Effect of different Tilapia feeds on the species diversity, richness and evenness of benthos community

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By

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DECLARATION

Effect of different Tilapia feeds on the species diversity, richness and evenness of benthos community

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

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MY BELOVED PARENTS AND HUSBANDS

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Contents

List of figures

List of Tables

Abstract

Aquatic macro-invertebrates play significant role in responding to a variety of environmental conditions of rivers and streams and therefore may be used as bio-indicators for water quality assessment. They are important for the assessment of water quality of rivers, lakes and streams as bio-indicators because they can be easily caught and seen with naked eyes. This study was conducted to assess the effect of different experimental diets on the species diversity, richness and evenness of benthos community in tilapia ponds. This study was conducted in total 36 ponds where two different feed experiments (two diets for experiment-1 and two other diets for experiment-2) (feed-1, feed-2) with two doses (single dose and double doses) were applied in tilapia culture to assess its effects on the macro-benthos community structure in culture system. There were two control groups (C1, C2) and four treatments (T1, T2, T3, T4) on the type of feed and number of doses with 6 replications for each. Benthos species diversity, richness and evenness were determined by seven sampling in which 4 samples were taken under experiment-1 and 3 samples were taken under experiment-2 in the tilapia culture period using Ekman dredger. The study revealed the presence of three major groups of macro-benthos; gastropods, Oligochaeta and Bivalvia comprising 9 species under 2 phylum. Gastropoda included 5 species (*Pila globosa, Septaria porcallana, Indoplanorbis exustus, Melanoides tuberculata and Bellamya chinensis*), Bivalvia included 3 species (*Anondonta californiensis, Neritina reclivata and Mercenaria campechiensis*) and Oligochaeta included 1 species *Nais simplex.* Shannon diversity index demonstrated that in experiment-1, higher diversity in macro-benthos community was found in C2 compared to T2 and T3 ($P<0.05$) as well as other treatments. However, in experiment-2 C2 also showed higher species diversity than that of T2 ($P<0.05$). In experiment-2, Menhinick's richness of macro-benthos species was found higher in C2 compared to T2 and T4 ($P<0.02$). In contrast, it was found insignificant in experiment-1. Finally, Pielou's evenness indices was found higher in T3 compared to T1 and T2 in experiment-1 ($P<0.05$). Nevertheless, it was higher in C2 in experiment-2 than that of T2 and T4. In conclusion, different diets or even aquatic environment with no diets might contribute in community structure of macro-benthos species. Therefore, further studies should be conducted to go for more micro-community structure of macro-benthos.

CHAPTER ONE

INTRODUCTION

1 Introduction

Benthic macroscopic organisms are bottom dwelling organisms who live on or inside the deposit at the bottom in aquatic environment (Barnes *et al.*, 2006; Idowu and Ugwumba, 2005). They are considered as important and integral biodiversity in aquatic ecosystem (Hossain, 2011). During their different life stages (e.g. larvae to adult stages) they are used as food by other animals. Most macro-benthos process and oxygenate the underlying sediments as well as break down organic matter before bacterial demineralization. They play significant roles in the energy pathway and nutrient cycling and they also constitute an important link in the aquatic food chain as food resource for fishes and other animals (Mustafa *et al.*, 2015). Most importantly, macrozoobenthos have been extensively used for the assessment of the ecological integrity and bio-monitoring of aquatic habitats (Nazarova *et al.*, 2004). That means macrobenthos response to the environmental changes in aquatic habitats, therefore are considered as good indicators of hydrologic stress and aquatic ecosystem health in general (Nazarova *et al.*, 2004). Benthic communities are often used as biological indicators because they can provide information on environmental conditions either due to the sensitivity of single species (indicator species) or because of some general feature that makes them integrate environmental signals over a long period of time. Bioindicators include biological processes, species, or communities and are used to assess the quality of the environment and how it changes over time.

Benthic organisms are rich in different essential nutrients, including amino acid, fatty acid, vitamins and mineral, and are generally consumed by humans. Besides different types of worms are used for recreational purposes as fishing bait in addition to using them in ornamental and recreational purposes (Belal and Ghobashy, 2014). In the present days it is a concern that aquaculture and its inputs have significant impact on the benthic community in the culture environment.

Aquaculture can impact the environment in a number of ways. This sector can strongly affect the natural fish population through larvae and broodfish collection, use of trash fish for fish as the source of fish meal, disposal of toxic chemicals, and high loads of nutrients and organic matter (Naylor *et al.*, 2000; Hargrave *et al.*, 1993; Holmer and Kristensen, 1992; Karakassis *et al.*, 1998; Christensen *et al.*, 2000). It can generate user conflicts, alter the hydrological regime, induce exotic species to the wild, and pollute water resources (Meghea *et al.*, 2009; Petrovici *et* *al*., 2010; Paulovits *et al*., 2007). Nutrition and feeding play a central and essential role in the sustainable development of aquaculture and, therefore, fertilizers and feed resources continue to dominate aquaculture needs. An intensive feeding of fish increases the amount of organic matters in the ecosystem, which together with fish excrete largely contribute to the aquatic environment pollution downstream to the farms. Deterioration of the water quality is indicated by the increased nitrogen and phosphorus fractions, decreased dissolved oxygen, and enhanced biological organic discharge (Kirkagaç *et al*., 2004; Romanescu and Cojocaru, 2010). The macro-invertebrates usually serve to measure the water degradation degree in the fish farms due to the fact that these organisms are very sensitive to this type of pollution (Dascalu *et al*., 2009; Heilskov and Holmer, 2001; Murugesan *et al*., 2009 ; Paulovits *et al*., 2007).

The changes observed in sediment chemistry affect benthic communities, usually by changing the abundance values, biomass, species diversity and richness (Weston, 1990). This is a consequence of the enhancement of both aerobic and anaerobic microbial activity in organically enriched sediments, resulting in dissolved oxygen depletion and production of toxic products such as H_2S (Holmer and Kristensen, 1992; Heilskov and Holmer, 2001). They also reported that benthic fauna plays an important role both in supplying and mineralizing organic matter (Heilskov and Holmer, 2001). In other study Gamito (1997) reported that macrobenthos can act as important food source for the aquaculture species (Gamito, 1997). They play an important role in transitional ecosystem, by filtering phytoplankton and then acting as a food source for larger organisms such as fish, thereby linking primary production with higher tropic levels (Anbuchezhian *et al*., 2009). They also structure and oxygenate the bottom by reworking sediments and play a fundamental role in breaking down organic material before bacterial remineralization (Tagliapietra *et al*., 2010). Cichlids (Tilapia) are one of the easiest and most profitable fish to farm due to their omnivorous diet, mode of reproduction tolerance of high stocking density, and rapid growth. Tilapia from aquaculture contains especially high ratios of omega-6 to omega-3 fatty acids (Fessehaye, 2006). Therefore, the analysis of benthic communities in Tilapia fish farm sediments may be valuable to assess the evolution of environmental quality within fish ponds and settlement ponds as well as the importance of the latter. Moreover, fish production in land-based fish farms involves large costs, namely in terms of water demand, oxygen supply, food, antibiotics and electricity. They are also costly in terms of human resources, as fish ponds require daily management due to the rapid growth of several types of primary producers that must be removed to allow water circulation and avoid oxygen depletion (Bartoli *et al.*, 2005). The diversity, richness and evenness of benthic community also differ naturally according to bioclimatic region, the type of ecosystem, types of feeding rate and the specific features of the habitat in which the organisms live such as bottom water salinity, water renewal rate and sediment type (Kripa *et al.*, 2013; Muralidharan *et al.*, 2010; Tagliapietra and Sigovini, 2010).

The distribution and abundance of benthic invertebrates are influenced by a wide variety of physical parameters, such as substrate, water temperature, dissolved oxygen, pH, salinity, and hydrodynamics, as well as disturbance and pollution (Cristini, 1991; Watson and Barnes, 2004). Benthic organisms are good indicators of local environmental conditions and anthropogenic disturbance since they live and feed on the sediment and have limited mobility, thus they cannot avoid exposure to pollution or disturbance (Dauer, 1993). Benthic communities generally respond in stages to habitat disturbance. Response stages include an increase or decrease in abundance; increase or decrease in diversity, and a shift to (or from) a pollution-tolerant to pollution-intolerant assemblage (San Francisco, 2009). When a benthic community is physically disturbed, the community may re-colonize through natural succession to pre-disturbance conditions within approximately one to five years following the cessation of the disturbance (Blake *et al.*, 1996; Van Dolah *et al.*, 1992). However, recovery may take longer if physical characteristics (e.g. sediment, hydrology, etc.) are changed and different species re-colonize (Schaffner *et al.*, 1996; Van Dolah *et al.*, 1994; Wilber and Stern, 1992).

Objective of the study

- To evaluate the effect of different Tilapia feeds on the species diversity, richness and evenness of benthos community.

Chapter

REVIEW OF LITERATURE

1.1 Literature Review

1.1.1 Overview of present aquaculture

Aquaculture has been considered as one of the fastest growing food producing sector with the growing global population and increased demand of fish worldwide (Tidwell and Allan, 2001). Aquaculture is becoming a more popular fish production method as it has an annual increase of 6 percent and is projected to produce over half of the fish consumed by 2025 (FAO, 2016). In contrast, 'capture fisheries' production rates are steadily declining and are predicted to collapse by 2048 (Skillington, 2016). In addition to providing relief for exploited fish populations, the success of aquaculture systems is attributed to the ability to selectively breed and rear fish that have a higher growth rate (Hindar *et al.*, 2006). This increased growth rate can increase fish production and ultimately reduce fish price by almost 2 percent by the year 2030 (World Bank, 2013).

Global fish production in 2016 rose to 171 MT, of which 88% (151MT) were used for human consumption. Aquaculture contributed 53% of fish produced for human consumption. Per capita fish consumption grew to 20.3 kg in 2016. The highest consumption, >50 kg/year, is found in SIDS. Between 1961 and 2016, the average annual increase in global food fish consumption outpaced population growth by a factor of 2. Global capture fisheries production was 90.9 MT in 2016 (79.3mT from marine capture), a small decrease from 2015, largely due to anchoveta, under the influence of China, Indonesia, USA, Russia and Peru are the top producers. Average annual growth in aquaculture was 5.8% in 2000–2016. China has produced >50% of world's aquaculture every year since 1991. In 2016, 37 countries produced more farmed than wild-caught fish- collectively they account for 50% of the world's population.

Fish and other aquatic animals make an 'irreplaceable' contribution to food and nutrition security in many Asian and African countries where large numbers of people are poor and undernourished (Kent, 1987). Fish are a rich source of high quality protein, a range of micronutrients, and fatty acids essential for human brain development (Tacon and Metian, 2013). They are also often the cheapest and most frequently consumed animal-source food in low income food deficit countries (World Bank, 2006), making an important contribution to diversity in otherwise monotonous diets dominated by starchy staples (Thilsted, 2013; Leroy and Frongillo, 2007). This role is particularly important in countries such as Bangladesh, Cambodia, Ghana, Nigeria, and the Pacific islands, where many people are impoverished and fish is by far the most frequently consumed animal-source food (Belton *et al.*, 2011; Hortle, 2007; Biederlack and Rivers, 2009; Gomna and Rana, 2007; Bell *et al.*, 2009). Among them Tilapia has become the third most important fish in aquaculture after carp and salmon, whose worldwide production exceeded 1,500,000 metric tons in 2002 (Fessehaye, 2006) and continues to increase annually. Because of their high protein content, large size, rapid growth (6 to 7 months to grow to harvest size), and palatability, a number of tilapiine cichlids specifically, various species of *Oreochromis*, *Sarotherodon*, and *Tilapia* are the focus of major aquaculture efforts. Overall, increasing aquaculture production, can prevent large amounts of malnutrition in the human population by providing an inexpensive protein source (FAO, 2016). Thus, increasing food production from aquaculture has been considered a main solution to meet the rising demand for seafood (Belton and Thilsted, 2014).

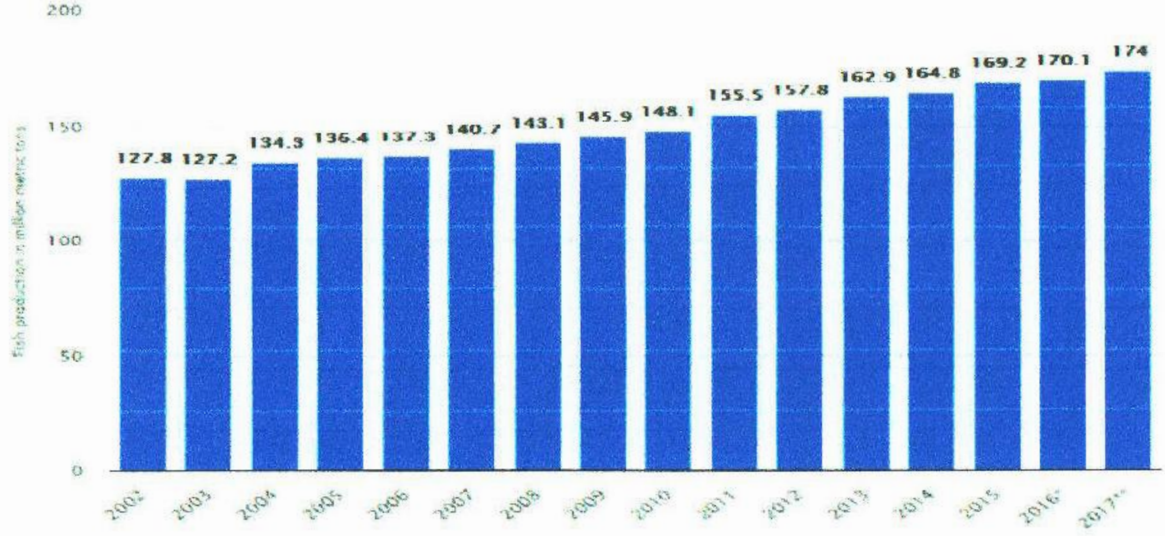


Figure 1. Global fish production from 2002 to 2017 (FAO, 2017).

Bangladesh is considered as one of the most suitable regions for fisheries in the world with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India. The diversified fisheries resources of the country are divided into two groups as Inland and Marine fisheries. The inland aquaculture sector is contributing more than 55% of the total production (Shamsuzzaman *et al.*, 2017). The fishery sector is contributing 3.61 percent to country's total gross domestic product (GDP) and 24.41 percent to country's agricultural GDP.

According to FAO, (2017) country's annual fish production reached 41.34 lakh tonnes in 2016-17, surpassing the target of 40.50 lakh tones. This sector represents one of the most productive and dynamic sectors in Bangladesh. According to the Preliminary Report on Household Income and Expenditure Survey (HIES) 2016 of Bangladesh Statistic Bureau, per capita average fish consumption of Bangladeshi people is 62.58 g a day, which is higher than the daily requirement. Bangladesh has been declared as the 5^{th} of global fish production in 2017 and this sector contributing 60% of national daily animal protein consumption, representing a crucial source of micro-nutrients. More than 17 million people including about 1.4 million women depend on fisheries sector for their livelihoods through fishing, farming, fish handling, and processing (Shamsuzzaman *et. al.,* 2017).

Table 1. Fisheries resources of Bangladesh (DoF, 2016).

Sector of fisheries	**2015-16**			**2014-15**			**Prod. increase**	**Growth rate %**
	Water area (Ha)	**Prod. (MT)**	**Productivity (kg/Ha)**	**Water area (Ha)**	**Prod. (MT)**	**Productivity (kg/Ha)**		
i)Inland Open Water (capture)	3918608	1048242	268	3906434	1023991	262	24251	2.37
1.River and Estuary	853863	178458	209	853863	174878	205	3580	2.05
2.Sundarbans	177700	16870	95	177700	17580	99	710	4.03
3. Beel								
(a) Natural	101492	81338	801	101621	79358	781	1980	2.50
(b) Beel nursery	12669	14115	1114	12540	13320	1062	795	5.96
Beel Total	**114161**	**95453**	**836**	**114101**	**92676**	**812**	**2777**	**3.00**
4. Kaptai Lake	68800	9589	139	68000	8645	126	944	10.92
5. Floodplain								
(a) Subsistence fisheries	2317175	611334	264	2317175	600876	259	10458	1.74
(b) Fry released program	158086	43121	273	145912	40478	277	2643	6.53
(c) Haor	228823	93417	408	228823	88856	388	4561	5.13
Floodplain Total	**2704084**	**747872**	**276**	**2691910**	**730210**	**271**	**17662**	**2.42**
ii) Inland close water (culture)	795841	2203554	2877	794361	2060408	2594	143146	6.94
6. pond	372405	1719783	4618	372397	1613240	4332	106543	6.61
7.Seasonal cultured waterbody								
(a) Paddy field/ Floodplain	126329	193191	1529	124868	187073	1498	6118	3.27
(b) Boropit	8547	14467	1692	8462	14207	1679	260	1.83
Seasonal waterbody Total	**134876**	**207658**	**1539**	**133330**	**201280**	**1510**	**6378**	**3.17**
8. Baor	5488	7729	1408	5488	7267	1324	462	6.36

9.Shrimp prawn farm								
(a) Shrimp prawn production		125699	456		126077	457	378	0.30
(b) Fish production		114099	414		97505	354	16594	17.02
Shrimp farm total	**275509**	**239798**	**870**	**275583**	**223582**	**811**	**16216**	**7.25**
(c) Crab	19408	13160	678	--	--	--	--	--
10. pen culture	7553	13364	1769	7553	13070	1730	294	2.24
11. cage culture	10	2062	21 kg/cum	10	1969	20 kg/cum	93	4.72
iii)Marine fisheries	--	**626528**	--	--	**599846**	--	**26682**	**4.49**
12. industrial		105348			84846		20502	24.16
13. Artisanal		521180			515000		6180	1.20
Total fish production		**3878324**			**3684245**		**194079**	**5.27**

In 2014–2015, total fishery production of Bangladesh was 3,684,245 metric tons, of which 1,023,991 metric tons (27.79%) was obtained from inland capture fisheries, 2,060,408 metric tons (55.93%) from inland aquaculture and 599,846 metric tons(16.28%) from marine water production (Shamsuzzaman *et al.*, 2017). Among them, only Carps species contributed 33.57% in total annual production, followed by pangas (11.404%), and tilapia (8.44%) (FRSS, 2016).

1.1.2 Farming of Tilapia

With increasing popularity among consumers, tilapia has become the world's second most important cultured fish after carps (Beveridge, 2010). Tilapia aquaculture in Bangladesh has developed significantly since 1999, based on the Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia (*Oreochromis niloticus*) introduced from Malaysia and on the significant genetic improvement research work by the Bangladesh Fisheries Research Institute. The Mozambique tilapia (*Oreochromis mossambicus*) was introduced to Bangladesh from Thailand in 1954 (Saha and Khatun, 2015). However, this species was not widely accepted for aquaculture because of its early maturation and prolific breeding leading to overcrowded ponds. To overcome this problem the Chitralada strain of Nile tilapia (*O. niloticus*) was introduced to Bangladesh from Thailand by UNICEF (United Nations International Children's Emergency Fund) in 1974 (Ahmed *et al.*, 2012). Nevertheless, Nile tilapia farming was slow to develop as most farmers remained more interested in carp. Gradually, the red tilapia (hybrid of *O. mossambicusx, O. niloticus*) was imported to Bangladesh from Thailand. The Bangladesh Fisheries Research Institute (BFRI) reintroduced Nile tilapia and Red tilapia from Thailand in 1987 and 1988, respectively (Gupta, 1992). Thereafter, Genetically Improved Farmed Tilapia

(GIFT) was introduced to Bangladesh by ICLARM (International Centre for Living Aquatic Resources Management, now known as the World Fish Centre) and BFRI in 1994 (Hussain *et al.*, 2004; Ponzoni *et al.*, 2010). The performance of the GIFT strain was found to be significantly superior to that of other tilapia in many respects (Hussain *et al.*, 2000; Hussain, 2009). Technology was developed to produce sex-reversed male tilapia (i.e. monosex tilapia), to avoid the unwanted reproduction and benefit from the faster growth rate of males compared to female. Since 2000, interest in tilapia farming has grown because of its observed success in other Asian producers and increasing consumer acceptance both within Bangladesh and internationally (Ahmed, 2009).

According to the Extension Department, the total annual production of tilapia in Bangladesh was estimated at 177,682 tonnes in 2010-11 whereas FRSS data estimated 98,750 tonnes production for the same period. Over the last three years, total tilapia production in Bangladesh has increased more than twice, from 88,210 tonnes in 2008-09 to 177,682 tonnes in 2010-11, 231,570 tonnes in 2013-14 which reached highest 311,022 tonnes in 2016-17. In 2015-16, the highest tilapia production was found in Khulna division (146,686 tonnes), followed by Chittagong (90,330 tonnes) and Dhaka (45,731 tonnes) (FAO, 2017).

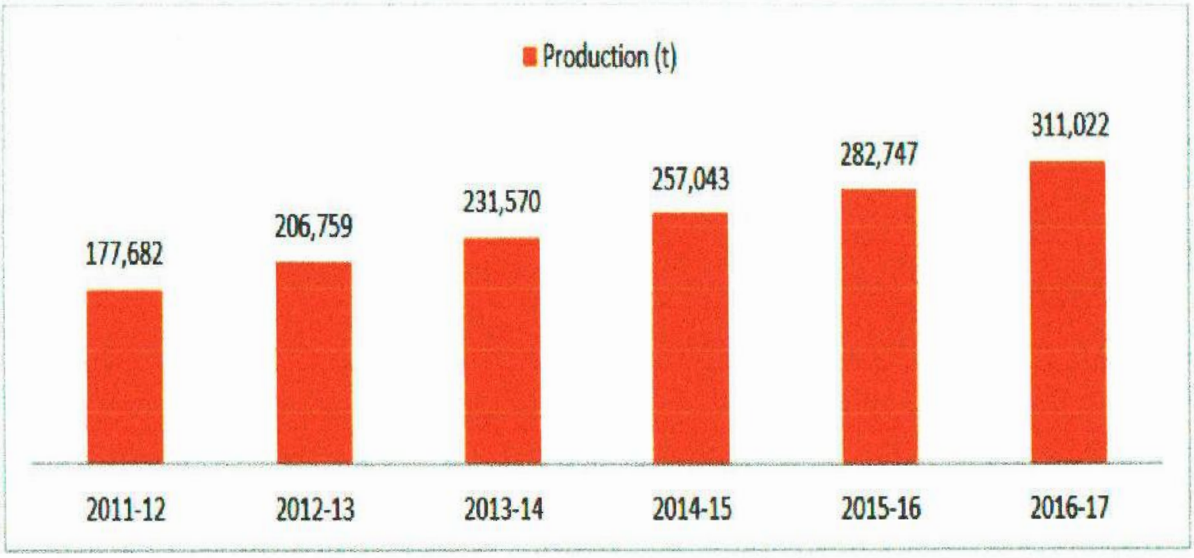


Figure 2. Tilapia production in Bangladesh in different consecutive years (FAO, 2017).

1.1.3 Tilapia as food source

Tilapia is one of commercially important aquaculture species susceptible to off-flavors. These 'muddy' or 'musty' flavors are normally caused by geosmin and 2-methylisoborneol, organic products of ubiquitous cyanobacteria that are often present or bloom sporadically in water bodies and soil (Robin *et al.,* 2006).These flavors are no indication of freshness or safety of the fish, but they make the product unattractive to consumers. Tilapia have very low levels of mercury, (Stern and Korn, 2011) as they are fast-growing, lean and short-lived, with a primarily vegetarian diet, so do not accumulate mercury found in prey. Tilapia are low in saturated fat, calories, carbohydrates and sodium, and are a good protein source. They also contain the micronutrients phosphorus, niacin, selenium, vitamin B_{12} and potassium. Thus tilapia has great potential in terms of food security and nutritional benefit. Tilapia is becoming popular among consumers because of taste and less bones making it easier to eat. With increasing popularity among consumers, tilapia has become one of the most important food fish in Bangladesh (Ahmed, 2009). The consumption of tilapia at household level has been increasing. Tilapia is becoming popular among all groups of people including women, children, young and old due to taste and less bones which easy to eat. Tilapia seems to be accepted by all religious, social and economic groups in Bangladesh. Restaurants, roadside hotels, highway motels, guesthouses, resorts and institutional consumers (university halls, patients of medical hospitals, and participants in training centres) also buy tilapia due to increasing popularity (Ahmed *et al.,* 2012). It is realized that tilapia is becoming an important component of the Bengali diet.

1.1.4 Environmental impacts of aquaculture

Like food production in agriculture, aquaculture can affect the environment by modifying natural habitats, wildlife, soil, water, and landscape. Aquaculture (Finfish and shellfish culture) causes impact on the environment in different ways. It involves an addition of solids and nutrients to the environment and is recognized as potentially causing environmental degradation. A building up of organic material beneath fish farms can impact on the flora and fauna of an area, in some cases causing major changes to sediment chemistry and in turn affecting the overlying water column (Bergheim *et al.,* 1982). Additional impacts may occur as a result of other farm discharges and waste products, for example from shore based stun and bleed operations. Because of narrow dikes, a considerable number of ponds get inundated with floodwater during the monsoon in every year. Preventing fish escape is very difficult during the flood season, especially for small-scale farmers who are reluctant to raise their pond dikes (Gozlan *et al.*, 2010). The escaping of exotic species, transmission and control of disease, and control of predatory species are also areas of concern in this type of aquaculture (McGeoch, 2010). Other potential negative impacts from farming include physical impacts associated with farming structures and farm operations, reductions in native stocks caused by the collection of result wild seed and impacts associated with the introduction of exotic species (Holmer *et al.*, 2007). Excessive use of feed, growth hormones, antibiotics, probiotics and additives has adverse impacts on ecosystems and concerns for bio-safety of environment (Goudswaard and Katunzi, 2002). Tilapia also causes negative impacts on aquatic communities through grazing or predation, interference competition (overcrowding) for food and space, as a vector of disease-causing pathogens, or by activities which lead to changes in the abiotic environment (e.g., loss of water quality which may affect survivorship of resident species) (Vicente and Fonseca-Alves, 2013).

A green water technology could be utilized for sustainable tilapia farming in Bangladesh. Tilapia has been used as biological controls for certain aquatic plant problems. It has a preference for a floating aquatic plant, duckweed (*Lemna* sp.) but also consumes some filamentous algae. In the United States and countries such as Thailand, they are becoming the plant control method of choice, reducing or eliminating the use of toxic chemicals and heavy metal-based algaecides. Tilapia rarely compete with other "pond" fish for food. Instead, because they consume plants and nutrients unused by other fish species and substantially reduce oxygen-depleting detritus; adding tilapia often increases the population, size and health of other fish. They are used for zoo ponds as a source of food for birds. Tilapia can be farmed together with shrimp in a symbiotic manner positively enhancing the productive output of both. In Kenya tilapia were introduced to control mosquitoes which were causing malaria, because they consume mosquito larvae, consequently reducing the numbers of adult female mosquitoes, the vector of the disease (*Petr,* 1993). In Brazil, tilapia fish skin is being used in a new clinical trial to treat burn injuries. In the United States, tilapia skin has been used to successfully treat third-degree wounds to the paws of two black bears caught in California's Thomas wildfire. Tilapia also provide an abundant food source for aquatic predators. Research conducted by Whetstone (2002) and Schwartz *et al.,* (1986) examined the effectiveness of tilapia at controlling aquatic plants in small ponds. Both studies were conducted using Blue Tilapia. Whetstone concluded that stocking rates of 200-400 fingerlings per acre provided control of filamentous algae in as little as one month. Whetstone also found that tilapia controlled water-meal (*Wolffia* spp.) at these stocking densities. Schwartz *et al.,* (1986) concluded that stocking rates of 500 to 2500 adult Blue Tilapia per hectare could control Najas and Chara. These benefits are, however, frequently outweighed by the negative aspects of tilapia as an invasive species.

1.1.5 Biology of Tilapia

1.1.5.1 Taxonomy

"Tilapia" is the generic name of a group of cichlids endemic to freshwater habitats. In the past this was a very large genus including all species with the common name 'tilapia', but today the vast majority are placed in other genera (Dunz and Schliewen, 2013). The cichlids group consists of three important genera – *Oreochromis, Sarotherodon* and *Tilapia.* Several characteristics distinguish these three genera, but possibly the most critical relates to reproductive behavior. All tilapia species are nest builders; fertilized eggs are guarded in the nest by a brood parent. Species of both *Sarotherodon* and *Oreochromis* are mouth brooders; eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them through incubation and for several days after hatching. In *Oreochromis* species only females practice mouth brooding, while in *Sarotherodon* species either the male or both male and female are mouth brooders (Pompa and Masser, 1999).

1.1.5.2 Scientific classification

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Cichliformes
Family:	Cichlidae
Genus:	*Oreochromis*
Species:	*Oreochromis niloticus*

1.1.5.3 Characteristics of Tilapia

Tilapia typically have laterally compressed, deep bodies. Like other cichlids, their lower pharyngeal bones are fused into a single tooth-bearing structure. A complex set of muscles allows the upper and lower pharyngeal bones to be used as a second set of jaws for processing food, allowing a division of labor between the "true jaws" (mandibles) and the "pharyngeal jaws". This means they are efficient feeders that can capture and process a wide variety of food items. Their mouths are protrusible, usually bordered with wide and often swollen lips. The jaws have conical teeth. Typically tilapia have a long dorsal fin, and a lateral line which often breaks towards the end of the dorsal fin, and starts again two or three rows of scales below. Among them the Nile tilapia has distinctive, regular, vertical stripes extending as far down the body as the bottom edge of the caudal fin, with variable coloration. Adults reach up to 60 cm (24 in) in length and up to 4.3 kg (9.5 lb). It lives for up to nine years (Froese *et al.*, 2015).

Figure 3. Tilapia species, *Oreochromis niloticus*

1.1.5.4 Habit and habitat

Tilapias inhabit a variety of fresh and, less commonly, brackish water habitats from shallow streams and ponds through to rivers, lakes, and estuaries. Although cichlids are found in freshwater and occasionally brackish water environments in Africa, Madagascar, Israel, Syria, India, Sri Lanka, South America, Central America, North America (one native species), and West Indies (Ehrhardt and Fitchett, 2016), true tilapias are native only to Africa and the Middle East (Chapman, 2000). However, introduced species of tilapias are established in many environments, including several states in the United States (Chapman, 2000). Tilapia are found throughout the tropical and sub-tropical regions of the world. They are unable to survive in temperate climates because they require warm water. The pure strain of the blue tilapia, *Oreochromis aureus*, has the greatest cold tolerance and dies at 45 °F (7 °C), while all other species of tilapia will die at a range of 52 to 62 °F (11 to 17 °C). As a result, they cannot invade temperate habitats and disrupt native ecologies in temperate zones; however, they have spread widely beyond their points of introduction in many fresh and brackish tropical and subtropical habitats, often disrupting native species significantly. Mozambique tilapias generally live in rivers and lagoons, they can also colonize fast-flowing areas of water such as creeks and streams. Due to their robust nature, Mozambique tilapias often over-colonize the habitat around them, eventually becoming the most abundant species in a particular area. The Nile tilapia thrives on the warmer temperatures commonly found in shallow waters compared to the colder environment of the deep lake which included in brackish water and survives temperatures between 8 and 42 °C (46 and 108 °F) (Froese *et al.,* 2015).

1.1.5.5 Food and feeding habit

Feeding patterns vary depending on which food source is the most abundant and the most accessible at the time. Tilapia ingest a wide variety of natural food organisms, including diatom, plankton, some aquatic macro-phytes (ranging from macro-algae to rooted plants), planktonic and benthic aquatic invertebrates, larval fish, detritus, and decomposing organic matter. This broad diet helps the species thrive in diverse locations. During commercial feeding, the fish may energetically jump out of the water for food. For their sustenance, newly hatched fry depend on their yolk sacs until consumed. Then, they eat the smallest phytoplankton present in the pond. Early juveniles and young fish are omnivorous, feeding mainly on zooplankton and zoobenthos but also ingest detritus and feed on aufwuchs and phytoplankton. At around 6 cm the species becomes almost entirely herbivorous feeding mainly on phytoplankton, using the mucus trap mechanism and its pharyngeal teeth (Moriarty and Moriarty, 1973; Moriarty *et al.*, 1973). As the fry become bigger, they eat larger organisms and supplemental feeds such as rice-bran, fishmeal and others. They are cannibalistic and will feed on their fry if food is not abundant. Tilapia are often considered filter feeders because they can efficiently harvest plankton from the water. However, Tilapia does not physically filter the water through gill rakers as efficiently as true filter feeders such as gizzard shad and silver carp. The gills of tilapia secrete a mucous that traps plankton. The plankton-rich mucous, or bolus, is then swallowed. Digestion and assimilation of plant material occurs along the length of the intestine (usually at least six times the total length of the fish).The Mozambique tilapia is less efficient than the Nile or Blue tilapia at harvesting planktonic algae. The Nile tilapia is an omnivore that feeds on both plankton and aquatic plants. It generally feeds in shallow waters, as harmful gases (such as carbon dioxide, hydrogen sulfide, and ammonia) and temperature fluctuations found in deep waters create problems for the physiology of the fish (Pfenning *et al.,* 2011). In general, tilapias are macrophyte-feeders, feeding on a diverse range of filamentous algae and plankton (Toguyeni *et al.,* 1997).The Nile tilapia typically feeds during daytime hours. Nile tilapia can filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although its main source of nutrition is obtained by surface grazing on periphyton mats. Due to their fast reproductive rate, however, overpopulation often results within groups of Nile tilapia. To obtain the necessary nutrients, night feeding may also occur due to competition for food during the daylight hours. Hence, although males and females eat equal amounts of food, males tend to grow larger due to a higher efficiency of converting food to energy (Toguyeni *et al.,* 1997).

1.1.5.6 Social behaviours

Groups of Nile tilapia establish social hierarchies in which the dominant males have priority for both food and mating. Circular nests are built predominantly by males through mouth digging to become future spawning sites. These nests often become sites of intense courtship rituals and parental care (Castro *et al.,* 2009). Like other fish, the Nile tilapia travels almost exclusively in schools. Although males settle down in their crafted nesting zones, females travel between zones to find mates, resulting in competition between the males for females. Like other tilapias, such as Mozambique tilapia, dominance between the males is established first through non-contact displays such as lateral display and tail beats. Unsuccessful attempts to reconcile the hierarchy results in contact fighting to inflict injuries. Nile tilapia has been observed to modify their fighting behavior based upon experiences during development. Thus, experience in a certain form of agonistic behavior results in differential aggressiveness among individuals *(Barki, Assaf; Gilson L. Volpato)*. Once the social hierarchy is established within a group, the dominant males enjoy the benefits of both increased access to food and an increased number of mates. However, social interactions between males in the presence of females results in higher energy expenditures as a consequence of courtship displays and sexual competition (Toguyeni *et al.,* 1997).

1.1.5.7 Physiology of Tilapia

1.1.5.7.1 Reproduction

Tilapia are known to be a mouth breeding species. Mouth breeding means they carry the fertilized eggs and young fish in their mouths for several days after the yolk sac is absorbed. *O. mossambicus* is a polygynous, sexually dimorphic, maternal mouthbrooder. Mature males aggregate at the start of the breeding season in shallow margins of waterbodies and establish courtship arenas. Each male digs a shallow circular pit which is aggressively defended and used for display to attract a receptive female. After fertilization of eggs within the pit, the female collects the eggs in her mouth and establishes a brooding territory elsewhere. Reproduction is regulated by temperature with spawning thresholds for the species reported between 18° and 25°C. Spawning is a seasonal in higher, subtropical latitudes (4-6 months), about 9-10 months in the tropics and virtually contious in equatorial regions where temperatures remain year-round above at least 24°C. Brood size is related to female body size and can range between a few hundred for stunted (early maturing) females to between 2000 and 4000 for large females > 25cm SL. The species is a multiple spawner and can produce several broods during a season, the frequency strongly influenced by temperature -spawning intervals have been reported of between 30 and 40 days, resulting in about 5 broods in subtropical regions and about 8-10 broods in the tropics. Because of the reproductive mode, survivorship of eggs and fry can be very high, with rates (at least under laboratory conditions) reported of between 50 and 95%, thus allowing for very rapid population increase under favourable conditions. Some species such as the Mozambique tilapia are mouth brooders. Tilapia will excavate a nest on gravel and defend the site against predators. As with many cichlid species, both parents protect the nest and young. Blue and Mozambique tilapia may become quite aggressive and will chase trespassers 20 or 30 feet from the nest before returning. During breeding season, Tilapia build nests in the shallow areas along shorelines. Spawning begins when water temperatures reach 20° C (68°F). Commercially grown tilapia are almost exclusively male. Because tilapia are prolific breeders, the presence of female tilapia results in rapidly increasing populations of small fish, rather than a stable population of harvest-size animal.

1.1.5.7.2 Sexual reproduction

Sexual maturity in ponds is reached at an age of 5-6 months. Spawning begins when the water temperature reaches 24 °C. The breeding process starts when the male establishes a territory, digs a craterlike spawning nest and guards his territory. The ripe female spawns in the nest, and immediately after fertilization by the male, collects the eggs into her mouth and moves off. The female incubates the eggs in her mouth and broods the fry after hatching until the yolk sac is absorbed. Incubating and brooding is accomplished in 1 to 2 weeks, depending on temperature. After fry are released, they may swim back into her mouth if danger threatens. Being a maternal mouth brooder, the number of eggs per spawn is small in comparison with most other pond fishes. Egg number is proportional to the body weight of the female. A 100 g female will produce about 100 eggs per spawn, while a female weighing 600-1 000 g can produce 1 000 to 1 500 eggs. The male remains in his territory, guarding the nest, and is able to fertilize eggs from a succession of females. If there is no cold period, during which spawning is suppressed, the female may spawn continuously. While the female is brooding, she eats little or nothing. Nile tilapia can live longer than 10 years and reach a weight exceeding 5 kg. In such an arrangement, territoriality and sexual competition amongst the males lead to large variations in reproductive success for individuals among a group. The genetic consequence of such behavior is reduced genetic variability in the long run, as inbreeding is likely to occur among different generations due to differential male reproductive success (Fessehaye *et al.,* 2006). Perhaps driven by reproductive competition, tilapias reproduce within a few months after birth. The relatively young age of sexual maturation within Nile tilapia leads to high birth and turnover rates. Consequently, the rapid reproductive rate of individuals can actually have a negative impact on growth rate, leading to the appearance of stunted tilapia as a result of a reduction in somatic growth in favor of sexual maturation (Pena-Mendoza *et al.,* 2005).

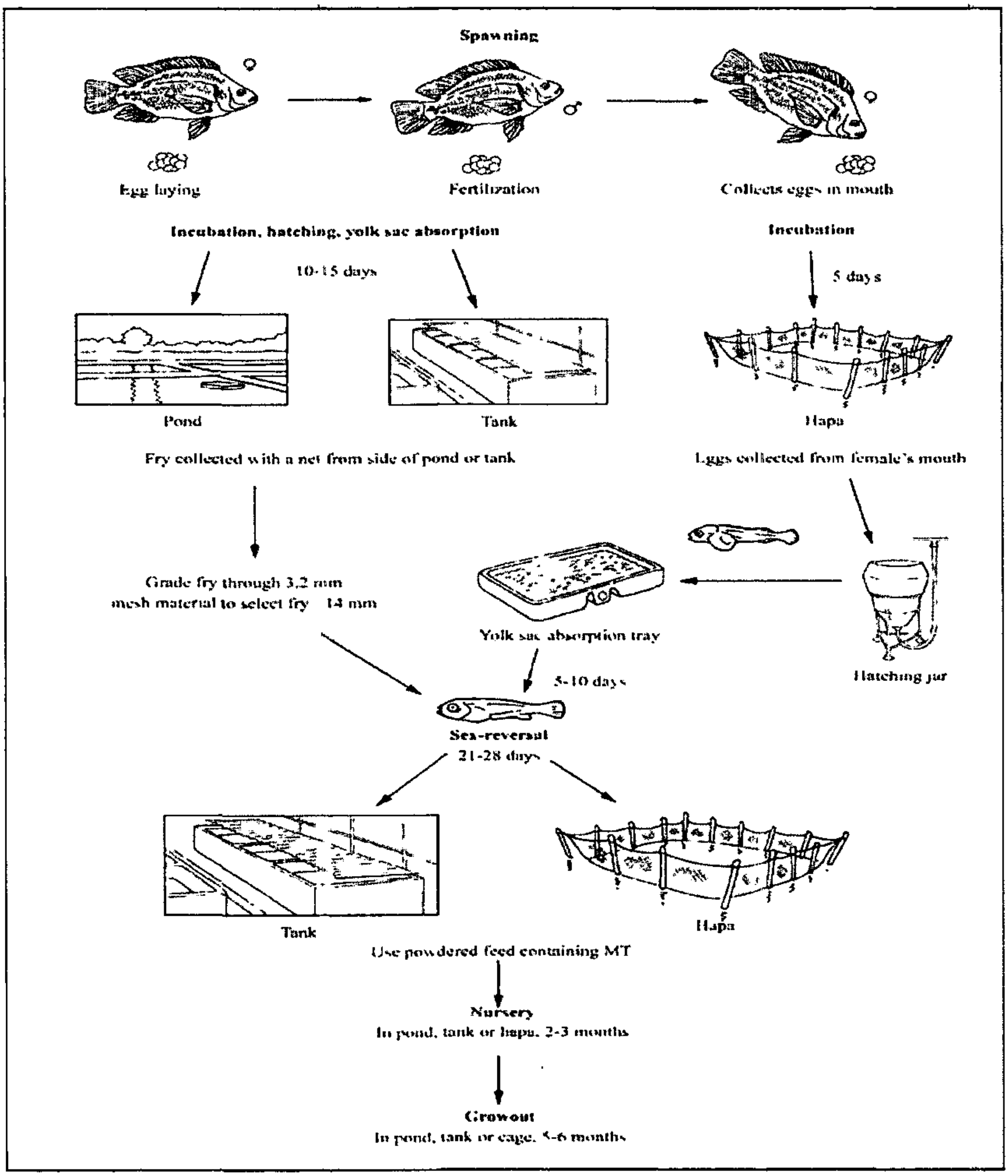


Figure 4. Reproduction cycle of *Oreochromis niloticus*

1.1.5.8 Parental care

Typically, Mozambique tilapias, like all species belonging to the *Oreochromis* genus are maternal mouthbrooders, meaning that spawn is incubated and raised in the mouth of the mother. Similar to other tilapia, Nile tilapia are maternal mouthbrooders and extensive care is therefore provided almost exclusively by the female. Parental care is therefore almost exclusive to the female. Males do contribute by providing nests for the spawn before incubation, but the energy costs associated with nest production is low relative to mouthbrooding. Compared to non-mouthbrooders, it is not energetically feasible to both mouthbrood and grows a new clutch of eggs. After spawning in a nest made by a male, the young fry or eggs are carried in the mouth of the mother for a period of 12 days. Sometimes, the mother will push the young back into her mouth if she believes they are not ready for the outside. Nile tilapias also demonstrate parental care in times of danger. When approached by a danger, the young often swim back into the protection of their mother's mouth. However, mouth-brooding leads to significant metabolic modifications for the parents, usually the mother, as reflected by fluctuations in body weight and low fitness. On one hand, protection of the young ensures passage of an individual's genes into the future generations; however, caring for the young also reduces an individual's own reproductive fitness (*Pena*-Mendoza *et al.*, 2005*)*.

1.1.5.9 Digestive system

Digestion in tilapias is a two-step process with distinct gastric and intestinal components. The mechanism for gastric digestion found in tilapias appears to be unique among animals. In other animals, the pH of fluids in an actively digesting stomach ranges from about 2.0 to 2.2 (Barrington, 1957). This is the pH at which vertebrate gastric digestive enzymes, including those of tilapias (Fish, 1960; Nagase, 1964; Moriarty, 1973) show maximum activity. The second step in the digestive process occurs in the intestine. The common bile duct which opens into the intestine on the back of the pyloric sphincter adds bile salts that maintain the pH between 6.8 and 8.8 (Fish, 1960; Nagase, 1964). Trypsin, chymotrypsin, amylase and esterase activity have all been identified in intestinal fluid (Fish, 1960; Nagase, 1964; Moriarty, 1973). The tilapia has a short esophagus leading to a small sac-like stomach with an exceptionally long intestine (4x the body long). The *O. niloticus* has firm pharyngeal teeth set on a triangular blade. Its role is to prepare food for digestion, shredding the coarser materials and breaking some of the cell walls before passing it on to the stomach (Bowen, 1981). However, it appears to have a gastric function because its contents are at a low pH, and the pH optima of its proteases are the same as the pH of the stomach contents (Bowen, 1981). The pH of the stomach varies with the degree of fullness and when full can be as low as 1.4, such that lysis of blue-green and green algae and diatoms is facilitated (Moriarty, 1973). Enzymatic digestion occurs in the intestine where pH increases progressively from 5.5 at the exit of the stomach to 8 near the anus. Nile tilapia exhibit a diel feeding pattern. Ingestion occurs during the day and digestion occurs mainly at night (Trewavas, 1983). The digestive tract of Nile tilapia is at least six times the total length of the fish, providing abundant surface area for digestion and absorption of nutrients from its mainly plant-based food sources (Opuszynski and Shireman, 1995).

1.1.5.10 Immunity

Tilapia (*Oreochromis mosambicus*) is one of fresh water fish that can survive in brackish water or in relative muddy water, and has a good adaptation level and resistance in the unfavorable environment. Mucous which coats or covers the outer side of fish is known as a non-specific mechanical and chemical defense against environmental change and any pathogenous agent. Mucous from each species of fish is different in its physical and chemical natures, and quantitatively, the mucous production is also different in each species of fish. Mucous has several functions; many of these are as a mechanical protection, osmoregulation, and barrier to colonization of parasites, fungus and bacteria. Mucous contains several substances, such as immunoglobulin (Purnomo and Purbomartono, 2013), lysozyme (Fletcher and White, 1973), C-reactive protein (CRP) (Ramos and Smith, 1978) and lectine (Suzuki, 1995). According to Drenner *et al.*, (1984), tilapia (*Oreochromis esculeritus*) was reported as a type of "size-selective suspension feeder" fish and the mucous that disposed from digestion duct was related to feeding digestion activity. Furthermore, Sanderson *et al.*, (1996) reported that in Tilapia (*Oreochromis niloticus*) the mucous secretion speed was determined by stimulus response toward particle size of feed. Lectine of PNA type found in mucous cells of Tilapia intestine according to Pajak and Danguy, (1993) was contained residual of b-galactose and β-N-acetylgalactosamine that plays as a viscoelastic barrier that covers mucous from acid and proteolyses environment.

1.1.6 Feeding adaptation in Tilapia

Nile Tilapia (*Oreochromis niloticus*) has been known as one of commercially important fish species for aquaculture, ranked as the second most important fish species after the carp due to their high growth rates, tolerance to environmental stress, high market demand, etc. (Yuan *et al.*, 2017). Several factors affect feed requirement of Nile tilapia, including biological factors (size, age, condition, and reproductive state of the fish), environmental factors (temperature, dissolved oxygen, water quality, and photoperiod) as well as feeding management (in term of the amount and frequency of feeding) (Subakti *et al.*, 2017). They quickly grow accustomed to new habitats, and their resistance is absolutely incredible. Because of their ability to adapt to different habitats and, above all, their food value, tilapia have been introduced into many areas both purposefully and accidentally. Tilapia are also daytime and surface feeder. It means they eat at the water surface and eat more during the day. At night there is little or no feeding activity (Riche and Garling, 2003).

1.1.7 Benthos community structure

Species diversity is the number of different species that are represented in a given community (a dataset). The effective number of species refers to the number of equally abundant species needed to obtain the same mean proportional species abundance as that observed in the dataset of interest (where all species may not be equally abundant). Species diversity consists of three components: species richness, taxonomic or phylogenetic diversity and species evenness. Species richness is a simple count of species or the number of species within an ecosystem. Environments that can support large numbers of species, such as tropical areas, tend to have greater species richness. Taxonomic or phylogenetic diversity is the genetic relationship between different groups of species, whereas species evenness quantifies how equal the abundances of the species are Hill, (1973) and Tuomisto, (2010). The Encyclopedia of Earth defines species evenness as "the variation in the abundance of individuals per species within a community." If a community has a large disparity between the numbers of individuals within each species it has low evenness. If the number of individuals within a species is fairly constant throughout the community it has a high evenness. The more even the number of animals per species within an ecosystem, the greater the species diversity (Balun *et al.*, 1996).

2 Materials and Methods

2.1 Study area and period

The study was conducted in Tilapia fish pond situated in Shahas village at Dumuria Upazilla of Khulna district. The study was conducted throughout six months from April 2017 to September 2017. During the experimental period all together seven sediment samples were collected from each pond for benthic community analysis at Fish Biology Laboratory of Khulna University.

Figure 5. Experimental ponds under different Tilapia feed treatment.

2.2 Pond Experimental Design

The study was conducted in total 36 ponds where two different feeds (feed-1, feed-2) with two doses (single dose and double doses) were applied in Tilapia culture. Here, C1 and C2 are control ponds having no requirements of feed with 6 replication of each and four treatments (T1, T2, T3 and T4). So, the treatments were assigned as no feed (C1), Feed-1 (T1), Feed-1 with double doses (T2); no feed (C2), Feed-2 (T3) and Feed-2 with double doses (T4). The experiment was conducted in two different trials with four different diets.

Table 2. Experimental layout of two different feed application in Tilapia culture.

C1	T1	T2	C2	T3	T4

$D_1F_0R_1$	$D_1F_1R_1$	$D_1F_2R_1$	$D_2F_0R_1$	$D_2F_1R_1$	$D_2F_2R_1$
$D_1F_0R_2$	$D_1F_1R_2$	$D_1F_2R_2$	$D_2F_0R_2$	$D_2F_1R_2$	$D_2F_2R_2$
$D_1F_0R_3$	$D_1F_1R_3$	$D_1F_2R_3$	$D_2F_0R_3$	$D_2F_1R_3$	$D_2F_2R_3$
$D_1F_0R_4$	$D_1F_1R_4$	$D_1F_2R_4$	$D_2F_0R_4$	$D_2F_1R_4$	$D_2F_2R_4$
$D_1F_0R_5$	$D_1F_1R_5$	$D_1F_2R_5$	$D_2F_0R_5$	$D_2F_1R_5$	$D_2F_2R_5$
$D_1F_0R_6$	$D_1F_1R_6$	$D_1F_2R_6$	$D_2F_0R_6$	$D_2F_1R_6$	$D_2F_2R_6$

*** D1-Diet-1, D2-Diet-2, F1-Normal feeding, F2-Double feeding

2.3 Diet Compositions

Two types of diet depending on different feed ingredients were applied in tilapia ponds. Two separate diets were formulated for each independent trial. The diets were prepared by the joint effort of WorldFish and Vietnam.

Table 3. Diet composition for experiment-1

Diet	Moisture	Crude protein	Crude fibre	Ash	Fat
1	8.03%	27.47%	3.55%	6.57%	8.30%
2	9.54%	24.99%	3.51%	6.21%	3.41%

Table 4. Diet composition for experiment-2

Diet	Moisture	Crude protein	Crude fiber	Ash	Fat
1	6.92%	22.41%	4.54%	6.53%	5.22%
2	6.38%	25.85%	6.09%	6.85%	7.53%

2.4 Pond preparation

Experimental ponds were cleaned and prepared by drying method before stocking fish. Pump machine was used for drying the ponds. After dewatering the bottom mud was dried by sunlight and the excess mud (about 10-12 cm) was removed by excavation. Then lime (1 kg per decimal), TSP (200g per decimal), and cow-dung (8-10 kg per decimal) were applied. If the plankton was not grow in pond water then second time lime (200-300g per decimal), TSP (100g per decimal) and cow-dung (2-3kg per decimal) were applied. All ponds were rectangular in shape with a maximum depth of about 1.5m and the size of each pond was $1m^2$. All the ponds were fully exposed to prevailing sunlight.

2.5 Stocking

In this experiment nursery reared Tilapia fingerlings (GIFT, Generation-XI) were stocked. For the first experiment the stocking rate was 40 individuals/m2 with the total weight of 3600g. In the second experiment same number of fingerlings with the weight of 3080g were stocked. All the fingerlings were collected from Bagerhat ASHA hatchery.

2.6 Benthos sample collection

A total of 4 samples were collected from experiment-1 and 3 samples were collected from experiment-2. Samples were collected from two point of each pond with Ecman dredger. For collecting sample the sampler was pushed into the sediment and transferred in labeled polyethylene bag. The collected sediment samples were brought to the Fish Biology Laboratory of Khulna University for further studies. Collected samples with labeled polyethylene bag were kept to a bucket and mixed. Then mixed layer was sieved using different mesh sizes sieve (0.075, 0.2, 0.92, 2.0 and 4.0 mm). After sieving, the organisms were isolated and kept into separated labeled vials with a forceps or spoon. The collected benthos samples were preserved in 50% ethyl alcohol.

2.7 Sample analysis

For identification the benthos samples were kept in glass Petri dish which was placed on a tissue paper for easy contrast of vision. Identifications were made to the species level and the organisms were enumerated under major taxa. Binocular microscope with digital camera was used to identify and capture the image of benthos.

2.7.1 Species Diversity

A diversity index is a mathematical measure of species diversity in a community. To understand the community structure the species diversity was calculated by Shannon-Weiner index, (H). Interestingly Shannon, a physicist, developed the index as a formula for measuring the entropy of matter in the universe. It turns out that the mathematical relationships hold true whether one is dealing with molecules in solution or species in an ecological community

According to Shannon and Weiner W (1949), the Species Diversity,

$$(H) = -\sum_{i=1}^{s} p_i \ln p_i$$

In the Shannon-Weiner diversity index, "p_i" is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), "ln" is the natural logarithm, "Σ" is the sum of the calculations, and "s" is the number of species.

2.7.2 Species Richness

Species richness is a measure of the number of species found in a sample. Since the larger the sample, the more species we would expect to find. This particular measure of species richness is known as D, the Menhinick's index is the ratio of the number of species to the square root of the number of individuals in the sample (Menhinick, 1964).

$$D = s/\sqrt{N}$$

Where 's' equals the number of different species represented in sample, and N equals the total numbers of individual organisms in sample.

2.7.3 Species evenness

Species evenness refers to how close in numbers each species in an environment is. Species evenness tells us how evenly the species are distributed in the ecosystem, or the relative abundance of each species in an area. Mathematically it is defined as a diversity index, a measure of biodiversity which quantifies how equal the community is numerically. The evenness of a community can be represented by Pielou's (1966) evenness index

$$\text{Species evenness } (J) = (H/|\ln D|)$$

Here, 'H' is species diversity index and 'D' is Species richness index. So to calculate the species evenness divide Shannon's diversity index H by natural logarithm of species richness ln (D).

2.8 Statistical analysis

Data were tested for normal distribution by Shapiro-Wilk test and homogeneity of variance by Levene test. Effect of different tilapia feeds on the benthic community structure (Shannon-Weiner's diversity index (H), Pielou's Evenness index (J) and Species richness Menhinick's index (D)) was determined by one-way ANOVA using SPSS software (version 16). Tukey-HSD post hoc test was done to understand the actual variation between the treatments.

Chapter Three

RESULTS

3 Results

The study was conducted to observe the effect of different tilapia feeds on the species diversity, richness and evenness of benthos community. Benthos species diversity, richness and evenness were determined by seven sampling in the tilapia culture period.

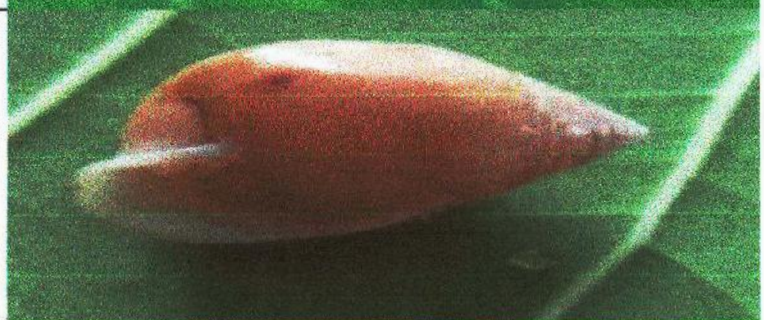
3.1 Macro-benthos community in tilapia ponds under different feeds

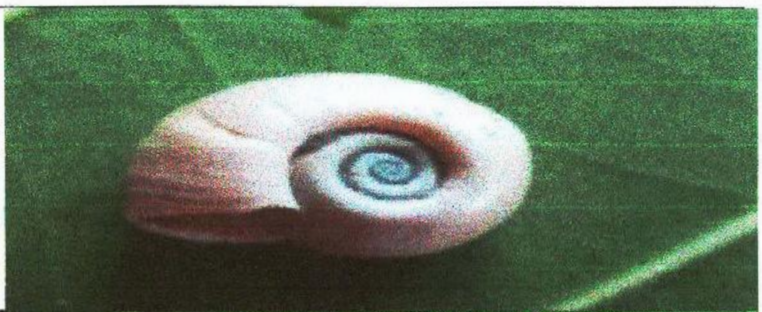
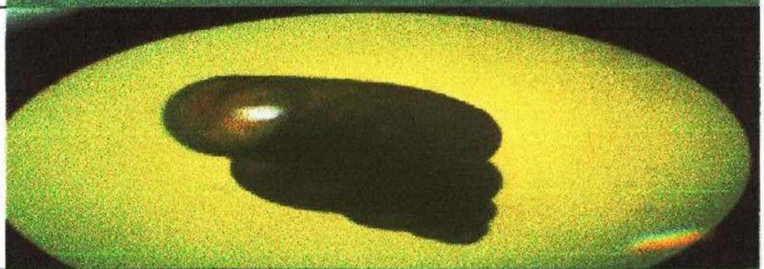
The occurrence of various groups of benthic invertebrates in the ponds have been presented in Table 5. A total of 9 different genera belonging to 2 phylum (Mollusca and Annelida) were found from the tilapia ponds. The Mollusca belonged to the classes: Gastropoda and Bivalvia when Annelida encountered belonged to the class Oligochaeta.

Table 5. Different groups of macro-benthos community identified from different feed treated tilapia ponds.

No	Genus	Species	kingdom	Phylum	Class	Order	Family
1	*Anondonta*	*Anodonta californiensis*	Animalia	Mollusca	Bivalvia	Unionoida	Unionidae
2	*Pila*	*Pila globosa*	Animalia	Mollusca	Gastropoda		Ampullariidae
3	*Septaia*	*Septaria porcellana*	Animalia	Mollusca	Gastropoda		Neritidae
4	*Neritina*	*Neritina reclivata*	Animalia	Mollusca	Bivalvia		Neritidae
5	*Indoplanorbis*	*Indoplanorbis exustus*	Animalia	Mollusca	Gastropoda		Planorbidae
6	*Melanoides*	*Melanoides tuberculata*	Animalia	Mollusca	Gastropoda		Thiaridae
7	*Mercenaria*	*Mercenaria campechiensis*	Animalia	Mollusca	Bivalvia		Veneridae
8	*Bellamya*	*Bellamya chinensis*	Animalia	Mollusca	Gastropoda		Viviparidae
9	*Earthworm*	*Nais simplex*	Animalia	Annelida	Oligochaeta	Lumbriculida	Acanthodrilidae

Table 6. Photos of different macro-benthos communities in different feed treated tilapia ponds.

Scientific name	Figure
Anodonta californiensis	
Pila globosa	
Septaria porcellana	
Neritina reclivata	

Indoplanorbis exustus	
Melanoides tuberculata	
Mercenaria campechiensis	
Bellamya chinensis	
Nais simplex	

3.2 Benthos species diversity, richness and evenness in experiment-1

3.2.1 Species diversity

Macro-benthos diversity was estimated in tilapia aquaculture ponds under two feed treatments in experiment-1. The results of this study revealed that higher macro-benthos diversity was found in C2 compared to T2 and T3 ($P<0.05$). The value of benthos diversity in treatment ponds was found to be fluctuated from 0.46-0.71. Diversity was found to be lowest (0.46±0.34) in T2 and highest (0.71±0.22) in C2 (Figure 6).

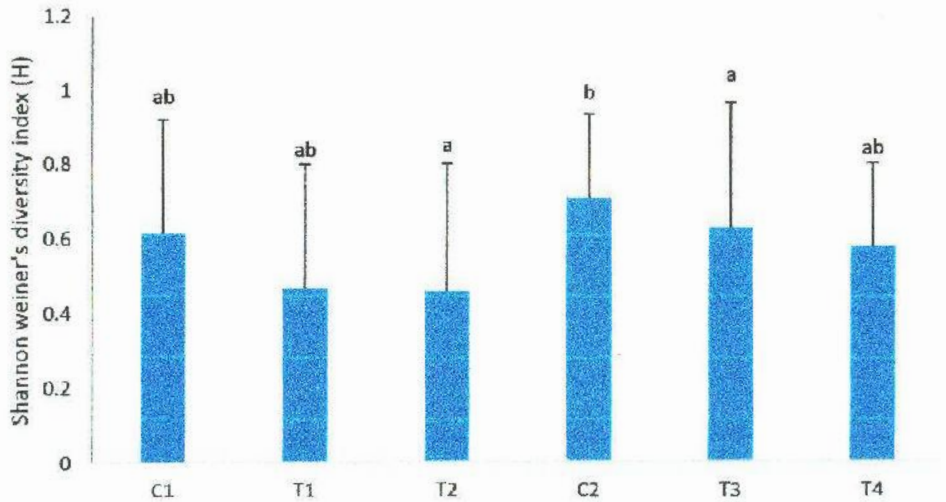


Figure 6. Shannon-Weiner's diversity index (H) of macro-benthos community in tilapia ponds in experiment-1. The error bars represent the standard deviations of one hundred and forty four samples. Different letters indicate significant difference among the treatments (one way ANOVA, $P<0.05$).

3.2.2 Species richness

Macro-benthos richness was estimated in tilapia aquaculture ponds in experiment-1 under two different diets at various feeding levels. The results of this study did not show any significant difference in species richness among the treatment ($P<0.05$). The value of benthos diversity in treatment ponds was found to be fluctuated from 0.40-0.52. Diversity was found to be lowest (0.40±0.16) in T4 and highest (0.52±0.22) in C2 (Figure 7).

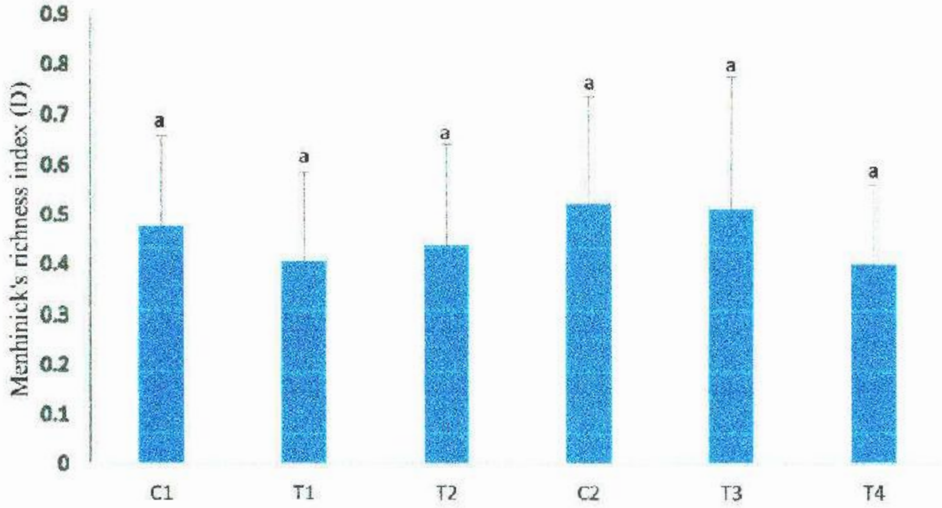


Figure 7. Menhinick's richness index (D) of macro-benthos community in tilapia ponds in experiment 1. The error bars represent the standard deviations of one hundred and forty four samples. Different letters indicate significant difference among the treatments (one way ANOVA, $P<0.05$).

3.2.3 Species evenness

Macro-benthos studies of experiment-1 indicated that higher species evenness was observed in T3 compared to T1 and T2 ($P<0.05$). The value of benthos evenness in treatment ponds was found to be fluctuated from 0.63-1.60. Diversity was found to be lowest (0.63±0.64) in T2 and highest (1.60±1.96) in T3 (Figure 8).

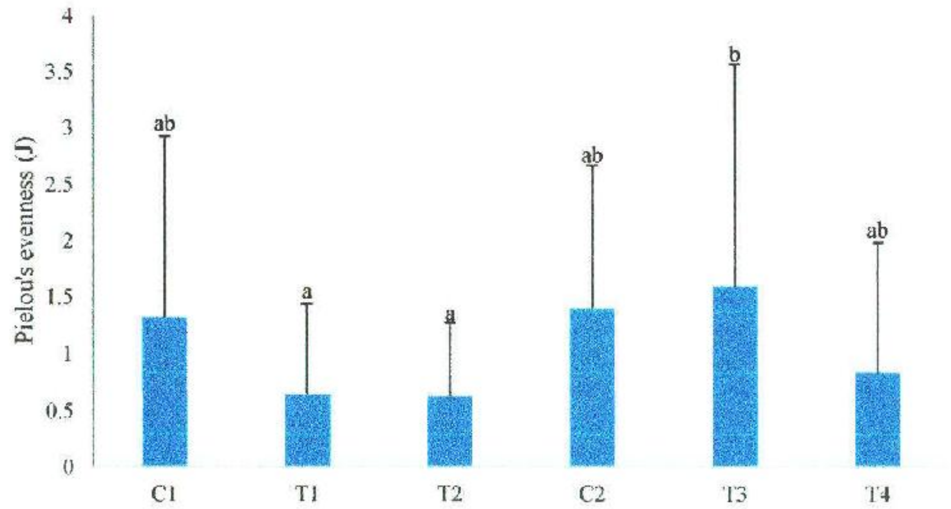


Figure 8. Pielou's evenness indices (J) of macro-benthos community in tilapia ponds in experiment 1. The error bars represent the standard deviations one hundred and forty four samples. Different letters indicate significant difference among the treatments (one way ANOVA, P<0.05).

3.3 Benthos species diversity, richness and evenness in experiment-2

3.3.1 Species diversity

In experiment-2 while Tilapia was fed with other two types of diets than experiment 1, then the higher diversity index was observed in C2 compared to other treatment groups, especially from T2 ($P<0.05$). The lowest value was found to be 0.45 ± 0.29 in T2 and the highest was found to be 0.72 ± 0.15 in C2 (Figure 9).

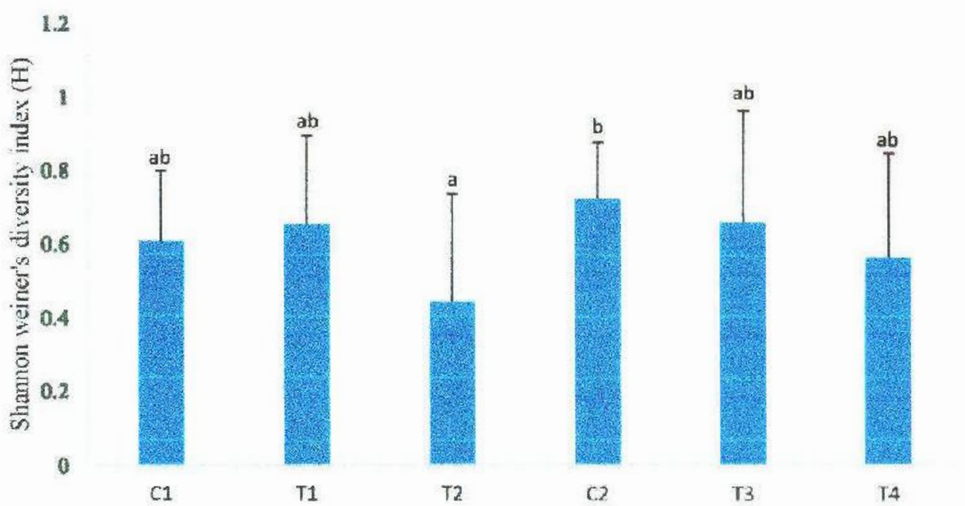


Figure 9. Shannon-Weiner's diversity index (H) of macro-benthos community in tilapia ponds in experiment-2. The error bars represent the standard deviations of one hundred and eight samples. Different letters indicate significant difference among the treatments (one way ANOVA, P<0.05).

3.3.2 Species richness

Similar to species diversity index in experiment-2, the species richness was found to be significant in C2 compared to other groups, especially T2 ($P<0.05$). The highest species richness was observed as 0.45±0.17 in C2, followed by T3 and T1. However, the lowest species richness was found to be 0.31±0.11 in T2, followed by C1 and T4 (Figure 10).

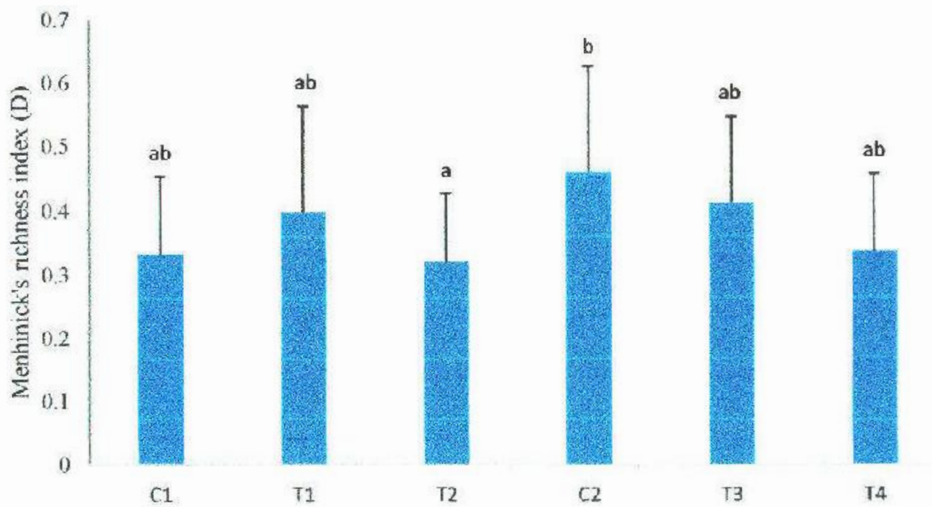


Figure 10. Menhinick's richness indices (D) of macro-benthos community in tilapia ponds in experiment-2. The error bars represent the standard deviations of one hundred and eight samples. Different letters indicate significant difference among the treatments (one way ANOVA, P<0.05).

3.3.3 Species evenness

Species evenness of macrobenthos community under Tilapia feed treatment in experiment-2 was found significantly higher in C2 compared to T2 and T4 ($P<0.05$). The value of benthos evenness in treatment ponds was found to be fluctuated from 0.45-1.12. Species evenness was found to be lowest (0.45±0.42) in T2, followed by T4 and C1, and highest (1.12±0.69) in C2, followed by T1 and T3 (Figure 11).

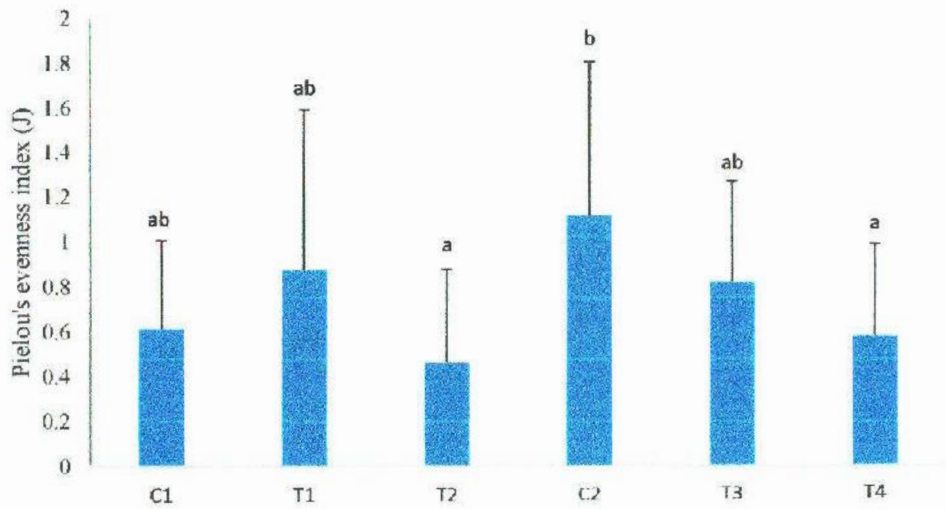


Figure 11. Pielou's evenness index (J) indices of macro-benthos community in tilapia ponds in experiment-2. The error bars represent the standard deviations one hundred and eight samples. Different letters indicate significant difference among the treatments (one way ANOVA, P<0.05).

Chapter Four

DISCUSSION

4 Discussions

Benthic communities are largely composed of macro-invertebrates, such as annelids, mollusks, and crustaceans. These organisms inhabit the bottom substrates of water bodies and play a vital role in maintaining sediment. Macro-benthos are also important food source for bottom-feeding fish, invertebrates, and birds. They are also important for enhancing of aquatic resources and also play an important role in food chain and biological purification of water (Quasin *et al.,* 2009). Filter feeders benthic community pump large amount of water through their bodies. As they filter water for food, they remove sediments and organic matter, cleaning the water. Organic matter that is not used within the water-column is deposited on the bottom of the water. It is then re-mineralized by benthic organisms into nutrients which are given back into the water column. This re-mineralization of organic matter is an important source of nutrients to the water body and is critical in maintaining the high primary production rates. Communities of benthic organisms are important indicators of environmental stress because they are particularly sensitive to pollutant exposure (Holland *et al.,* 1987). This sensitivity arises from the close relationship between benthic organisms and sediments which can accumulate environmental contaminants over time and the fact that these organisms are relatively immobile, which means they receive prolonged exposure to contaminants in their immediate habitat (Sanders *et al.*, 1980; Nixon *et al.*, 1986). Hence, in this experiment macro-benthos community was investigated under appliance of different diets from the realization of a previous experiment that tilapia feed may affect the benthos diversity, richness and evenness. Macro-benthos diversity was estimated in tilapia aquaculture ponds under two feed treatments in experiment-1. The results of this study revealed that higher macro-benthos diversity was found in C2 compared to T2 and T3 ($P<0.05$). The value of benthos diversity in treatment ponds was found to be fluctuated from 0.46-0.71. Diversity was found to be lowest (0.46±0.34) in T2 and highest (0.71±0.22) in C2 (Figure 5).

Through identification and quantification studies we found a total of nine different macro-benthos under two phylum (Table 5 and 6). From first experiment with two different feeds (Table 3) higher macro-benthos diversity in control group 2 compared to T2 ($P<0.05$). On the other hand although not significant, but C1 and C2 had higher macro-benthos diversity compared to normal and double feeding for both the diets. This results indicate the tilapia culture environment without nutrient loads support to develop more stable macro-benthos community structure (Figure 6).

Results indicate that the effects of changes in benthos biodiversity are variable over space and time and frequently depend on specific biological traits or functional roles of individual species. Abiotic and biotic factors of freshwater environment probably affect the diversity of macro-benthos. Biotic factors were competition for space and food, predation, reproduction, substrate settlement preference and the abiotic factors were temperature, air and light exposure, dissolved O_2 (Ramamurthy, 1953; Weyl, 1970). In the ponds, there was a strong dominance by a few species, the diversity was very low and some single species reached a very high abundance. The abundance of mollusks (Gastropoda and Bivalvia) were available in all the examined ponds and significantly higher ($P < 0.05$) receiving the feed, possibly due to direct benefits of micronutrients. It indicated that the environment of the examined ponds were polluted with both organic and inorganic pollution as mollusks could survive in the environments where large amount of organic and inorganic materials are present. The main reasons of this observation that mollusks also feed on organic material in water and are common in environments where large amount of organic materials are present due to their ability to survive in the resultant low oxygen levels and other reducing conditions better than most other macro-benthos species (Yankson and Kendall, 2001).

In the case of experiment 2, we observed similar results like experiment 1, where it is realized that no feeding controls (C1 and C2) have higher impact on the diversity of macro-benthos community in tilapia ponds except T1 (Figure 9). Like most tilapia ponds in the experiment, among mollusks the gastropods were the most abundant group in all treatments, but due to their tough shells, they are not readily available as food for the tilapia. The gastropod populations did not decrease significantly probably due to being protected by their shells. Bivalve populations also declined, probably due to the unsuitability of the pond bottom environment for their filter-feeding habit. An accumulation of organic matter results in active microbial decomposition, which can lead to oxygen depletion in the sediment. Under reduced conditions, anaerobic decomposition products, such as ammonia, nitrite and sulphide (Shishehchian, 2001) are harmful to benthos. However, the organic enrichment of sediments tends to be accompanied by the development of reducing conditions on the bottom sediment and the deoxidization of bottom water, as a result of the decomposition of abundant organic matter (Tsutsumi, 1990). Thus the application of commercial feed products in aquaculture ponds is the main negative impact of aquaculture activities resulting organic enrichment in the bottom sediments, due mainly to the accumulation of fish faeces, uneaten feed and dead plankton (Focardi, *et al.,* 2005).

In the study it was also observed that some species of macro invertebrates were found to decrease in number or disappeared from the pond. This could be attributed to the intolerant nature of the concerned benthic fauna. This might be due to input of large quantity of leaf litter from surrounding areas into the pond by wind action. In addition, the double doses feed might have possibly reduced the growth of macro-invertebrates during the experimental period due to huge organic load.

In experiment 1, we did not find any significant variation on the species richness, although two different control groups represented higher trend of species richness compared to normal and double feeding in tilapia ponds (Figure 7). This similarity in species richness might be due to adaptation of different macro-benthos in different feed treated ponds, which might control their distribution and abundance in the couture system. In contrast, in experiment 2, species richness was found significant in C2 compared to T2 ($P<0.05$), which was also higher than that of other experimental groups (Figure 10). In second experiment, the higher richness in C2 was might be due to better pond environmental conditions. On the other hands, feed ingredients in two different diets might have effect on the experimental pond water and sediment chemistry, which might have significant effect on the species richness. Apparently, single feeding had less effect compared to the double feeding on the reduction of species richness in both the experiment. Exceptionally, C1 had lower species richness than that of T1 might be due to some other factors which may affect to increase the number of species in pond condition.

Gastropods are one of the most diverse groups of animals, both in form, habit, and habitat. They are by far the largest group of mollusks, with more than 62,000 described living species, and they comprise about 80% of living molluscs. Molluscs are also one of the larger invertebrate groups in the fresh water habitat of Bangladesh (Baby *et al.,* 2010). Gastropods live in every conceivable habitat on Earth. Favourable ecological conditions for *M. tuberculata* are permanent and shallow waters, rivers, streams, ponds, marshes, emergent plants and well-oxygenated niches (Pointier and McCullough, 1989) but also several anthropized aquatic environments such as garden ponds, irrigation systems or artificial lakes. (Roessler *et al.*, 1977) found this species in waters up to 30ppt. It lives usually in water between 18 and 25°C (Murray, 1971). *M. tuberculata* has no negative impact on aquatic macrophytes (Madsen, 1992) although it has been reported to displace several gastropods where introduced (Murray, 1971; Jacobson, 1975; Pointier, 1999). Roessler *et al.*, (1977) reported competition for trophic resources with *Neritina virginea* in Florida. Hence, the richness of macro-benthos community might increase or decrease due to application of different diets in tilapia culture ponds.

Species evenness was found to be fluctuated in different tilapia feed treatment both in experiment 1 and experiment 2. In experiment 1, single dose of diet 2 was found to have significant species evenness compared to normal and double feeding of diet 1 ($P<0.05$) (Figure 8). In general view, it was also higher than that of T4, as well as C1 and C2. Lower evenness in control groups might be due to over-dominance of particular macro-benthos, which might responsible for competitive exclusions of other species. In another concept, lower evenness in T1, T2 and T4 might be due to effect of feed ingredients to favour or disfavour of particular benthos organisms in the pond sediments. In contrast, in experiment 2, higher species evenness was found in C2 compared to T4 and T2, which indicate higher organic load might create toxic environment in double feeding ponds that might responsible for disappearance of many macro-benthos species from the ponds. Despite of that accumulation of higher nitrogenous species in pond environment might cause death of several macro-benthos from double feeding diets treated experimental ponds.

Strzelec and Krolczyk, (2004) indicated that many gastropod species are tolerant to most physico-chemical parameters and their occurrence is affected by the quality of bottom sediments and abundance of vegetation and reported that the most suitable substrate for snails in rivers is a sandy bottom covered with thin layer of organic silt. (Lacoursiere *et al.*, 1975) and (Vincent *et al.*, 1982) suggested that gastropod variability may be explained by abiotic factors like depth, current and sediments. Temperature seems to be an important factor for the distribution and relative abundance of molluscs. Bivalves, however, exhibited a negatively significant correlation with water temperature. Further, temperature plays an important role in abundance and evenness of *Lymnaea* sp. (Canete *et al.*, 2004). Michael (1968); Dutta and Malhotra, (1986) and Malhotra *et al.*, (1996) also recorded a positive correlation between molluscs and temperature, while a negative correlation between temperature and molluscs was noticed by Ricker, (1952), Shrivastava, (1956) and Vasisht and Bhandal, (1979). In a survey conducted by Giovanelli *et al.*, (2005), the water flow and rainfall were designated as variables responsible for the distribution of population of *Physa marmorata* and *M. tuberculata*. p^H recorded by Garg *et al.*, (2009) suggests that molluscs were found to be independent of fluctuations with respect to p^H value. The findings of Martins-Silva and Barros, (2001) revealed that acidic p^H is unfavorable to the occurrence of molluscs. Both gastropods and bivalves had a negative insignificant relationship with DO which finds support from Garg *et al.* (2009) report. Cheatum (1934) and Sharma (1986) documented that some molluscs can survive in very low oxygen conditions. Therefore, environmental factors might significantly control the distribution and macro-benthos species evenness in aquatic system.

Chapter Five

Conclusion

5 Conclusion

From the present study the following conclusions can be drawn:

1. Control groups as well as different feed treatments have significant effect on the macro-benthos community structure; especially, species diversity, richness and evenness in tilapia culture ponds.
2. Single feeding and double feeding can also contribute in distribution and community structure in aquatic environment.

Recommendations:

1. Further research should be conducted to understand the micro-community structure of macro-benthos in aquaculture system.
2. Combinations of many different feeding practices should be studied to understand the habitat preference of key indicator macro-benthos species.

Chapter Six

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Chapter Seven

APPENDIXES

7 Appendices

7.1 Photo gallery

Fig: collection of samples in labeled polyethylene bag from tilapia ponds

Fig: Sieving the samples using different mesh sizes sieve

Fig: Preservation of benthos in labeled vials containing 50% ethyl alcohol

Fig: Identification of benthos kept in round transparent Petri dish in naked eye

Fig: Heavy loading of benthos in collected samples

Fig: Binocular microscope for identification of small sizes benthos

7.2 Table of Diversity in experiment-1

Oneway ANOVA

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C1	24	.6154	.30599	.06246	.4862	.7446	.00	1.18
T1	24	.4701	.33071	.06751	.3305	.6098	.00	.96
T2	24	.4639	.33962	.06933	.3204	.6073	.00	1.06
C2	24	.7118	.22424	.04577	.6171	.8065	.21	1.01
T3	24	.6353	.33213	.06779	.4951	.7755	.00	1.08
T4	24	.5837	.22312	.04554	.4895	.6779	.00	.87
Total	144	.5800	.30491	.02541	.5298	.6303	.00	1.18

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.134	5	.227	2.575	.029
Within Groups	12.161	138	.088		
Total	13.295	143			

Tukey HSD

Treatment1	N	Subset for alpha = 0.05	
		1	2
T2	24	.4639	
T1	24	.4701	.4701
T4	24	.5837	.5837
C1	24	.6154	.6154
T3	24	.6353	.6353
C2	24		.7118
Sig.		.347	.060

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Tukey HSD

(I) Treatment1	(J) Treatment1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	T1	.14529	.08569	.537	-.1024	.3930
	T2	.15155	.08569	.489	-.0961	.3992
	C2	-.09642	.08569	.870	-.3441	.1512
	T3	-.01990	.08569	1.000	-.2676	.2278
	T4	.03172	.08569	.999	-.2160	.2794
T1	C1	-.14529	.08569	.537	-.3930	.1024
	T2	.00626	.08569	1.000	-.2414	.2539
	C2	-.24171	.08569	.060	-.4894	.0060
	T3	-.16519	.08569	.390	-.4129	.0825
	T4	-.11357	.08569	.771	-.3612	.1341
T2	C1	-.15155	.08569	.489	-.3992	.0961
	T1	-.00626	.08569	1.000	-.2539	.2414
	C2	-.24797*	.08569	.050	-.4956	-.0003
	T3	-.17145	.08569	.347	-.4191	.0762
	T4	-.11983	.08569	.728	-.3675	.1278
C2	C1	.09642	.08569	.870	-.1512	.3441
	T1	.24171	.08569	.060	-.0060	.4894
	T2	.24797*	.08569	.050	.0003	.4956
	T3	.07652	.08569	.948	-.1711	.3242
	T4	.12814	.08569	.668	-.1195	.3758
T3	C1	.01990	.08569	1.000	-.2278	.2676
	T1	.16519	.08569	.390	-.0825	.4129
	T2	.17145	.08569	.347	-.0762	.4191
	C2	-.07652	.08569	.948	-.3242	.1711
	T4	.05161	.08569	.991	-.1961	.2993
T4	C1	-.03172	.08569	.999	-.2794	.2160
	T1	.11357	.08569	.771	-.1341	.3612
	T2	.11983	.08569	.728	-.1278	.3675
	C2	-.12814	.08569	.668	-.3758	.1195
	T3	-.05161	.08569	.991	-.2993	.1961

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

7.3 Table of Richness in experiment-1

Oneway ANOVA

Descriptives

Richness

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
C1	24	.4776	.18069	.03688	.4013	.5539	.20	.87
T1	24	.4075	.17930	.03660	.3318	.4832	.16	1.00
T2	24	.4385	.20417	.04168	.3523	.5247	.12	1.00
C2	24	.5227	.21554	.04400	.4317	.6137	.22	1.22
T3	24	.5115	.26620	.05434	.3991	.6239	.00	1.22
T4	24	.4020	.15934	.03253	.3347	.4692	.10	.87
Total	144	.4600	.20577	.01715	.4261	.4939	.00	1.22

ANOVA

Richness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.323	5	.065	1.558	.176
Within Groups	5.731	138	.042		
Total	6.055	143			

Tukey HSD

Treatment2	N	Subset for alpha = 0.05 1
T4	24	.4020
T1	24	.4075
T2	24	.4385
C1	24	.4776
T3	24	.5115
C2	24	.5227
Sig.		.319

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Tukey HSD

(I) Treatment2	(J) Treatment2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	T1	.07013	.05883	.840	-.0999	.2402
	T2	.03913	.05883	.985	-.1309	.2092
	C2	-.04508	.05883	.973	-.2151	.1249
	T3	-.03383	.05883	.993	-.2039	.1362
	T4	.07567	.05883	.792	-.0944	.2457
T1	C1	-.07013	.05883	.840	-.2402	.0999
	T2	-.03100	.05883	.995	-.2010	.1390
	C2	-.11521	.05883	.372	-.2852	.0548
	T3	-.10396	.05883	.490	-.2740	.0661
	T4	.00554	.05883	1.000	-.1645	.1756
T2	C1	-.03913	.05883	.985	-.2092	.1309
	T1	.03100	.05883	.995	-.1390	.2010
	C2	-.08421	.05883	.708	-.2542	.0858
	T3	-.07296	.05883	.816	-.2430	.0971
	T4	.03654	.05883	.989	-.1335	.2066
C2	C1	.04508	.05883	.973	-.1249	.2151
	T1	.11521	.05883	.372	-.0548	.2852
	T2	.08421	.05883	.708	-.0858	.2542
	T3	.01125	.05883	1.000	-.1588	.1813
	T4	.12075	.05883	.319	-.0493	.2908
T3	C1	.03383	.05883	.993	-.1362	.2039
	T1	.10396	.05883	.490	-.0661	.2740
	T2	.07296	.05883	.816	-.0971	.2430
	C2	-.01125	.05883	1.000	-.1813	.1588
	T4	.10950	.05883	.430	-.0605	.2795
T4	C1	-.07567	.05883	.792	-.2457	.0944
	T1	-.00554	.05883	1.000	-.1756	.1645
	T2	-.03654	.05883	.989	-.2066	.1335
	C2	-.12075	.05883	.319	-.2908	.0493
	T3	-.10950	.05883	.430	-.2795	.0605

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

7.4 Evenness in experiment -1

Oneway ANOVA

Descriptives

Evenness

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C1	24	1.3213	1.60106	.32682	.6452	1.9973	.00	7.15
T1	24	.6503	.78912	.16108	.3171	.9835	.00	3.76
T2	24	.6307	.64978	.13264	.3563	.9051	.00	2.83
C2	24	1.4100	1.26184	.25757	.8772	1.9428	.18	4.98
T3	24	1.6043	1.95753	.39958	.7777	2.4309	.00	7.61
T4	24	.8402	1.14299	.23331	.3576	1.3229	.00	6.03
Total	144	1.0761	1.34596	.11216	.8544	1.2978	.00	7.61

Tukey HSD

Treatment3	N	Subset for alpha = 0.05
		1
T2	24	.6307
T1	24	.6503
T4	24	.8402
C1	24	1.3213
C2	24	1.4100
T3	24	1.6043
Sig.		.112

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Tukey HSD

(I) Treatment3	(J) Treatment3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	T1	.67096	.37894	.488	-.4242	1.7662
	T2	.69057	.37894	.455	-.4046	1.7858
	C2	-.08876	.37894	1.000	-1.1840	1.0064
	T3	-.28309	.37894	.976	-1.3783	.8121
	T4	.48102	.37894	.801	-.6142	1.5762
T1	C1	-.67096	.37894	.488	-1.7662	.4242
	T2	.01961	.37894	1.000	-1.0756	1.1148
	C2	-.75972	.37894	.345	-1.8549	.3355
	T3	-.95405	.37894	.126	-2.0493	.1412
	T4	-.18994	.37894	.996	-1.2851	.9053
T2	C1	-.69057	.37894	.455	-1.7858	.4046
	T1	-.01961	.37894	1.000	-1.1148	1.0756
	C2	-.77933	.37894	.316	-1.8745	.3159
	T3	-.97365	.37894	.112	-2.0689	.1215
	T4	-.20955	.37894	.994	-1.3047	.8857
C2	C1	.08876	.37894	1.000	-1.0064	1.1840
	T1	.75972	.37894	.345	-.3355	1.8549
	T2	.77933	.37894	.316	-.3159	1.8745
	T3	-.19433	.37894	.996	-1.2895	.9009
	T4	.56978	.37894	.663	-.5254	1.6650
T3	C1	.28309	.37894	.976	-.8121	1.3783
	T1	.95405	.37894	.126	-.1412	2.0493
	T2	.97365	.37894	.112	-.1215	2.0689
	C2	.19433	.37894	.996	-.9009	1.2895
	T4	.76411	.37894	.338	-.3311	1.8593
T4	C1	-.48102	.37894	.801	-1.5762	.6142
	T1	.18994	.37894	.996	-.9053	1.2851
	T2	.20955	.37894	.994	-.8857	1.3047
	C2	-.56978	.37894	.663	-1.6650	.5254
	T3	-.76411	.37894	.338	-1.8593	.3311

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

7.5 Diversity in experiment-2

Oneway ANOVA

Descriptives

Diversity2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C1	18	.6085	.19166	.04517	.5132	.7039	.37	1.11
T1	18	.6565	.24131	.05688	.5365	.7765	.26	1.05
T2	18	.4466	.29366	.06922	.3006	.5926	.00	.82
C2	18	.7281	.15359	.03620	.6517	.8044	.52	1.00
T3	18	.6640	.30447	.07176	.5126	.8155	.00	1.07
T4	18	.5705	.28346	.06681	.4295	.7114	.00	1.00
Total	108	.6124	.26073	.02509	.5626	.6621	.00	1.11

ANOVA

Diversity2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.851	5	.170	2.701	.025
Within Groups	6.423	102	.063		
Total	7.274	107			

Tukey HSD

Treatment4	N	Subset for alpha = 0.05	
		1	2
T2	18	.4466	
T4	18	.5705	.5705
C1	18	.6085	.6085
T1	18	.6565	.6565
T3	18	.6640	.6640
C2	18		.7281
Sig.		.107	.418

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Tukey HSD

(I) Treatment4	(J) Treatment4	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
C1	T1	-.04800	.08365	.993	-.2910	.1950
	T2	.16194	.08365	.387	-.0810	.4049
	C2	-.11952	.08365	.709	-.3625	.1234
	T3	-.05550	.08365	.985	-.2985	.1875
	T4	.03808	.08365	.997	-.2049	.2810
T1	C1	.04800	.08365	.993	-.1950	.2910
	T2	.20994	.08365	.131	-.0330	.4529
	C2	-.07152	.08365	.956	-.3145	.1714
	T3	-.00750	.08365	1.000	-.2505	.2355
	T4	.08608	.08365	.907	-.1569	.3290
T2	C1	-.16194	.08365	.387	-.4049	.0810
	T1	-.20994	.08365	.131	-.4529	.0330
	C2	-.28146*	.08365	.013	-.5244	-.0385
	T3	-.21744	.08365	.107	-.4604	.0255
	T4	-.12386	.08365	.677	-.3668	.1191
C2	C1	.11952	.08365	.709	-.1234	.3625
	T1	.07152	.08365	.956	-.1714	.3145
	T2	.28146*	.08365	.013	.0385	.5244
	T3	.06402	.08365	.973	-.1789	.3070
	T4	.15760	.08365	.418	-.0854	.4006
T3	C1	.05550	.08365	.985	-.1875	.2985
	T1	.00750	.08365	1.000	-.2355	.2505
	T2	.21744	.08365	.107	-.0255	.4604
	C2	-.06402	.08365	.973	-.3070	.1789
	T4	.09358	.08365	.873	-.1494	.3365
T4	C1	-.03808	.08365	.997	-.2810	.2049
	T1	-.08608	.08365	.907	-.3290	.1569
	T2	.12386	.08365	.677	-.1191	.3668
	C2	-.15760	.08365	.418	-.4006	.0854
	T3	-.09358	.08365	.873	-.3365	.1494

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

7.6 Richness in experiment-2

Oneway ANOVA

Descriptives

Richness2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C1	18	.3315	.11983	.02824	.2719	.3911	.15	.64
T1	18	.3964	.16633	.03920	.3137	.4791	.18	.72
T2	18	.3199	.10660	.02512	.2669	.3730	.17	.63
C2	18	.4598	.16570	.03906	.3774	.5422	.26	.76
T3	18	.4113	.13383	.03154	.3447	.4778	.14	.64
T4	18	.3362	.12028	.02835	.2764	.3960	.15	.56
Total	108	.3759	.14345	.01380	.3485	.4032	.14	.76

ANOVA

Richness2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.277	5	.055	2.937	.016
Within Groups	1.925	102	.019		
Total	2.202	107			

Tukey HSD

Treatment5	N	Subset for alpha = 0.05	
		1	2
T2	18	.3199	
C1	18	.3315	.3315
T4	18	.3362	.3362
T1	18	.3964	.3964
T3	18	.4113	.4113
C2	18		.4598
Sig.		.353	.065

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Tukey HSD

(I) Treatment5	(J) Treatment5	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	T1	-.06489	.04579	.717	-.1979	.0681
	T2	.01156	.04579	1.000	-.1214	.1446
	C2	-.12833	.04579	.065	-.2613	.0047
	T3	-.07978	.04579	.507	-.2128	.0532
	T4	-.00467	.04579	1.000	-.1377	.1283
T1	C1	.06489	.04579	.717	-.0681	.1979
	T2	.07644	.04579	.555	-.0566	.2094
	C2	-.06344	.04579	.736	-.1964	.0696
	T3	-.01489	.04579	1.000	-.1479	.1181
	T4	.06022	.04579	.776	-.0728	.1932
T2	C1	-.01156	.04579	1.000	-.1446	.1214
	T1	-.07644	.04579	.555	-.2094	.0566
	C2	-.13989*	.04579	.033	-.2729	-.0069
	T3	-.09133	.04579	.353	-.2243	.0417
	T4	-.01622	.04579	.999	-.1492	.1168
C2	C1	.12833	.04579	.065	-.0047	.2613
	T1	.06344	.04579	.736	-.0696	.1964
	T2	.13989*	.04579	.033	.0069	.2729
	T3	.04856	.04579	.896	-.0844	.1816
	T4	.12367	.04579	.084	-.0093	.2567
T3	C1	.07978	.04579	.507	-.0532	.2128
	T1	.01489	.04579	1.000	-.1181	.1479
	T2	.09133	.04579	.353	-.0417	.2243
	C2	-.04856	.04579	.896	-.1816	.0844
	T4	.07511	.04579	.574	-.0579	.2081
T4	C1	.00467	.04579	1.000	-.1283	.1377
	T1	-.06022	.04579	.776	-.1932	.0728
	T2	.01622	.04579	.999	-.1168	.1492
	C2	-.12367	.04579	.084	-.2567	.0093
	T3	-.07511	.04579	.574	-.2081	.0579

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

7.7 Table of Evenness in experiment-2

Oneway ANOVA

Descriptives

Evenness2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C1	18	.6133	.38766	.09137	.4206	.8061	.26	1.60
T1	18	.8756	.71409	.16831	.5205	1.2307	.22	2.77
T2	18	.4594	.41607	.09807	.2525	.6663	.00	1.66
C2	18	1.1164	.68816	.16220	.7742	1.4586	.44	2.44
T3	18	.8230	.44514	.10492	.6016	1.0443	.00	1.64
T4	18	.5807	.40989	.09661	.3768	.7845	.00	1.51
Total	108	.7447	.56044	.05393	.6378	.8516	.00	2.77

ANOVA

Evenness2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.166	5	1.033	3.705	.004
Within Groups	28.442	102	.279		
Total	33.608	107			

Tukey HSD

Treatment5	N	Subset for alpha = 0.05	
		1	2
T2	18	.4594	
T4	18	.5807	
C1	18	.6133	.6133
T3	18	.8230	.8230
T1	18	.8756	.8756
C2	18		1.1164
Sig.		.179	.056

Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Chapter TWO

MATERIALS AND METHODS

Tukey HSD

(I) Treatment5	(J) Treatment5	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	T1	-.26228	.17602	.671	-.7735	.2490
	T2	.15392	.17602	.952	-.3573	.6652
	C2	-.50309	.17602	.056	-1.0144	.0082
	T3	-.20961	.17602	.840	-.7209	.3016
	T4	.03268	.17602	1.000	-.4786	.5439
T1	C1	.26228	.17602	.671	-.2490	.7735
	T2	.41620	.17602	.179	-.0951	.9275
	C2	-.24081	.17602	.746	-.7521	.2705
	T3	.05266	.17602	1.000	-.4586	.5639
	T4	.29496	.17602	.551	-.2163	.8062
T2	C1	-.15392	.17602	.952	-.6652	.3573
	T1	-.41620	.17602	.179	-.9275	.0951
	C2	-.65701*	.17602	.004	-1.1683	-.1457
	T3	-.36353	.17602	.314	-.8748	.1477
	T4	-.12124	.17602	.983	-.6325	.3900
C2	C1	.50309	.17602	.056	-.0082	1.0144
	T1	.24081	.17602	.746	-.2705	.7521
	T2	.65701*	.17602	.004	.1457	1.1683
	T3	.29348	.17602	.556	-.2178	.8047
	T4	.53577*	.17602	.034	.0245	1.0470
T3	C1	.20961	.17602	.840	-.3016	.7209
	T1	-.05266	.17602	1.000	-.5639	.4586
	T2	.36353	.17602	.314	-.1477	.8748
	C2	-.29348	.17602	.556	-.8047	.2178
	T4	.24229	.17602	.741	-.2690	.7536
T4	C1	-.03268	.17602	1.000	-.5439	.4786
	T1	-.29496	.17602	.551	-.8062	.2163
	T2	.12124	.17602	.983	-.3900	.6325
	C2	-.53577*	.17602	.034	-1.0470	-.0245
	T3	-.24229	.17602	.741	-.7536	.2690

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