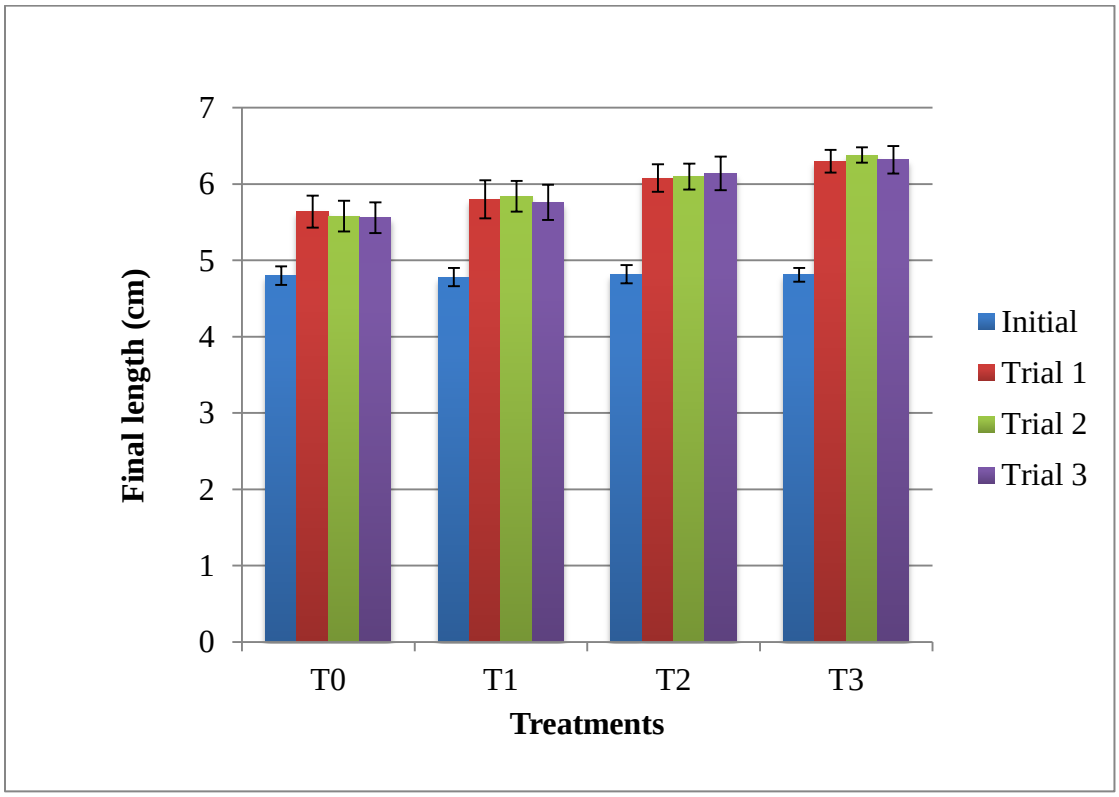


Fig 4: Final carapace length (cm) of mud crab, *Scylla serrata*

4.1.4 Carapace length gain (%)

The average carapace length gain (%) of *S. serrata* in three trials reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is showed in Table 9 and Fig. 5. At the time of moulting of the crab, the average carapace length gain (%) of T_3 was significantly higher ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average carapace length gain (%) which was significantly greater ($P<0.05$) than that of T_1 and T_2, whereas the lowest average carapace length gain (%) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in carapace length gain % between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 10).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract given the maximum carapace length gain percentage (29-34 %) than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 9: Average carapace length gain (%) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average length gain (%)			
	T_0	T_1	T_2	T_3
1st Trial	16.85 ± 1.74^{d}	21.16 ± 1.90^{c}	25.56 ± 0.99^{b}	29.59 ± 0.41^{a}
2nd Trial	16.12 ± 1.45^{d}	22.54 ± 2.16^{c}	27.04 ± 1.38^{b}	34.08 ± 1.00^{a}
3rd Trial	16.24 ± 0.85^{d}	19.86 ± 1.27^{c}	27.34 ± 2.80^{b}	31.06 ± 2.57^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 10: ANOVA for carapace length gain (%) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	454.273	3	151.424	15.429	.000
	Within Groups	157.031	16	9.814		
	Total	611.303	19			
Trial 2	**Between Groups**	857.273	3	285.758	23.379	.000
	Within Groups	195.564	16	12.223		
	Total	1052.836	19			
Trial 3	**Between Groups**	689.268	3	229.756	10.924	.000
	Within Groups	336.520	16	21.032		
	Total	1025.788	19			

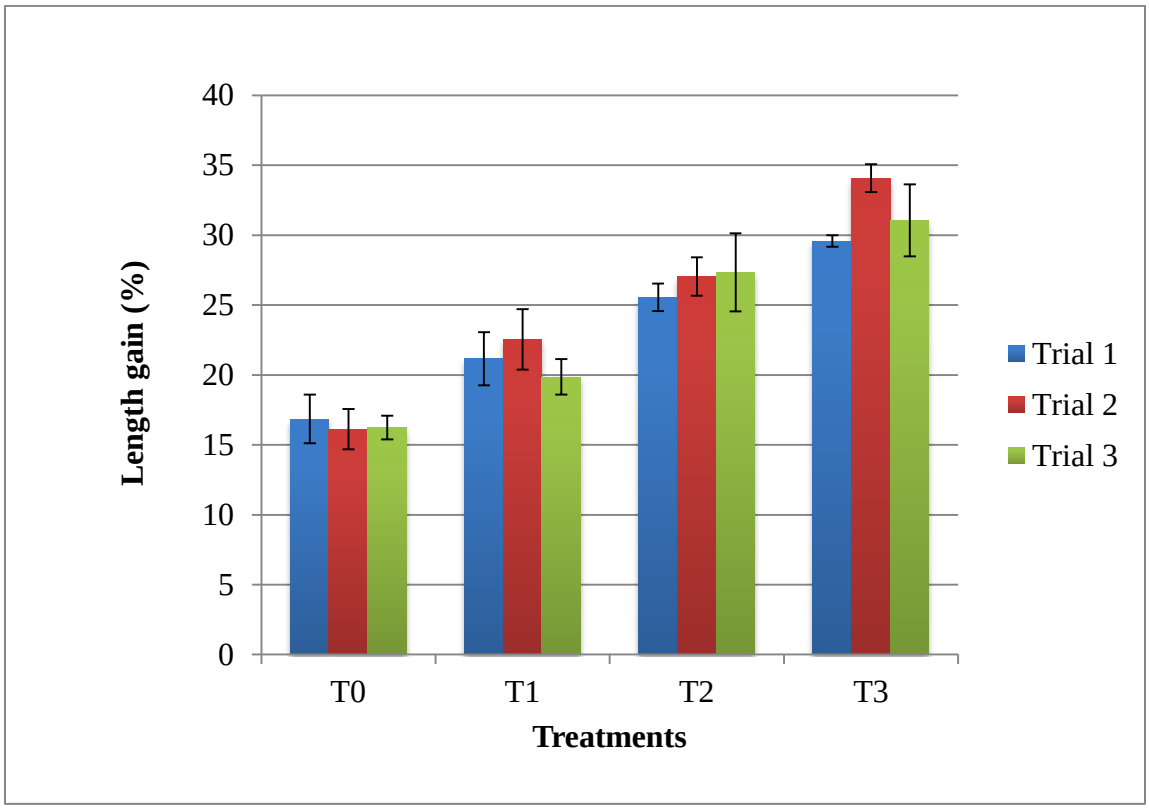


Fig 5: Carapace length gain (%) of mud crab, *Scylla serrata*

4.1.5 Final carapace width (cm)

The average final carapace width (cm) of *S. serrata* in three trials reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is showed in Table 11 and Fig. 6. At the time of moulting of the crab, the average final carapace width (cm) of T_3 was significantly higher ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average final carapace width (cm) which was significantly greater ($P<0.05$) than that of T_1 and T_2, whereas the lowest average final carapace width (cm) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in final carapace width between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 12).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract given the higher gain in carapace width than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 11: Average final carapace width (cm) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average final width (cm)			
	T_0	T_1	T_2	T_3
Initial	6.46 ± 0.16^{d}	6.46 ± 0.17^{c}	6.53 ± 0.17^{a}	6.50 ± 0.14^{b}
1st Trial	7.60 ± 0.21^{d}	7.78 ± 0.22^{c}	8.04 ± 0.17^{b}	8.22 ± 0.17^{a}
2nd Trial	7.52 ± 0.21^{d}	7.88 ± 0.17^{c}	8.04 ± 0.16^{b}	8.26 ± 0.13^{a}
3rd Trial	7.50 ± 0.21^{d}	7.82 ± 0.21^{c}	8.12 ± 0.19^{b}	8.24 ± 0.17^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 12: ANOVA for final carapace width (cm) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	1.130	3	.377	1.879	.174
	Within Groups	3.208	16	.201		
	Total	4.338	19			
Trial 2	**Between Groups**	1.458	3	.486	3.186	.052
	Within Groups	2.440	16	.152		
	Total	3.898	19			
Trial 3	**Between Groups**	1.644	3	.548	2.733	.078
	Within Groups	3.208	16	.200		
	Total	4.852	19			

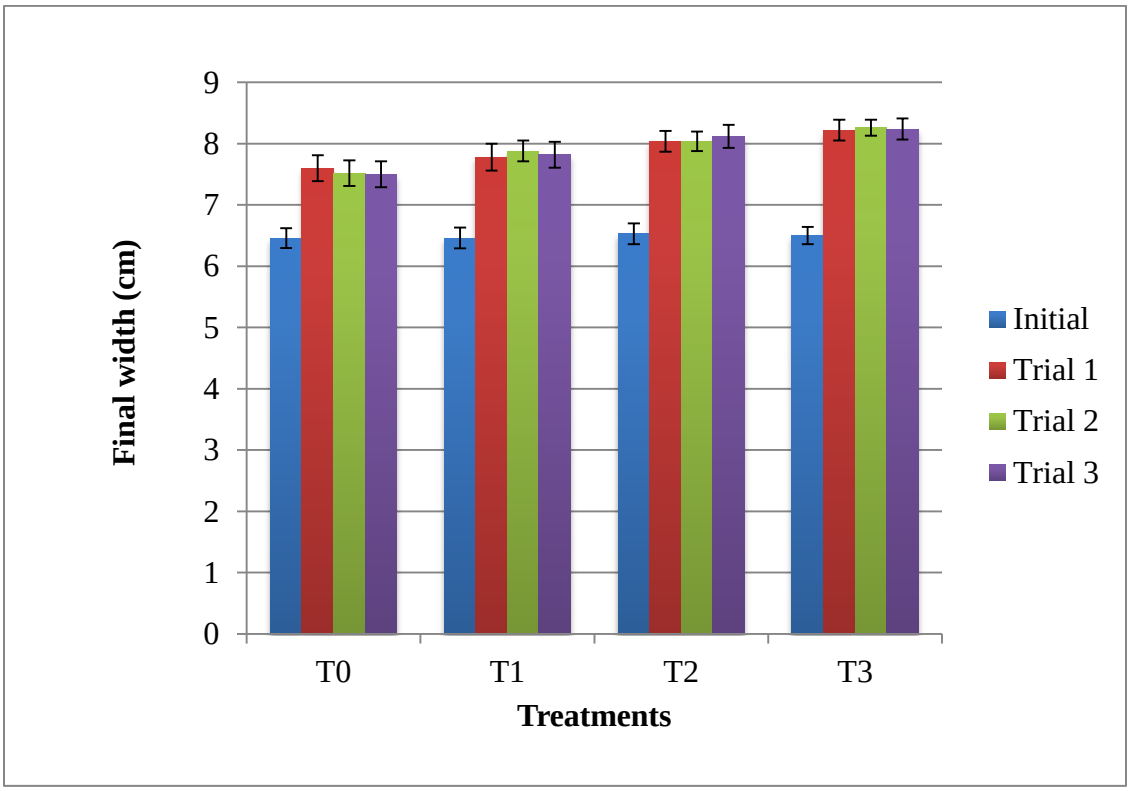


Fig 6: Final carapace width (cm) of mud crab, *Scylla serrata*

4.1.6 Carapace width gain (%)

The average carapace width gain (%) of *S. serrata* in three trials reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is showed in Table 13 and Fig. 7. At the time of moulting of the crab, the average final carapace width (cm) of T_3 was significantly higher ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the highest average carapace width gain (%) which was significantly greater ($P<0.05$) than that of T_1 and T_2, whereas the lowest average carapace width gain (%) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in carapace width gain % between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 14).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract given the maximum carapace width gain percentage (25-27 %) than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract.

Table 13: Average carapace width gain (%) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average width gain (%)			
	T_0	T_1	T_2	T_3
1st Trial	16.54 ± 1.00^{d}	20.83 ± 1.04^{c}	22.99 ± 0.86^{b}	26.52 ± 0.67^{a}
2nd Trial	16.38 ± 0.51^{d}	21.98 ± 0.67^{c}	23.79 ± 1.03^{b}	27.49 ± 0.42^{a}
3rd Trial	16.79 ± 0.66^{d}	20.70 ± 1.03^{c}	23.83 ± 1.23^{b}	26.37 ± 0.14^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 14: ANOVA for carapace width gain (%) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	261.447	3	87.149	21.143	.000
	Within Groups	65.949	16	4.122		
	Total	327.396	19			
Trial 2	**Between Groups**	321.008	3	107.003	43.680	.000
	Within Groups	39.195	16	2.450		
	Total	360.203	19			
Trial 3	**Between Groups**	256.380	3	85.460	22.334	.000
	Within Groups	61.224	16	3.826		
	Total	317.604	19			

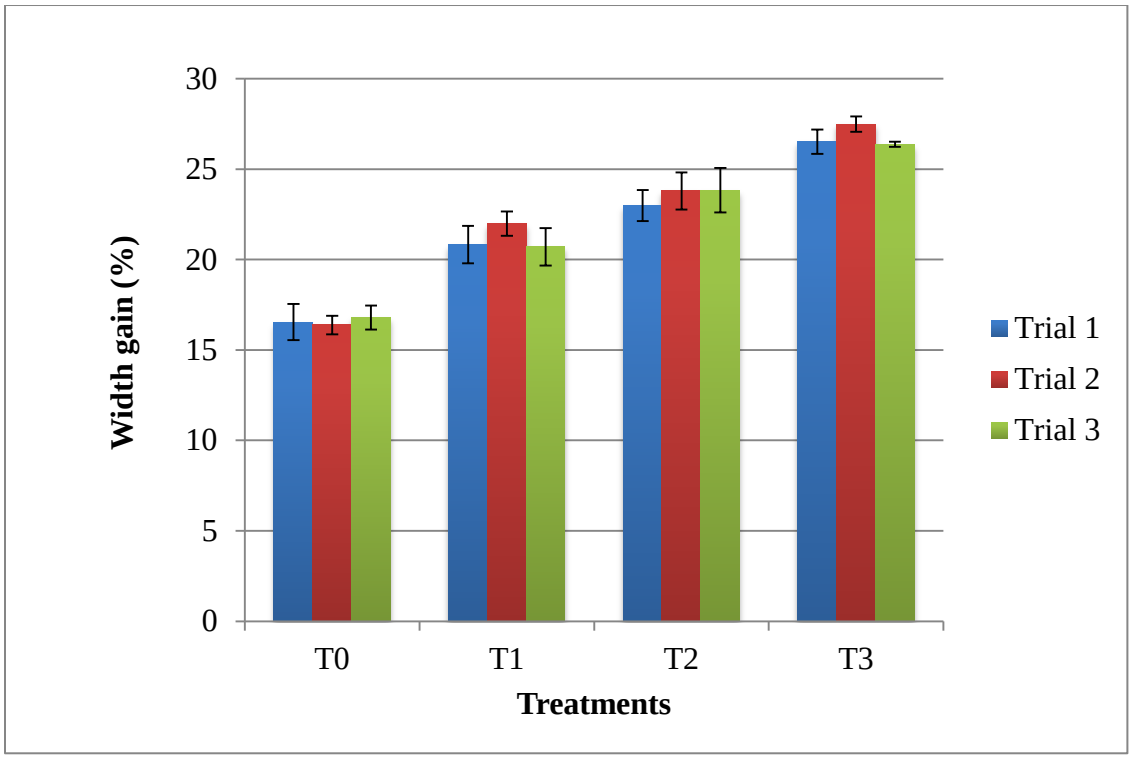


Fig 7: Carapace width gain (%) of mud crab, *Scylla serrata*

4.2 Moulting period (days)

The average period of moulting in three trials of *S. serrata* reared at four different doses of spinach extract of 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3) is showed in Table 15 and Fig. 8. The average period of moulting T_3 was significantly lower ($P<0.05$) than that of T_0, T_1 and T_2. At the end of all the three trials, T_3 showed the lowest moulting period (days) which was significantly lower ($P<0.05$) than that of T_1 and T_2, whereas the highest moulting period (days) was observed in T_0.

One way ANOVA indicated significant difference ($P<0.05$) in moulting period between control and different doses treatments. Tukey multiple comparison test showed a significant difference ($P<0.05$) between control and treatments of spinach extract of *S. serrata* in (Table 16).

Thus, at the end of experiment it reveals that 0.025 mg/g dose of spinach extract reduce the moulting period (30-32 days) of mud crab than the dose injected of 0.015 mg/g (38-40 days), 0.02 mg/g (34-36 days) and 0 mg/g control (43-44 days) spinach extract.

Table 15: Average moulting period (days) of *Scylla serrata* in polypropylene boxes during the experimentation

Treatments	Average moulting period (days)			
	T_0	T_1	T_2	T_3
1st Trial	43.60 ± 0.50^{d}	40.20 ± 0.37^{c}	36.80 ± 0.58^{b}	32.00 ± 0.70^{a}
2nd Trial	43.60 ± 0.50^{d}	40.20 ± 0.66^{c}	36.40 ± 0.50^{b}	31.40 ± 0.92^{a}
3rd Trial	44.00 ± 1.00^{d}	38.20 ± 0.96^{c}	34.60 ± 1.20^{b}	31.00 ± 0.70^{a}

*Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 16: ANOVA for moulting period (days) of *Scylla serrata*

ANOVA

Trials		Sum of Squares	df	Mean Square	F	Sig.
Trial 1	**Between Groups**	367.750	3	122.583	79.086	.000
	Within Groups	24.800	16	1.550		
	Total	392.550	19			
Trial 2	**Between Groups**	411.400	3	137.133	60.278	.000
	Within Groups	36.400	16	2.275		
	Total	447.800	19			
Trial 3	**Between Groups**	460.950	3	153.650	31.518	.000
	Within Groups	78.000	16	4.875		
	Total	538.950	19			

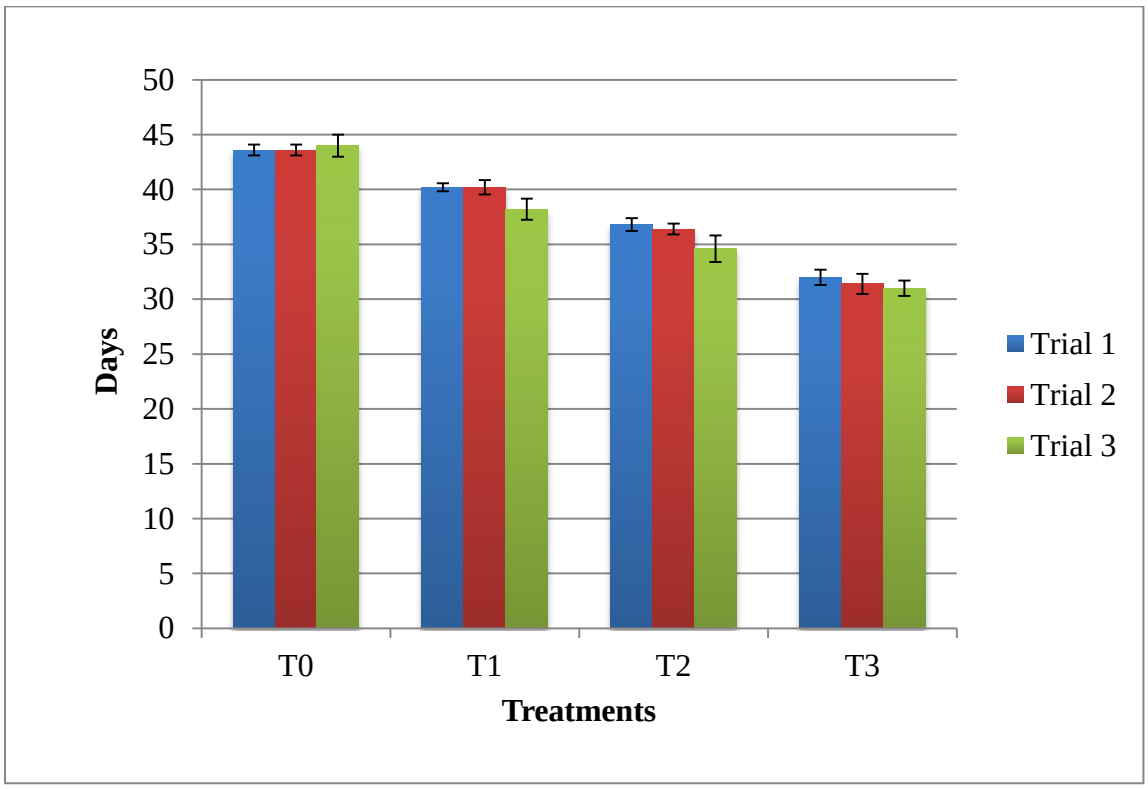


Fig 8: Moulting period (days) of mud crab, *Scylla serrata*

DISCUSSION

5. Discussion

Mangrove crab, *Scylla serrata* are well known for their great aquaculture potential (Mwaluma, 2002). Recently soft shell crab production has proved one of the most profitable system in their commercialization. Soft shell mangrove crab (*Scylla* spp.) farming in Asia started during the early 1990's when there was a strong demand for soft shell crabs in domestic and international markets (Quinitio and Lwin, 2009). The methods of production of soft shell crab depend on the maintenance of crab premoult stage either in open, semi-closed or closed system, until the moment of moulting. In recent year large scale, soft shell crab production was done through closed system rearing (Tavares *et al.*, 2017). But it was observed that moulting cause of mortality due to failure to secure energy for shedding the exoskeleton when due, or immediately after moulting due to vulnerability from predation or injury and other physical stresses (Mirera and Mtile, 2009).

Considering the above aspect and getting early production of soft shell crabs number of experiment were conducted on induced moulting and feed formulation by many Scientists in the world (Drach and Tchernigovtzeff, 1969; Sheen and Wu, 1999; Sheen, 2000; Catacutan, 2002; Stevens, 2002 & 2012; Watnabe *et al.,* 2004; Nagaraju *et al.,* 2004; Bakrim *et al.,* 2008; Reddy *et al.*, 2004; Aslamyah and Fujaya, 2010, 2011, & 2014; Fujaya, 2011; Fujaya *et al.,* 2014; Allayie *et al.,* 2011; Baldwin and Johnsen, 2011; Soundarapandian *et al.,* 2013; Nguyen *et al.,* 2014; Chamchuen *et al.,* 2014; Herlinah *et al.,* 2015; Tahya *et al.,* 2016; Tavares *et al.,* 2017; Nikhalan and Sukarti, 2017).

Among these Fujaya (2011) and Herlinah *et al.,* (2015) both have reported that the injection of Vitomolt (15 μg/g) and Mulberry extract (100 ppm) gave the highest production of soft crab respectively. Some of the interesting observations were made by scientists on feeding experiment by preparing the feed using additives for inducing the moulting of crab. For example feed additives 700 μg/g spinach extract (Aslamyah and Fujaya, 2010) 4 mg/100g Mulberry extract (Fujaya *et al.,* 2014), 2 mg/100g phytoecdysteroid (Nikhalan and Sukarti, 2017) were used in artificial diets.

In the present studies, spinach extract was used for inducing the moulting and early period for getting soft shell crab. The experiment was conducted by injecting spinach extracts to the mangrove crab in the closed system where 0.025 mg/g doses of spinach extract (T_3) showed the better result of the soft shell crab production than 0 mg/g (T_0), 0.015 mg/g (T_1) and 0.02 mg/g (T_2).

In case of gain in weight and weight gain percentage after moulting of crab where studied. The final average weight in T_3 treatment was significantly higher than that of T_0, T_1 and T_2. At the end of three trials, T_3 showed the same highest average final weight which was significantly greater than that of T_1 and T_2, whereas the lowest average final weight was observed in T_0. The maximum weight gain percentage in soft shell crab i.e. 70-72% was recorded in the dose given of 0.025 mg/g of spinach extract injection. Fujaya (2011) reported that Vitomolt (moulting stimulant made from spinach extracts) injection dose of 15 μg/g to the mud crab (*S. serrata*) gave the highest weight gain (53.6 %) in the soft shell production. Aslamyah and Fujaya (2011), found that artificial feed with spinach extract gave highest percentage of moulting (74 %) but lowest weight gain (23.20 %). Tahya *et al.,* (2016) also investigated that the mandibular organ (MO) are the important

role in moulting and growth in mud crab, *Scylla olivacea*. Soundarapandian *et al.,* (2013) described that at salinity 30 ppt, the trash fish feeding performed the higher weight gain (90 %) in swimming crab, *Charybdis natator*. This result was supported by Nguyen *et al.,* (2014) in their experiments where 90% weight gain was observed in *Scylla serrata*.

In gain in final carapace length and percentage after the time of moulting of the crab, it showed that 0.025 mg/g dose of spinach extract gave the higher gain in carapace length than the dose of 0.015 mg/g and 0.02 mg/g spinach extract. The maximum carapace length gain percentage in soft shell crab i.e. 29-34 % was recorded in the dose of 0.025 mg/g of spinach extract injection. Chamchuen *et al.,* (2014) development of digestive enzymes and activity ratio of trypsin to chymotrypsin (T/C ratio) for growth in blue swimming crab during moulting. He remarked that since the T/C ratio level at the end of moulting cycle probably indicate a high carapace growth after moulting. But remark given by Aslamyah and Fujaya (2014) that growth parameters such as weight and carapace width did not differ by frequency of feeding with vitomolt. Mirera and Mtile (2009) reported that new moult crabs increased in size between 15 and 25 % based on carapace width gain in mud crab, *Scylla serrata* in cage culture system.

In case of gain in carapace final width and percentage after the time of moulting of the crab, it showed that 0.025 mg/g dose of spinach extract gave the higher gain in carapace width than the dose of 0.015 mg/g and 0.02 mg/g spinach extract. The maximum carapace width gain percentage in soft shell crab i.e. 26-27 % was recorded in the dose of 0.025 mg/g of spinach extract injection. Catacutan (2002) observed different dietary protein and lipid levels and protein to energy ratios for growth and body composition of mud crab *S. serrata.* He observed that since the carapace width and final

weight of crab fed with protein/lipids 40/6 (P/E ratio, 27.5 mg protein/kJ) were not enhanced with further increase in lipid to 12% (P/E ratio, 24.5 mg protein/kJ), or protein level to 48%. Tahya *et al.,* (2016) mandibular organ (MO) are playing important role in moulting and growth in mud crab, *S. olivacea*. The body weight increased on an average of 31.88 g and carapace width increased on an average of 12.24 mm when injected with mandibular organ extract. Herlinah *et al.,* (2015) that injected mulberry extract concentration 100 ppm gave highest carapace width gain 6.0 mm and the body weight gain 32.98 g of mud crab *S. olivacea*.

During the study of moulting period (days) of the mud crab (*S. serrata*), it revealed that 0.025 mg/g dose of spinach extract reduced the moulting period up to 30-32 days than the dose injected of 0.015 mg/g and 0.02 mg/g spinach extract. Aslamyah and Fujaya (2010) stated in their result that on artificial diet with 30.62 % protein and carbohydrates 49.13 % enriched with spinach extract 700 ng/g gave the moulting percentage (20 %) for time duration was 41-50 days, and (26.67 %) for time duration is 51-60 days of mud crab (*Scylla* spp.). Further Aslamyah and Fujaya (2011) added in their result on artificial diet enriched by spinach extract that the moulting percentage (74 %) was achieved in duration of 41-50 days for moulting in mud crab. in natural condition Aslamyah and Fujaya (2014) frequency of feeding artificial feed used that contain vitomolt, crab reared in box and placed in pond gave the percentage of moulting as 66.67 % in mud crab *Scylla* spp. Stevens (2002) observed the moulting of red king crab (*Paralithodes camtschaticus*) by time-lapse video in the laboratory. He observed that the average time require for ecdysis from splitting the carapace to complete extraction was 20 minutes, 17 seconds. Herlinah *et al.,* (2015) conclude in their observation that

moulting result in injected with mulberry (*Morus* spp.) leave extract dose of 125 ppm gave fastest moulting time 29 days and slowest 44 days at 100 ppm and 150 ppm mulberry extract dose respectively to mud crab *S. olivacea*. Tahya *et al.,* (2016) the mandibular organ extract injection increased the maximum moult percentage (80-90 %) and minimum time required for moulting 26-34 days of mud crab *Scylla olivacea*.

SUMMARY

6. Summary

Soft-shell production of mangrove crab farming in Asia started during the early 1990's when there was a strong demand for soft-shell crabs in domestic and international markets (Quinitio and Lwin, 2009). The soft-shell mud crab can be eaten whole (carapace and all limbs) when cooked. Soft-shell crabs are available year round. Because of its profitability, there is an increasing interest to engage in this type of crab farming business venture in Asia (Shelley and Lovatelli 2011). Thailand is one of the leading soft-shell crab producers in the world. Production of mud crabs in 2015 was around 226,390 metric tons with a farm-gate value of US$1.06 billion (Quinitio *et. al.,* 2015 and FAO 2015) Commercial markets were primarily driven by live or frozen soft-shell crab sales.

Presently, the soft-shell production of mud crab (*Scylla* spp.) are generally carried out in indoor (Polypropylene boxes) or outdoor culture system (ponds). The advantages of indoor soft shell production are maintenance of water parameters such as temperature, salinity, pH, dissolve oxygen (DO) through filtered and recirlculated water system can be done in controlled condition. Aslamyah and Fujaya, 2010 and 2014 also Fujaya 2011, had developed the spinach extraction method for inducing the moulting and growth of mud crab by adding stimulant in artificial feed or injecting the different hormone to crab. Thus, the present study was carried out to standardize the optimum dose of spinach extract for production soft shell.

The objective of the study was to optimize the dose of spinach extract for inducing the moulting of mud crab. And also to study the moulting period required during the injection of spinach extract dose to the mud crab.

Spinach extract contains phytoecdysteroid, a substance which is well known to stimulate moulting in crabs. The study was conducted using four different doses of spinach extract viz. 0 mg/g control (T_0), 0.015 mg/g (T_1), 0.02 mg/g (T_2) and 0.025 mg/g (T_3). The spinach extract was developed as per the method given by Fujaya (2011) and was administrated through injection at base of coxa of last leg of periopod of mud crab. The water parameters ranges were maintained in the polypropylene boxes such as temperature-27-32°C, salinity-16-30 ppt, dissolved oxygen- ≥ 5ppm and pH- 7.5-8.5 in the polypropylene boxes. The growth parameters (weight, weight- gain%, carapace length, carapace length-gain%, carapace width, carapace width-gain %) and moulting period recorded during this experiment.

The highest growth parameter observed as average final weight (103.40±5.39 g) and average weight gain percentage (70-72%) in dose of 0.025 mg/g. Average final carapace length (6.32±0.18 cm) and average carapace length gain percentage (29-34%) in dose of 0.025 mg/g. The average final carapace width gain (8.24±0.17 cm) and average carapace width gain percentage (26-27%) in dose of 0.025 mg/g. The period required for moulting was observed higher in (44-45 days) in dose of 0 mg/g, while the lowest period required for moulting in (30-32 days) was observed with the dose of 0.025 mg/g.

The spinach extraction dose of 0.025 mg/g injected to the crab was found to be better with respect to growth i.e. better weight, carapace length, carapace width as well as weight gain, carapace length gain and carapace width gain. It was also revealed that of the same dose given (minimum period) i.e. 30-32 days for moulting of crab. Thus, soft shell production can be expedite by this technique in future.

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