Effect of Time Restricted Feeding and Re-feeding Regimes on Compensatory Growth, Body composition and Feed Utilization in Prawn (*Macrobrachium rosenbergii*) Culture System

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A Thesis By

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DECLARATION

Effect of Time Restricted Feeding and Re-feeding Regimes on Compensatory Growth, Body composition and Feed Utilization in Prawn (*Macrobrachium rosenbergii*) Culture System

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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Dedicated to

My Beloved Parents

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Abstract

A fourteen (14) weeks feeding experiment was conducted to determine the effect of time restricted feeding and refeeding regime on compensatory growth, body composition and feed utilization in improved traditional prawn (*Macrobrachium rosenbergii*) culture system. Three groups of prawn with an initial body weight of 1 g were used in which the control group (C) received feed regularly at *ad libitum* throughout the experimental period and the other two groups, Treatment 1 (T_1) and Treatment 2 (T_2), feed was given in 4 and 5 days per week respectively throughout first 9 weeks then satiation was given in last 5 weeks. At the end of experiment, final weight of C, T_1 and T_2 were found 17.23±6.56g, 16.04±6.18g and 15.64±6.34 g respectively which was insignificant among experimental groups. Highest survival rate was found in C but did not vary significantly ($P<0.05$) with T_2. FCR decreased with increased restriction of feeding while, PER and NPU increased with feeding restriction which is a good indication for the better utilization of feed by restricted groups. Moisture content increased significantly while protein and lipid content decreased significantly with increased restriction time of feeding. After satiation period, only T_2 was able to replenish the lipid content. Water quality parameters did not vary significantly among experimental groups and remained within optimum level. The present study showed that time restriction could be applied in *Macrobrachium rosenbergii* culture without detrimental effects on growth, feed efficiency and nutrient utilization.

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Chapter- I

Introduction

1 Introduction

1.1 Background of the project

Prawns and shrimps are considered one of the most popular foods globally (Banu *et al.*, 2016). It contains good amount of organic and inorganic constituents which includes protein, carbohydrate and lipid in addition to that, it also contains a significant proportion of minerals (Ca, P, Mg, Mn and Cl) and vitamins (A, C, and D) (Abulude *et al.*, 2006). Shrimp and prawn together contributes 15.3 % of global fisheries trade by means of value (FAO, 2016). Prawn has become a prominent species for small scale as well as large-scale inland aquaculture farming all over the world (Nandlal and Pickering, 2005). Bangladesh is considered one of the most suitable countries in the world for giant freshwater prawn (*Macrobrachium rosenbergii* De Man 1879) culture, because of its favorable water resources, availability of post larvae and agro-climatic conditions which provide a unique opportunity for the production of *Macrobrachium* spp. (Akand and Hasan, 1992; Muir, 2003). Freshwater prawn (*Macrobrachium rosenbergii*) farming is currently one of the most important sectors of the national economy of Bangladesh and during the last two decades, its development has attracted considerable attention because of its export potential. The prawn and shrimp sector as a whole is the second largest export industry in Bangladesh after ready made garments (Ahmed *et al.*, 2008) contributed 3937.6 crore TK in 2014-15 (DoF, 2016 a). Prawn farming in Bangladesh provides livelihood opportunities for the coastal poor, with over 8,35,000 farmers are involved in prawn and shrimp production (DoF, 2016 b). Around two – third of the total production of freshwater prawn comes from aquaculture in Bangladesh (DoF, 2013).

For successful aquaculture, feed management is a key factor affecting water quality (Boyd and Tucker, 1998) and production economics (Jolly and Clonts, 1993). Feed cost is generally the largest single operating cost in any animal production system, ranging from 30-70% depending on the intensity of production (Dickerson, 1978; Bickel, 1988; Campo and Turrado, 1998) including aquaculture (Shang, 1990; Tacon *et al.*, 1995). Due to intensification of production methods, production from feed dependent aquaculture has increased from 12.2 – 50.0 million tonnes during the period 1995 – 2015 (FAO, 2017). Now and then, one of the major problems faced by fish culturists is to attain a balance between rapid fish growth and efficient use of the supplied feed. A substantial number of studies were conducted to determine the effects of feeding level/ration level on growth rates and food conversion efficiencies (Deng *et al.*, 2003; Bureau *et al.*, 2006; Oakes *et al.*, 2007). These studies suggested that overfeeding could result in increasing feed conversion ratios (FCR; Talbot *et al.*, 1999), wastage of expensive feed (Thorpe and Cho, 1995) and deterioration of water quality (Cho and Bureau, 1998). On the other hand, underfeeding can cut back growth rate and FCR (Cho, 1992) resulting in lower yields and profits. Successful cultured fish production therefore requires optimization of feeding practices to ensure the most economically effective growth rates and feed utilization efficiencies. Feed costs for culture practices in practical manner, can be minimized by taking the advantage of the phenomenon of restricted feeding strategies in two different ways: (1) by decreasing daily feed allotment or (2) by decreasing the time for feeding (Pirhonen and Forsman, 1998).

Restricted feeding assists compensatory growth, which has been revealed in various fish species, such as cicheidis (Wang *et al.*, 2000), cyprinids (Russell and Wootton, 1992; Xie *et al.*, 2001; Zhu *et al.*, 2001), gadoids (Jobling *et al.*, 1994), pleuronectids (Paul *et al.*, 1995) and salmoinids (Johansen *et al.*, 2001). These fishes showed different response for compensatory growth either complete or partial (Jobling and Baardvik, 1994). Compensatory growth or catch up growth or recovery growth of fish refer to the unusually fast/rapid growth that follows a period of reduced growth resulting from restricted food availability or some unfavorable environmental conditions (Jobling 2010; Sevgili *et al.*, 2013). Compensatory growth offers the possibility of improving the growth rates of fish by a careful choice of feeding protocol in which periods of feed deprivation are followed by periods of satiation feeding (Nikki *et al.*, 2004; Wang *et al.*, 2005; Montserrat *et al.*, 2007; Mattila *et al.*, 2009). The response or degree of compensatory growth is varied with the fish species, age, sex, diet composition, feed allowances, feeding regime, duration and severity of feed deprivation imposed prior to re feeding, duration of feeding trial, nutritional/ physiological status of fish, water temperature, and fish rearing system (Cho, 2011). There is an increasing body of evidence from work with some other animals that the compensatory growth response also influenced by similar factors (Wilson and Osbourn, 1960, Plavnik and Hurwltz, 1985, Summers *et al.,* 1990, Marais *et al.,* 1991, Jones and Farrell, 1992). Fish deplete their energy reserves during starvation and replenish them during hyperphagia (Alvarez and Nicieza, 2005). Stored protein and lipid are the main source of energy during starvation (Schafer, 1968). Species faces nutritional stress during starvation as a result, their body composition undergo various changes. For example, a reduction in dry weight and corresponding increase in tissue water-content in response to starvation was reported in the lobster *Panulirus longipes* (Dall, 1974), reduction in RNA:DNA ratios in response to starvation was observed in postlarvae of the lobster *Homarus americanus* (Juinio *et al.,* 1992), the crab *Callinectes sapidus* (Wang and Stickle 1986), and juvenile of *Penaeus vannamei* (Moss 1994). In *Penaeus esculentus,* the effects of starvation were characterized by significant reductions in total lipid and protein (Barclay *et al.,* 1983). Rapid utilization of triacylglycerol (TAG) reserves in response to starvation has been reported in sub-adult *Penaeus esculentus* (Chandumpai *et al.,* 1991) and larval *Homarus americanus* (Sasaki, 1984). Therefore, the degree of change in body composition during starvation and refilling percentage after satiation period is needed to be known from nutritional point of view. In cannibalistic species, feed restriction should have to be properly maintained as an increase in cannibalism has reported with increased feed deprivation (Polis, 1981; Hecht and Pienaar, 1993) which, leads to yield loss in large number of marine and freshwater fishes. Although several studies have been carried out to observe the compensatory growth of different fish species, from an exhaustive literature search we found that, almost 90% of them were undertaken in the indoor rearing system. So, actual compensatory growth due to restricted feeding and refeeding in commercial or mass production of fish in pond or other culture system are not known (Mohanta *et al.,* 2017) as well as less is known about compensatory growth in crustaceans, an exceptional type of animal, as they grow intermittently accompanied with molting activities (Wu *et al.,* 2001). Therefore evidence for compensatory growth in *Macrobrachium rosenbergii* needed to be established as restricted feeding without growth suppression is preferable for economic and environmental reason as well as for better end product quality (Gaylord and Gatlin, 2001). The good production from pond is rely on good water quality that is various physical, chemical and biological parameters of water (Moses, 1983).

Now a days in Bangladesh, many farmers are practicing improved traditional culture system (Chandra *et al.*, 2010) where artificial feed is used which affects the water quality. Dissociation of protein in fish feed, releases a substantial amount of nitrogen compounds (Hopkins *et al.*, 1995). Nutrients released from uneaten feed and faces causes eutrophication (Wetzel, 2001 b) or could be directly toxic to aquatic fauna (Ebeling and Michael, 2002; Boardman *et al.*, 2004). Microbial action on uneaten feed increase biological oxygen demand (BOD) and decrease pH which adversely affects exoskeleton formation during molting period (Chen *et al.*, 2003). Good production from aquaculture ponds can be achieved when cultured species get sufficient nutrition and physical and chemical parameters of the pond remain optimum and an equilibrium is achieved. Since the main objective of aquaculture is to maximize fish production at minimum cost, so knowing the appropriate feeding regimes that achieve compensatory growth of fish is necessary before the practical application to aquaculture (Tian and Qin, 2004). Proper development of feeding rate based on the compensatory growth phenomenon is not only to help in reducing the feed cost, but also to minimize the nitrogen/organic load responsible for water pollution in fish culture environment as a result, small scale fish farmers in Bangladesh will be encouraged to get engaged in freshwater prawn farming which will increase overall fisheries production, create self-employment and improve the socioeconomic condition of small scale prawn farmers. Considering the above importance, the present study has been undertaken.

1.2 Objectives of the project

- To evaluate the compensation growth and production performance of prawn under time restricted and re-feeding regimes; and
- To assess the effect of time restricted feeding on body composition (protein, fat, moisture and ash) and feed utilization of prawn.

Chapter-2

Literature Review

2 Review of literature

2.1 Overview of aquaculture

To establish food safety for increasing population (7.3 billion in 2014), UN member States adopted the 2030 Agenda for Sustainable Development. It sets aims for the contribution and conduct of fisheries and aquaculture towards food security and nutrition in the use of natural resources so as to ensure sustainable development in economic, social and environmental terms (FAO, 2017). Fish accounted for about 17 percent of the global population's intake of animal protein and 6.7 percent of all protein consumed (FAO, 2013). Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita intake of animal protein. In 2015, global aquaculture production reached 106 million tonnes, 76.6 million tonnes of aquatic animals and 29.4 million tonnes of aquatic plants, growing at an average annual rate of 6.6 percent since 1995 (FAO, 2017).

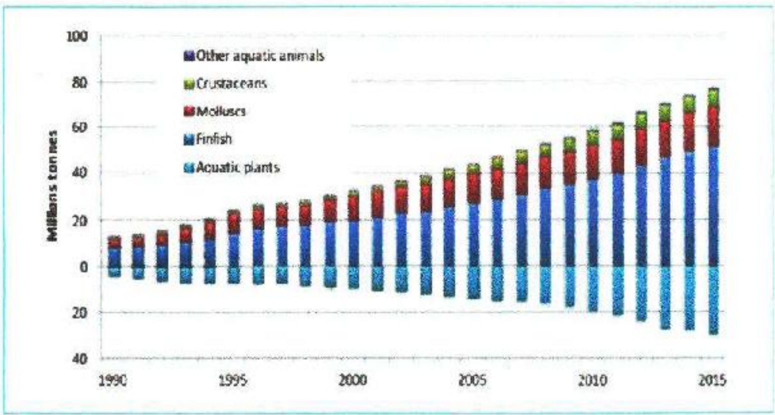

Figure 1: World aquaculture production of farmed aquatic animals and plants (1990-2015) (FAO, 2017)

Within 15 years in the new millennium, the aquaculture's contribution to the world production of aquatic animals (captured and farmed combined) has increased from 25.7% in 2000 to 45.3% in 2015 (FAO, 2017). With almost all farmed aquatic animals destined for human consumption, aquaculture supplied 10.42 kg of food fish for human consumption on world average in 2015, a level further up by 0.28 kg from 10.14 kg in 2014 (FAO, 2017). Due to over exploitation, destruction of habitat, pollution and many other causes wild fish stocks are decreasing day by day. Under these considerations, aquaculture is an effective way to ensure food security for millions of people. Bangladesh in endowed with vast water area with a good diversity of fisheries resources. A large number of people of the country depends on fish or other fisheries resources as their livelihood since immemorial time. These sectors have also contribution on protein supply, solution of unemployment problem, earning foreign currencies and and socio-economic development of Bangladesh. It contributes 3.61 percent to national GDP and around one-fourth (24.41 percent) to the agricultural GDP. More than 11 percent of total population of Bangladesh are engaged with this sector on full time and part time basis for their livelihoods (DoF, 2017). Bangladesh has achieved self-sufficiency in fish production with an annual output of 41.34 lakh MT against a demand of 40.50 lakh MT in 2016-17. The country earned about BDT 42876.40 million by exporting almost 68.31 thousand MT of fish and fisheries products in 50 countries (DoF, 2017). Bangladesh ranked fifth in world aquaculture production (FAO, 2016). In 1983-84, the contribution of inland capture and culture fisheries to total fish production were 62.59 percent and 15.53 percent respectively; whereas in 2016-17, inland capture fisheries contributes only 28.14 percent and inland culture fisheries contributes 56.44 percent to total fish production (DoF, 2017). During the last eight years, the aquaculture production became more than double (10.63 lakh MT in 2008-09 to 23.33 lakh MT in 2016-17).

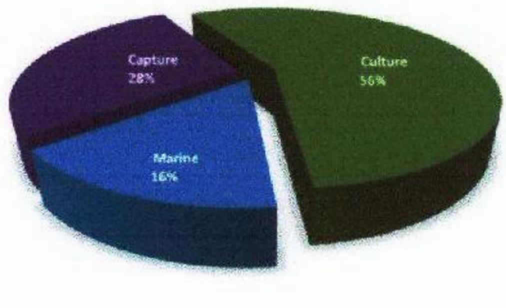

Figure 2: Annual fish production in Bangladesh (DoF, 2017)

Climatic condition of Bangladesh is suitable for culture of giant fresh water prawn (*Macrobrachium rosenbergii*). Among the twenty four freshwater prawns, *Macrobrachium rosenbergii* has significant aquaculture potential for its commercial value throughout the world (Akand and Hasan, 1992; Muir, 2003). The prawn and shrimp sector have gradually grown over the last three decades and provide direct and indirect employment opportunity for around 1.5 million people (Ahmed *et al.*, 2008). Total area of prawn and shrimp farm was 272717 ha and production was 246406 MT in 2016-17 which has contributed 5.96 % of country's total production as well as nearly double in terms of volume compared to total production in 2002-03 which was 1.60 lakh MT (DoF, 2017). In 2016-17. Bangladesh exported 6627.37 MT freshwater prawn with a value of about 820.28 crore BDT (DoF, 2017). Despite the great potentiality of freshwater prawn culture in Bangladesh, there have been some concerns in recent years for sustainable prawn farming which provides less stress on environment as well as economically profitable.

2.2 Use of artificial feed in aquaculture and its impact on environment

Feeding of fish during culture aims at producing the maximum weight of marketable fish within the shortest time at least cost. Feed enhance growth and supply the energy for movement and all other activities the fish engages in. In advance aquaculture practices, fish nutrition and energy is completely depends on artificial feed as there environment is manipulated (Giri *et al.*, 2002). Artificial feed could be defined as the complete or supplemental diet prepared from different feed ingredients containing necessary nutrients for growth and energy. Fish meal is one of the most common source of fish nutrient in fish feed, mostly made from fish that are not generally used for human consumption; a small portion is made from the bones and offal left over from processing fish used for human consumption. while the larger percentage is manufactured from wild caught, small marine fish; either unmanaged by-catch (Jacob, 2017) or sometimes sustainable fish stocks. (Miles and Champman, 2006). Fish meal production is a significant contributor of over-fishing, and risks pushing fisheries beyond their replacement rate. Some areas of the world, such as Western Africa, have seen a large increase in fish meal production which in turn is hurting local fisheries and driving fisheries into collapse (Jacob, 2017). Four or five tonnes of fish are needed to manufacture one tonne of dry fishmeal (Miles and Champman, 2006). Due to fulfilling the increasing demand of fish meal, fishing beyond sustainable rate leads to reduction of marine biodiversity and yield. In 2016, global fish meal production was 4.5 million MT where in 2013, it was 4.9 million MT (IFFO, 2017). Excessive use of artificial feed hampers water quality as dissociation of protein in fish feed releases substantial amount of nitrogenous compounds (Hopkins *et al.,* 1995). Nutrients released from uneaten feed and faces causes eutropication (Wetzel, 2001 b) or could be directly toxic to aquatic fauna (Ebeling *et al.,* 2002; Boardman *et al.,* 2004) as well as decomposition of uneaten feed leads to oxygen deficiency in water (Rice, 1994). On the other hand use of excessive feed leads to economic loss (Hopkins *et al.,* 1995).

2.3 Freshwater Prawn (*Macrobrachium rosenbergii*)

Macrobrachium rosenbergii, also known as the Giant Freshwater Prawn, Malaysian prawn, Freshwater Scampi (especially in India), is a species of freshwater shrimp. This species is commercially important for its value as a food source. Species of the freshwater prawn genus *Macrobrachium* are distributed throughout the tropical and subtropical zones of the world. They are found in most inland freshwater areas including lakes, rivers, swamps, irrigation ditches, canals and ponds as well as estuarine waters. Most species require brackish water in the initial stages of their life cycle although some complete their life cycles in inland fresh water lakes. *Macrobrachium rosenbergii* is indigenous in the whole of the South and Southeast Asian area as well as in Northern Ocenia and in the Western Pacific island (New, 2002). It is the largest known of all *Macrobrachium* species, adult males having been reported with a total body length of up to 33 cm, and adult females of up to 29 cm (New, 2002). The larvae are carnivorous and in culture they are fed on live, newly hatched brine shrimp (artemia). Juvenile and adult prawns are omnivorous, and feed on a wide variety of food items such as aquatic worms, insects and their larvae, small mollusks and crustaceans, flesh and offal of fish and other animals, grains, nuts, seeds, fruits, algae, tender leaves and stems of aquatic plants (Nandlal and Pickering, 2006).

2.4 Compensatory growth

Compensatory growth (CG), a phenomenon of growth spurt following a growth retardation period, has been suggested as a rearing tactic in numerous aquatic organisms (Ali *et al.*, 2003). Many organisms exhibit faster growth during recovery from total or partial feed deprivation than they do during the periods of continuous food availability (Wilson and Osbourn, 1960; Jobling, 1994). The consequence is that animals experiencing a period of food depression may achieve the same size at age as conspecifics experiencing environmental conditions that are more favorable. The response which tends to restore the original growth trajectory is called compensatory or catch up growth (Ali *et al.*, 2003).

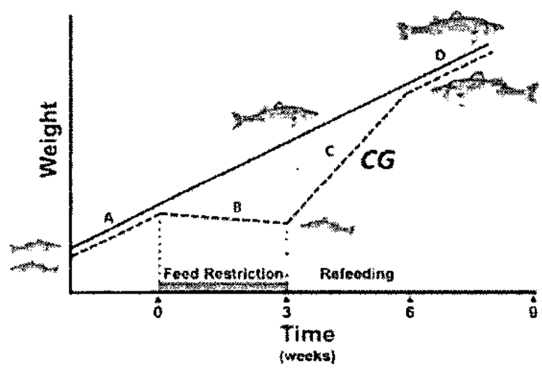

Figure 3: Compensatory growth

Dietary restriction and starvation with single phase and subsequent satiation feeding or cycled restriction and full feeding are the strategies that have been mostly resorted (Hayward *et al.*, 1997; Qian *et al.*, 2000; Nikki *et al.*, 2004; Eroldoğan *et al.*, 2006). The compensatory growth response may be species specific. Compensatory growth in fish has aroused interest because of its potential application as a management in commercial fish production (Wang *et al.*, 2005). It tends to enhance growth and feed efficiency (FE), potentially reducing production costs and assisting in water quality management (Turano *et al*, 2008). Appropriate exploitation of this phenomenon may allow the design of feeding regimes that improve growth rate and feed efficiency (Jobling *et al.*, 1994; Hayward *et al.*, 1997). Depending on the degree of recovery/compensation, the compensatory growth due to restricted feeding and re feeding is categorized into four types: (i) no growth compensation; (ii) partial growth compensation; (iii) full compensation; and (iv) overcompensation as compared to normal fed group (Ali *et al.,* 2003; Tian and Qin, 2003).

2.5 Feed restriction

It entails offering the animal's limited amounts of feed, normally lower than the amount that they are able to eat. Restricted feeding strategies, seemed to be closely related to the duration and severity of feed limitation imposed in any culture techniques (as aquaculture). Feed restriction does not always result in growth retardation of fish (Känkänen and Pirhonen 2009; Rosauer *et al.,* 2009; Cho, 2011). Feed restriction could be imposed in two different ways, 1) Time restriction (daily/ weekly/ monthly cycle). 2) Rate restriction (25%, 50%, 75% etc.). Development of a feed restriction method is not only required for economical reasons but also to address environmental concerns (Cho *et al.,* 2006 a). Several studies have shown that organic matter from fish farms can negatively affect the environmental sea bottom situation. This holds for sediment chemistry (Findlay and Watling 1997; Hargrave *et al.,* 1997; Yokoyama *et al.,* 2009), community dynamics of marine seagrasses (Pergent *et al.,* 1999), benthic macrofauna (Hargrave *et al.,* 1997), meiofauna (La Rosa *et al.,* 2001; Mirto *et al.,* 2002), and bacteria (La Rosa *et al.,* 2001). Properly organized feed restriction should therefore, be helpful to limit the amount of feed input to the aquatic system which will minimize the pollution of the water column and underlying sea-bottom as well as improve the long term success of fish farming activities.

2.6 Effect of feed restriction on compensatory growth

Compensatory growth represents increased growth rate, or, more correctly increased weight gain, as a result of growth suppression during an earlier period of food restriction. It has long been recognized that animals, even organs, have propensity to compensate or 'catch-up' following loss or deprivation. The ensuing catch-up following restricted feeding has usually been characterized by an increase in rate and efficiency of weight gain during the recovery period (Lawrence and

Pearce, 1964; Winick and Noble, 1966; Reid and White, 1977). A number of studies were carried out to determine the effect of feed restriction on compensatory growth in different fish and crustacean species. Nunes *et al.* (2006) conducted a study to evaluate the effect of time- (TR) and rate-restricted feeding (RR) on the growth performance of *Litopenaeus vannamei* raised in controlled conditions which indicated that, it is possible to moderately reduce daily feeding rates without detrimental effects in *L. vannamei* survival, growth and feed efficiency. The gibel carp, *Carassius auratus gibelio*, could achieve complete compensatory growth when fish were refed to satiation after 1- or 2-week feed deprivation (Qian *et al.,* 2000; Xie *et al.,* 2001). Barramundi, *Lates calcarifer*, experiencing 1-week feed deprivation exhibited notable compensatory growth after refeeding and caught up with the body weight of fish fed to satiation daily throughout the feeding trial, but fish fasted for more than 1 week exhibited partial compensatory growth (Tian and Qin, 2003). Wang *et al.* (2000) in their 8 weeks study on hybrid tilapia (*Oreochromis mossambicus* × *O.niloticus*), found feed intake and specific growth rate values during refeeding period in fish starved for 1, 2 or 4 weeks significantly higher compared to the control group and reported that these fish showed some compensative reactions. Cho (2005) and Cho *et al.* (2006 b) showed that olive flounder, *Paralichthys olivaceus*, achieved full compensatory growth when fed for 6 weeks after 2-weeks feed deprivation during winter and summer season, respectively. Kim and Lovell (1995) reported that feeding channel catfish, *Ictalurus punctatus* every third day for 3 weeks did not significantly alter final weight, providing evidence of compensatory growth. Weatherley and Gill (1981) reported that small rainbow trout fed a restricted ration for 16 weeks or starved for 3 weeks weighed less than control fish which were full-fed; however, the restricted and starved groups caught up with the control group in body weight after 12-15 weeks on full-feeding. Russell and Wooton (1992) found that European minnows, *Phoxinus phoxinus* starved for 16 weeks showed hyperphagia and increases in growth and feed efficiency when placed on full feeding, as compared to continuously fed control fish. The compensatory increases in appetite and growth were sustained for about 3 weeks until the starved and refed fish had attained the weight of the control. In turbots, *Scophthalmus maximus* (L.), different restricted feeding regimens were carried out during 41 days and weight loss was observed. Afterwards, all groups were fed without any restricted feeding through 34 days and complete compensation was determined in restricted feeding groups (Saether and Jobling, 1999). In rainbow trouts starved in 0, 2, 4, 8 or 14 days, it was determined that all groups except the 8 day starvation group showed similar growth performance like control group. The results regarding full compensatory growth in starved fish during their refeeding periods depending on high feed consumption were supported by the strong relationship between body weight gain and feed consumption (Nikki *et al.*, 2004). Red sea breams which were exposed to one, two or three weeks of starvation showed full compensatory growth at the end of a 9 weeks study (Oh *et al.*, 2007). There is no compensation in a restricted feeding regimen (Eroldoğan *et al.*, 2008) but partial compensation following different starving periods in gilthead sea breams, *Sparus aurata*, has been reported (Eroldoğan *et al.*, 2006). Two weeks starvation and 6 weeks refeeding with olive flounders showed better improvement as compared with a continuous feeding group (Cho and Cho, 2009). From above discussions it is clear that, appropriate restriction level followed by proper refeeding could accelerate compensatory growth.

2.7 Effect of feed restriction on body composition

It was seen that during starvation, the reserves (especially lipid composition) in the fish and other crustaceans are decreased. In many studies, the growth of fish during compensation growth was found to be faster. In compensatory growth, it has been observed that somatic growth parameters and lipid levels return to their prior levels compared to those during starvation periods (Ali *et al.*, 2003). Oh *et al.* (2008), in their study on juvenile black rockfish (*Sebastes schlegeli*) applied 3 different starvation periods (fasted for 5 days, 10 days and 14 days) followed by a 5 day re feeding period. On the 14 day of the study, lipid ratios in lean body mass in 10 day starvation and 14 day starvation groups were lower than those in control and 5 day starvation groups. However, on the 49 day, the 14 day starvation group showed lower lipid ratios compared to the other three groups while no differences were determined among the other three groups. Mattila *et al.* (2009), in their study on pikeperch (*Sander lucioperca*) measured visceral fat (%), total body fat (%) and energy content in 1+3 (one-day feeding followed by a three-day feed deprivation) and 1+6 (fed once a week) groups. At the end of the study, the researchers reported that the compensation ability improved also, the fish showed only a partial compensation tendency in lower feeding frequencies and during decreasing feeding duration periods. Conflict exists in the literature on the relative abundance and order of utilization of major energy reserves in decapod crustaceans during feed restriction or starvation. Major depletion of lipid reserves in the starved shore crab *Carcinazus maenas* (Heath and Barnes, 1970) and southern king crab *Paralomis granulosa* (Comoglio *et al.*, 2005) contrasts with the predominant use of protein reserves in *Hemigrapsus nudus* (Neiland and Scheer, 1953) and in the lobsters *Panulirus longipes* and *Nephrops nowegicus* (Dall 1974). Although lipid was considered the primary reserve of the crayfish *Orconectes nair* (Armitage *et al.* 1972), protein was metabolized predominantly in *Orconecses virilis* (Hazlett *et al.*, 1975) whereas both lipid and protein were used during progressive starvation of *Orconectes limosus* (Speck and Urich, 1969). PL of starved prawn (*Macrobrachium rosenbergii*) firstly utilize protein and then lipid (Sing and Balange., 2007). The shrimp *Crangon crangon* starved for 4 weeks consumed mainly carbohydrate, then lipid, and finally protein reserves (Cuzon and Ceccalldi, 1972), yet from oxygen to nitrogen ratios of this starved species, lipid and protein were considered the principal sources of energy (Regnault, 1981). Composition of the hepatopancreas of the pink shrimp *Penaeus duoranum* starved for 12 days suggested initial use of lipid, then protein, and finally carbohydrate reserves (Schafer, 1968). Biochemical changes in starved *Penaeus japonicus* implied a reserve utilization of carbohydrate, lipid, and then protein (Cuzon *et al.*, 1980). Yet another order of reserve utilization was indicated from biochemical changes in tissue sections of the tiger prawn *Penaeus esculentus*, with protein from the abdomen considered the major source of energy and lipid of secondary importance (Barclay *et al.*, 1983). Resuming satiation level after feed deprivation or restriction, fish and crustaceans are able to replenish or partially replenish the deficiency of nutrient composition in body. Four groups of Chinese shrimp, *Fenneropenaeus chinensis* were subjected to feed restriction at the rate of 4%, 8% and 12% of body weight per day (% B.W.day−1) and satiation. After 10 days of restricted feeding, the shrimp showed decreased body contents of lipid, protein, and energy compared with the controls. After a satiation period of 30 days, restricted groups were able to replenish the nutrient deficiency (Wu *et al.,* 2001). Sing and Balange (2007) reported that regain percentage of the protein and lipid content in PL of prawn (*Macrobrachium rosenbergii*) starved for 1 and 2 weeks were more than those are starved for 4 weeks compared to control. Oh *et al.* (2007) conducted an experiment on red sea bream *Pagrus major* throughout 9 weeks where four different feeding strategies were considered - one group with continuous feeding for 9 weeks namely control (C). In rest of three groups, feed was deprived for 1 week, 2 weeks and 3 weeks in first 3 weeks namely F1, F2 and F3 respectively. Fishes of deprived groups showed lower crude protein, lipid and energy content than control after deprivation period. After satiation feeding over 6 weeks, F1 and F2 were able to replenish the lipid content where only F2 was able to replenish protein content like C.

Chapter-3

Methodology

3 Methodology

3.1 Site selection and time frame

The study was conducted in the experimental pond complex II of Fisheries and Marine Resource Technology Discipline of Khulna University.The area of the ponds were 240 m^2 for each. All ponds were rectangular in shape with a maximum depth of 1.5 m. All the ponds were fully exposed to prevailing sunlight. The experiment was conducted throughout July to December, 2017.

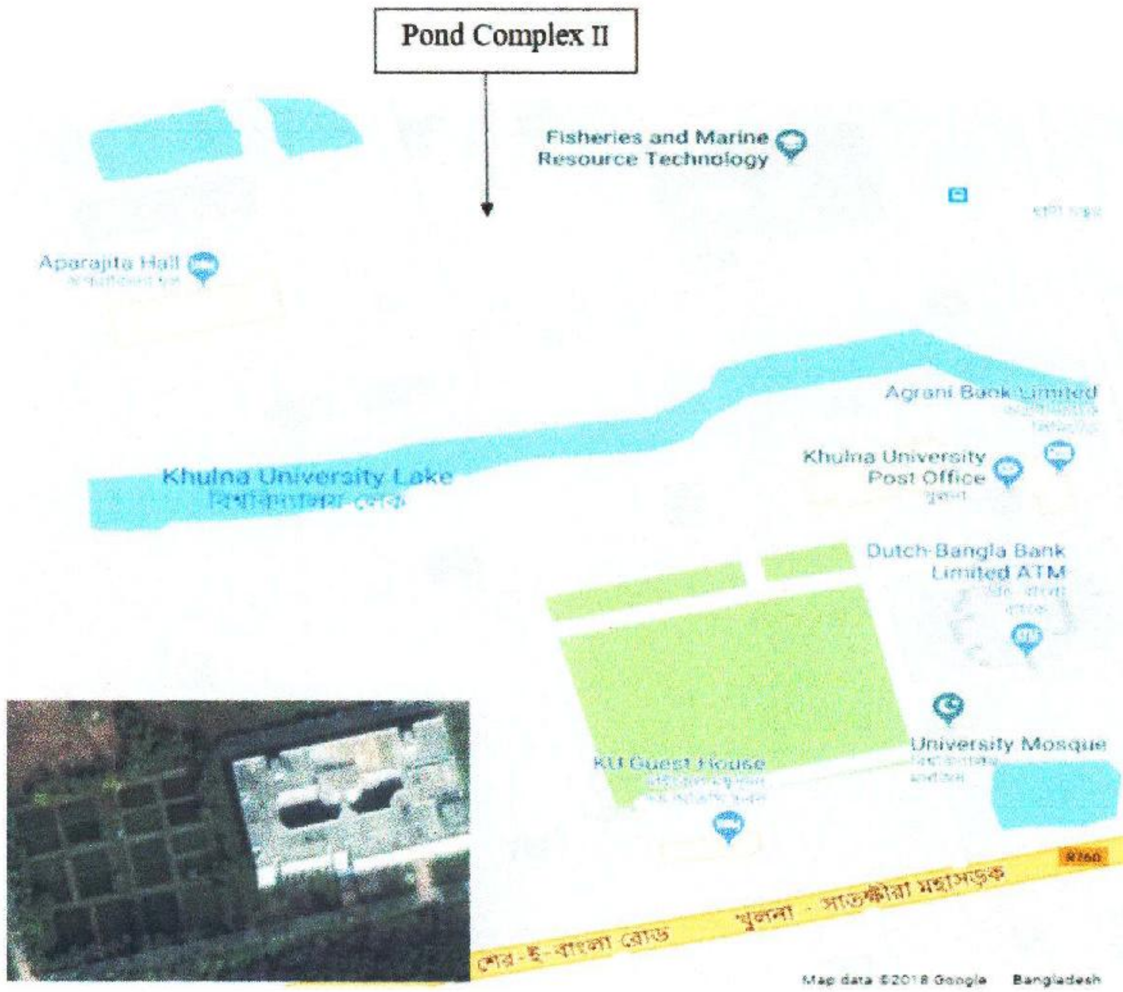

Figure 4: Representative Map of Study Area (Google Map, 2018) and experimental ponds are shown up, right side of the map.

3.2 Experimental Design

This experiment had one control and two treatments on the basis of time restricted feeding and re-feeding protocol and was conducted throughout 14 weeks. Feed restriction was imposed on treatment in first 9 weeks then satiation was given in last 5 weeks. Each treatments had two replications.

Table 1: Feeding strategy

Treatments	Replications	Feeding strategy
Control	C1	Regular feeding with satiation.
	C2	
Treatment 1	T1R1	Feeding for 4 days followed by no feeding for 3 days.
	T1R2	
Treatment 2	T2R1	Feeding for 5 days followed by no feeding for 2 days.
	T2R2	

3.3 Pond preparation

The ponds were first dewatered with a pump and then were dried in the sun for one month, by this way all unwanted organisms in the sediment were killed. The embankments of the ponds were reconstructed and repaired. Liming ($CaCO_3$) was done at a rate of 250kg/hectare on the bottom of ponds. The ponds were watered by a pump up to a level of 1.5 m. After watering, rotenone was applied at the rate of 6kg/hectare/feet of water depth to eradicate the unnecessary organisms. The total area of the ponds was finally fenced with a net to prevent the entering of unwanted organisms. The water level of the experimental ponds was found good enough during rainy season but during winter season, underground water was added to maintain the water level. Each pond had its own individual supply from a central water distribution channel and did not receive the outflow from another pond.

3.4 PL collection and stocking of prawn

The post larvae (PL) of prawn (hatchery produced) were collected from Chuknagar, Dumuria, Khulna. After pond preparation, PL was reared in the nursing pond for four weeks with a stocking density of 25 PL/m^2. After nursing PL, it became juvenile which was stocked in grow-out ponds with a stocking density of 2/m^2 while the average individual weight was 1±0.021g. Before releasing juveniles, acclimatization to the new environment was done by keeping the juveniles-containing-pot in the water of pond for a while and then mixing pond's water gradually with the pot to reduce mortality.

3.5 Feeding

Feed (industrially produced) contained 29% protein was applied. PL of *Macrobrachium rosenberii* in three different treatments were fed by three different restricted feeding and re feeding strategies. Feeding frequency was 3 times/ day and was given according to body weight (Tacon *et al.*, 2002). Feed was provided in feed trays made up of split bamboos. The feed trays were tied to bamboo poles and suspended in the pond water at a level of 45 cm from the water surface. Trays were cleaned each time before supplementing the feed.

3.6 Growth monitoring

Sampling was conducted every 7 days interval for growth monitoring by using seine net. Thirty individuals were randomly collected during sampling from each replication. The length of the samples were measured by using scale and the weight was measured by an electric balance with 0.001 g accuracy. At the end of the experiment, all the ponds were dewatered by water pump and all the fishes were harvested completely and were counted to determine the survival rate, FCR and other production performance parameters.

3.6.1 Growth parameters determination

Feeding efficiency (FE)=Biomass gain(kg)/ feed used (kg) × 100

Survival (%) = $\frac{N_t}{N_o}\times100\%$

Where N_t is the number of shrimp at the end of the experiment and N_0 the initial number of shrimp.

Average individual Growth (G) = $W_t - W_o$

Where W_t is the final body weight and W_0 the initial body weight (in grams).

Individual Growth rate (GR) = $\frac{(W_t-W_o)}{t}$

Where t is the duration of the growth interval (in g/day)

Specific growth rate (SGR) =$\frac{\{\ln(W_t)-\ln(W_o)\}}{t}\times100$

Where t is the duration of the experiment in days, W_t is the final average individual body weight and W_0 the initial average individual body weight (in % BW/day)

Feed conversion ratio (FCR) =$\frac{TFG}{TWG}$

where TFG = Total feed given (dry matter basis) and TWG =Total weight gained (Wu *et al.*, 2001; Sevgili *et al.*, 2013).

3.7 Body composition analysis

Body composition was measured in fish nutrition laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University three times throughout the experimental period during stocking, end of the restriction period and after harvesting respectively. The proximate composition of prawn was analyzed for the content of protein, lipid, ash and moisture, following the method of AOAC (1983).

3.7.1 Determination of moisture

The weighed quantity of sample were dried in a thermostat controlled oven at 105°C for 24 hours. Then the sample were kept in a dessicator until to get optimum room temperature. After cooling, the dry sample were weighed by an electric balance and the differences between the two weights gave the weight of the sample.The percentage of the moisture content was calculated by the following equation:

% Moisture = (Weight of sample-Weight of the dried sample $\times$100)/(Weight of the sample)

3.7.2 Determination of Protein

Protein was determination by using micro Kjeldahl method. Firstly 0.2-0.4 gm of sample was weighed and inserted into a kjeldahl flask and the percentage of nitrogen was determined by the standard Kjeldahl method. Then the %N was multiplied by the conversion factor 6.25 to get the percentage of crude protein.Two step were used to complete the total process. First digestion and second distillation. The distilled solution stored in the conical flask was taken and titrated against 0.102 (N) HCl solutions. The percentage of gross portentous nitrogen was calculated by the following formula:

%N= (Volume of HCl $\times$ normality of HCl $\times$ 0.014)/(weight of sample (gm)) $\times$100

%Protein= %N $\times$ 6.25 (conversion factor).

3.7.3 Determination of lipid

Most method of measuring lipid content depend on extracting the lipid by dissolving it in a suitable solvent. Crude lipid of the sample was determined by extracting a weighted quantity of sample (2-5g) with methanol and chloroform (1:2) solution which was mixed by using a mixer and filtered by a filter paper. Then 50 ml water was added with the sample solution to turned into milky color solution and it was kept in separation funnel for 8 hours. When three layers were appeared in the separation funnel a dried and weighted beaker was used to collect the solution only the bottom layer of the separation funnel. After collection of the separated solution from separation funnel, it was kept in the hot plate for drying. After evaporating all the chloroform, a simple amount of sticky liquid left in the bottom of the beaker. Then it was taken out from the hot plate and then allowed to cool down.After cooling down it was weighed and deducting the weight of the beaker that was measure quickly from the cooled beaker containing lipid to obtain lipid. The percentage of the lipid content was calculated by the following formula:

% Lipid = (Weight of lipid ×100)/(Weight of the sample)

3.7.4 Determination of ash

The weighed quantity of sample was muffled at 550˚C for 8 hours.Then it was transferred to the dissector to cool down at room temperature for a few minutes. Finally ash weight was taken by subtracting the crucible weight from ash with weight. The percentage of ash was determined by the following formula:

% Ash = (Weight of ash ×100)/(Weight of the sample)

3.8 Water quality measurement

Water samples were taken every 7 days interval from each treatments and control. Following parameters were measured by following methods,

Table 2: Water quality measurement

Parameters	Methods/ Equipments were Used
Temperature	Thermometer (Degree Celcius)
pH	pH meter (DPH-2 ATAGO)
Dissolved oxygen DO (mg/L)	Winkler method
Alkalinity (mg/L)	Titrimetric
Hardness (mg/L)	Titrimetric
Ammonia nitrogen	Nessler method

3.9 Data analysis

All data were presented as means ± standard error of the mean and were tested for homogeneity of variances using Levene's test. After confirming homogeneity of variances, one-way analysis of variance (ANOVA) was applied to determine the differences in growth of prawn, body composition, feed utilization efficiencies and percentage survival among the experimental groups.

Chapter-4

Result

4 Results

4.1 Growth performance parameters of prawn

4.1.1 Increment of body weight

The body weight of prawn in different samplings was measured and calculated which was shown by (Figure 5). Initially at the time of stocking, the average body weight was 1g. At the end of restriction period, the mean weight of C, T_1 and T_2 was found to be 9.89±4.29g, 7.89±2.93g and 9.65±3.32 g respectively. No significant difference was found among C and T_2 where T_1 varied significantly from other experimental groups. After re feeding throughout last 5 weeks, the harvesting weight of the prawn was found to be 17.23±6.56g, 16.04±6.18g and 15.64±6.34 g in C, T_1 and T_2 respectively. Highest final average weight was found in control group and lowest final weight was found in T_2 but no significant difference was found among the experimental groups. The individual growth of prawn decreased with increasing restriction of artificial feeding. The mean weight gain after 14 weeks of culture was found to be 16.23±6.56g, 15.04±6.18g and 14.64±6.34 g in C, T_1 and T_2 respectively which was not statistically significant ($P>0.05$).

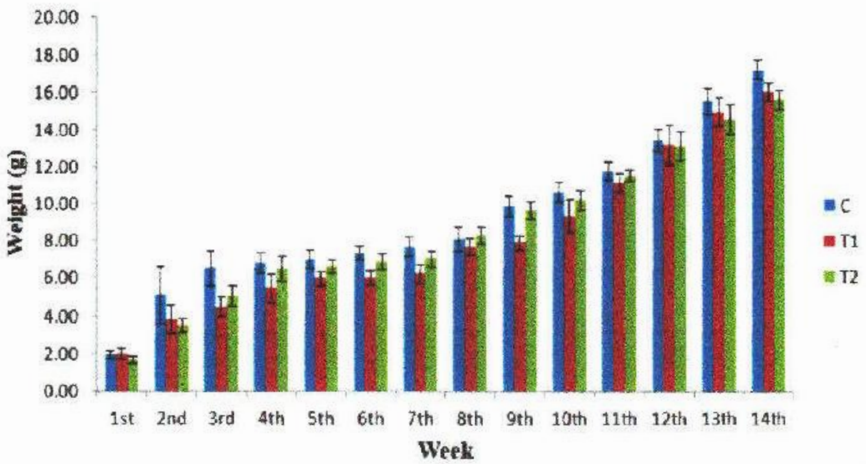

Figure 5: Mean weight of prawn in different samplings in different experimental groups. The error bar represents the SE of six replicates.

4.1.2 Increment of body length

Length increased in each sampling (Figure 6). The final length was found to be 11.67±7.68cm, 11.16±6.03cm and 11.09±1.48 cm in C, T_1 and T_2 respectively. The highest mean final length was found in control and lowest final length was found in T_2 which was insignificant (P>0.05) among the experimental groups. The length was decreased with increasing of restricted artificial feeding.

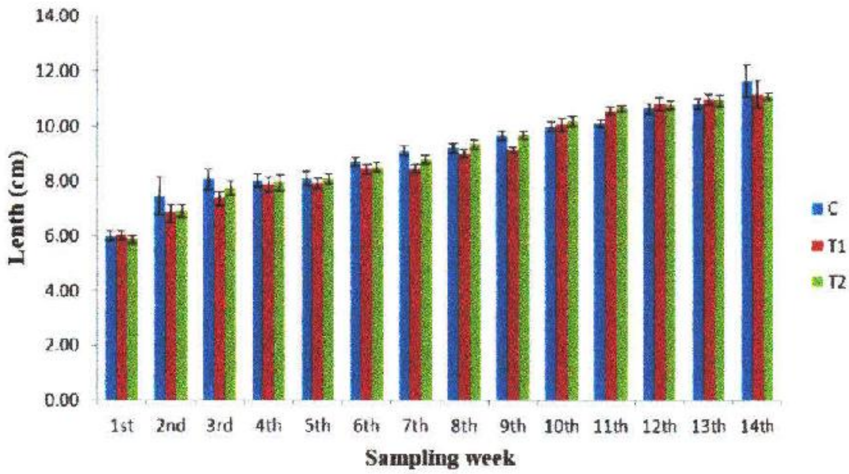

Figure 6: Mean length of prawn in different samplings and in different groups. The error bar represents the SE of six replicates.

4.1.3 Survival rate

At the end of the experiment the survival rate of prawn in C, T_1 and T_2 was found to be 77±14.14, 66±12.96 and 69±11.78 % respectively (Table 3). Highest survival rate was found in C in which the prawn was fed at satiation and lowest survival rate was found in T_1. No significant difference was found among the experimental groups except C and T_1 (P>0.05) .

4.1.4 Specific growth rate (SGR)

The mean specific growth rate of prawn in C, T_1 and T_2 was found to be 2.53±0.45, 2.52±0.43 and 2.55±0.42 (BW%/day) respectively (Table 3). Highest SGR was found in T_2 and lowest SGR was found in T_1 but no significant difference was found among the experimental groups (P>0.05).

SGR increased with increasing restriction of feeding.

4.2 Feed Utilization

4.2.1 Feed Conversion Ratio (FCR)

The FCR value was found to be 2.31±0.23, 2.04±0.23 and 2.24±0.18 in the experimental groups of C, T_1 and T_2 respectively (Table 3). Highest FCR was found in C (control) which was statistically significant ($P<0.05$) than lowest FCR in T_1. FCR decreased with increasing feeding restriction.

4.2.2 Protein Efficiency Ratio (PER)

The PER value in C, T_1 and T_2 was 1.44±0.27, 1.62±0.18 and 1.48±0.24 respectively (Table 3). Highest PER value was found in T_1 which was statistically significant ($P>0.05$) and lowest PER value was found in C. The results indicated that when PER was high then FCR was low. The protein efficiency ratio increased with increasing restriction of artificial feeding.

Table 3: Growth performance parameters and feed utilization of *Macrobrachium rosenbergii* during the experiment (mean ± SD).

	C	T1	T2
Final weight (g)	17.23 ± 6.56^{a}	16.04 ± 6.18^{a}	15.64 ± 6.34^{a}
Final Length (cm)	11.67 ± 7.68^{a}	11.16 ± 6.03^{a}	11.09 ± 1.48^{a}
Survival rate (%)	77 ± 14.14^{a}	66 ± 12.96^{b}	69 ± 11.78^{ab}
Specific growth rate(SGR)	2.53 ± 0.45^{a}	2.52 ± 0.43^{a}	2.55 ± 0.42^{a}
Feed conversion ratio (FCR)	2.31 ± 0.23^{a}	2.04 ± 0.23^{b}	2.24 ± 0.18^{ab}
Protein efficiency ratio (PER)	1.44 ± 0.27^{a}	1.62 ± 0.18^{b}	1.48 ± 0.24^{ab}

4.3 Body composition

Body composition of cultured prawns were measured in three different steps. During stocking, at the end of restricted feeding and after satiation feeding (after harvesting). At the end of restricted feeding, moisture content was found 70.81±0.12, 72.9±0.13 and 70.8±0.55 percent (%); protein was found 68.3 ±0.04, 65.9±0.08 and 67.58±0.05 percent (%); lipid content was found

14.11±0.02, 12.44±0.02 and 13.6±0.02 percent (%) as well as ash content was found 16.9 ±0.05, 17.5±0.09 percent (%) and 17.4±0.02 in C, T_1 and T_2 respectively (Table 4). Protein and lipid content significantly varied among experimental groups. In case of moisture content, no significant differences was found among C and T_2; where, T_1 showed significant difference ($P<0.05$) with rest of experimental groups. Ash content of both treatments significantly varied from control. At the end of re feeding period at satiation level, highest and lowest level of - moisture was found in T_1 (69.74±0.08 %) and C (68.09±0.05 %) respectively; protein content was found in C (68.48±0.05 %) and T_1 (66.55±0.01 %) respectively; lipid content was found in T_1 (14.47±0.01 %) and T_2 (14.27±0.01 %) respectively; ash content was found in T_1 (16.84±0.01 %) and T_2 (16.17±0.02 %) respectively. Moisture, protein and ash content vary significantly among experimental groups. Lipid content of T_2 showed significantly similar value with C (Table 4).

Table 4: Changes in body composition of *Macrobrachium rosenbergii* during the experiment (mean ± SD).

Time	Groups	Moisture (%)	Protein(%)	Lipid(%)	Ash(%)
Initial	-	81.64±1.06	67.46±0.84	13.88±0.07	18.45±0.08
At the end of restriction	C	70.81±0.12[a]	68.3 ±0.04[a]	14.11±0.02[a]	16.9 ±0.05[a]
	T1	72.9±0.13[b]	65.9±0.08[b]	12.44±0.02[b]	17.5±0.09[b]
	T2	70.8±0.55[a]	67.58±0.05[c]	13.6±0.02[c]	17.4±0.02[b]
After refeeding period	C	68.09±0.05[a]	68.48±0.05[a]	14.32±0.03[a]	16.34±0.03[a]
	T1	69.74±0.08[b]	66.55±0.01[b]	14.47±0.01[b]	16.84±0.01[b]
	T2	68.32±0.03[c]	67.82±0.01[c]	14.27±0.01[a]	16.17±0.02[c]

4.4 Water quality parameters

In the present study, important water quality parameters, like pH, temperature, dissolved oxygen (DO), alkalinity, hardness, ammonia salinity were monitored (Table 5). No significant difference ($P>0.05$) was found among the groups for the parameters. All water quality parameters were within the acceptable range for freshwater prawn culture.

Table 5 : Mean values and ranges of water quality parameters in different treatments.

Water quality Parameters	Experimental groups		
	C	T_1	T_2
	Mean±SD	Mean±SD	Mean±SD
Temperature (°C)	26.91±4.53	27.11±4.48	26.87±4.34
DO (mg/L)	4.90±0.67	5.09±0.70	5.03±0.67
pH	7.58±0.17	7.62±0.17	7.66±0.18
Salinity (ppt)	0.53±0.32	0.59±0.21	0.56±0.23
Alkalinity ($CaCO_3$ mg/L)	115.22±14.6	128.78±21.43	113.67±9.01
Hardness ($CaCO_3$mg/L)	176.3±43.7	182.8±34.2	170.4±42.8
Ammonia (NH_3mg/L)	0.095±.001	0.092±.002	0.093±.001

Chapter-5

Discussion

by inter molts during their whole life history (Hartnoll, 1982). As found by Bordner and Conklin (1981) for juvenile lobsters (*Homarus americanus*). During re-feeding period, food consumption rate also affected by molting cycle in crustaceans characterized by loss of appetite prior to and shortly after shedding the exoskeleton (Wu *et al.*, 2001). When conducting aquaculture in pond, its very difficult to determine the exact compensation rate because plankton always present there so, there always lies a chance that, prawn could consume those during starvation. To minimize this within present experiment, no fertilizer was used during pond preparation and within culture period as well as feeding tray was used to remove unused food particles. So, chances of nutrient leaching was minimized which lead to restrict planktonic growth.

Survival is the first biological priority of any organism (Nunes *et al.*, 2005). Consequently, the absorbed nutrients are apportioned first to ensure life and all other biological processes are supplied only afterwards (e Sá *et al.*, 2004). Lower survivability leads to lower yield and profit. In this experiment, highest survivability was found in control but no significant differences was found among treatments. Survivability in T_1 was significantly lower than control. Survivability of crustaceans under prolonged feed restriction level are more than fishes because they are generally able to withstand and recover from propagated periods of starvation. Adult crayfish (Speck and Urich, 1969) and lobsters (Dall, 1974) have been maintained in the laboratory without food for 28 and 41 days, respectively. The adult shore crab *Carcinus maenas* can cope up with up to 3 months of starvation with only 50% mortality (Wallace, 1973), and juvenile blue crabs, *Callinectes sapidus*, have a high capability for recovery from prolonged starvation (Wang and Stickle, 1986). Vogt *et al.* (1985) reported that 50 days old postlarval *Penaeus monodon*, were able to withstand an absence of food for a maximum of 15 days, with moralities first appearing after 5 days. Its tough to determine the actual mortality rate due to feed restriction as freshwater prawn is cannibalistic in nature and in pond environment there lies more chances of fatality due to infection by pathogens and aquatic insects (for post larvae).

In this experiment, higher SGR (2.55±0.42) was found in T_2 compared to C and T_1 where, no significant difference was found among experimental groups. That was because, fishes under feed restriction or deprivation have been reported to have higher SGR and feed intake compared to a control group during the refeeding period (Nikki *et al.*, 2004; Turano *et al.*, 2007; Wang *et al.*, 2009). There lies a tendency toward an increased specific growth rate with the increased duration of feed deprivation up to certain limit which varies between species, as reported by Wieser *et al.* (1992) in cyprinid species and Wang *et al.* (2000) in hybrid tilapia. When feed is provided in satiation within a limited period of time, it is shared more evenly among individuals under restriction or deprivation compared to those individuals who have access to food continuously (Pirhonen and Forsman, 1998).

Feed conversion ratio (FCR) is the mathematical relationship between the input of the feed that has been fed and the weight gain of a population (Rice, 1994). It depends on the animals genetics (Arthur *et al.*, 2014), age (Varley, 2009), type of feed ingredients, the conditions in which the animal is kept, and storage as well as use of feed by the firm workers (National Research Council, 1981). It reflects the overall efficiency of any culture system the lower the FCR, the higher the weight gain obtained from the feed (Robinson and Li, 2015). In present experiment, highest FCR in control (2.31±0.23) indicates that, control need more amount of feed for per gm weight gain than others. Lowest FCR was found in T_1 (2.04±0.23) which indicates that, feed was utilized more efficiently in T_1 compared to other experimental groups, which might be occurred due to the higher digestive enzymatic activities which likely occurred in restricted feeding regimes as suggested by Jobling (1994) and Barrington (1957). A number of studies showed that, starvation or restriction of food may lead to an increase in enzymatic activity of different section of intestine (Harpaz *et al.,* 2005; Krogdahl and Bakke-McKellep, 2005).

The protein efficiency ratio (PER) values indicate how efficiently the dietary protein is being utilized by the fish species for its growth (Mohanta *et al.*, 2017). In many fish species, where the full growth compensation is achieved following the restricted feeding and re feeding protocol, the PER values were reported to be varied between 1.17 and 3.01. As found within range 1.47–1.8 by Abdel-Tawwab *et al.*, (2006) in *O. niloticus*; 2.71 by Mattila *et al.*, (2009) in *S. lucioperca*; 1.17 by Eslamloo *et al.*, (2012) in *B. schwanenfeldii*; 2.10–2.50 by Sevgili *et al.*, (2012, 2013) in

O. mykiss; 2.92–3.01 by Azodi *et al.*, (2013) in *O. mykiss*; 1.58 by Yengkokpam *et al.*, (2014) in *L. rohita*. Present PER values 1.62±0.18 and 1.48±0.24 for T_1 and T_2 falls within the range of previously reported values (as indicated above) for several fish species that had achieved the full compensatory growth which indicates that, full compensation growth was achieved in T_1 and T_2, where the PER values did not vary significantly among experimental groups. Also, no significant change was found in PER in single and multiple cycle fed atlantic halibut (Heide *et al.*, 2006), varied cycle fed olive flounder (Cho and Cho, 2009) and single cycle fed rainbow trout (Sevgili *et al.*, 2013). Comparatively slightly higher PER value was found in T_1 which might be due to the low metabolic expenditure, which possibly occurred during the period of recovery (Ali and Jauncey, 2004).The maximum PER value in T_2 indicates that, the supplemented dietary protein was utilized more efficiently as compared to other treatments in this specific feeding protocol.

One of the most remarkable responses to feed restriction we observed was a significant increase in water content in restricted groups where highest amount was found in T_1 and lowest amount was found in C at the end of restriction period. Similar responses to feed deprivation have been reported in lobster (Dall, 1974; Sasaki, 1984) and penaeid shrimp (Cuzon *et al.*, 1980; Barclay *et al.*, 1983). In crustaceans, during starvation energy is derived exclusively from endogenous resources and tissues are eroded due to catabolic activities (Stuck *et al.*, 1996). Crustacean's body are fixed by exoskeleton. So, to maintain the necessary body volume and internal turgescency during starvation, the eroded tissue mass generally replaced by water (Cuzzon and Ceccaldi, 1973; Dall, 1974; Wilcox and Jeffries, 1976; Cuzon *et al.*, 1980; Sundarapandian *et al.*, 1997). Moisture content in experimental groups tended to decrease after *ad libitum* feeding. Similar result was found by Sing and Balange (2007) and Wu e*t al.* (2001). So, hydration can be used as a short term indicator of nutritional stress in crustaceans as stated by (Wilcox and Jeffries, 1976).

Protein is an important component for the evaluation of physiological standards (Diana, 1982). They are utilized to form various cell structures (Babsky *et al.*, 1989). Generally crustaceans muscles contain high concentration of free amino acid (Cobb *et al.*, 1975). In present study at the end of restriction period, it was observed that the rate of protein levels decline with increased restriction period. Similar result was found during starvation in some crustaceans (Barclay *et al.*, 1983; Wang and Stickle, 1986; Juinio *et al.*, 1992; Moss, 1994; Sing and Balange, 2007). The consecutive loses in protein level in restricted groups might be attributed to increased proteolytic activity in fish tissues due to restricted diet regimes where, amino acid basically used for energy (De Silva and Anderson, 1995). At the end of re feeding period, restricted groups were not able to replenish the lost protein level. That might be due to the short re feeding period as, duration of satiation feeding was not sufficient enough to regain the protein level lost during restricted period. In present study, the level of total lipid was found vary significantly among experimental groups where, highest and lowest amount was found in C and T_1 respectively. A reduction in lipid content as a response to feed deprivation has also been reported for adult and sub adult *Peneaus duodorum*, *Oeneaus japonicas*, *Peneaus esculentus*, *Fenneropeneaus chinensis* (Schafer, 1968; Cuzon *et al.*, 1980; Barclay *et al.*, 1983; Wu *et al.*, 2001). This was because, in crustaceans, lipids are catabolized during starvation to produce energy (Heath and Barnes, 1970; Bourdier and Amblard, 1989). At the end of satiation feeding in present study, T_2 was able to replenish its lost lipid content which indicates significantly similar fattening of body cavities and internal organs to C. Lower lipid content in T_1 proves that, long term starvation causes less fattening and higher energy consumption. Which agrees with the result found by Adaklı and Taşbozan (2015). At the end restriction, body ash tended to be higher in restricted groups than control. This result complies with the findings by Sing and Balange (2007) in *Macrobrachium rosenbergii* and Wu *et al.* (2001), in *Fenneropeneaus chinensis*. When prawns were subjected to *ad libitum* feeding from restriction, body ash decreased in treatments than before but remained slightly higher than control.

Water quality is determined by various physico-chemical and biological factors (Moses, 1983). Water quality parameters, such as water temperature, hardness, alkalinity, ammonia, dissolve Oxygen (DO), pH etc. play an important role for the fish health and pond production in the water. Thus it is important to monitor water quality according to the cultured species in aquaculture (Andrew, 2007). It is supposed that, in-spite of being a crustacean species, *Macrobrachium rosenbergii* and fish show similar tolerance capacity of water where recommended temperature range for prawn culture in pond is 21.9-33.5°C (Fair and Fofner, 1981). To get the year round production of freshwater prawn the temperature range should be 25-31°C but the best result found if the water temperature exist within the range between 28- 31°C (New, 2002).

In freshwater environment, the concentration of DO can be changed markedly specially on the pond bottom where prawns live most of the time in their life (Chen and Chen, 2003). The optimum concentration of DO in the water of freshwater prawn pond should be in the range of 3-7 mg/L (New, 2002). The suitable DO content in the earthen ponds for monoculture system of *M. rosenbergii* is 5-8.1 mg/L (Hossain *et al.*, 2000). In present experiment, DO of all the experimental groups were within the optimum limit and showed no significant differences among them. Water pH is the measure of acidity and alkalinity of any aqueous solution. It is the negative logarithm of the activity of the solved hydronium ion, more often expressed as the measure of hydronium concentration (Wurts and Durborow, 1992). According to Boyd and Tucker (1998) water pH values between 6.5-8.5 is required to maintain the productivity of water and normal physiology of aquatic life. In present experiment, no significant differences was found in pH level among experimental groups. The range in all experimental groups were lied within the optimum range for prawn culture whose ranges are 6.8-8.4 and 7.5-9.5 as mentioned by Hossain *et al.* (2000) and Ali *et al.* (1982) respectively.

Hardness means the total of Calcium and Magnesium content, but mainly expressed by the quantity of Calcium Carbonate. Freshwater fish need to spend less amount of energy for osmoregulation in hard water (alkalinity level 100-150 mg $CaCO_3$/L) resulting an increase in growth but the higher limit has an effect on the osmoregulation when the ion concentration is high (Department of Water Affairs and Forestry, 1996). Less than 20 ppm of $CaCO_3$ in water cause to fluctuation and greater than 300 ppm of $CaCO_3$ can cause unproductive of pond water due to the lacking of CO_2 (Colt and Bouck, 1984). In the present study hardness level among experimental groups were found optimum for prawn growth which is similar as the recommended range which is 50-200 mg/L $CaCO_3$ (Wetzel, 2001 a). Wickins (1972) reported the suitable range of hardness for *M. rosenbergii* culture is to be 50-200 mg/L $CaCO_3$. Ammonia (NH_3) is a critical parameter for successful prawn production. Free ammonia can be toxic to many fish as well as many shrimp species which may cause gill irritation and respiratory problems (Mayer, 2012). Ammonia >1 mg/L is harmful for freshwater prawn (New, 2002). In present experiment, ammonia level in all experimental groups were lied below the toxic level where no significant differences were found among experimental groups but slightly higher mean value was found in control which might be due to the more availability of feed ingredients in control compared to treatments (Hopkins *et al.*, 1995).

Chapter-6

Conclusion

6 Conclusion

In the present work there were no hampering of growth performance of restricted groups as they showed similar specific growth rate and final growth compared to control where feed was available throughout the experimental period which indicates, restricted groups were able to show compensatory growth. Protein efficiency ratio and NPU increased with feeding restriction while FCR decreased with restriction of feeding which is a good indication for the better utilization of feed by restricted groups. Body composition of restricted groups showed negligible differences from control. From present study, results imply that, feed restriction throughout two days in a week would not hamper the growth of *Macrobrachium rosenbergii* and followed by proper re feeding, feed could be restricted three days in a week in improved traditional prawn culture as it does not hamper final body mass and showed better feed efficiency. Our findings have implications for aquaculture management procedures, because farmers are sometimes forced not to feed their shrimp at full ration during the course of farming based on economic, labor or other circumstances such as weather condition, water quality, etc.

Chapter-7

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7 References

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Photo Gallery

Photo Gallery

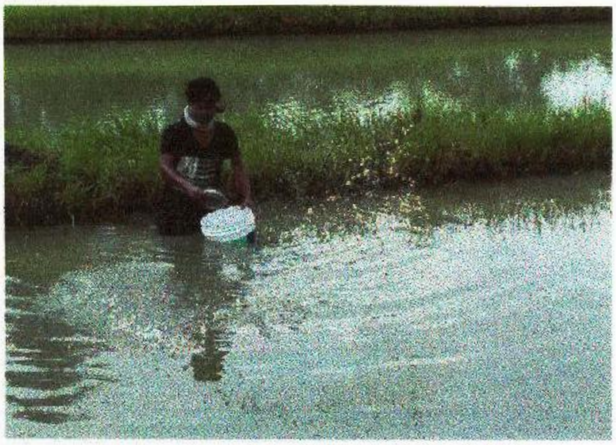

Fig: Liming

Fig: PL Stocking

Fig: Feed on feed Tray

Fig: Feed tray placement.

Fig: Measurement of length after harvesting.

Fig: Titration during protein determination.