Effectsof feeding frequency on growth performance and RNA:DNA ratio in *Mystuscavasius* (Hamilton, 1882) fingerlings

A thesis submitted to the Department of Fisheries, University of Dhaka in the partial fulfillment of the requirements for the degree of Master of Science (MS) in Fisheries.

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Declaration

I hereby declare that the dissertation entitled "**Effects of feeding frequency on growth performance and RNA:DNA ratio in *Mystuscavasius* (Hamilton, 1882) fingerlings**" submitted to the Department of Fisheries, University of Dhaka for the degree of Master of Science (MS) is based on self-investigation, carried out under the supervision of Dr. Md. Golam Rabbane, Associate professor, Department of fisheries, University of Dhaka, Dhaka-1000, Bangladesh.

I also declare that this or any part of this work has not been submitted for any other degree anywhere. All sources of knowledge used have been duly acknowledged.

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This is certified that the research study entitled "**Effects of feeding frequency on growth performance and RNA:DNA ratio in*Mystuscavasius* (Hamilton, 1882) fingerlings''**was done by Mosheur Rahman, Roll No:Curzon-804,Registration No: 2011-412-775,MS session: 2015-16,under mysupervision.

This is further to certify that it is an original work and suitable in partial fulfillment of the requirements for the degree of **Master of Science(MS) in Fisheries** from University of Dhaka, Dhaka-1000, Bangladesh.

I wish every success in his life.

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Abstract

Mystuscavasius(Hamilton, 1882)is one of the most important aquaculture fish species and it is popularly cultured in all over Bangladesh. The fish is widely consumed by the people for their rich protein content and essential fatty acids. So a study was conducted to estimate the growth performance like growth performance, survival rate, condition factor, average daily gain (ADG), specific growth rate (SGR) and percentage of weight gain (%) of *Mystuscavasius*.To estimate the effects of different feeding frequency on growth performance of gulsha an experiment was conducted for 90 days in the 12aquarium under four treatments located at the Department of Fisheries, University of Dhaka. Each aquarium was filled with 18 fingerlings. The fingerlings were fed with one .two, three and four times feeding in a day respectively. The fingerlings were fed at the rate of 5% of their body weight.The study was conducted during the month August to October 2016.

Water quality parameters were observed weekly and the results were more or less similar in four treatments and remained within the suitable ranges for aquaculture. During the rearing and feeding trail in the laboratory condition, the change in growth and feed utilization by the gulsha fish feed on four times different feeding frequency have been assessed by the determination of condition factor (K), average daily gain(ADG), specific growth rate (SGR), survival rate and feed conversion ratio (FCR).The treatmentsare significantly differentwas found in feeding frequency. The highest FCR (2.69 ± 0.26) was found in treatment 4 (four times feeding per day) while the lowest FCR(1.68 ± 0.04) was found in the treatment 2(two times feeding per day).The value of Average Daily Gain(ADG) was found highest (0.30 ± 0.017) g/day in treatment 3 (three times feeding per day) and the lowest ADG (0.08 ± 0.008) g/day was found in treatment 4. The values of SGR were highest (11.90 ± 0.02) % for the treatment 3 and lowest (0.76 ± 0.02)% in treatment 4.The condition factor was highest (1.61 ± 0.018) in treatment 2 while the lowest (1.05 ± 0.02) condition factor was found in treatment 1.Condition factor of treatment two was significantly diferent than others treatment.The highest survival rate (93.25 ± 0.47)% was observed in the treatment 4 belonged to 60^{th} day culture period and the lowest survival rate (83.32 ± 5.55)was found in the treatment 4 belonged to 30^{th} day culture period. Result from RNA: DNA ratio indicates the growth performance of gulsha fish. The highest RNA:DNA ratio (0.93 ± 0.07)was observed in the treatment 2 (TR2) whereas the lowest(0.57 ± 0.11) ratio was observed in the treatment 1(TR1).

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Chapter 1

Introduction

Introduction

1.1 Background

Fisheries is a vital sector contributing substantially to the Bangladesh economy. It is a major provider of employment next to agriculture and the much needed inexpensive wholesome protein food to the mass. Fish is also in vitamins and variable quantities of fat, carbohydrates, calcium,phosphorus and other nutrients, which are important for human health and growth.

Bangladesh is a land of rivers and it is astorehouse of varieties of resources. The country has a vast area of water bodies that indicates rivers, ponds, canals, streams, beels, haors,baorsetc.Bangladesh is unique for agriculture and fisheries resources management. It is the richest country in the world in terms of freshwater diversity (Shafi and Quddus, 1982).Inland water fish used in to be caught practically by most of the people, practically those living in rural areas.

The growth in biomass of fish in intensive and semi-intensive culture system depends on various factor notably feeding regimes. Fish growth at different stages in largely governed by the kind of feed, feeding frequency, feed intake and its ability to absorb the nutrients. One problem confronting by fish culturists is to obtain a balance between rapid fish growth and optimum use of the supplied feed. Among these, feeding frequency is an important factor for the survival and growth of fish at the early stages (Hung et al., 2001; Dwyer et al., 2002).

The feeding rate and feeding frequency should be determined for individual feeds and carefully monitored for feed consumption rate and growth period. Optimum feeding frequency may provide maximum utilization of diet. It is evident from earlier studies that due to starvation. The fishes should have enough to feed up to satiation for their optimum growth. However, overfeeding leads not only to areduction in feed conversion ratio and anincrease in input cost but results in accumulation of wastes that adversely affects the water quality.

Time of feeding and feeding frequency have been reported to affect to feed intake and growth performance (Neoske and Spieler, 1984) i.e. Indian catfish, *Heteropneustes fossilis*(Sundararaj etal., 1982), rainbow trout , *Oncorhyncus mykiss* (Reddy et al., 1994), Rohu , *Labeo rohita* (Choudhury et al ., 2002) .Therefore, it is important to standardize the feeding and feeding rate for the target species in aquaculture for optimum production. When fish are fed with at optimal

feeding frequency, growth and feed conversion ratio are expressed to improve because regulates their feed intake in relation to their energy demand (Kaushik and Meadale, 1994) and their feeding rhythms (Dada et al., 2002).Food is the source of energy for fish to carry out basic biochemical functions such as growth, reproduction, and movement. Fish growth is influenced by feed availability and intake, genetics, age and size, environment and nutrition. Of these factors, feed intake is perhaps the principal factor affecting thegrowth rate of fish (Li et al., 2014). Feed management in terms of optimization of feeding rate and frequency has become one of the crucial areas of research in the field of aquaculture. Overfeeding and leftover food disrupt the water quality (Ng et al., 2000)while inadequate food supply has adirect impact on production cost (Mihelakakis et al., 2002).

Mystuscavasius is very popular in Bangladesh due to its culture characteristic which has endeared it to many fish farmers. To improve the culture of *Mystuscavasius* there is aneed for more information on the management method in the area of feeding and feeding frequency in order to produce fish within a shortest possible time and at minimum cost with good quality. The effect of feeding frequency on the survival rate of *Mystuscavasius* fish and suggesting an aquaculture practice that will minimize losses and maximize profit in rearing these fish (Asuwaju et al., 2014). Bangladesh is a country with a lotof rivers and canals. Once upon a time fish was abundant in the open water of our country. But with the increasing rate of population, pollution, environmental change; hence production rate and production quality are declining in the hatcheries. As the gulsha is a commercial importance fish, it has alarge scope yet. It can major source of protein. People also can find their employment and change their socio-economic condition. Foreign currency can be earned by culturing this fish in theproper cultivation process. So further research is needed on gulsha fish.

The demand for fish, on the other hand, has been rising as a result of anincrease in population, income per capita in exports and in the prices of alternative sources of animal protein. With supply lagging and demand rising, the prices of fishery products have increased rapidly withadverse effects on the welfare of low-income people whose protein intake derived mainly from fish, the traditionally lowest-priced source of animal protein. So if the production of fish can be increased, it can fulfill our existing demand as well as earn foreign currency.

1.1.1Significance of Fisheries in Bangladesh

Bangladesh is a riverine country. Bangladesh is blessed with numerous natural gifts and about 230 rivers are the best gifts of nature. 230 rivers including tributaries flow through the country constituting a waterway of total length around 24,140 kilometers (15,000 mi). It's not that the river is theonly resource of the water for fishes in our country, it covered with vast recourses of water of water excluding river like tributaries, estuaries, long cost lines, haors, bells, wetland, floodplains etc. fisheries sector of Bangladesh has been playing a very significant role and diverse potential for future development in Bangladesh. Fisheries production in Bangladesh is increasing rapidly. Recently advanced technologies are applied and new species introduction in aquaculture.Bangladesh has tremendous fisheries potential. There is a total of 264 species of freshwater fin fishes, 65 spp of prawn and shrimp (Hossain, 1985). Inland open water is the major source of production in the country, where open water capture fisheries contribute 28.07% and inland culture fisheries contribute 55.15% of the total production during 14 (DOF, 2015).Though Bangladesh has vast water body but the production of fish through aquaculture is low compared to another many country of the world. Bangladesh has gained thefourth position in theinland open water and fifth position in inland culture fisheries in the world (FAO, 2014). Fisheries contribute 3.69% of total GDP and about 22.60% of agricultural GDP. Fisheries provide about 60% of animal protein in the daily diet of people of Bangladesh (DOF, 2015).

1.1.2Current status of fisheries in Bangladesh

Declining of wild fish capture with the increasing of thepopulation it becomes very important to theculture of fishes in control condition. At thefirst time pond culture is begun for freshwater fish culture but with the time for the increasing of demand, fish culture is started in every water body like lakes, river, bills, haor, baor, ditch etc. At present fish, culture becomes very popular in themarine area. Fisheries sector of Bangladesh can be broadly divided into four major sub-sectors: Inland capture or open water fisheries, inland culture or closed water fisheries, marine industrialfisheries and marine artesian fisheries. Open water fishery is a self-sustaining system although human interventions 2have significantly deteriorated its healthy and productivity

recently. Culture fishery, on the other hand, is primarily an economic venture managed by private individuals and farms. The inland fisheries contribute about 83.22% of total catch and the remaining 16.78% comes from themarine fisheries (DoF, 14). The inland aquatic Bangladesh is rich in faunal diversity containing 266 species of freshwater and brackish water fin fishes. There are 54 species of fin fishes as threatened in Bangladesh, among these 12 species are vulnerable. Nowadays, many exotic species such as silver carp, catfish, tilapia, pangas, piranha etc. The brackish water Shrimp culture has been encouraged as it earns valuable foreign currency.

Table 1: Capture water fisheries resources in Bangladesh

Inland Fisheries	**Water Area (ha)**	**Production (m.ton)**	**Percentage (%)**
1.River and Estuaries	853,863	167373	4.72
2.Sundorbans	1777700	18366	0.52
3.Beels	114161	88911	2.51
4.Kaptai Lake	68800	8179	0.23
Flootplains	2695529	712976	20.09
Subtotal	3910053	995805	28.07

Table 2: Capture water fisheries resources in Bangladesh

Inland Fisheries	**Water Area (ha)**	**Production (m.ton)**	**Percentage (%)**
1.Ponds	371309	1526160	43.01
2.Streams	130488	193303	5.45
3.Baors	5488	6514	0.18
4.Shrimp Farming	275274	216447	6.10
5.Pen Culture	6775	13054	0.37
6.Cage Culture	7	1447	0.04
Subtotal	789341	1956925	55.15

Sources:Fish catch statistics of Bangladesh. DoF, 14.

1.1.3Current status of fisheries in the world

Tilapia has developed into the second most important cultured freshwater fish, behind the carp. Tilapia production is growing exponentially with the global output standing at 2.5 million tonnes annually, and has, therefore, been dubbed as the twenty-first century's most culturable fish (Shelton and Popma, 2006; Fitzsimmons, 2010). The world's total tilapia production in 2010 was

3.49 million tons (FAO, 2012). The last three decades have seen significant developments in farming of tilapias worldwide. They are being farmed in about 85 countries worldwide. Egypt has been expanding its culturing industry in recent years and is now producing 200000 tons (FAO, 2008). Currently, tilapia is farmed commercially in almost 100 countries worldwide, with over 98 percent of the production occurring outside their original habitats (FAO, 2011).

Global inland waters capture production reached 11.6 million tonnes in 2012. Although its upward trend seems continuous, its share in total global capture production does not exceed 13 percent. "Inland waters" remains the most difficult subsector for which to obtain reliable capture production statistics. Several countries in Asia, the continent that accounts for two-thirds of the global total, are believed to either under- or over-estimate their inland water catches. The total catch reported by India is very variable and that from Myanmar has increased 4.3 times in a decade (FAO, 2014).

Table 3: Capture water fisheries resources in Bangladesh

Ten major countries producing highest inland water fish production

Position	**Country**	**Continent**	**Production,2012(Tonnes)**
1	China	Asia	22,97,839
2	India	Asia	14,60,456
3	Myanmar	Asia	12,46,460
4	Bangladesh	Asia	9,57,095
5	Cambodia	Asia	4,49,000
6	Uganda	Africa	4,07,638
7	Indonesia	Asia	3,93,533
8	United Rep. of Tangania	Africa	3,14,945
9	Nigeria	Africa	3,12,009
10	Brazil	American	2,66,042

Sources: (FAO, 2012) **Total 1,16,30,320 T**

1.2 Biology and history of *Mistuscavasius*

Mystuscavasius, the Gangetic mystus, is a species of catfish of the family Bagridae. In the wild, it is found in Indian Subcontinent countries such asIndia, Pakistan, Sri Lanka, Nepal, and

Myanmar. Reports of this species from the Mekong basins, Malaysia, and Indonesia are misidentifications of the species *Mystusalbolineatus* or *Mystussingaringan*.

Few populations occur in Thailand, but only in the Salween basin. It grows to a length of 40 cm. The pectoral spine of the species may give painful wounds and sometimes can be venomous. The population is known to be decreasing in recent past, due to catching, pet trading and habitat destruction.

Mystuscavasius was described from the Atrai River (Hamilton, 1822). This species was previously thought to occur throughout the Indian subcontinent and Myanmar, but Chakrabarty et al. (2005) showed that the name should be restricted to the populations from thenorthern part of the subcontinent, those from the southern part are referable to *M. seengtee* and those from Myanmar are referable to *M. falcarius*.

Although Mishra et al.(2009) report a mean decline of 27.6% in acatch for this species in southwestern Bengal (lower Ganges-Brahmaputra system and Subarnarekha River) for the period 1960-2000, current evidence indicates that it is still relatively widespread and abundant across the majority of its range. Despite being caught in artisanal fisheries, the level of exploitation is not deemed high enough to be a threat to long-term survival of *Mystuscavasius*; hence assessed as Least Concern.

Known from the Ganges, Brahmaputra, Mahanadi and Godavari river drainages in northern India, Nepal, and Bangladesh. The conspecificity of thematerial identified as this species from the Indus River drainage awaits further verification (Chakrabarty et al., 2005).

But the native countries are Bangladesh; India (Assam, Bihar, Chattisgarh, Delhi, Haryana, Himachal Pradesh, Jharkand, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Punjab, Rajasthan, Tripura, Uttaranchal, Uttar Pradesh, West Bengal); Nepal.Although Mishra et al. (2009) reported a mean decline of 29.9% in catch for this species in southwestern Bengal (lower Ganges-Brahmaputra system and Subarnarekha River) for the period 1960-2000, and an average decline of 57% each decade from 1980-2000; there is insufficient data from other areas where this species is naturally distributed. Data from throughout the Ganges-Brahmaputra system suggests that this species is still relatively common.

This species is heavilyutilized as a food fish in some parts of its range and is occasionally caught and exported as an ornamental fish.

Although there is a marked decline in the population in southern West Bengal due to overfishing, the threats to this species in other areas of its distribution are unknown. Since there is no information on the biology of this species, the impact of potential threats (especially those of an anthropogenic nature) remains unknown. The current threats to aquatic biodiversity in all of its known distribution have also not been adequately identified. Although IUCN Bangladesh (2000) identify habitat loss as a major threat to this species, this has not been verified by an empirical evidence.

1.3 Quality of feed

Chowdhury (1998) studied the growth of an Indian major carps *Labeo rohita*at room temperature for 50 days using formulated diet compassion of fish meal, soyabean oil , wheat flour ,vitamin premix (943.00%, 4.00%, 30.00% and 26.52 g) recorded at 5% level of feeding with SGR, FCR, PER,ANPU and APD and value of 1.40, 1.53, 1.97,22.40 and 77.77 respectively.

Islam et al. (1996) carried out an experiment to find out suitable feed and density to rear shingi fry in laboratory condition with three different types of feeds and they observed that growth rate and survival rate of shingi fry were significantly high in life *tubifex.*

Jingran et al. (1982) pointed out that *H. fossilis* fingerlingsstocked at 250000 /ha and fed with rice dust. Cut weed-fish-mixed with cattledung yielded a production equivalent to 4.4 t/ha in 4 months at 80 % survival.

Ruohonen et al. (1998) investigated that one –year- old rainbow trout weight range 400 – 700 g were fed for 18 weeks on low- fathering or commercial dry pellets 1, 2 or 4 times in a day. Quadratic regression analysis indicated that at least three feedings were required for maximum growth and fish fed a dry diet could benefit from even more frequent feeding. The proportion of lipid in growth increased a number of feedings, but the protein content was not affected mean 18.6% protein wet basis. Food consumption dry weight was affected frequently than maximum

growth. It is suggested that thenumber of feedings required for maximum growth may be dependent on the nutrientdensity of thediet.

Shapawi et al. (2011) developed the pelleted feed forcultured fish is an important aspect of theaquaculture industry. Two frame – made feedswere formulated with a blend of alternative ingredients in the place of amarinefish meal and fish oil and fed to humpback grouper (Cromileptes actively)fingerlingsfor14 weeks. The performance of the poultry by –product-basedfarm-made feeds was compared with a local commercial marine fish, an importedcommercial marine fish feed and with trash fish (*Sardinella spp*.).Growth performance of groupers fed the farm-made wasbetter thanorcomparable to fish fed the commercial feedsor trash fish.Feed conversion ratios (FCR) in fish fed pelleted feeds (1.3 to 2.4) were significantly better than the FCR of fish fed trash fish (5.0)

1.3.1 Effect of feed on growth

Abid et al. (2009) worked on the effect of feeding frequency on the growth and survival of *Labeo rohita* (rohu), during fingerlings rearing was studied in 280 L glass aquaria. Feeding frequency one, two and three times daily as three treatments were evaluated against a control without feeding in triplicate glass aquaria in a trial of 60 days.Single breed fingerlings range from mean length (6.8$\pm$.3) cm, mean weight (4.18$\pm$0.02) g were stocked at the density of 25 /aquarium. Survival rate was recorded as 100%,similarly, the growth in terms of length and feed conversion ratio did no differ significantly (P<.05) among treatments and were in the ranges of 7.92 to 13.6 cm and 1.39 to 1.54 respectively. The study suggested that feeding frequency of three times daily is sufficient for under intensive culture of *Labeo rotita* fingerlings.

Abid et al. (2009) investigated the feeding trial to determine the efficiency of varying dietary protein regimes of *Labeo rohita*fingerlings under intensive rearing for a period of six months. Stocking density was 25 fish/g glass aquarium-2(280-liter water volume).Seven different diets were tested. Fish were fed daily @ 4% of wet body weight twice a day. Mean weight gain, mean length gain, mean specific growth rate, feed conversion ratio, and survival rate were evaluated to determine the growth performance in different treatment. The survival rate was 100% at all feeding levels. In aquaria fish fingerlings fed with 45 % low coat based diet showed significantly higher (P<0.05) weight gain (26.17g) than other diet and highly significantly to control diet (9.77g).Ali et al. (2006) conducted to evaluate the effect of feeding cycling on the specific

growth rate, condition factor and RNA/DNA ratio of *Labeo rohita* . Fingerlings *L. rohita* were divided into control, 5 daysand 10 days feed cycling. Specific growth rate,body composition, condition factor and RNA/DNA ratio of *L. rohita* of individual fish and of each group were calculated. There was a highly significant ($P<0.001$) effect of feed cycling on specific growth rate and RNA/DNA ratio of *L. rohita*.however, the effect on condition factor was insignificant.A gradual decline was observed in specific growth rate and RNA/DNA ratio with increasing length of starvation. *L. rohita* was able to maintain it's main body constituents such as fat, protein, organic and inorganic contents indicating a compensatory growth, which was independent of theduration of starvation.

Carlos (2006) conducted a study to assess theeffect of different levels of dietary intake and feeding frequencies on the growth and survival of bighead Carp fry. He concluded that a significant effect or higher feeding rate using higher frequency and growth manifested in the final mean weight and SGR indicate that the increasing feeding rate resulted in increased growth.He also investigated that feeding frequency significantly influenced fry survival with highest values observed with feeding once or 3 times daily. In the same experiment, Carlos also showed that specific growth rate differed in relation to feeding rate but no to feeding frequency. Nandeesha et al. (1993) studied the evaluation of mixed feeding schedules I the two major carps*Catla catla*and *Labeo rohita* about 12 and 45 g size respectively and were fed on diets containing 13.9 (low) and 31.8%(H) protein. The study showed the possibility of protein diet proved uneconomical and did not promote growth in proportion to protein input. Also, there was no significant difference between the final mean weights attained by calta and rohu in various diets.

Sarder (1992) carried out an experiment on the growth and survival of *Pangasius pangasius* under three different dietary conditions each containing thesame percentage of protein (30 %) in which fish meal was the main protein source. However in diets, 17.5% of the total protein was replaced by sesame- oil cake and in diets 2, thesame percentage was replaced by mustard oil cake while diet 3 was commercial feed having thesame percentage of protein. He observed that the growthand survival rate were higher in thecase of diet 1 and 2 than did but there was no significant difference between diet 1 and diet 2.

Wang et al. (1998) determined four treatment groups of age 0 hybrid sunfish (female green *Lepomis cyanellus*× male bluegill*L. macrochirus*) were fed to satiation at one of four frequencies one,two,three or four meals per day for day 30 days. Fish fed three and four times daily showed the greatest consumption and growth rates; food conversion ratios did not differ among the four treatments (P>0.05).Because growth and food conversion were not enhanced when feedings wereincreased from three to four times daily, the optimal feeding frequency for growth was considered to be three times per day.

1.3.2 Growth of fish

Akand et al. (1989) reported on the dietary protein requirement of the stinging catfish, *Hereropneustes fossilis* (Bloch) while they worked on the effect of dietary protein on the growth, food conversion and body composition of shingi catfish.

Azzaydi et al. (1999) worked on the combined effect of feeding time and meal size on the growth performance and feeding rhythms were studied in European sea bass maintained under natural summer- autumn conditions. Three feeding strategies were compared: a modulated automatic- MF, a fixed automatic –feeding FF and a free access to self-feeders- SF. Under SF, fish showed a diurnal self – feeding pattern, with the greatest percentage of self- feeding activity concentrated in the evening. The trigger activation of both treatments MF and FF was associated with the time of feed delivery. Feeding strategies affected biomass increase, SGR and feed conversion ratio (FCR), the greatest biomass increase and highest SGR being obtained with MF and the poorest FCR with FF.

Kohinoor et al. (1998) studied on the growth and production performance of the red tilapia and Nile tilapia under low- input culture system. They found that the gross fish production 0f 3218 and 3017 kg/ha were obtained from Nile tilapia and red tilapia ponds respectively. They also reported that cost and benefits showed higher benefit from Nile tilapia culture.

1.4 RNA:DNA ratio as a condition factor

RNA:DNA ratios have been used as a short term growth and condition factor in thewide range of marine organisms. For example, RNA:DNA ratio has been used a useful indicator of the nutritional condition in juveniles of Ruditapes decussates (Luis et al., 1995). Determination of the nutritional condition using RNA:DNA ratio has been conducted on a wide range of marine

organisms, but mainly on fish (Bullow, 1970). For example, RNA:DNA ratios have been used in studies of short-term growth and condition in Atlantic cod *Gadus mohhua* (Buckley, 1979).

1.5 Research needs

Bangladesh has depended on the wild aquatic resources for their diets and economic security (Rahman, 1989; Rainboth, 1990).Rural families consume fish an average of 3.5 days per week (Minkin et al., 1997) and more than 70 percent of animal protein in the diet comes from fish (Institute of Nutrition and Food Science, 1983).The freshwater population of Bangladesh largely depends on the seasonal variations in rivers and floodplain ecosystems. But the flood control projects and the construction of embankments have blocked the migration route and destroyed the natural spawning and feeding ground of many fishes. Pollution also the major cause of decreasing the freshwater population of Bangladesh. Increasing of overfishing and lack of scientific knowledge also a barrier to fish production. Fisheries management policies have focused on increasing the production of a limited number of commercially valuable species (Minkin and Boyce, 1994).

Due to climate change, the stock of gulsha fish affects greatly in our natural water body. The inadequate knowledge in the field of feeding system also affects the growth performance of the gulsha fish. Destruction of the natural wetland and natural water body also decrease of thediversity of the gulsha fish. Although all other countries take initiative this adverse condition but our country is far away from the track. So it is a great opportunity to open up a new window of the improvement of gulsha culture technique in our country. With this point of view, I made thedecision to conduct my M.S research work on the effect of feeding frequency on growth performance of the gulsha.

Determination of the nutritional condition using the RNA:DNA ratios has been conducted on a wide range of marine organisms, but mainly on fish (Bullow, 1970; Buckley, 1984 and Robinson and ware, 1988).RNA:DNA ratios have been used as short-term growth and condition factor in Bluegill *Lepomis macrochirus* (Bullowet et al., 1978; Bullow et al., 1981), Atlantic *cod Gadus morhua* (Buckley, 1979), channel catfish *Ictalurus punctatus* (Peterson et al., 1992). But nobody used RNA:DNA ratio as an indicator of physiological state of calta (*Calta catla*) fingerlings elsewhere in the world.

1.6 Problem statement

The biggest problem was the feed habit of the gulshafish. The fingerlings of gulshado not prefer the powder feed. They do notconsume the powder feed. As a result, the feed pollutes the water qualityand deplete the oxygen.

The cost of feed is one of the principle factorsdeciding the profitability of the intensive fish culture. As the intensiveculturetotally depends on the suppliedfeed, the cost of the feed is expensive according to the perspective of ourcountry.

The lack of the feeding instrument like automaticfeeder affects the growth of the fish. The fish does not consume the totalsupplied feed. As a result, the water pollutes gradually which deplete theoxygen concentration, DO etc.

The disease is the another major problemof culturing gulsha fish. Fish can infectwith fungal, bacterial, ulcer or worm diseases (Plumb and john (Editor), 1985).When environment change unusually, the disease effect easily.

Stress is the inability of the two adapts to change. Thereare several causes of stress such as dissolved oxygen, water temperature, pH,rough handling, chemical toxicants, poor water quality increase in a number ofdisease organisms and reduced ability of fish to resist infection.

1.7 Rationale

The largest share of protein comes from fish. Gulsha is consumed by the people in Bangladesh; however, it is commercially important due to its high protein demand. But the population of these freshwater species is declining day by day due to the heavy fishing pressure and the urbanization. There are many systems of artificial propagation developed to save this freshwater fish from extinction. The farmers of our country don't know the proper way of culturing of this fish species. Though the vast possibility of this fish, there are many developed cultures of our country in other species but gulsha culture is not done proper way.There is a little work on this fish. With the point of view, I took the decision to determine the best feeding frequency and growth performance of gulsha.

1.8 Objectives

Theoverall objective ofthe presentstudy was to estimate the growth performance and best feeding frequency of gulsha fish with micro-pelleted feed.

The specific objectives of the study were

- to estimate the best feeding frequency of the supplied feed;
- to determine the average daily gain (ADG), specific growth rate (SGR),food conversion ratio (FCR), weight gain and percentage of weight gain of gulsha;
- to determine the survival rate of gulsha;
- to determine the ratio of RNA:DNA as an indicator of thephysiological state of gulsha fingerlings.

Chapter 2

Materials and Methods

2.1 Experimental fish

Gulsha fish (*Mystus cavasius*) was selected for the purpose of experimental due to several reasons.The culture of this fish would prove a more profitable venture for the following reasons:

- The gulsha fish have good market demand
- Comparatively moderate in market price
- Low risk and simple management
- They can tolerate high stocking density
- They can toleratepoor oxygen level

Scientific classification of Gulsha fish *Mystuscavasius(*Hamilton, 1822)

Kingdom:Animalia

Phylum Chordata

SubphylumVertebrata

Class Actinopterygii

Order Siluriformes

FamilyBagridae

Genus: *Mystus*

Species *M. cavasius*

Local name Gulsha

Figure 1:*Mystuscavasius*(Hamilton,1822)

2.1.1Collection of samples

Fingerlings of gulsha fingerlings (*Mystus cavasius*) were collected from "Bhai Bhai AdarshoMatsho Hatchery" at Trishal in Mymensingh. Fingerlings were carried by oxygenated polyethenebags with pure water.Fingerlings were placed into the tank for conditioning.After measuring initial length and weight,the fingerlings were released into the aquariums.

2.1.2Study place

The whole experimental was carried out Aquatic Laboratory of Fisheries Department,University of Dhaka.

2.1.3Study period

The study was carried out from 31 July to 5 October 2016.

Figure 2:Experimental Aquariums in the aquatic lab. at Dept. of Fisheries

2.1.4Experimental Design

The experiment was designed on four treatments with three replicates. Twelve aquariums were needed to complete the experiment.

The aquariums were filled with the tap water.Each Aquarium was filled with about 30 liters water and labeled according to their sequence.Each aquarium was filled with 18 fingerlings.

Continuous oxygen was provided by the aerator.Oxygen was provided 24 hours during the experiment.After conditioning the fingerlings,they were released into the aquariums with

providing oxygen with the help of aerator.No feed was provided on the first day.Water quality was monitored within seven days interval.Sampling was carried out within one-month interval.

The experiment was designed by four treatment like treatment-1, treatment-2, treatment-3, treatment-4. Treatment-1, treatment-2, treatment-3, treatment-4 were provided feed once, twice, thrice and four times respectively.5% the feed was provided on the basis of their body weight.

2.2Feeding trail of fish

The fingerlings were supplied by "Optimum Micro Pellet" as their daily feed. At first, the feed was weighted by the electric balance. The calculated feed was weighed by the balance.Then the weighted feed was provided according to their body weight. The proximate composition of the feed was-

Proximate composition of supplied feed	Amount (%)
Crude protein	32
Crude fat	4
Moisture	10
Crude fiber	4

Figure 4: Commercial Pellet feed Figure

Figure 3: Supplied feed cover

2.2.1 Characteristic of the feed

1. Color enhancer by spirulina
2. Complete nutrition

3. Non-water fouling
4. Rich in vitamins C &D

2.2.2 Ingredient of supplied feed

The ingredients of supplied feed were-

- Fish meal
- Wheat flour
- Yellow corn
- Shrimp meal
- Spirulina
- Fish oil
- Vitamins and
- Minerals

2.3Apparatus and Materials

1. pH meter
2. DO meter
3. Digital electric balance
4. Aerators
5. Measuring scale
6. Aquariums
7. Multiplug
8. Gulsha catfish fingerlings
9. Micro-pellet feed
10. Fresh tap water
11. Scoop net for collecting the sample

2.3.1 Fish sampling procedure

For the purpose of the sampling, the fishes were collected by the fine mesh scoop net. They were collected every month interval. The individual length and weight were recorded carefully.

During sampling, freezing water was used for reducing the stress.After sampling, they were released carefully into the aquarium.The final length and weight were carried out after three months.The final length and weight were important to compare with the initial status of the fingerlings.

2.4Analysis of experimental data

Condition factor(K)

This is the factor through which condition of the fish is expressed in numerical terms i.e. thedegree of plumpness or flatness is usually estimated as the condition factor.

It was calculated by the following formula as suggested by Hile(1936)

Condition factor,K= $\frac{W}{L^3}\times 100$

Where

K= condition factor

W= body weight(g)

L= body length(cm)

Average Daily Gain(ADG,g/day)

ADG is called the development of body weight per day. ADG was determined by the following formula as suggested by Jones(1967)

$$ADG=\frac{\text{Mean final weight}-\text{Mean initial fish weight}}{T2-T1}$$

Where

T1=Initial time

T2= Final time

Specific Growth Rate (SGR,%)

The percentage of the increase of body weight per day is called SGR.SGR(%) was calculated by the following formula as suggested by Hopkins(1992)

SGR (%) = $\frac{\ln W_f-\ln W_i}{T-t}\times 100$

Where

T=Final time

t=initial time

Feed Conversion Ratio(FCR)

The ratioof feed consumed by the fish and weight gain of the fish is called the FCR. Feed conversion ratio was determined by the following formula as suggested by Payne(1987).

$$FCR=\frac{\text{Feed(g) consumed by the fish}}{\text{Weight (g) the gain of the fish}}$$

Against each respectivevalue, all statistical analysis were carried out by using the computer package SPSS (version 24)

Survival rate(%)

Survivability of Gulsha (Mystus cavasius) fish for each treatment was estimated on the basis of the number of fish gathered at the end of one month,two months and three months respectively of rearing of the fishes in the experimental tanks.Survivability of gulsha was calculated by counting the actual number of the fish survived,divided by the initial number stocked and multiplying by 100 and thus

$$\text{Survival rate(\%)} = \frac{\text{No. of actual fish survived}}{\text{No. of actual stocked}} \times 100$$

2.5 Isolation of RNA:DNA

For determination of RNA:DNAratio,many isolation methods were used for the rapid isolation of high molecular fish DNA which is free of contaminations (protein and other enzymes).But the CTAB method of optimized as it gave better DNA quantity. Here, DNAzol and CATB method are described.

2.5.1 CTAB method

The search for a more efficient means of extracting DNA of both higher quality and yield has led to doing the CTAB method. This method gives the optimum result. The non-ionic detergent Cetyltrimethylammonium bromide (CTAB) method provides a less expensive way to liberate cellular nucleic acid from asmall amount of tissue.CTAB was first developed by Murry and Thompsion in 1980.

Materials

- CTAB buffer
- Tissue lysis buffer
- 2- Mercaptoethanol (2-ME)
- Absolute Ethanol (ice cold)
- 70 % Ethanol (ice cold)
- 65 degree Celsius water bath
- Phenol, Chloroform, Iso Amyl Alcohol (PCI)
- Distilled water
- Frozen fish tissue from liver, muscle or gill
- Forceps
- Microcentrifuge tube
- Micro-pipette
- CTAB buffer 100 ml
- 2.0 g CTAB (Hexadecyl trimethylammonium bromide)
- 10.0 ml 1 M tris p^H 8.0
- 4.0 ml 0.5 M EDTA p^H 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)
- 28.0 ml 5 M NaCl
- 40.0 ml water
- I g PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidone homopolymer) Mw 40,000)

All p^H was adjusted to 5.0 with HCl and makeup to 100 ml with water.

2.5.2 Procedures

- 0.48 g fish tissue was grinded to a fine paste with approximately 2000 µl of warm CTAB buffer
- CTAB/fish extract mixture was transferred to a microcentrifuge tube
- 1.4 µl 2-ME solutions was added to the homogenized tissue
- CTAB /fish extract mixture was incubated for about 1 h at 65 degree Celsius in a recirculating water bath
- After incubated, the CTAB / fish extract was homogenate with an equal volume of 25:24:1 phenol :chloroform: isoamyl alcohol and mixed well by inversion

- Centrifuged at 14000 rpm for 10 min to spin down cell debris. The supernatant was transferred to clean microcentrifuge tubes
- To each tube equal volume of phenol:chloroform: isoamyl alcohol (PCI) was added and the solution was mixed by inversion. After mixing, another centrifugation was done at 14000 rpm for 10 min
- The upper aqueous phase was transferred only (contains the DNA) to a clean microcentrifuge tube
- To each tube double volume of ice cold absolute ethanol was added
- The tubes were inverted slowly several times to precipitate the DNA
- The precipitate was added isolated by spinning the tube at 14000 rpm for 5 min to form a pellet. The supernatant was removed and the DNA pellet was washed by adding two changes of ice cold 70 % ethanol.
- After the wash, DNA was spinned into a pellet by centrifuging at 14000 rpm for 10 min
- All the supernatant was removed and DNA pellet was allowed to dry (approximately 15 min). The DNA was not allowed to over dry or it would be hard to re-dissolve.
- The DNA was then resuspended in 10 µl TBE buffer
- After resuspension, the DNA was stored at 4 degrees Celcius temperature

2.5.3 Quantification of DNA and RNA

The quantity of DNA and RNA was measured by the Thermo Scientific Nanodrop 2000 Spectro-photometer.

2.5.4Data analysis

All data were analyzed by using SPSS (version 24) with the level of significance at <0.05

2.6 Physico-chemical parameters of the aquarium

Water Quality Monitoring

Water quality refers to the chemical, physical, biological, and radiological characteristics of water. Water quality was determined by the dissolved oxygen(DO), temperature, pH. DO was measured by the DO meter and pH was determine by the pH meter and the temperature was measured by aconductivitymeter**.**

Water temperature

The temperature of the aquarium water was recorded by the help of conductivity meter between 10 to 11 am and 3 to 4 pm respectively.

Dissolved oxygen(DO)

The dissolved water of the water was determined by the DO meter between 10 to 11 am and 3 to 4 pm respectively.

Hydrogen ion concentration(pH)

pH was measured by the pH meter between 10 to 11 am to 3 to 4 pm respectively.

Figure 5: DO, p^H and temperature meter

Chapter 3

Results

Results

3.1 Growth performance of fish

.Different growth performance parameters (Condition factor K, Average daily gain ADG, Specific growth rate SGR, Food conversion ratio FCR and survival rate) were observed in different feeding frequency.

3.1.1 Average daily gain (ADG)

The average daily gain was observed for gulsha fingerlings in the 90 daysrearing period (table 4). After the end of the 90 days rearing period,the highest ADG value was achieved by the fish at treatment TR3by(0.30±0.017) g/daybelonged to 90th day culture period. On the other hand, the lowest ADG was found at the treatment TR1by (0.08±0.008) g/day belonged to 30th day culture period.

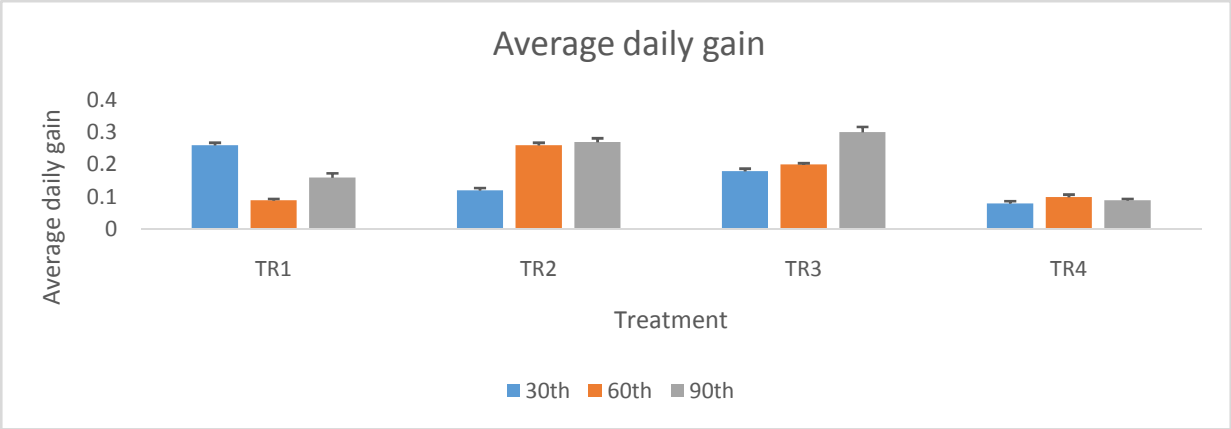

Figure 6: Average Daily Gain (Mean± SEM) of gulsha fish in four different treatments cultured for 90 days

Table 4 :Average daily gain (Mean± SEM) of gulsha fish for different treatments at 90 day

Treatment	ADG (g/day)		
	30th day	60th day	90 day
1	0.026±0.008^{a}	0.09±.0.005^{a}	0.16±0.014^{a}
2	0.12±0.008^{b}	0.26±0.008^{b}	0.27±0.012^{b}
3	0.18±0.008^{c}	0.20±0.005^{c}	0.30±0.017^{b}
4	0.08±0.008^{d}	0.10±0.008^{a}	0.09±.005^{d}

Values are Mean ± SEM (n=9).Means in the same column with different superscripts are significantly different at P<.05

3.1.2 Specific growth rate (SGR)

The average daily gain was observed for gulsha fingerlings in the 90 days rearing period (table 5). After the end of the 90 days rearing period,the highest SGR value was achieved by the fish at treatment TR3(11.90±0.02)% belonged to 30th day culture period. On the other hand, the lowest ADG was found at the treatment TR4by(0.76±0.02)% belonged to 90th day culture period.

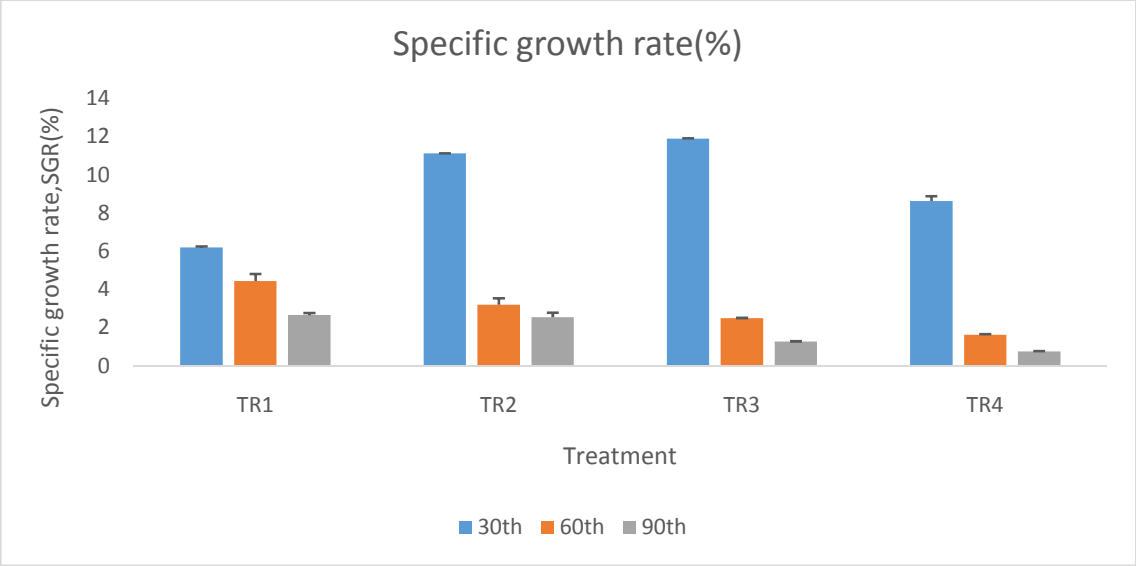

Figure 7:Specific Growth rate,(SGR,%) (Mean ± SEM) of gulsha in four different treatments cultured for 90 days

Table 5 : SGR (%) of gulsha fish for different treatments at 90-day rearing

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Treatment	SGR (%)		
	30th day	60th day	90 day
1	6.21±0.05^{a}	4.45±0.37^{a}	2.66±0.11^{a}
2	11.13±0.006^{b}	3.21±0.33^{b}	2.55±0.24^{b}
3	11.90±0.02^{c}	2.5±0.01^{bc}	1.27±0.02^{c}
4	8.63±0.26^{d}	1.62±0.04^{d}	0.76±0.02^{d}

Values are Mean ± SEM (n=9).Means in the same column with different superscripts

are significantly different at P<0.05.

3.1.3 Food conversion ratio(FCR)

Foodconversion ratio (FCR) value of the feed used for feeding fingerlings at different frequencies of 1 times,2 times, 3 times,4 times a day was found in table6. The lowest i.e the best FCR (1.68±0.04) was observed in treatment TR2 two times per day feeding belonged to 60th day culture period and the highest i.e the worst FCR value (2.69±0.26)was recorded in treatment TR4with feeding frequency four times per day belonged to 90th day culture period. A low value of FCR is an indicator of better food utilization efficiency of supplemental feeds.

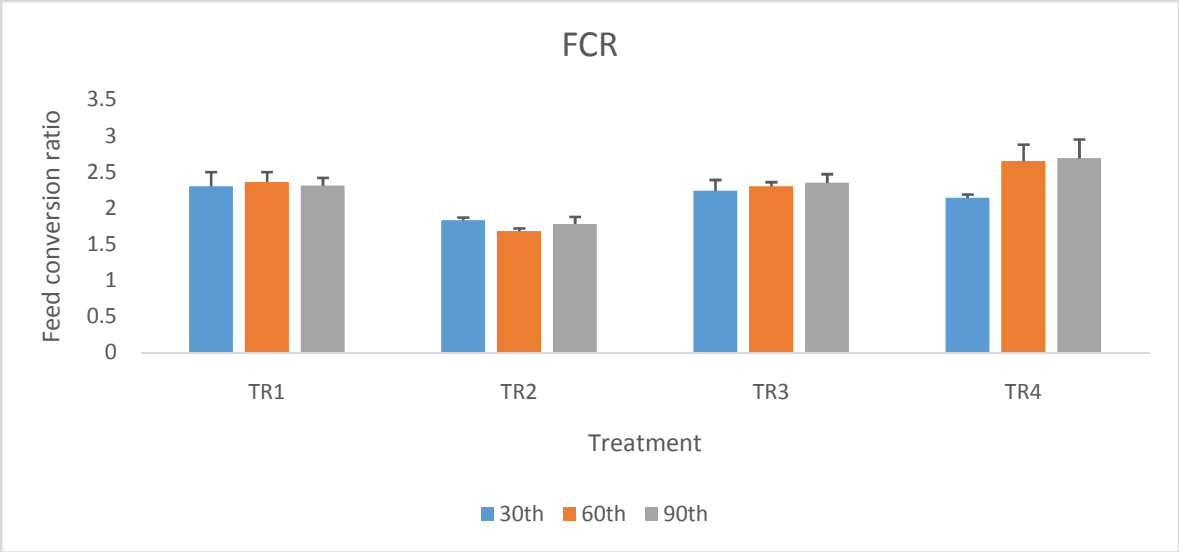

Figure 8:Feed Conversion ratio (Mean ± SEM) of gulsha cultured for 90 days with four different treatments

Table 6:FCR at 90 days rearing period ((Mean± SEM)

Treatment	FCR		
	30th day	**60th day**	**90th day**
1	2.30±0.20	2.36±0.14	2.31±0.11
2	1.83±0.04	1.68±0.04	1.78±0.10
3	2.24±0.15	2.30±0.06	2.35±0.12
4	2.14±0.05	2.65±0.23	2.69±0.26

Values are Mean ± SEM (n=9).Means in the same column with different superscripts are significantly different at P<0.05.

3.1.4 Condition factor (K)

The observed condition factor (k) was showed in table 9.After 90 days rearing condition, the highest condition factor was observed (1.61±0.018)in the treatment TR2with two times feeding frequency per day. The lowest FCR was observed(1.05±0.02) in the treatment TR3 with three times feeding frequency per day.

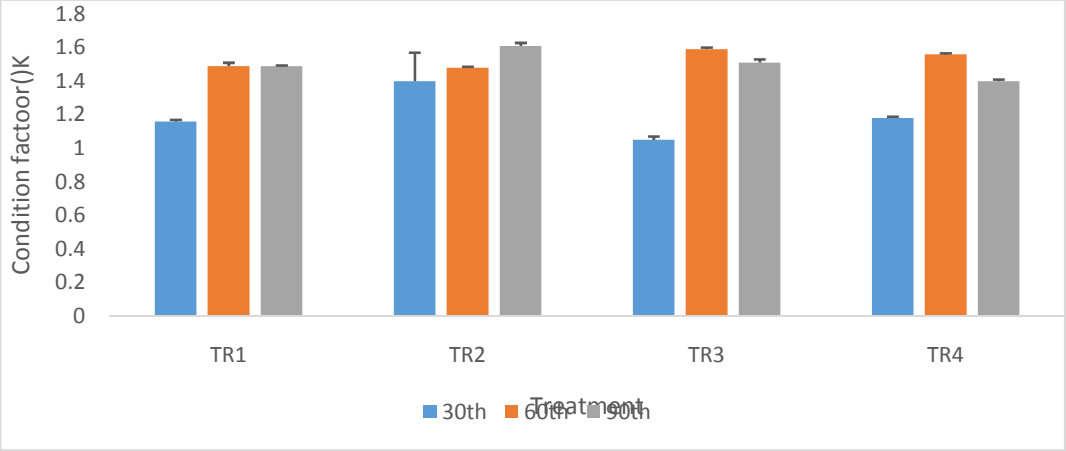

Figure 9:Condition factor (Mean± SEM) of gulsha cultured for 90 days with fourdifferent treatments

Table 7 :Condition factor (K) at 90 days rearing period (Mean± SEM)

Treatment	K		
	30th day	**60th day**	**90th day**
1	1.16±0.01[a]	1.49±0.02[a]	1.49±0.003[a]
2	1.40 ±0.17[a]	1.48±0.006[a]	1.61±0.018[b]
3	1.05±0.02[a]	1.59±0.01[b]	1.51±0.020[a]
4	1.18±0.008[a]	1.56±0.006[b]	1.40±0.01[c]

Values are Mean ± SEM (n=9).Means in the same column with different superscripts are significantly different at P<0.05.

3.1.5 Survival rate (%)

The values of thesurvival rate of the experiment fish gulsha (*Mystus cavasius*) in the four different aquarium like TR1,TR2,TR3 and TR4 respectively. The values of thesurvival rate of

fish are expressed in percentage showing in figure and table. The highest value of survival rate estimated in treatment 3 (93.45%) and lowest in treatment 4 (83.32%)in 30th days culture period.

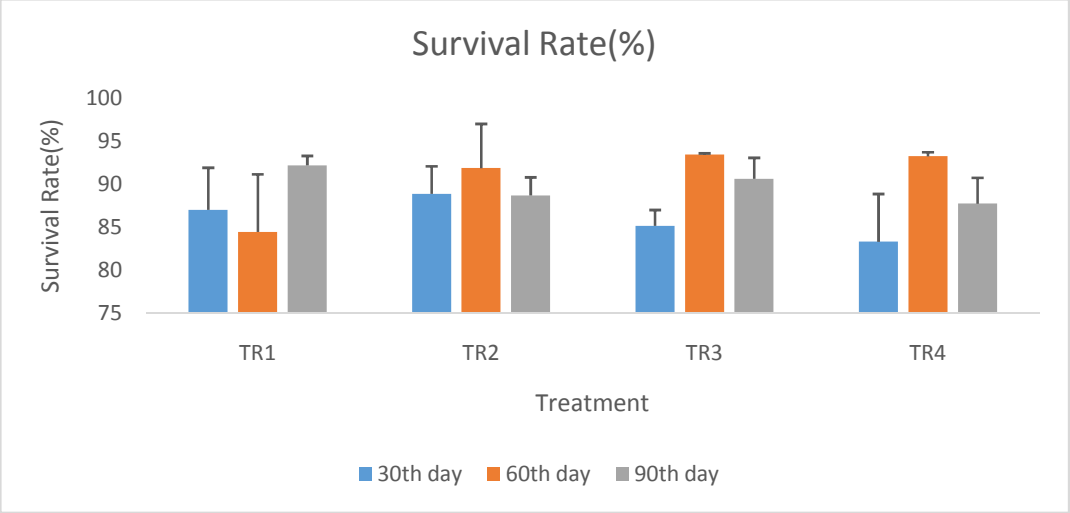

Figure 10:Survival rate (Mean ± SEM) of gulsha cultured for 90 days with four different treatments

Table 8 : Survival rate at 90 days rearing period (Mean± SEM)

Treatment	Survival rate		
	30th day	**60th day**	**90th day**
1	87.03±4.89	84.46 ±6.71	92.20±1.10
2	88.88±3.20	91.88±5.14	88.69±.2.12
3	85.17±1.84	93.45±0.15	90.62±2.45
4	83.32±5.55	93.25±0.47	87.75±2.99

Values are Mean ± SEM (n=9).Means in the same column with different superscripts are significantly different at P<0.05.

3.1.6 RNA:DNA ratio

The highest RNA:DNA ratio (0.93±0.07) was observed in the treatment 2 (TR2) whereas The lowest (0.57±0.11) RNA:DNA ratio was observed in the treatment 1(TR1).The values are significantly differents.

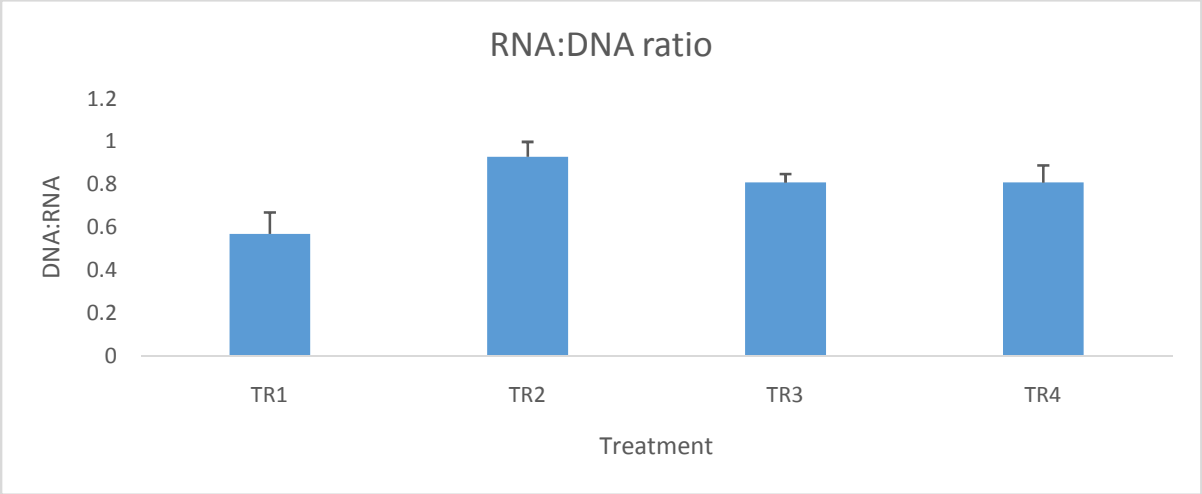

Figure 11:RNA:DNA (Mean ± SEM) of gulsha cultured for 90 days with four different treatments.

Table 9 : DNA, RNA content, and RNA:DNA ratio in the muscle of *Mystus cavasius*

Treatment	DNA (ng/µl)	RNA(ng/µl)	RNA:DNA
1	55.96±7.39	30.70±2.67	0.57 ± 0.11^{a}
2	34.70±14.20	19.50±6.90	0.93 ± 0.07^{b}
3	62.33±16.73	50.26±13.35	0.81 ± 0.004^{ab}
4	25.43±3.92	20.73±3.38	0.81 ± 0.008^{ab}

Values are Mean ± SEM (n=9).Means in the same column with different superscripts are significantly different at P<0.05.

3.2 Physico-chemical parameter of tank water

The water quality parameter should be maintained carefully; otherwise, it may be detrimental to the fish health. Poor water quality may reduce the growth rate of fish and may also make fish diseased condition which ultimately the expected production. Most important water quality parameters are water temperature, dissolved oxygenand p^{H}.

3.2.1Temperature

The average water temperature was found in the four treatments were (23.7±.10°C), (23.83 ± 0.06°C), (23.7 ± 0.05°C), (23.67 ± 0.03°C) respectively. The maximum temperature (23.83 ± 0.06°C) was recorded in the treatment 2 (TR2) whereas, the minimum temperature was recorded

(23.67 ± 0.03°C) in the treatment 4 (TR4). No significant difference was found among four treatments during the study period.

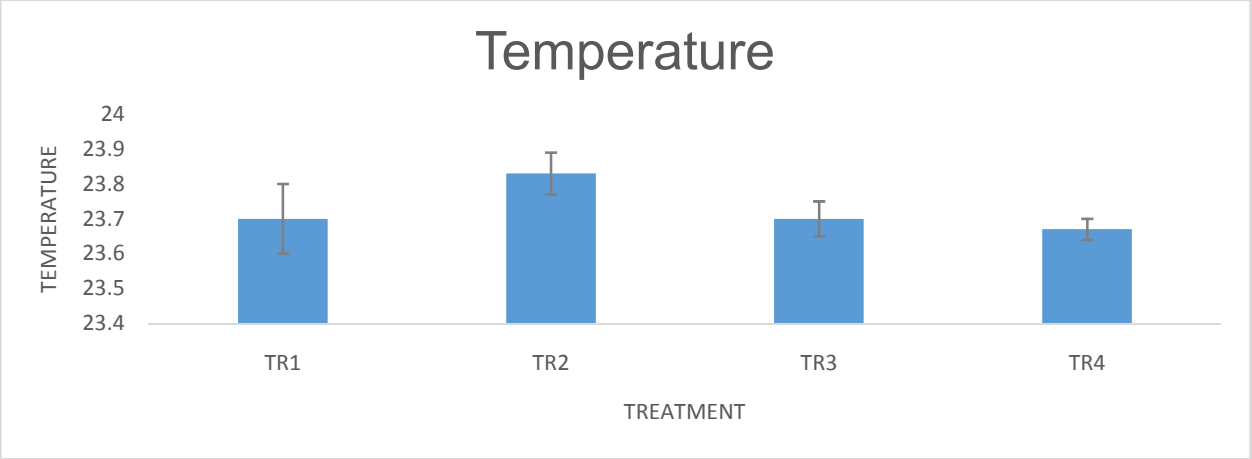

Figure 12:Temperature (Mean± SEM) of gulsha cultured for 90 days with four different treatments

3.2.2Dissolved oxygen(DO)

During the experiment period, dissolved oxygen content in the tank water within the range (7.53 ±0.26) mg/L,(7.51 ±0.14)mg/L,(7.81 ±0.17) mg/L and (7.95 ±0.07) mg/L respectively.The highest value of dissolved was (7.95 ±0.07 mg/L) found in treatment (TR4) and lowest value of dissolved was (7.51 ±.14 mg/L) found in the treatment 2 (TR2). No significant difference was found among four treatments during the study period.

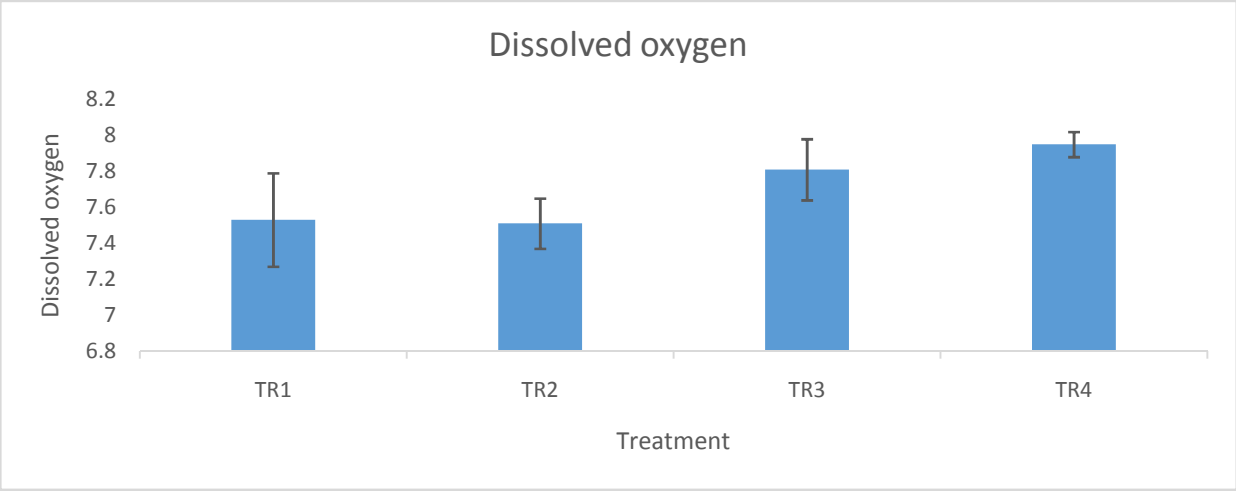

Figure 13:Dissolved oxygen (Mean± SEM) of gulsha cultured for 90 days with four different treatments

3.2.4P^{H} of culture water

During the experiment period, the mean values of p^{H} content of the water in treatments were (8.12±0.008), (8.02±0.077), (8.05±0.01), (8.05±0.01) respectively.No significant difference was found among four treatments during the study period.

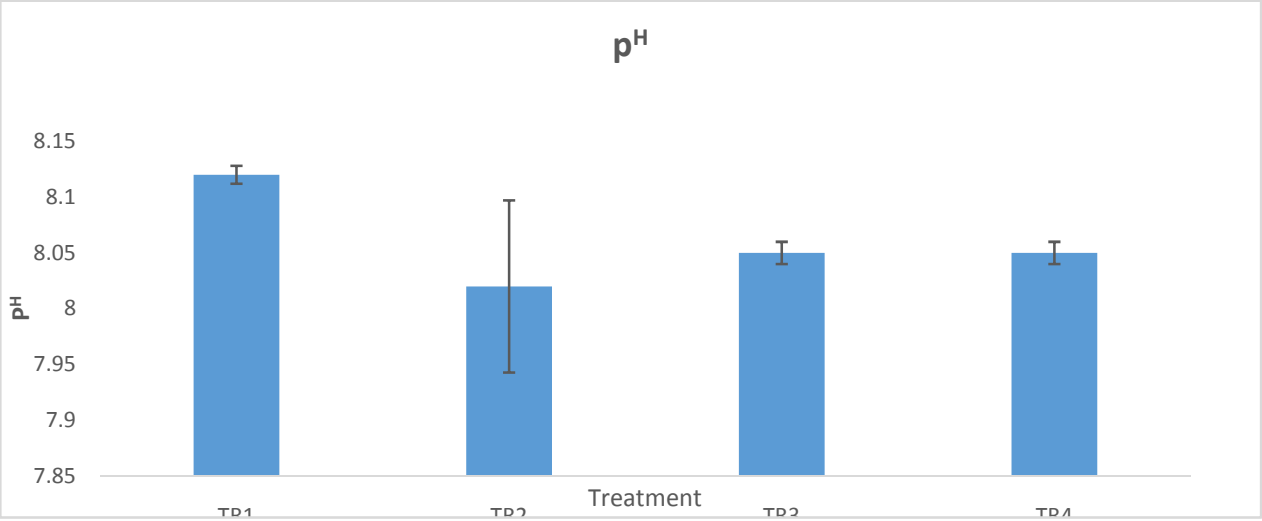

Figure 14:pH (Mean± SEM) of gulsha cultured for 90 days with four different treatments

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Chapter 4

Discussion

Discussion

This investigation described the growth performance, survival rate and fecundity of gulsha*(Mystus cavasius)* through different feeding frequency during 90 days culture period. In present study,gulsha was selected for analysis because of its availability and easy culture and also because of its great acceptance by the poor people of Bangladesh to meet their nutritional requirements. As gulsha is omnivorous fish, different kinds of feeds are supplied to determine the effect of feeding regime on the growth performance the fish.

4.1 Growth performances

Growth performances in terms of total length (TL), body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), average daily gain (ADG) and condition factor (K) were recorded among four types of treatment.

4.1.1 Condition factor

Condition factor of this study showed the high variation and better performance in treatment TR3. At the 90 days culture, the highest value of condition factor (K) of gulsha was observed in treatment TR2 (K= 1.61) and the lowest value was found in treatment TR3 (K=1.05). Condition factor found in treatment TR2 is found significantly higher than that ofTR3 at 90 days culture period. Rahman et al. (1997) in a study on the survival and growth of catfish giving selected supplemental feeds got the values of condition factor between 0.51 to 0.87.Besra (1997) observed K value nearly 1.0 in *Anabas testudineus*. So the Condition factor of treatment TR_3 (K= 1.61) is higher than other treatment.

4.1.2 Average Daily Gain (ADG)

The average daily gain was observed highest value in treatment TR3 (0.30 ± 0.17) belonged to 90 days culture period and lowest was observed in treatment TR3 (0.08 ± 0.008) belonged to 30 days culturing period.ADG indicates the growth performance of fish taking daily provided feed. Increased ADG of the fish suggested that the fish were able to regulate osmotic pressure of the body fluid; this was in agreement with 50 suggestions of Nilkolsky (1963), the more the osmoregulatory adaptation, lesser the difference between the compositions and pressures of the internal fluid of the organism and its external environment.

4.1.3 Specific Growth Rate (SGR, %)

From this study, Specific Growth Rate was found in significantly different variation. Highest specific growth rate (11.90±0.02) %was observed by treatment TR3 and the lowest value (0.76±0.02)%was observed by the treatment TR4. Specific growth rate of treatment TR3 (11.90±0.02) significant difference than treatment TR4 (0.76±0.02) at 90 days of sampling. This finding resembles the Medawar's (1945) fifth law "the specific growth rate declines more and more slowly as the organism increases in age" at the various conditions. Minot (1990) was the person to recognize that for most animals the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero in some animals. Fishes usually have a range of requirements (i.e., temperature, DO, PH, Light intensity, turbidity and so on.) for a growth and successful spawn.

4.1.4 Feed Conversion Ratio (FCR)

From This investigation, Feed Conversion Ratio of gulsha was differentin the four treatments. The highest feed conversion ratio (2.69±0.26) in treatment TR4 and lowest Feed Conversion Ratio (1.68±0.04)in treatment TR2. The lowest value of FCR indicates the better growth performance of fish. There is no significant difference among all treatments at 0.05 levels during 90 days culture period. Doolgindachabaporn (1994) found from an investigation that the FCR value of *Crypinus carpio*ranges from 1.8 to 3.0 and Akand et.al.(1989) found FCR value 2.0 to 2.7 of *Heteroneustes fossilis*. So the TR1was indicated the better growth performance and the TR2 was indicated the worst growth performance of gulsha fish.

4.1.5 Survival Rate (%)

The values of survival rate highest in treatment TR3(93.45±0.15) and lowest in treatment TR1 (83.32±5.55) % after 30 days culture period. Patricio (2004) investigated the high survival of blue tilapia strain 90% at with rapid changes in temperature used for Egyptian (Lake Manzala). These results are in contrast with Dan and Little (2000) who revealed that tilapia showed similarly high survival rates in deep and shallow ponds (97-100%). So the survival rate of gulshais highly and better performance.

4.1.5 RNA:DNA

The highest value of RNA:DNAratio was observed in the (0.93±0.07) the treatment 2 (TR2).The lowest value of RNA:DNA ratio was observed (0.57±0.11) in the treatment 1 (TR1).RNA/DNA ratio is an indicator of the fish growth.Gupta et al.(2015) were observed the RNA:DNA ratio range from 0.16 to 0.32. Wilder and Stanley, (1983) demonstrated that fishes fed restricted rations have decreased RNA:DNA ratios up to 30% D. Derek et al, (2005) have been observed significant reductions in RNA:DNA ratios of Bluegill exposed to hypoxia in the field study. Effects of environmental factor such food availability (bastrop et al., 1992) and temperature (Mathers et al., 1993) on growth and condition of fishes also has been successfully evaluated with RNA:DNA ratios.

4.2 Water quality parameters

The water quality parameter should be maintained carefully; otherwise, it may be detrimental to the fish health. Poor water quality may reduce the growth rate of fish and may also make fish dise**ase** condition which ultimately reduced the expected production.Most important water quality parameters are water temperature, dissolved oxygen**,** and P^H.

4.2.1 Dissolved Oxygen (mg/L)

Dissolved oxygen is the another important water quality parameter, on which fish depends on to live. If dissolved oxygen storage found in the aquarium water fish start gasping and in that case, aeration should be provided immediately, otherwise severe fish mortality may occur. In the present study dissolved oxygen content in the aquarium water were within the range (7.53±0.26 mg/L,7.51± 0.14 mg/L, 7.81±0.17mg/L and 7.95±0.07 mg/L respectively.

Ali et al. (2005) observed the DO value between 4.0 mg/L to 7.4 mg/L. More or less similar results were reported by Hossain (2000) where they recorded DO values of fish ranged from 3.8 to 6.9 mg/L and 2.04 to 7.5 mg/L respectively.

4.2.2 Hydrogen ion concentration (P^H)

Hydrogen ion concentration (P^H) is very important factor in fish life. The P^H range was found in the four treatment 8.12 ±0.008, 8.02 ± 0.077, 8.05 ± 0.01, 8.05±0.01 respectively.

Ali et al. (2005) also found the P^H values range from 6.8 to 8.0.Foyd (1990) observed the acceptable range required for fish culture 6.5-9.0. The P^H in the system in thenearly neutral zone and found no harmful effect on the fish growth.

4.2.3 Temperature

During the experimental period, recorded water temperature was more or less similar in different treatments. The average water temperature was found in the four treatment were (23.7±0.10°C), (23.83 ± 0.06°C), (23.7 ± 0.05°C), (23.67 ± 0.03°C) respectively .

These findings were more or less similar to the findings of Rahman (1982) who reported that the water temperature ranged from 26.06 to 31.97°C was suitable for fish culture. Mollah and Haque (1978) recognized water temperature during their studies and reported the water temperature range 26.06-31.97°C.

In the summary, it can be concluded that optimum feeding frequency is very important for fish growth and sustainable aquaculture production. This study was done to determine the optimum feeding frequency and growth performance for *Mystus cavasius*that is an important indigenous cultured fish in Bangladesh. In the study, we observed the highest growth performance by feeding two times per day. The highest growth performance was confirmed by measuring length-weight and RNA:DNA ratio.

Chapter 5

Conclusions and Recommendations

Conclusions and recommendation

The experiment was conducted in the laboratory of department of Fisheries, University of Dhaka.The experiment period was 90 days and the experiment started on from 31 July and it was finished inOctober2016 .For the experiment, 12 tanks were divided into four treatments and for each treatment, three replicas were set .fingerlings stocking was maintained 18 pieces for each tank.Four different feedings frequencies were practiced in four treatment, to know which feeding frequency play best on the growth performance of gulsha (*Mystus cavasius*) fingerling. In treatment (TR1), one time in a day was maintained; in treatment 2 (TR2), two times feeding daily was maintained; in treatment 3 (TR3), three times feeding daily was maintained and in treatment 4 (TR4); four times feeding daily was maintained. The amount of feed that was given in each treatment was calculated according to the weight of fish. The growth of fish in case of weight was monitored 30 days interval and water quality was monitored weekly to adjust the feeding rate.Growth parameters were calculated after completion of the experiment and one-way analysis of variance (ANOVA) was used for statistical analysis of data and was followed by Tukey's HSD test and Post Hoc test.

The water quality parameters such as water temperature, dissolved oxygen, and P^H of aquariums were monitored at theweekly interval and the value of water quality parameters have been found to be within the acceptable range for the growth of the gulsha (*Mystus cavasius*) fingerlings. No significant variation of water quality parameters was observed among different treatments.

Fingerlings of gulsha were habituated with the supplemental feed with 1 to 4 times per day. During the experimental period, four feeding frequency were applied to observe the effect of feeding frequency on the growth performance of the gulsha (*Mystus cavasius*) fingerlings. Different growth parameters like Condition factor (K), ADG, SGR, FCR were calculated by using thestandard formula. The final result of thegrowth rate of gulsha fingerlings among four feeding frequency, the highest Average daily gain(ADG) was observed in treatment 3 (TR3), where fingerlings were fed three times feeding daily in 90^{th} days feeding period.The best

Specific Growth rate (SGR) was recorded in the treatment 1 (TR1) where fingerlings were fed once feeding daily in 30th days feeding period. Lowest FCR indicates the better growth performance for fish. The lowest FCR was recorded in the treatment 2 (TR2) in 60th days feeding period. Condition factor (K) was recorded in the treatment 2 (90th days feeding daily). The result of the present study showed that the maximum utilization for higher growth did not occur by increasing daily feeding times only, definite feeding frequency for feed should be needed to perform thebest result. There was a believe that if fishes are given 1% feed off their body weight at atime perform good growth, but nowadays we found that increased feeding frequency play better result on the growth and production of gulsha (*Mystus cavasius*) fingerlings.

However, further investigation should be carried out for suitable gulsha (*Mystus cavasius*) culture in our country and hope it will also to evolve a definite aquarium culture technology of gulsha (*Mystus cavasius*) culture in our country. Therefore, it can be calculated that feeding frequency played a vital role on the growth performance of gulsha (*Mystus cavasius*) fingerlings.

References

References

Abid, M., Ahmed, M.S., 2009. Efficacy of feeding frequency on growth and survival of *labeo rohita* (ham) fingerlings under intensive rearing. The J. Animal & Plant Sciences 19, 111-113.

Abid, M., Ahmed, M.S., 2009. Growth response of *Labeo rohita* fingerlings fed with different feeding regimes under intensive rearing. The J. Animal & Plant Sciences 19, 45--49.

Akand, A.M., Mia, M.L., Haque, M.M., 1989. Effect of dietary protein level on growth, food conversion and body composition of Shingi, *H. fossilis*(Bloch). Aquaculture 77, 175-180.

Ali, M., Iqbal, R., Rana, S. A., Athar, M., Iqbal, F., 2005. Effect of feed cycling on specific growth rate, condition factor and RNA/DNA ratio of *Labeo rohita*. African J. Biotech. 5, 1551-1556.

Asuwaju,F.P., Onyeche,V.O., Ogbuebunu, K.E., Moradun, H.F., Robert, E.A., 2014. Effect of Feeding Frequency on Growth and Survival Rate of *Clarias gariepinus* Fingerlings Reared in Plastic Bowls.Journal of Fisheries and Aquaculture Science 9, 425-429.

Azzaydi, M., Martinez, F.J., Zamora, S., Sanchez-Vazquez, F.J., Madrid, J.A., 1999. Effect of meal size modulation on growth performance and feeding rhythms in European sea bass (*Dicentrarchus labrax,* L.). Aquaculture 170, 253-266.

Bastrop, J.B., Allen, J.L., 1992. The influence of P^H on the efficacy and residues of quinaldine. Trans. Am. Fish. Sco. 100, 544-545

Bullow, F.J., 1970. DNA-RNA ratios as anindicator of recent growth rates of a fish. Journal of the Fisheries Research Broad of Canada. 27, 2343-2349.

Chakrabarty, P., Ng, H.H., 2005. The identity of catfishes identified as *Mystus cavasius* (Hamilton, 1822) (Teleostei: Bagridae), with a description of a new species from Myanmar. *Zootaxa* 1093, 1-24.

Chowdhury, B.B.P., 1998. The relationship between feeding frequency and growth of one Indian major carp (*Labeo rohita*) fingerlings fed on differently formulated diets. M.S.Thesis.Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. pp 66.

Chowdhury, B.B.P., Das, D.R., Ibrahim, M., Chakraborty, S.C., 2002. The relationship between feeding frequency and growth of one Indian Major carp *Labeo rohita* (Ham) Fingerlings fed on differently formulated diets. Pak. J. Biol. Sci. 500, 1120-1122.

Dada, A.A., Fagbenro, A., Fasakin, E.A., 2002. Determination of Optimum feeding frequency for *Heterobranchus bidorsalis* try in outdoor concrete tanks. J. Aqua. in the Tropics 17, 167-174.

Dan, N.C., Little, D.C., 2000. Over-wintering performance of Nile-tilapia, *Oreochromis niloticus* (L.) brood fish and seed at ambient temperatures in northern Vietnam. Aquacult. Research. 31(6), 485-493.

Derek, A., Allen, R., William, E.K., 2005. Field and laboratory determination of hypoxic effects on RNA:DNA ratio of Bluegill. American Midland Naturalist.143(2), 433-442.

DoF, 2015. National Fish Week 2015 Compendium (in Bangla). Department of fisheries, Ministry of Fisheries and Livestock, Bangladesh. pp.148.

Doolgindachabaporn, 1994. Physiological responses during stress and subsequent recovery at different salinities in adult pejerryodontesthesbonariensis. Aquaculture 200, 349-362

Dwyer, K.S., Brown, J.A., Parrish, C., Lall, S.P., 2002. Feeding frequency affects food consumption, feeding pattern, and growth of juvenile yellowtail flounder *(Limanda ferruginea).* Aquaculture 213(1-4), 279-292.

FAO, 2008. The yearbook of fishery and aquaculture statistics. Fisheries and aquaculture department. Food and Agriculture Organization of the United Nations, Rome, Italy. pp 176.

FAO, 2011. FAO Factsheet -Global Importance of a Growing Sector: Fisheries and Aquaculture. FAO, Rome. pp128.

FAO, 2012. The yearbook of fishery and aquaculture statistics, fisheries, and aquaculture department. Food and Agriculture Organization of the United Nations, Rome, Italy, 239.icus (L.) cultured in concrete tanks. Aquaculture Research 36 (2), 163-171.

FAO, 2012. The yearbook of fishery and aquaculture statistics, fisheries, and aquaculture department. Food and Agriculture Organization of the United Nations, Rome, Italy, 239.icus (L.) cultured in concrete tanks. Aquaculture Research36(2),163-171.

FAO, 2014. The state of world Fisheries and Aquaculture 2014, Rome. pp .223.

Fitzsimmons, K., 2010. Potential to Increase Global Tilapia Production.Global Outlook for Aquaculture Leadership. Kuala Lumpur. The Global Aquac. Alliance 13, 21-28.

Graig.S., and Helfrich, A.L., 1998.Understanding fish nutrition, feed, and feeding. pp 256-268.

Gupta, S.K., Pal, A.K., Sahu, N.P., Jha, A.K., Kumar, S., 2015. Effects of dietary microbial levan on growth performance, RNA/DNA ratio and some physio-biochemical responses of *Labeo rohita*(Hamilton) juveniles. Aquaculture Nutrition 21, 892-902.

Hamilton, F., 1822. An account of the fishes found in the river Ganges and its branches. Constable and Co, Edinburgh, UK.

Hung, L.T., Tuan, N.A., Lazard, J., 2001. Effect of supplementary feed on the growth of shing fish (*Heteropneustes fossilis,* Bloch). Bangladesh J. Aquac Trop. 16(2), 171-184.

Islam, M.A.S.M., Rahmatullah., Salam, M.A., 1996. Effect of food and stocking density on the rearing of shingi (*Heteropneustes fossilis*) fry. Bang. J.Sc.. 25, 73-78.

IUCN Bangladesh, 2000. Red Book of Threatened Fishes of Bangladesh. IUCN Bangladesh, Dhaka.

IUCN, 2010. IUCN Red List of Threatened Species (ver. 2010.4). Available at: http://www.iucnredlist.org. (Accessed: 27 October 2010).

Jingran, V.G., 1982. Fish and Fisheries of India. Hindustan publ. Crop.(India), pp.666.

Johnstore, J., 1918. The value of the herring as food. Nature 102, 6-7.

Kaushik, S.J., Meadale, F., 1994. Energy requirements, utilization, and supply to salmonids. Aquaculture 124, 81 -94.

Kohinoor, A.H.M., Modak, P.C., Hussain, M.G., 1998. Growth and production performance of red Tilapia and Nile tilapia under low input culture system Bangladesh J. Fish Res. 3, 11-17.

Li, M.H., Manning, B.B., Robinson, E.H., 2004. Effect of daily feed intake on feed efficiency of juvenile channel catfish. N. Am. J. Aquacult. 66, 100-104.

Luis, M.Z., Chicharo, A.T., Alexandra, M., 1995. The DNA/RNA ratio as a useful indicator of the nutritional conditional in juveniles of acumara, *Alganseslacustris* Steindachner 1995, from Lake Patzuaro, Michoacan, Mexico. Aquacult. Fish. Man. 22, 15-18

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., Burren, L.J., 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Onchorynchus mykiss*. Effects of age and temperature. J. Fish. Biol. 43, 546-563.

Medawars, 1945. Physiology of salinity tolerates in tilapia: an update of basic and applied aspects. Aquatic Living Resources. 2, 91-97.

Mihelakakis, A.C., Tsolkas, Yoshimatsu, T., 2002. Optimization of feeding rate for hatchery-produced juvenile gilthead sea bream *Sparus aurata*. J. World Aquacult. Soc. 33, 169-175.

Minkin., Stephen, F., James, K., Boyce., 1994. 'Net Losses: "Development" Drains the Fisheries of Bangladesh,' Amicus Journal (New York: Natural Resources Defense Council) 16, 36-40.

Minot, J.F., 1990. developmental rates of Menidiaaudens with notes on salt tolerance.Transactions of the AmericalFisheries Society. 100, 603-610.

Mishra, S. S., Acherjee, S. K., Chakraborty, S. K., 2009. Development of tools for assessing conservation categories of siluroid fishes of fresh water and brackish water wetlands of South West Bengal, India. Environmental Biology of Fishes 84, 395-407.

Mollah, M.F.A., Haque, A.K.M.A., 1978.Studies on monthly variations of plankton in relation to the physicochemical condition of water a bottom soil of two ponds zooplankton. Bangladesh Journal of Fish 1(2), 99-103.

Nandeesha, M.C., Silba, S.S., Krishnamurthy, D., Kaushik, S.J., Lequet, P., 1993.Evaluation of mixed feeding schedules in two Indian major carps. Catla (*Catla Catla*) and rohu (*Labeo rohita*). Fish nutrition in practice: 4th international symposium on fish nutrition and feeding. Biurritz, France. 69,753-765.

Neoske, T.A., Spieler, R.E., 1984. Circadian feeding time affects thegrowth of fish..Transactions of the AmericalFisheries Society. 113, 540-544.

Ng, K.W., Lu, K.S., Hashim, R., Ali, A., 2000. Effects of feeding rate on growth, feed utilization and body composition of a tropical Bagrid catfish. Aquacult. Int. 8, 19-29.

Nilkolsky, G.V., 1963. The Ecology of Fishes. Academic press, London. pp. 352.ISBN:597.0500000000000.

Plumb., John, A.,(Editor) 1985. Principal Diseases of Farm Raised Catfish. Southern Cooperative Series Bulletin No.225, Alabama Agricult. Experiment station, Auburn University.pp 76.

Rahman, A.K.A., 1989. Freshwater Fishes of Bangladesh. Zoological Society of Bangladesh pp 1.

Rahman, M.A., Bhadra, A., Hussain, M.G., 1997.Effects of **the**culture of Tilapias, 2nd Ed.University Press **C**ambridge. pp 529-547.

Rahman, M.S., Choudhury, M.Y., Fiaque, A.K.M.A., Haque, M.S., 1982.Limnological studies for four ponds. Bangladesh Journal of Fish 5, 25-35.

Rainboth, W., 1990.The Fish communities and Fisheries of the Sundarbans. Agriculture and human values **7(**2**)**, 61-67.

Reddy, P.K., Leather, J .F., Khan, M.N., Boujard, T., 1994. Effect of the daily meal time on the growth of rainbow trout fed different ration levels. Aqua. Int. 2, 165-179.

Ruohonem, K., Vielma, J., Grove,D.J., 1998.Effects of feeding frequency on growth and food utilization of rainbow trout (*Oncorhynchus mykiss*) fed low-fatherrings or dry pellets. Aquaculture 165, 111-121.

Sarder, M.R.I., 1992. Culture feasibility of pangus (*Pangosiuspangosius,* Ham) in floating net cages. MS. Thesis submitted to the Department of Fisheries Biology and Limnology, BAU,Mymensingh. pp 99.

Shafi, M., Quddus, M.M.A., 1982. Bangladesh Matshya sampad (in Bangla).Dhaka, bangla Academy pp 314-319 .

Shapawi, R., Mustafa, S., Ng, W.K.,2011. A comparison of the Growth performance and Body Composition of the Humpback grouper, (*Cromileptes altivelis*) Fed on farm-made feeds, Commercial Feeds or Trash Fish. Aqua. Sci. 6(5) , 523-534.

Shelton, W.L., POPMA, T.J., 2006. Biology.Culture and Nutrition. Food Products Press, an imprint of The Haworth Press, Inc, NY 139041580, USA. pp 645.

Sundararaj, B.I., Nath, P., Halberg, F., 1982. Circadian meal timing in relation to the lighting schedule optimizes catfish body weight gain. J. Nutr. 112,1085-1097.

Wang, N., Hayward, R.S., Noltie, D.B., 1998. Effect of feeding frequency of food consumption, growth, size variation, and feeding pattern of age-0 hybrid sunfish. Aquaculture 165, 261-267.

Wilder, I.B., Stanley, J.G., 1983. RNA:DNA ratio as an index of growth in salmonid fishes in the laboratory and in streams contaminated by carbaryl. J.Fish.Biol. 22, 165-172.

Appendices

Appendices

Appendix A

Condition factor

30th days

Descriptives

Condition factor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	1.1633	.03215	.01856	1.0835	1.2432	1.14	1.20
TR2	3	1.4000	.30000	.17321	.6548	2.1452	1.10	1.70
TR3	3	1.0533	.03786	.02186	.9593	1.1474	1.01	1.08
TR4	3	1.1267	.01528	.00882	1.0887	1.1646	1.11	1.14
Total	12	1.1858	.18774	.05419	1.0666	1.3051	1.01	1.70

ANOVA

Condition factor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.202	3	.067	2.910	.101
Within Groups	.185	8	.023		

Total	.388	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Condition factor

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-.23667	.12430	.299	-.6347	.1614
	TR3	.11000	.12430	.813	-.2880	.5080
	TR4	.03667	.12430	.990	-.3614	.4347
TR2	TR1	.23667	.12430	.299	-.1614	.6347
	TR3	.34667	.12430	.090	-.0514	.7447
	TR4	.27333	.12430	.203	-.1247	.6714
TR3	TR1	-.11000	.12430	.813	-.5080	.2880
	TR2	-.34667	.12430	.090	-.7447	.0514
	TR4	-.07333	.12430	.932	-.4714	.3247
TR4	TR1	-.03667	.12430	.990	-.4347	.3614
	TR2	-.27333	.12430	.203	-.6714	.1247
	TR3	.07333	.12430	.932	-.3247	.4714

Homogeneous Subsets

Condition factor

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
TR3	3	1.0533
TR4	3	1.1267
TR1	3	1.1633
TR2	3	1.4000
Sig.		.090

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

60th day

Descriptives

Condition factor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	1.4933	.03786	.02186	1.3993	1.5874	1.45	1.52
TR2	3	1.4867	.01155	.00667	1.4580	1.5154	1.48	1.50
TR3	3	1.5967	.02517	.01453	1.5342	1.6592	1.57	1.62
TR4	3	1.5633	.01155	.00667	1.5346	1.5920	1.55	1.57
Total	12	1.5350	.05283	.01525	1.5014	1.5686	1.45	1.62

ANOVA

Condition factor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.026	3	.009	14.876	.001
Within Groups	.005	8	.001		
Total	.031	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Condition factor
Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound

TR1	TR2	.00667	.01972	.986	-.0565	.0698
	TR3	-.10333*	.01972	.003	-.1665	-.0402
	TR4	-.07000*	.01972	.031	-.1332	-.0068
TR2	TR1	-.00667	.01972	.986	-.0698	.0565
	TR3	-.11000*	.01972	.002	-.1732	-.0468
	TR4	-.07667*	.01972	.019	-.1398	-.0135
TR3	TR1	.10333*	.01972	.003	.0402	.1665
	TR2	.11000*	.01972	.002	.0468	.1732
	TR4	.03333	.01972	.387	-.0298	.0965
TR4	TR1	.07000*	.01972	.031	.0068	.1332
	TR2	.07667*	.01972	.019	.0135	.1398
	TR3	-.03333	.01972	.387	-.0965	.0298

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Condition factor

Tukey HSD[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
TR2	3	1.4867	
TR1	3	1.4933	
TR4	3		1.5633
TR3	3		1.5967
Sig.		.986	.387

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

90th day

Descriptives

Condition factor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	1.4967	.00577	.00333	1.4823	1.5110	1.49	1.50
TR2	3	1.6133	.03215	.01856	1.5335	1.6932	1.59	1.65
TR3	3	1.5133	.03512	.02028	1.4261	1.6006	1.48	1.55
TR4	3	1.4000	.01732	.01000	1.3570	1.4430	1.39	1.42
Total	12	1.5058	.08196	.02366	1.4538	1.5579	1.39	1.65

ANOVA

Condition factor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.069	3	.023	35.226	.000
Within Groups	.005	8	.001		
Total	.074	11			

Multiple Comparisons

Dependent Variable: Condition factor

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-.11667*	.02082	.002	-.1833	-.0500
	TR3	-.01667	.02082	.852	-.0833	.0500
	TR4	.09667*	.02082	.007	.0300	.1633
TR2	TR1	.11667*	.02082	.002	.0500	.1833
	TR3	.10000*	.02082	.006	.0333	.1667
	TR4	.21333*	.02082	.000	.1467	.2800
TR3	TR1	.01667	.02082	.852	-.0500	.0833
	TR2	-.10000*	.02082	.006	-.1667	-.0333
	TR4	.11333*	.02082	.003	.0467	.1800
TR4	TR1	-.09667*	.02082	.007	-.1633	-.0300
	TR2	-.21333*	.02082	.000	-.2800	-.1467
	TR3	-.11333*	.02082	.003	-.1800	-.0467

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Condition factor

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
TR4	3	1.4000		
TR1	3		1.4967	
TR3	3		1.5133	
TR2	3			1.6133
Sig.		1.000	.852	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Average daily gain

30th day

Descriptives

Average daily gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	.0267	.01528	.00882	-.0113	.0646	.01	.04
TR2	3	.1267	.01528	.00882	.0887	.1646	.11	.14
TR3	3	.1833	.01528	.00882	.1454	.2213	.17	.20
TR4	3	.0867	.01528	.00882	.0487	.1246	.07	.10
Total	12	.1058	.06112	.01764	.0670	.1447	.01	.20

ANOVA

Average daily gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.039	3	.013	56.036	.000
Within Groups	.002	8	.000		
Total	.041	11			

Multiple Comparisons

Dependent Variable: Average daily gain

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-.10000*	.01247	.000	-.1399	-.0601
	TR3	-.15667*	.01247	.000	-.1966	-.1167
	TR4	-.06000*	.01247	.006	-.0999	-.0201
TR2	TR1	.10000*	.01247	.000	.0601	.1399
	TR3	-.05667*	.01247	.008	-.0966	-.0167
	TR4	.04000*	.01247	.050	.0001	.0799
TR3	TR1	.15667*	.01247	.000	.1167	.1966
	TR2	.05667*	.01247	.008	.0167	.0966
	TR4	.09667*	.01247	.000	.0567	.1366
TR4	TR1	.06000*	.01247	.006	.0201	.0999
	TR2	-.04000*	.01247	.050	-.0799	-.0001
	TR3	-.09667*	.01247	.000	-.1366	-.0567

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
TR1	3	.0267			
TR4	3		.0867		
TR2	3			.1267	
TR3	3				.1833
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

60th day

Descriptives

Average daily gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	.0900	.01000	.00577	.0652	.1148	.08	.10

TR2	3	.2633	.01528	.00882	.2254	.3013	.25	.28
TR3	3	.2000	.01000	.00577	.1752	.2248	.19	.21
TR4	3	.1067	.01528	.00882	.0687	.1446	.09	.12
Total	12	.1650	.07453	.02151	.1176	.2124	.08	.28

ANOVA

Average daily gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.060	3	.020	119.533	.000
Within Groups	.001	8	.000		
Total	.061	11			

Multiple Comparisons

Dependent Variable: Average daily gain

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-.17333*	.01054	.000	-.2071	-.1396
	TR3	-.11000*	.01054	.000	-.1438	-.0762
	TR4	-.01667	.01054	.439	-.0504	.0171
TR2	TR1	.17333*	.01054	.000	.1396	.2071
	TR3	.06333*	.01054	.001	.0296	.0971
	TR4	.15667*	.01054	.000	.1229	.1904
TR3	TR1	.11000*	.01054	.000	.0762	.1438
	TR2	-.06333*	.01054	.001	-.0971	-.0296
	TR4	.09333*	.01054	.000	.0596	.1271
TR4	TR1	.01667	.01054	.439	-.0171	.0504
	TR2	-.15667*	.01054	.000	-.1904	-.1229
	TR3	-.09333*	.01054	.000	-.1271	-.0596

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Average daily gain

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05

		1	2	3
TR1	3	.0900		
TR4	3	.1067		
TR3	3		.2000	
TR2	3			.2633
Sig.		.439	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

90th day

Descriptives

Average daily gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	.1633	.02517	.01453	.1008	.2258	.14	.19
TR2	3	.2733	.02082	.01202	.2216	.3250	.25	.29
TR3	3	.3000	.03000	.01732	.2255	.3745	.27	.33
TR4	3	.0900	.01000	.00577	.0652	.1148	.08	.10
Total	12	.2067	.09049	.02612	.1492	.2642	.08	.33

ANOVA

Average daily gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.086	3	.029	55.441	.000
Within Groups	.004	8	.001		
Total	.090	11			

Multiple Comparisons

Dependent Variable: Average daily gain
Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound

TR1	TR2	-.11000*	.01856	.002	-.1694	-.0506
	TR3	-.13667*	.01856	.000	-.1961	-.0772
	TR4	.07333*	.01856	.018	.0139	.1328
TR2	TR1	.11000*	.01856	.002	.0506	.1694
	TR3	-.02667	.01856	.513	-.0861	.0328
	TR4	.18333*	.01856	.000	.1239	.2428
TR3	TR1	.13667*	.01856	.000	.0772	.1961
	TR2	.02667	.01856	.513	-.0328	.0861
	TR4	.21000*	.01856	.000	.1506	.2694
TR4	TR1	-.07333*	.01856	.018	-.1328	-.0139
	TR2	-.18333*	.01856	.000	-.2428	-.1239
	TR3	-.21000*	.01856	.000	-.2694	-.1506

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Average daily gain

Tukey HSD[a]

		Subset for alpha = 0.05		
Treatment	N	1	2	3
TR4	3	.0900		
TR1	3		.1633	
TR2	3			.2733
TR3	3			.3000
Sig.		1.000	1.000	.513

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Specific Growth Rate(SGR)

30th day

Descriptives

Specific growth rate

N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
TR1	3	6.2133	.09074	.05239	5.9879	6.4387	6.11	6.28
TR2	3	11.1367	.01155	.00667	11.1080	11.1654	11.13	11.15
TR3	3	11.9033	.04509	.02603	11.7913	12.0153	11.86	11.95
TR4	3	8.6367	.45611	.26333	7.5036	9.7697	8.20	9.11
Total	12	9.4725	2.34394	.67664	7.9832	10.9618	6.11	11.95

ANOVA

Specific growth rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.998	3	19.999	366.230	.000
Within Groups	.437	8	.055		
Total	60.434	11			

Multiple Comparisons

Dependent Variable: Specific growth rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-4.92333*	.19080	.000	-5.5343	-4.3123
	TR3	-5.69000*	.19080	.000	-6.3010	-5.0790
	TR4	-2.42333*	.19080	.000	-3.0343	-1.8123
TR2	TR1	4.92333*	.19080	.000	4.3123	5.5343
	TR3	-.76667*	.19080	.016	-1.3777	-.1557
	TR4	2.50000*	.19080	.000	1.8890	3.1110
TR3	TR1	5.69000*	.19080	.000	5.0790	6.3010
	TR2	.76667*	.19080	.016	.1557	1.3777
	TR4	3.26667*	.19080	.000	2.6557	3.8777
TR4	TR1	2.42333*	.19080	.000	1.8123	3.0343
	TR2	-2.50000*	.19080	.000	-3.1110	-1.8890
	TR3	-3.26667*	.19080	.000	-3.8777	-2.6557

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Specific growth rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
TR1	3	6.2133			
TR4	3		8.6367		
TR2	3			11.1367	
TR3	3				11.9033
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

60th day

Descriptives

Specific growth rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	4.4500	.65000	.37528	2.8353	6.0647	4.05	5.20
TR2	3	3.2133	.58654	.33864	1.7563	4.6704	2.85	3.89
TR3	3	2.5200	.03000	.01732	2.4455	2.5945	2.49	2.55
TR4	3	1.6200	.07000	.04041	1.4461	1.7939	1.55	1.69
Total	12	2.9508	1.14273	.32988	2.2248	3.6769	1.55	5.20

ANOVA

Specific growth rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.819	3	4.273	22.131	.000
Within Groups	1.545	8	.193		
Total	14.364	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Specific growth rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	1.23667*	.35878	.036	.0877	2.3856
	TR3	1.93000*	.35878	.003	.7811	3.0789
	TR4	2.83000*	.35878	.000	1.6811	3.9789
TR2	TR1	-1.23667*	.35878	.036	-2.3856	-.0877
	TR3	.69333	.35878	.288	-.4556	1.8423
	TR4	1.59333*	.35878	.009	.4444	2.7423
TR3	TR1	-1.93000*	.35878	.003	-3.0789	-.7811
	TR2	-.69333	.35878	.288	-1.8423	.4556
	TR4	.90000	.35878	.133	-.2489	2.0489
TR4	TR1	-2.83000*	.35878	.000	-3.9789	-1.6811
	TR2	-1.59333*	.35878	.009	-2.7423	-.4444
	TR3	-.90000	.35878	.133	-2.0489	.2489

*. The mean difference is significant at the 0.05 level.

Homogenous subsets

Specific growth rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
TR4	3	1.6200		
TR3	3	2.5200	2.5200	
TR2	3		3.2133	
TR1	3			4.4500
Sig.		.133	.288	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

90th day

Descriptives

Specific growth rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	2.6667	.20207	.11667	2.1647	3.1686	2.55	2.90
TR2	3	2.5533	2.15361	1.24339	-2.7965	7.9032	1.29	5.04
TR3	3	1.2767	.04619	.02667	1.1619	1.3914	1.25	1.33
TR4	3	.7600	.03606	.02082	.6704	.8496	.72	.79
Total	12	1.8142	1.25714	.36291	1.0154	2.6129	.72	5.04

ANOVA

Specific growth rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.020	3	2.673	2.284	.156
Within Groups	9.365	8	1.171		
Total	17.384	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Specific growth rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	.11333	.88339	.999	-2.7156	2.9423
	TR3	1.39000	.88339	.443	-1.4389	4.2189
	TR4	1.90667	.88339	.214	-.9223	4.7356
TR2	TR1	-.11333	.88339	.999	-2.9423	2.7156
	TR3	1.27667	.88339	.509	-1.5523	4.1056
	TR4	1.79333	.88339	.254	-1.0356	4.6223
TR3	TR1	-1.39000	.88339	.443	-4.2189	1.4389
	TR2	-1.27667	.88339	.509	-4.1056	1.5523
	TR4	.51667	.88339	.934	-2.3123	3.3456
TR4	TR1	-1.90667	.88339	.214	-4.7356	.9223

	TR2	-1.79333	.88339	.254	-4.6223	1.0356
	TR3	-.51667	.88339	.934	-3.3456	2.3123

Homogenous subsets

Specific growth rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05 1
TR4	3	.7600
TR3	3	1.2767
TR2	3	2.5533
TR1	3	2.6667
Sig.		.214

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Feed Conversion Ratio

30th day

Descriptives

Feed conversion ratio

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	2.3000	.34641	.20000	1.4395	3.1605	1.90	2.50
TR2	3	1.8333	.12583	.07265	1.5208	2.1459	1.70	1.95
TR3	3	2.2467	.26274	.15169	1.5940	2.8994	2.09	2.55
TR4	3	2.1400	.08718	.05033	1.9234	2.3566	2.08	2.24
Total	12	2.1300	.27250	.07866	1.9569	2.3031	1.70	2.55

ANOVA

Feed conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.392	3	.131	2.459	.137
Within Groups	.425	8	.053		
Total	.817	11			

Post Hoc Tests

Homogenous subsets

Multiple Comparisons

Dependent Variable: Feed conversion ratio

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	.46667	.18818	.138	-.1359	1.0693
	TR3	.05333	.18818	.991	-.5493	.6559
	TR4	.16000	.18818	.830	-.4426	.7626
TR2	TR1	-.46667	.18818	.138	-1.0693	.1359
	TR3	-.41333	.18818	.204	-1.0159	.1893
	TR4	-.30667	.18818	.416	-.9093	.2959
TR3	TR1	-.05333	.18818	.991	-.6559	.5493
	TR2	.41333	.18818	.204	-.1893	1.0159
	TR4	.10667	.18818	.939	-.4959	.7093
TR4	TR1	-.16000	.18818	.830	-.7626	.4426
	TR2	.30667	.18818	.416	-.2959	.9093
	TR3	-.10667	.18818	.939	-.7093	.4959

Homogeneous Subsets

Feed conversion ratio

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05 1
TR2	3	1.8333
TR4	3	2.1400
TR3	3	2.2467
TR1	3	2.3000
Sig.		.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

60th day

Descriptives

Feed conversion ratio

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	 Upper Bound	Minimum	Maximum
TR1	3	2.3667	.25166	.14530	1.7415	2.9918	2.10	2.60
TR2	3	1.6833	.07638	.04410	1.4936	1.8731	1.60	1.75
TR3	3	2.3000	.10536	.06083	2.0383	2.5617	2.19	2.40
TR4	3	2.6500	.40927	.23629	1.6333	3.6667	2.30	3.10
Total	12	2.2500	.42503	.12270	1.9799	2.5201	1.60	3.10

ANOVA

Feed conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	1.492	3	.497	8.027	.009
Within Groups	.496	8	.062		
Total	1.987	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Feed conversion ratio

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	-.00667	.04055	.998	-.1365	.1232
	Treatment3	-.01667	.04055	.975	-.1465	.1132
	Treatment4	-.05000	.04055	.625	-.1799	.0799
Treatment2	Treatment1	.00667	.04055	.998	-.1232	.1365
	Treatment3	-.01000	.04055	.994	-.1399	.1199
	Treatment4	-.04333	.04055	.717	-.1732	.0865
Treatment3	Treatment1	.01667	.04055	.975	-.1132	.1465
	Treatment2	.01000	.04055	.994	-.1199	.1399
	Treatment4	-.03333	.04055	.843	-.1632	.0965
Treatment4	Treatment1	.05000	.04055	.625	-.0799	.1799
	Treatment2	.04333	.04055	.717	-.0865	.1732
	Treatment3	.03333	.04055	.843	-.0965	.1632

Homogeneous Subsets

Feed conversion ratio

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
TR2	3	1.6833	
TR3	3	2.3000	2.3000
TR1	3		2.3667
TR4	3		2.6500
Sig.		.063	.373

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

90th day

Descriptives

Feed conversion ratio

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	2.3000	.20000	.11547	1.8032	2.7968	2.10	2.50
TR2	3	1.7833	.17559	.10138	1.3471	2.2195	1.60	1.95
TR3	3	2.3500	.22113	.12767	1.8007	2.8993	2.18	2.60
TR4	3	2.6967	.46715	.26971	1.5362	3.8571	2.36	3.23
Total	12	2.2825	.42132	.12162	2.0148	2.5502	1.60	3.23

ANOVA

Feed conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.277	3	.426	5.037	.030
Within Groups	.676	8	.084		
Total	1.953	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Feed conversion ratio

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	.51667	.23733	.209	-.2434	1.2767
	TR3	-.05000	.23733	.996	-.8100	.7100
	TR4	-.39667	.23733	.396	-1.1567	.3634
TR2	TR1	-.51667	.23733	.209	-1.2767	.2434
	TR3	-.56667	.23733	.157	-1.3267	.1934
	TR4	-.91333*	.23733	.020	-1.6734	-.1533

TR3	TR1	.05000	.23733	.996	-.7100	.8100
	TR2	.56667	.23733	.157	-.1934	1.3267
	TR4	-.34667	.23733	.501	-1.1067	.4134
TR4	TR1	.39667	.23733	.396	-.3634	1.1567
	TR2	.91333*	.23733	.020	.1533	1.6734
	TR3	.34667	.23733	.501	-.4134	1.1067

*. The mean difference is significant at the 0.05 level.

Homogenous subsets

Feed conversion ratio

Tukey HSD[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
TR2	3	1.7833	
TR1	3	2.3000	2.3000
TR3	3	2.3500	2.3500
TR4	3		2.6967
Sig.		.157	.396

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Survival rate(%)

30th day

Descriptives

Survival Rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Treatment 1	3	87.0300	8.48104	4.89653	65.9619	108.0981	77.78	94.44

Treatment 2	3	88.8800	5.55501	3.20718	75.0806	102.6794	83.33	94.44
Treatment 3	3	85.1767	3.19852	1.84667	77.2311	93.1222	83.33	88.87
Treatment 4	3	83.3200	9.61288	5.55000	59.4403	107.1997	72.22	88.87
Total	12	86.1017	6.48334	1.87158	81.9823	90.2210	72.22	94.44

ANOVA

Survival Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51.523	3	17.174	.334	.801
Within Groups	410.848	8	51.356		
Total	462.371	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Survival Rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	-1.85000	5.85127	.988	-20.5878	16.8878
	Treatment3	1.85333	5.85127	.988	-16.8845	20.5912
	Treatment4	3.71000	5.85127	.918	-15.0278	22.4478
Treatment2	Treatment1	1.85000	5.85127	.988	-16.8878	20.5878
	Treatment3	3.70333	5.85127	.919	-15.0345	22.4412
	Treatment4	5.56000	5.85127	.780	-13.1778	24.2978
Treatment3	Treatment1	-1.85333	5.85127	.988	-20.5912	16.8845
	Treatment2	-3.70333	5.85127	.919	-22.4412	15.0345
	Treatment4	1.85667	5.85127	.988	-16.8812	20.5945
Treatment4	Treatment1	-3.71000	5.85127	.918	-22.4478	15.0278
	Treatment2	-5.56000	5.85127	.780	-24.2978	13.1778
	Treatment3	-1.85667	5.85127	.988	-20.5945	16.8812

Homogenous subsets

Survival Rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment4	3	83.3200
Treatment3	3	85.1767
Treatment1	3	87.0300
Treatment2	3	88.8800
Sig.		.780

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

60th day

Descriptives

Survival Rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Treatment 1	3	84.4667	11.63096	6.71514	55.5738	113.3596	71.42	93.75
Treatment 2	3	91.8833	8.90987	5.14412	69.7500	114.0167	82.35	100.00
Treatment 3	3	93.4500	.25981	.15000	92.8046	94.0954	93.30	93.75
Treatment 4	3	93.2500	.82310	.47522	91.2053	95.2947	92.30	93.75
Total	12	90.7625	7.34681	2.12084	86.0946	95.4304	71.42	100.00

ANOVA

Survival Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	162.912	3	54.304	1.008	.438
Within Groups	430.820	8	53.853		
Total	593.732	11			

Post Hoc tests

Multiple Comparisons

Dependent Variable: Survival Rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	-7.41667	5.99180	.622	-26.6045	11.7712
	Treatment3	-8.98333	5.99180	.480	-28.1712	10.2045
	Treatment4	-8.78333	5.99180	.498	-27.9712	10.4045
Treatment2	Treatment1	7.41667	5.99180	.622	-11.7712	26.6045
	Treatment3	-1.56667	5.99180	.993	-20.7545	17.6212
	Treatment4	-1.36667	5.99180	.995	-20.5545	17.8212
Treatment3	Treatment1	8.98333	5.99180	.480	-10.2045	28.1712
	Treatment2	1.56667	5.99180	.993	-17.6212	20.7545
	Treatment4	.20000	5.99180	1.000	-18.9879	19.3879
Treatment4	Treatment1	8.78333	5.99180	.498	-10.4045	27.9712
	Treatment2	1.36667	5.99180	.995	-17.8212	20.5545
	Treatment3	-.20000	5.99180	1.000	-19.3879	18.9879

Homogenous subsets

Survival Rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1

Treatment1	3	84.4667
Treatment2	3	91.8833
Treatment4	3	93.2500
Treatment3	3	93.4500
Sig.		.480

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

90th day

Descriptives

Survival Rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Treatment 1	3	92.2000	1.90526	1.10000	87.4671	96.9329	90.00	93.30
Treatment 2	3	88.6933	3.67606	2.12238	79.5615	97.8252	85.71	92.80
Treatment 3	3	90.6200	4.25813	2.45843	80.0422	101.1978	85.71	93.30
Treatment 4	3	87.7567	5.08779	2.93744	75.1179	100.3954	83.30	93.30
Total	12	89.8175	3.78772	1.09342	87.4109	92.2241	83.30	93.30

ANOVA

Survival Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.493	3	11.831	.774	.541
Within Groups	122.322	8	15.290		

Total	157.815	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Survival Rate

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	3.50667	3.19272	.700	-6.7175	13.7309
	Treatment3	1.58000	3.19272	.958	-8.6442	11.8042
	Treatment4	4.44333	3.19272	.537	-5.7809	14.6675
Treatment2	Treatment1	-3.50667	3.19272	.700	-13.7309	6.7175
	Treatment3	-1.92667	3.19272	.928	-12.1509	8.2975
	Treatment4	.93667	3.19272	.991	-9.2875	11.1609
Treatment3	Treatment1	-1.58000	3.19272	.958	-11.8042	8.6442
	Treatment2	1.92667	3.19272	.928	-8.2975	12.1509
	Treatment4	2.86333	3.19272	.807	-7.3609	13.0875
Treatment4	Treatment1	-4.44333	3.19272	.537	-14.6675	5.7809
	Treatment2	-.93667	3.19272	.991	-11.1609	9.2875
	Treatment3	-2.86333	3.19272	.807	-13.0875	7.3609

Homogenous subsets

Survival Rate

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment4	3	87.7567
Treatment2	3	88.6933
Treatment3	3	90.6200
Treatment1	3	92.2000
Sig.		.537

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Temperature

Descriptives

Temperature

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Treatment 1	3	23.7000	.17321	.10000	23.2697	24.1303	23.50	23.80
Treatment 2	3	23.8333	.11547	.06667	23.5465	24.1202	23.70	23.90
Treatment 3	3	23.7000	.10000	.05774	23.4516	23.9484	23.60	23.80
Treatment 4	3	23.6667	.05774	.03333	23.5232	23.8101	23.60	23.70
Total	12	23.7250	.12154	.03509	23.6478	23.8022	23.50	23.90

ANOVA

Temperature

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.049	3	.016	1.157	.384
Within Groups	.113	8	.014		
Total	.163	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Temperature

Tukey HSD

(I) Treatment	(J) Treatment	Mean	Std. Error	Sig.	95% Confidence Interval

		Difference (I-J)			Lower Bound	Upper Bound
Treatment1	Treatment2	-.13333	.09718	.548	-.4445	.1779
	Treatment3	.00000	.09718	1.000	-.3112	.3112
	Treatment4	.03333	.09718	.985	-.2779	.3445
Treatment2	Treatment1	.13333	.09718	.548	-.1779	.4445
	Treatment3	.13333	.09718	.548	-.1779	.4445
	Treatment4	.16667	.09718	.376	-.1445	.4779
Treatment3	Treatment1	.00000	.09718	1.000	-.3112	.3112
	Treatment2	-.13333	.09718	.548	-.4445	.1779
	Treatment4	.03333	.09718	.985	-.2779	.3445
Treatment4	Treatment1	-.03333	.09718	.985	-.3445	.2779
	Treatment2	-.16667	.09718	.376	-.4779	.1445
	Treatment3	-.03333	.09718	.985	-.3445	.2779

Homogenous subsets

Temperature

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment4	3	23.6667
Treatment1	3	23.7000
Treatment3	3	23.7000
Treatment2	3	23.8333
Sig.		.376

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Dissolved oxygen

Descriptives

Dissolved oxygen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
Treatment 1	3	7.5333	.45092	.26034	6.4132	8.6535	7.10	8.00
Treatment 2	3	7.5167	.24664	.14240	6.9040	8.1294	7.35	7.80
Treatment 3	3	7.8167	.30551	.17638	7.0578	8.5756	7.55	8.15
Treatment 4	3	7.9500	.13229	.07638	7.6214	8.2786	7.80	8.05
Total	12	7.7042	.32506	.09384	7.4976	7.9107	7.10	8.15

ANOVA

Dissolved oxygen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.412	3	.137	1.466	.295
Within Groups	.750	8	.094		
Total	1.162	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Dissolved oxygen

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	.01667	.25000	1.000	-.7839	.8173
	Treatment3	-.28333	.25000	.681	-1.0839	.5173
	Treatment4	-.41667	.25000	.398	-1.2173	.3839
Treatment2	Treatment1	-.01667	.25000	1.000	-.8173	.7839
	Treatment3	-.30000	.25000	.644	-1.1006	.5006
	Treatment4	-.43333	.25000	.368	-1.2339	.3673
Treatment3	Treatment1	.28333	.25000	.681	-.5173	1.0839
	Treatment2	.30000	.25000	.644	-.5006	1.1006
	Treatment4	-.13333	.25000	.948	-.9339	.6673
Treatment4	Treatment1	.41667	.25000	.398	-.3839	1.2173

Treatment2	.43333	.25000	.368	-.3673	1.2339
Treatment3	.13333	.25000	.948	-.6673	.9339

Homogenous subsets

Dissolved oxygen

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment2	3	7.5167
Treatment1	3	7.5333
Treatment3	3	7.8167
Treatment4	3	7.9500
Sig.		.368

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Concentration of hydrogen ion(P^H)

Descriptives

Hydrogen ion concentration

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Treatment 1	3	8.1167	.01528	.00882	8.0787	8.1546	8.10	8.13
Treatment 2	3	8.0200	.13454	.07767	7.6858	8.3542	7.87	8.13
Treatment 3	3	8.0533	.02082	.01202	8.0016	8.1050	8.03	8.07
Treatment 4	3	8.0533	.02309	.01333	7.9960	8.1107	8.04	8.08
Total	12	8.0608	.06960	.02009	8.0166	8.1051	7.87	8.13

Test of Homogeneity of Variances

Hydrogen ion concentration

Levene Statistic	df1	df2	Sig.
6.670	3	8	.014

ANOVA

Hydrogen ion concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	3	.005	1.015	.435
Within Groups	.039	8	.005		
Total	.053	11			

Post Hoc tests

Multiple Comparisons

Dependent Variable: Hydrogen ion concentration

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment1	Treatment2	.09667	.05672	.381	-.0850	.2783
	Treatment3	.06333	.05672	.690	-.1183	.2450
	Treatment4	.06333	.05672	.690	-.1183	.2450
Treatment2	Treatment1	-.09667	.05672	.381	-.2783	.0850
	Treatment3	-.03333	.05672	.933	-.2150	.1483
	Treatment4	-.03333	.05672	.933	-.2150	.1483
Treatment3	Treatment1	-.06333	.05672	.690	-.2450	.1183
	Treatment2	.03333	.05672	.933	-.1483	.2150
	Treatment4	.00000	.05672	1.000	-.1816	.1816
Treatment4	Treatment1	-.06333	.05672	.690	-.2450	.1183
	Treatment2	.03333	.05672	.933	-.1483	.2150
	Treatment3	.00000	.05672	1.000	-.1816	.1816

Homogenous subsets

Hydrogen ion concentration

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment2	3	8.0200
Treatment3	3	8.0533
Treatment4	3	8.0533
Treatment1	3	8.1167
Sig.		.381

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix B

RNA:DNA

Descriptives

Ratio

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
TR1	3	.5783	.20185	.11654	.0769	1.0798	.39	.79
TR2	2	.9394	.10846	.07669	-.0350	1.9139	.86	1.02
TR3	3	.8081	.00838	.00484	.7873	.8289	.80	.82
TR4	3	.8130	.01506	.00870	.7756	.8504	.80	.82
Total	11	.7707	.16473	.04967	.6600	.8813	.39	1.02

ANOVA

Ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.178	3	.059	4.414	.048
Within Groups	.094	7	.013		
Total	.271	10			

Multiple Comparisons

Dependent Variable: Ratio

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TR1	TR2	-.36110*	.10570	.044	-.7110	-.0112
	TR3	-.22977	.09454	.158	-.5427	.0832
	TR4	-.23465	.09454	.148	-.5476	.0783
TR2	TR1	.36110*	.10570	.044	.0112	.7110
	TR3	.13133	.10570	.622	-.2185	.4812
	TR4	.12645	.10570	.648	-.2234	.4763
TR3	TR1	.22977	.09454	.158	-.0832	.5427
	TR2	-.13133	.10570	.622	-.4812	.2185
	TR4	-.00489	.09454	1.000	-.3178	.3081
TR4	TR1	.23465	.09454	.148	-.0783	.5476
	TR2	-.12645	.10570	.648	-.4763	.2234
	TR3	.00489	.09454	1.000	-.3081	.3178

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Ratio

Tukey HSD[a,b]

		Subset for alpha = 0.05	
Treatment	N	1	2

TR1	3	.5783	
TR3	3	.8081	.8081
TR4	3	.8130	.8130
TR2	2		.9394
Sig.		.178	.586

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.667.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.