EFFECTS OF NATURAL AND COMMERCIAL FEED ON YIELD, GROWTH AND REPRODUCTION OF ZEBRAFISH (*Danio rerio*)

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CERTIFICATE

This is to certify that this thesis entitled, **"Effects of natural and commercial feeds on yield, growth and reproduction of Zebrafish (*Danio rerio*)"** submitted by Md. Rakibur Rahman, Exam. Roll: Curzon- 4208, MS Session: 2013-2014, Registration No.: HA-2384, Session: 2009-2010 has been carried out under my supervision. This is further to certify that this is an original work and suitable in partial fulfillment for the degree of Master of Science in Fisheries, University of Dhaka.

I wish him every success in life.

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TO

MY BELOVED PARENTS

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ABSTRACT

Although zebrafish is used as a model species for understanding a number of biological functions and mechanisms, there is no practically successful information on the nutritional requirements. This study evaluated the effects of several natural and commercially available feeds and different feeding regimes on the growth, yield and reproductive performance of zebrafish (*Danio rerio*) and developed a standard diet. Zebrafish (n = 21) were stocked into each of 15 tanks (volume, 4 L); 3 tanks were assigned to each of 5 feeding combinations for a period of 62 days. Fish were fed with selective diet (e.g. diet 1: dried tubifex; diet 2: *Artemia*; diet 3: *Artemia* and commercial pellet feed; diet 4: Spirulina and commercial pellet feed; diet 5: Commercial pellet feed) twice daily for 62 days. Throughout the whole experimental period weight, length and survivability were recorded to determine the growth rate and compared among 5 different diets. The specific growth rate among various diets varied within (1.65±0.115%) to (0.39±0.102%) where highest value was found in fish fed with diet 5 (commercial pellet feed). Mean weight and length gain were greater in zebrafish fed diet 5 than diet 1, 2, 3 and 4. An excellent outcome happened for survival rate (%) in diets where highest survival rate was 100% in diet 4 and lowest value was (90.256±1.015) in diet 1. At the end of 62 days, 5 male and 5 female fish from each dietary treatment were pooled into breeding tanks and the effects of feeding combinations on reproductive performance were observed. Mean spawning success was significantly ($p<0.05$) greater in zebrafish fed with diet 5 (Commercial pellet feed only) than in those fed with diet 1, 2, 3 and 4. Mean fertilization and hatching rates were higher in zebrafish fed with diet 3, 4 and 5 than zebrafish fed with diet 1 and 2. Zebrafish consuming the commercial pellet feed only, resulted in more viable offspring and more growth rate and it is a simpler feeding regime compared to other diets. The author recommended the commercial pellet feed to use in zebrafish culture for maximum growth and production of viable offspring under laboratory condition.

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Introduction

1.1 General Background

1.1.1 History

The Zebrafish (*Danio rerio*) have become a widely used vertebrate model species for research associated with comparative and evolutionary biology as well as biomedical areas(Arunachalam *et al*., 2013). Zebrafish belong to the single largest vertebrate family Cyprinidae which contains over 2,400 species, including the goldfish. Zebrafish are among the smallest members of this family, with adults measuring 30-40 millimeters or about 1.5 inches long. Zebrafish get their name from the stripes running the length of their torpedo-shaped bodies. They originate from the Himalayan region, where they are found in slow-moving bodies of fresh water (Viljoen, 1999).

The zebrafish life cycle advances through 4 major developmental stages: Embryo, larva, juvenile and adult. The cycle begins when eggs and sperm are released by a mating pair. After fertilization, the initial stages of development progress rapidly, with embryos hatching into larvae by 3 days post fertilization. From this point, progression into a sexually mature adult requires an additional two to three months (Viljoen, 1999).

Zebrafish are very cheap and easy animal to maintain, have a relatively short breeding cycle. They take only three months until they begin reproducing, a high fecundity, and produce relatively large (~0.7mm) translucent embryos that can be obtained throughout the year. The optical clarity of the embryo allows direct visualization of individual cells and the cell movements that occur within the developing embryo. This visual accessibility coupled with the short life cycle and the external fertilization of the zebrafish egg make studying the developmental processes of the zebrafish a relatively easy task (Eisen, 1996; Streisinger *et al*., 1981). Interestingly, despite the importance and advantages of this species, little is known about the natural history, habitats, and native distribution (Arunachalam *et al*., 2013). Though zebrafish have recently been proposed as a possible model organism for nutrition and growth studies in fishes (Alestro *et al*., 2006).

1.1.2 Zebrafish as a model organism

Zebrafish as a model organism is very popular nowadays. There are some reasons being zebrafish a model organism. The fish is very convenient for the experiment for the following reasons (Chakraborty *et al*., 2009).

Large numbers of fish can be kept easily and cheaply in the laboratory and they are easy to differentiate the male and female (Spence *et al*., 2006). Generation time is very short typically 3 to 4 months which makes it suitable for experiment. *D. rerio* eggs are large relative to other fish (0.7 mm in diameter at fertilization) and optically transparent. Development is rapid, with precursors to all major organs developing within 36 hours, and larvae display food seeking and active avoidance behaviors within five days after fertilization. As it is a very hardy fish,high stocking density and poor oxygen level is tolerable for them (Chakraborty *et al*., 2009).

1.1.3 Morphology

Zebrafish have fusiform, laterally compressed bodies that reach an average length of 25 mm. The largest recorded zebrafish reached 64 mm in captivity. They have centrally located eyes and thin elongate mandibles with a protrusive lower jaw that causes the mouth to point upwards. Zebrafish have several defining features including an incomplete lateral line, two pairs of barbels, and several longitudinal stripes along the sides of their body. Like other cyprinids, zebrafish are stomachless and toothless. As a result, they rely on gill rakers to break up food. Additionally, they are obligate suction feeders. The degree of sexual dimorphism in zebrafish is minimal, as males tend to have more yellow coloration and tend to have larger anal fins than females (Albertson and Kocher. 2006). Zebrafish lives on average 3.5 years, with oldest individuals surviving up to 5.5 years. Gerhard *et al*., 2002)

1.1.4 Habitat

The natural range of the zebrafish is centred around the Ganges and Brahmaputra river basins in north-eastern India, Bangladesh and Nepal although in the past specimens have also been collected in the Indus, Cauvery, Pennar, Godavari and Mahanadi river basins (Talwar *et al*., 1991). Zebrafish is largely confined to and most frequently associated with habitats of low flow and with a sandy substrate in secondary and tertiary channels connected with the main channel of a stream/river (Arunachalam *et al*., 2013). They appear to be a floodplain rather than a true riverine species. They are most commonly encountered in shallow ponds and standing water bodies, or habitats adjacent to wetlands and paddy fields. The association with rice cultivation may relate to the use of fertilisers that may promote the growth of zooplankton, a major component of the zebrafish diet (Spence *et al.*, 2007). Habitat types are identified from various geographic locations. These connections can be natural channels or man-made irrigation canals, beels, or culture ponds (Arunachalam *et al*., 2013). It is a small and robust fish, so large numbers can be kept easily and cheaply in the laboratory, where it breeds all year round.

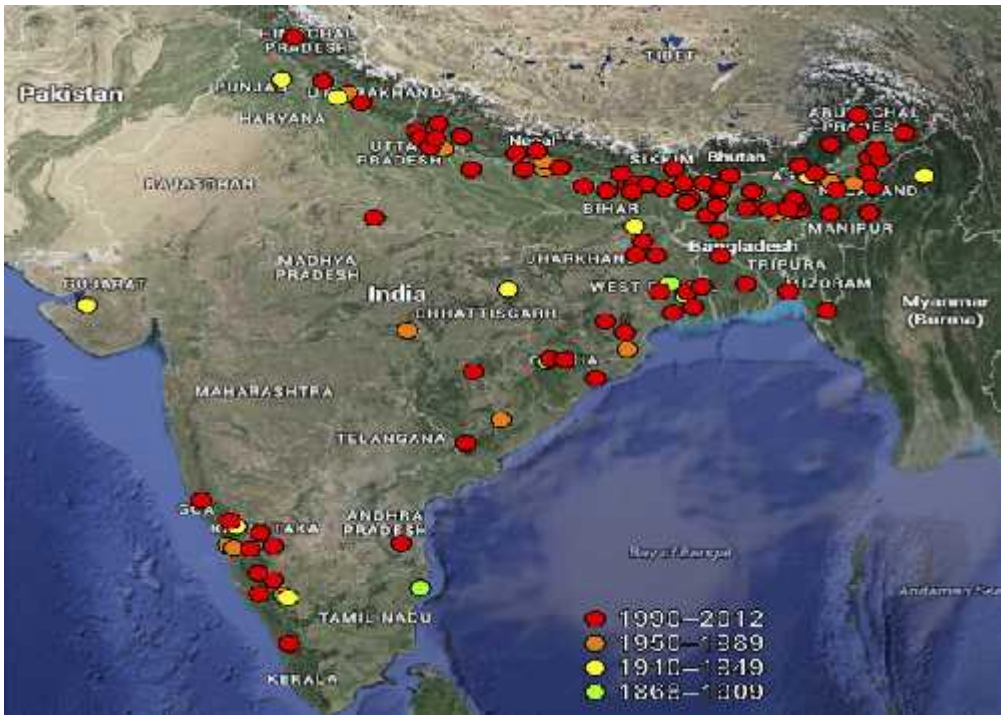

Fig. 1. Zebrafish and their geographic range. Historic and more recent sites where zebrafish have been reported in India, Nepal, Bangladesh and possibly Myanmar (Spence *et al*., 2006)

1.1.5 Diet

Zebrafish are omnivorous. The diet of zebrafish in Bangladesh primarily consisted of zooplankton and insects. Growth rates o zebrafish depend on diet and Growth rates also varied with age and season, with the period of most rapid growth in early life during the monsoon months (Spence *et al.*, 2007).

Though their natural diet consists primarily of zooplankton and insects, phytoplankton, filamentous algae and vascular plant material, spores and invertebrate eggs, fish scales, arachnids, detritus, sand and mud have also been reported from gut content analysis. The majority of insects identified in these studies were aquatic species, or aquatic larval forms of terrestrial species like dipterans, and it has been recommended that the zebrafish have some value in mosquito control (Dutta, 1993).

When planktonic items are high in their diet it indicates that zebrafish feed chiefly in the water column. However, terrestrial insects and arachnids are also consumed, suggesting surface feeding, while the presence of inorganic elements and detritus suggest they also feed from the substrate. They can eat a variety of other foods, such as worms and small crustaceans, if their preferred food sources are not readily available (Spence *et al*., 2007). However, zebrafish are not a universally ideal research model. There are a number of disadvantages to their scientific use. Absence of a standard diet is one of the major disadvantages (Engeszer *et al*., 2007).

1.2 Zebrafish husbandry

1.2.1 Chemistry of water

A primary cause for the rise of the zebrafish as an experimental model animal is their capability of tolerance of a vast range of environmental conditions in captivity. Their adaptive capability is a reflection of their scattered distribution in the wild (Talwar and Jhingran, 1991). It is important to identify that there is an energetic cost to fish in operating outside their optimum range of environmental parameters. Fishes maintained under sub-optimal conditions must devote an increasing proportion of energy towards maintaining homeostasis, rather than on growth and reproduction (Wooton, 1998). A consequence of sub-optimal conditions decreases growth rates, the number and quality of offspring, and ultimately, survival (Haywood, 1983). Thus, determination of optimal

ranges of water quality parameters is vital for zebrafish in captivity, so that mortalities can be minimized, fish growth and reproduction can be qualitative and rapid as well as.

One of the most vital physical parameters is temperature which is notable to consider in fish culture operations because of the profound effects it applies on chemical and biological processes in living systems (Boyd, 1979). Fish as a poikilothermic animal display varying degrees of tolerance to changes in temperature, as well as a more narrow optimum range in which they perform well (Kelsch and Neill, 1990). As zebrafish exhibit a tolerance for wide temperature ranges, they can be classified as eurythermal. Data from controlled laboratory experiments (Cortemeglia and Beitinger, 2005; Schaefer and Ryan, 2006) indicate that zebrafish have a maximal thermal tolerance range of 6.7–41.7 °C, which puts them in a similar class with the most eurythermal fish species (Bennett and Beitenger, 1997). Fish that are acclimated for a period of time at lower temperatures can extend their lower temperature tolerance further than fish acclimated to higher temperatures (Cortemeglia and Beitinger, 2005; Schaefer and Ryan, 2006). This pattern of temperature flexibility has also been documented in natural populations. Observations of water temperature from nine different sites at which zebrafish were collected in Bangladesh ranged from 16.5°C to 33°C (Spence *et al*., 2006). These data provide enough evidence that the broad tolerance shown by zebrafish in laboratory experiments is not an artificial response, but rather is representative of conditions they experience in natural environment. The optimum temperature for zebrafish has not been formally defined. The maintenance temperature of 28.5°C recommended by Westerfield (1995) is almost universally cited for zebrafish in culture.

The hydrogen ion concentration of water in aquatic environment has some effects on biological processes in fish and the function of the microbial community that related to them. In closed recirculating aquaculture systems, the recommended optimal pH range is 7-8 for the bacterial flora in biofilms that metabolize nitrogenous wastes excreted by fish (Masser *et al*., 1999). While most freshwater fish can tolerate a wider pH range of ~6.0–9.5, it is generally practical to maintain most freshwater fish at a pH in the 7–8 range in order to promote good health of biofilters and stable water quality (Timmons *et al*., 2002). However, all fishes display a specific range of preference where growth, food conversion, and reproduction is optimal, and consequently, the goal of pH management in culture is to balance these needs.

Very limited data from field studies suggest that zebrafish are encountered in slightly alkaline waters. Spence *et al*., (2006) reported an average pH of 8.0 across nine zebrafish habitats in Bangladesh, and McClure *et al*., (2006) found a similar mean pH of 8.0 at 3 sites in India. Waters in the Ganges River drainage have also been reported to be typically alkaline, with an average pH in excess of 8.0 from (Payne *et al*., 2003). The optimal pH for zebrafish in captivity has never been apparently determined. The maintenance pH that most zebrafish facilities strive for is between 7.0–8.0, which is within the general range recommended for freshwater fish and this clearly is convenient to successfully rear and breed zebrafish in laboratory (Brand *et al*., 2002).

Mainly calcium and magnesium are the parameters which are considered to measure water hardness and to a lesser extent, iron and selenium are also involved in calculating hardness in water (Wurts, 2002). Fish require these ions for biological function, and they must either be provided to fish in captivity in their water or diet. The most important of these ions, calcium, is required by fish for ossification, blood clotting, and a number of other biological and physiological processes (Wurts, 1993). Zebrafish have been classified as a "hard water" species, preferring hardness values in excess of 100 mg/L CaCO3 (Brand *et al*., 2002). However, experimental evidence documenting this is limited. Zebrafish showed decreased resistance to soft environments in a comparative study with goldfish (Carrasius auratus) and ayu (Plecoglossus altivelis), a pattern the authors attributed to the presumption that zebrafish were not often subjected to low calcium conditions in nature (Chen *et al*., 2003). A review of the literature does not reveal any recorded hardness values for habitats within the natural range for zebrafish. Systematic experiments investigating the effect of varying ranges of hardness on reproduction, growth, and disease resistance could further strengthen this classification and would be helpful in determining guidelines for zebrafish culture (Chen *et al*. 2003).

In fish cultivation, dissolved oxygen is a significant parameter to be considered (Boyd, 1979). Though zebrafish is a hardy fish, low levels of dissolved oxygen are responsible for more mortality of fish in culture than any other parameters (Timmons *et al*., 2002). Fish require oxygen for respiration, and demand depends upon a number of factors, including body size, feeding rate, activity levels, and temperature. The availability of

dissolved oxygen in the water is determined by water temperature, salinity, and water quality (Boyd, 1979).

The dissolved oxygen requirements of zebrafish have not been determined. In general, small-bodied, tropical fish such as zebrafish typically have high metabolic rates and, therefore, consume more oxygen per unit weight than larger fish (Helfman *et al*., 1997). This fact, coupled with their relatively high maintenance temperatures, stocking density, and levels of feed input that are typical of intensive zebrafish facilities necessitate that dissolved oxygen levels be maintained at or just under saturation (~7.8 mg/L at 28.0 °C) to ensure health of the fish. A number of warmwater species, such as tilapia (Popma and Masser, 1999), are tolerant of lower levels of dissolved oxygen, and it may be possible that zebrafish fall into this category, given that they are likely to encounter oxygen-poor environments in nature. There are requirements for detailed studies of zebrafish performance at varying levels of dissolved oxygen for developing standard dissolved oxygen range for culture.

1.2.2 Feeding

Feeding practices are also of crucial importance in fish husbandry. The amount of feed presented at each feeding and the frequency of application are both important components of feeding protocols, and are often specific to both the species and application of its culture (i.e. breeding versus meat production) (National Research Council, 1993). These parameters may also have profound impacts on feed efficiency, growth rates, and ultimately, gamete production in cultured fish (Lee *et al*., 2000). None of these parameters has been defined for zebrafish. In terms of ration size, there are two general approaches utilized in fish culture: feeding to satiation and body weight feeding. A derivative of the former method, the so-called “five-minute rule”, is commonly employed in zebrafish facilities (personal observation). This technique requires that no more (or less) food should be presented to fish at each feeding than they can fully consume within 5 min. However, this particular rule-ofthumb is suspect, given that there are seldom uniform numbers of fish in tanks and different feed types have different residence times and nutrient leaching rates in the water column. The result is that fish may be chronically over- or under fed using this scheme, a situation that can lead to decreases in water quality or depression of growth, reproductive function, and immune response.

Feeding by body weight involves providing a ration as a fixed percentage of fish body weight each day. In intensive culture systems, larval fish are typically fed more per day than adult fish, up to 50–300% of their body weight each day, compared to 1–10% for adults (Bryant and Matty, 1980, 1981). This method, which necessitates that managers have accurate estimates of total fish weight in the system, is commonly employed in commercial aquaculture, but rarely, if ever, utilized for zebrafish in research settings. However, it probably represents the most efficient and scientifically sound manner in which to determine feeding allowances for zebrafish.. Standards for frequency of feedings are also lacking for zebrafish. Data from other fishes generally indicate that the number of feedings required per day will generally decrease as fish get older (e.g. Pullin and Lowe-McConnell, 1982), but the number (as well as the amount of food in each) most appropriate for each life stage is often very species and environment (e.g. temperature) specific.

It may be reasonable to speculate that because zebrafish are small-bodied and lack a true stomach, it would be best to present them with frequent, small meals throughout the day to promote maximal assimilation, but this remains to be demonstrated. At any rate, an ideal frequency can be readily determined via a series of simple experiments, and should be combined with the results of ration size studies to determine the most suitable feeding regimen for zebrafish.

1.2.3 Breeding and reproduction techniques

1.2.3.1 Reproduction

Relatively little is known about zebrafish breeding and reproductive behavior, particularly in natural settings. However, results of a number of laboratory-based experiments, along with anecdotal observations stemming from years of use as a research model organism provide a reasonable picture of reproduction in this animal.

Zebrafish are asynchronous, batch spawners that, under favorable conditions, spawn continuously upon attainment of sexual maturation (Breder and Rosen, 1966). Females are capable of spawning on a nearly daily basis. Eaton and Farley found that females would spawn once every 1.9 days if continuously housed with a male (Eaton and Farley, 1974), and Spence and Smith (2005) showed that females in their experiments were capable of producing viable clutches every day over a period of at least 12 days, though

variance in egg production was substantial. This interval is likely to be greater when the environment (water quality, diet, social situation, etc.) is sub-optimal or if the fish are used for production frequently.

Olfactory cues play a vital role in zebrafish reproduction. The release of steroid glucuronides into the water by males induces ovulation in females (Chen and Nartinich, 1975; van den Hurk and Lambert, 1983). After ovulation, females release hormones that in turn prompt male mating behavior that immediately precedes and elicits oviposition and spawning (van den Hurk and Lambert, 1983). Pheromones also appear to have the ability to suppress reproduction, as holding water from "dominant" female zebrafish has been shown to inhibit spawning of subordinate females (Gerlach, 2006). This information should be considered in the design of broodstock management regimes; for example, a combination of increased chemical filtration and rate of water replacement on recirculating systems may help to reduce potential decreases in the reproductive output of broodstock brought about by pheromonally mediated dominance interactions between females.

Reproduction in zebrafish is strongly regulated by photoperiod. Zebrafish most commonly spawn at dawn, within the first few hours of daylight, in both the laboratory (Selman *et al*., 1993) and the wild (Spence *et al*., 2006). However, spawning does not seem to be strictly limited to this time period. In captivity, zebrafish will breed throughout the day, particularly during the evenings prior to an imposed dark period and it is also possible to strip viable eggs from females throughout the day (personal observation). In the wild, zebrafish have also been observed spawning during the afternoon following the onset of heavy rain (Spence *et al*., 2006). So while clearly there is a strong relationship between photoperiod, ovulation, and spawning, control is not absolute. There also appears to be some element of mate choice in zebrafish. Ritualized mating behavior and the establishment and defense of territories on the part of males suggest that females may be selective (Darrow and Harris, 2004; Spence and Smith, 2005). This supposition is supported by the fact that females have been shown to produce larger clutches and spawn more frequently when paired with certain males (Spence and Smith, 2006a). However, the selective basis of female choice is unclear. Both male and female zebrafish show a strong preference for ovipositor site, selecting and preferentially spawning over gravel versus silt in both laboratory and field-based experiments (Spence

et al., 2006). Fish also showed a preference for vegetated over non-vegetated sites. Therefore, male defense of desirable spawning locations, over which females are choosy, may be the basis to the zebrafish mating system. Females may also select males based on their genotype. Many fish, including zebrafish, use olfactory cues to differentiate between kin and non-kin, and this mechanism may be utilized during breeding to avoid mating with close relatives. For example, female rainbow fish Melanotaenia eachamensis and guppies Poecillia reticulata prefer unrelated over related males based on visual and olfactory cues (Hughes *et al*., 1999; Arnold, 2000).

Zebrafish also appear to use olfactory cues in social and mating contexts. Using odor plume tests, Gerlach and Lysiak showed that adult female zebrafish chose the odors of non-related, unfamiliar (reared and maintained separately) males over those of unfamiliar brothers for mating (Gerlach and Lysiak, 2006). The underlying genetic basis of this preference is unknown, but may be the major histocompatibility complex (MHC) genes that are important in kin recognition in other fish species (Apanius *et al*., 1997). Clearly, years of breeding zebrafish (in many cases, via sib-mating) in captivity demonstrates that female choice based on any one or all of these factors may be readily overridden. However, a more comprehensive understanding of reproductive behaviors may facilitate the design of improved spawning techniques and breeding programs that can ultimately help increase spawning efficiency in laboratory breeding facilities.

1.2.3.2 Breeding techniques

The most basic and the first formally described breeding technique for laboratory zebrafish involves placing marbles at the bottom of holding or special breeding tanks. When fish spawn over the marbles, the eggs drop into the spaces in between, preventing egg cannibalism and facilitating their subsequent collection (Westerfield, 1995; Brand *et al*., 2002). While this method may be effective to some extent, it is generally over-simplified and impractical for use in large culturing facilities with hundreds or thousands of tanks. Despite its shortcomings, it is still frequently cited in the methods sections of zebrafish papers, and is often used by investigators breeding zebrafish for the first time. The majority of zebrafish breeding facilities currently utilize a dedicated breeding tank technique that adheres to the following general principles: a small (typically b1 L) plastic mating cage or box with a mesh or grill bottom is placed inside a slightly larger container that is filled with water. Fish (pairs or small groups) are then added to the box in the evening. When the fish spawn (usually the following morning; see above discussion), the fertilized eggs fall through the "floor" of the inner box and are thereby protected from cannibalism by adults (Mullins et al., 1994). This technique has proven to be generally effective and, consequently, derivations of the trap design are manufactured by a number of aquaculture and laboratory product supply companies. Available products vary slightly in size, shape, depth, and total volume, as well as adjustability. Surprisingly, the effects of these parameters on reproductive success have not been formally investigated.

Clearly, the growing popularity of the zebrafish as an experimental model indicates that the ability of facility managers to induce reliable egg production in their zebrafish stocks does not appear to be a major problem in the field. However, it is unclear as to how efficient this process is, and should be, across rearing facilities. Although zebrafish appear to spawn under a wide range of conditions in the laboratory, reductions in the overall egg production may reflect sub-optimal husbandry parameters, and could constitute an unacceptable situation as far as fish welfare is concerned. Therefore, data on the average broodstock spawning parameters (i.e. spawning success, fertility, clutch size, inter-spawn interval, reproductive longevity for standard zebrafish wild-type strains, etc.) should be collected from a wide number of culturing facilities. Such information would facilitate the design of experiments that could be used to help establish standards in broodstock production and quality for the zebrafish research community.

1.3 Growth study of zebrafish

Zebrafish have some benifits as a model organism for nutritional and growth studies. Zebrafish possess the most developed genomic program compared to that of any other aquacultured fish, they are easy to maintain and breed, have short generational time and produce a large number of offspring. These advantages permit the use of the zebrafish as an excellent springboard for population studies and improving reproducibility of the experiments. However, zebrafish can feed from animal and vegetal protein sources, which allows us to infer that it has nutritional pathways similar to those of cultivated herbivores such as carp and tilapia, and to those of carnivores such as trout and salmon. Nevertheless, more studies on zebrafish nutrition and protein, carbohydrates and lipid requirements are needed to assess the nutritional requirement for optimal skeletal muscle growth in fishes (Ulloa *et al*., 2011).

1.4 Embryogenesis

Zebrafish are considered as the latest model for embryological development studies. These embryos have the great advantage that they develop as "see through" embryos, that is, all internal development can be clearly observed from the outside in the living embryo. That is the main reason of the use of zebrafish for embryogenic studies. There are seven broad periods of embryogenesis-the zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and hatching periods. These divisions highlight the changing spectrum of major developmental processes that occur during the first 3 days after fertilization (Kimmel *et al*., 1995).

1.5 Relationship between glofish and zebrafish

GloFish are zebrafish. Scientists added a fluorescence gene to the genetic code of zebrafish. Because GloFish are fluorescent fish, they absorb light and then re-emit it, so they appear brighter and more vibrant as the amount of light is increased (Darrow and Harris., 2004).

Glofish are the first genetically modified animals to become publicly available. GloFish are available in eight stunning colors: Starfire Red®, Electric Green®, Sunburst Orange®, Cosmic Blue®, Galactic Purple®, Red Danio, Green Danio, and Orange Danio. No differences between GloFish and Zebra Danios have been observed, aside from their coloration. They are shoaling fish, which should be kept in groups of three or

more; like Zebra Danios, they find security in numbers. The differences or descriptions of observable parts of glofish and zebrafish are given in table 1: (Devlin *et al*., 2006)

Table 1: Differences between zebrafish and glofish.

Observable part	**Zebrafish**	**GloFish**
Fins	Five fins	Five fins
Number of Horizontal Stripes	Five stripes	Five stripes
Gills	A pair of gills	A pair of gills
Eyes	Two black eyes	Two black eyes
Color	Black and white stripes	Red, green, white, blue or orange stripes

1.6 Research needs

The use of the zebrafish as a model organism has grown dramatically over the last two decades. The rise in popularity of the zebrafish in research can at least be partially attributed to the relative hardiness of the species. The zebrafish, native to the flood plains of South Asia, is an adaptable and tolerant species that develops quickly and produces offspring reliably when cultured in the laboratory. Ironically, due in part to the hardiness of the species, the development of optimal husbandry protocols for zebrafish has stagnated and understanding of their unique environmental and nutritional requirements is limited. Without this information, zebrafish culturists are often left to develop husbandry protocols based on educated guesses that can potentially result in sub-optimal culture conditions. Over the last few years though there has been a concerted effort by the zebrafish community to develop culture techniques that result in improved survival, growth and reproductive performance. Naturally, many of these studies have concentrated on determining proper feeds and feeding practices to improve growth, survival and reproductive performance of the zebrafish. Most research facilities culturing zebrafish rely upon some type of manufactured diet to provide the majority of nutrition to their zebrafish stocks. These diets are generally assumed to be nutritionally complete and can often be used as a sole source of nutrition throughout the life cycle of the

zebrafish (Carvalho *et al*., 2006; Siccardi et al., 2009). As it is said earlier that absence of a standard diet is one of the major disadvantages of zebrafish. It is important to note that the specific dietary requirements of the zebrafish are not well defined and results with manufactured diets can be highly variable. It has been demonstrated that growth, yield, generation time and reproductive performance of the zebrafish is improved by supplementing manufactured diets with live feeds organisms (Goolish *et al*., 1999). So research is needed to specify a standard diet for zebrafish.

1.7 Scope of the review

We begin with a summary of the taxonomic status of the zebrafish, which has recently undergone revision, together with a brief description of its external appearance, and a summary of the main laboratory lines currently used in research. We then review what is known of its natural ecology including distribution, habitat, natural diet, growth, yield, reproduction and mortality. Growth, yield, reproduction and mortality in domesticated zebrafish are compared among different diets as we attempt to review zebrafish development. The next section focuses on zebrafish reproductive ecology, including spawning behavior, which is largely known only from studies on domesticated strains although some information is available on wild fish. The majority of behavioral studies on zebrafish are concerned with their aggregation and shoaling. We conclude by suggesting potential future directions for research using the zebrafish as a behavioral model.

1.8 Objectives

The experiment was conducted to find out a standard diet for zebrafish which will be very effective on the growth, yield and reproduction of zebrafish. The specific objectives of this research are given below:

- **a.** identification of the effects of different natural and commercial feeds in zebrafish yield;
- **b.** observation of the growth of zebrafish for different commercial and natural feeds;
- **c.** identification of the effects of natural and commercial feeds in the reproduction of zebrafish;
- **d.** to find out the most effective diet for zebrafish's growth, reproduction and yield;
- **e.** to make a statistical data of the growth of zebrafish for different feeds during the whole life cycle.

CHAPTER 2: MATERIALS AND METHODS

Chapter 2

Materials and Methods

2.1 Experimental fish

The experiment was conducted with glofish (transgenic red fluorescent protein Glofish) as an alternative of zebrafish. Zebrafish are some of the easiest egg laying fish to breed in the tank and glofish should be no different. So the glofish was selected for the purpose of experiment due to several reasons. For many decades, it has been both a very popular aquarium fish and an important experimental model in fields of research. Hundreds of labs around the world now routinely use *D. rerio* in both basic and applied research.

Fig. 2. Zebrafish

2.2 Scientific classification of Zebrafish (*Danio rerio)* (Francis Buchanan-Hamilton, 1822)

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cyprinidae

Genus: *Danio*

Species: *Danio rerio* (F. Hamilton, 1822)

Common Name: Zebrafish

Local Name: Anju

2.3 Collection and Stocking of Fish

All the fish were collected from Bismillah Aquarium fish Center, Dhaka university market, Katabon, Dhaka. Fish were transported by plastic bags having oxygen facilities and transferred to all tanks respectively. During the stocking sufficient care was taken to reduce stress.

2.4 Study Area

The experiment was conducted in 15 (eight) experimental tanks which were located at the Department of Fisheries, University of Dhaka. All of those tanks were randomly placed in a steel made rack.

2.5 Study Period

The study was carried out from 20 June, 2015 to 8 September 2015. Altogether the whole study period was 78 days. All the experiment, procedures, processes were done during this period of time.

2.6 Experimental Design

The experiment was designed (3×5) with three replicates in the lab room of Department of Fisheries, University of Dhaka. There were fifteen tanks used to complete the purpose of the experiment.

All the tanks were filled up with tap water and labeled according to experimental design. Each of the tanks was filled up with tap water in the quantity of about 3 liters. In total 315 zebrafish were stocked in all of the tanks and each of the tanks containing about 21 fishes. Aerator was used for 24 hours during the experimental period. The fishes were released slowly into the experimental tanks for experiment with the help of scoop net and at the same time, oxygen was supplied with the help of Aerator. No feed was given on first day. Length and weight of all of the fishes were measured after stocking and must be before giving feed. Length was measured by measuring steel scale and weight was measured by digital electric balance.

Five different types of natural and commercial feeds were used for this experiment such as treatment 1 (diet-1) was fed with the Dried tubifex, treatment 2 (diet-2) was fed with the live *Artemia* and treatment 3 (diet-3) was fed with *Artemia* with commercial pellet feed as complementary feed, treatment 4 (diet-4) was fed with Spirulina with commercial pellet feed as complementary feed and treatment 5 (diet-5) was fed with commercial pellet feed only. All the feeds were given from the second day of stocking.

2.7 Apparatus and Materials

1. pH meter
2. Beaker
3. micro pipette
4. DO meter
5. Digital electric balance
6. Aerators
7. Bowls
8. Measuring steel scale
9. Small beakers
10. Tanks
11. Multiplug
12. Zebrafish
13. *Artemia* cyst
14. Special tank for Artemia culture
15. Dried tubifex
16. Spirulina powder feed

17. Pellet/flake feed (Commercial feed)
18. Fresh tap water
19. scoop net for collecting sample
20. Multiparameter water quality meter

2.8 Tank Construction

Tank construction is a concern in zebrafish research. Although there are no published standards for the size and shape of tanks used for zebrafish, commercially available options generally fall into a distinct size and space. So for the experiment, tanks were ordered with a targeted size regarding the primary factors in determining tank sizes and shapes. The size of all tanks was 24cm in length × 18.5cm in width × 20cm in deep. All the tanks were made of glass.

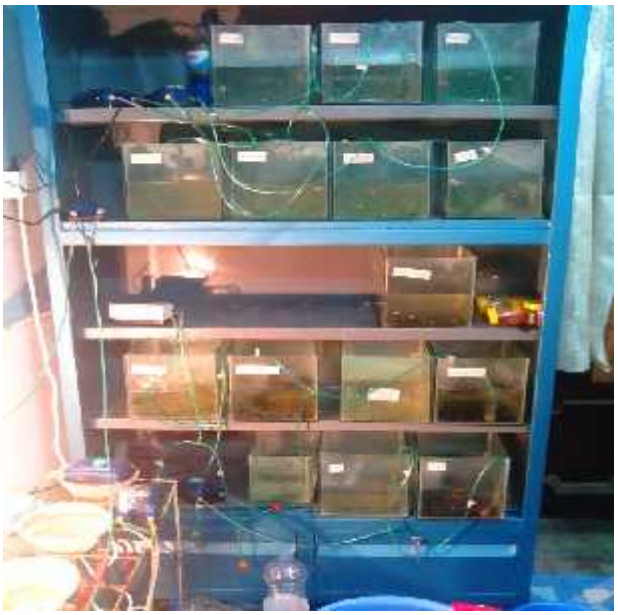

Fig. 3. Experimental tanks

2.9 Selection of Feed

There were five types of feeds used in the experiment these are as follows:

Table 2: Feeds used for the experiment.

Number of Diets	Feeds
D1	Dried tubifex
D2	Live *Artemia* worm
D3	*Artemia* + commercial pellet feed
D4	Spirulina + commercial pellet feed
D5	Commercial Pellet feed

Artemia cyst (GSL,USA) were collected, rinsed with filter–treated water, and collected in a squirt bottle containing culture water. The optimal salinity for artemia is 35-40 ppt (Verschuere et al., 1999). To keep the salinity level convenient for artemia culture 15g salt was mixed with 500ml water though the salinity was 30 ppt for *Artemia* culture throughout the study period. Oxygen was provided to *Artemia* by using an aquarium pump and plastic tubing until *Artemia* were fed to the zebrafish in the study.

Dried tubifex cube was collected from Aquarium fish market in a much fined box which was wrapped with aluminum foil to reduce food spoilage and other contaminations.

Spirulina powder was collected from BCSIR (Bangladesh Council of Science and Industrial Research, Dhaka) as it is widely used as both human and animal food. As well as Spirulina is highly nutritive.

The commercial feed was collected from Aquarium fish market in a much fined box which was wrapped with aluminum foil named “TetraBits Complete, Germany”. It is a Complete and balanced nutritional staple food ideal for all mid-water and bottom feeding fish.

Fig. 4. Commercial pellet feed

Fig. 5. *Artemia* cyst

Fig. 6. Dried tubifex

Fig. 7. Spirulina powder

2.9.1 Proximate composition of feeds

The proximate compositions of feeds used for the experiment are shown in table 3. The composition was collected from the manufacturer of the diet.

Table 3: Proximate composition of feeds used for the experiment.

Feeds	Protein (%)	Mineral components (%)	Fat (%)	Moisture (%)	Crude Fibre (%)
Tubifex	50-60	6.9	10	4	2
Artemia	39.4	28.1	4.96	9.6	5
Spirulina	60-70	8	0.27-0.47	7	10-20
Commercial pellet feed	47	9	10	6	3

2.10 Feeding Strategy

Each types of feed was given into each of those experimental tanks and observed till the point of satiation. Satiation is the point within a 5-min period, where zebrafish were no longer actively searching for food. The feed was supplied by manual spreading method. The experimental tanks were monitored everyday to observe the behavior of fishes. All the ponds were kept clean to provide hygienic condition.

2.11 Water Quality Parameters

Water quality was measured twice through the whole study period. But to keep the temperature suitable for fish the temperature was fully maintained by air-condition machine throughout the experiment period. pH, Temperature, dissolved oxygen (DO; mg/L), Turbidity, ORP, Resistivity (MΩcm) and conductivity were measured respectively.

2.11.1 Water temperature (ºC)

The temperature of tank water was recorded with help of Multiparameter water meter (Model: HI9828 multiparameter, HANNA instruments, woonsocket. RI-USA) between 9.30 to 10.30 a.m.

2.11.2 Dissolved oxygen (DO)

The dissolved oxygen (DO) of tank water was recorded with help of a DO meter between 9.30 to10.30 a.m.

2.11.3 Hydrogen ion concentration (pH)

pH meter was used to measure the pH of the tank water. It was recorded between 3:30 to 4:30 p.m.

2.11.4 Total dissolved solids in water (TDS)

TDS is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular suspended form. It was measured by Multiparameter water meter (Model: HI9828 multiparameter, HANNA instruments, woonsocket. RI-USA) between 9:30 to 10:30 a.m.

2.11.5 Oxygen Reduction Potentiality in water (ORP)

ORP stands for Oxidation-Reduction Potential. It was measured by Multiparameter water meter (Model: HI9828 multiparameter, HANNA instruments, woonsocket. RI-USA) between 3:30 to 4:30 p.m.

2.11.6 Conductivity (mS/cm)

Conductivity is a measurement of the electrical conductance per unit distance in an aqueous solution. Conductivity was recorded by Multiparameter water meter (Model: HI9828 multiparameter, HANNA instruments, woonsocket. RI-USA) between 9:30 to 10:30 a.m.

2.11.7 Resistivity (MΩcm)

Resistivity was recorded to measure the purity level of the water and it was recorded by Multiparameter water meter (Model: HI9828 multiparameter, HANNA instruments, woonsocket. RI-USA) between 9:30 to 10:30 a.m.

2.12 Water Exchange

Tap water was used for adding water in the tank. 4 L water was added in each tank per day at one day interval. This method mitigated pollution from excretory product of individuals and maintained water quality suitable for the growth of the experimental fish and as well as the productivity.

2.13 Fish Sampling Procedure

Fish sampling was done at thirty days interval started from the stocking of fish. From each tank 33.3% fish was collected for measurement. During each sampling, fish had been caught by fine mesh scoop net then they were kept in a concentration of 2-phenoxyethanol. The concentration was made of 18µl 2-phenoxyethanol and 60 ml water. It is anesthesia that allows them to be performed out of the water with decreased stress and it minimizes the movement of fish during length and weight measurement. Micro pipette was used take in anesthesia.

Fig. 8. Length measurement of fish with steel scale

Fig. 9. Weighting of fish with digital weighting balance (Model: EK600Dual, AND-Gulp)

Then weight was measured by digital weighting balance (Model: EK600Dual, AND-Gulp) and length was measured by steel scale. Their individual lengths and weights were recorded to the nearest centimeter and nearest gram respectively for the analysis of fish. Altogether length and weight of 105 fishes were measured from every sampling. The final length (cm) and weight (g) of the individual fish were carefully recorded.

2.14 Growth parameters

The following parameters are used to evaluate the growth of fish such as weight gain, average daily wight gain (g/day), percent weight gain (%), specific growth rate (SGR), Gender weight gain (g), Length enhancement (cm), food conversion ratio (FCR), survival rate (%).

2.14.1 Weight gain

Weight gain is an increase in body weight. This can involve an increase in muscle mass, fat deposits, excess fluids such as water or other factors (Gonzales, 2012).

Weight gain was calculated as

$$\text{Weight gain (g)} = \text{Mean final weight - Mean initial weight}$$

2.14.2 Gender weight gain

Gender weight ratio, defined as the ratio between the weight gains of female fish compared to male fish (Gonzales, 2012). It was determined by using the following equation:

$$\text{Gender weight gain (g)} = \frac{\text{Wf}}{\text{Wm}}$$

Wf = Female weight gain

Wm = Male weight gain

2.14.3 Specific growth rate

Specific growth rate determines was determined by using the following equation (Gonzales, 2012).

$$\text{Specific growth rate (g)} = \frac{(\ln W_2 - \ln W_1)}{(T_2 - T_1)} \times 100$$

Here,

W2= Mean final weight (g)

W1= Mean initial weight (g)

T2= Time at the end of the experiment

T1= Time at the start of the experiment

2.14.4 Condition factor

Fulton's condition factor, K, is another measure of an individual fish's health that uses standard weight. Proposed by Fulton in 1904, it assumes that the standard weight of a fish is proportional to the cube of its length (Nash *et al*., 2006).

Condition factor was determined by using the following equation:

$$\text{Condition factor, K(\%)} = \frac{\text{Fish weight}}{(\text{Fish length})^3} \times 100$$

2.14.5 Length gain

Length gain is an increase of body length.

Length gain of the fish was determined by using the following equation:

Length gain (cm) = Mean final length (cm) - Mean initial length (cm)

2.14.6 Food conversion ratio

Food conversion ratio is a measure of an animal's efficiency in converting feed mass into increases of the desired output. It is a function of the quality of the feed, and the conditions in which the animal is kept.

Food conversion ratio was calculated by following equation:

$$\text{Food conversion ratio, FCR} = \frac{\text{Feed (g) consumed by the fis}}{\text{Weight (g) gain of the fi} \quad (\overline{W_2} - \overline{W_1})}$$

2.14.7 Survival rate

Survival rate is a part of survival analysis, indicating the percentage of people in a study or treatment group who are alive for a given period of time after diagnosis (Gonzales, 2012).

Survival rate was measured by following equation:

$$\text{Survival rate (\%)} = \frac{\text{Number of fis harvested}}{\text{Number of fi stocked}} \times 100$$

2.15 Reproductive Evaluation

To determine the reproductive evaluation of the experimental fish for different diets, spawning was done twice.

2.15.1 Sampling procedure

During each spawning trial, male and female zebrafish from each dietary treatment were randomly placed in pair wise crosses (n=5) and allowed to spawn overnight in 0.5-L spawning tanks. Five pairs of fish were selected from each diet and kept in five different spawning tanks. Altogether there were 25 spawning tanks for five diets with 5 spawning tanks each.

Fig. 10. Breeding tanks

2.15.2 Spawning success

Spawning success, defined as the total number of females that spawn during a spawning trial. It was calculated at the end of each spawning trial as: (Gonzales, 2012).

$$\text{Spawning success (\%)} = \frac{\text{Number of spawning events per dietary treatment}}{\text{Number of pairs established}} \times 100$$

2.15.3 Fertilization rate

Fertilization rate is used to measure how many oocytes become fertilized by sperm cells. To calculate the fertilization rate of fish, determination of fecundity was needed and it was measured by counting the number of viable eggs released by a female zebrafish during a spawning event. Fertilization rate was determined using the following equation (Gonzales, 2012).

$$\text{Fertilization rate(\%)} = \frac{\text{Number of fertilized embryos}}{\text{Total number of embryos produced at a spawning event}} \times 100$$

2.15.4 Hatching rate

Hatching rate was determined using following equation:

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched eggs}}{\text{Total number of fertilized embryos}} \times 100$$

2.15.5 Embryogenesis

Embryogenesis is the development of a fertilized egg that occurs early on in pregnancy. After a sperm fuses with an egg, many changes occur in a specific order. The cells divide, reorganize and form layers of tissue that will eventually develop into specific organs. Zebrafish have seven broad periods of embryogenesis. All periods occur during the first 3 days after fertilization.

Table 4: Periods of embryogenesis of zebrafish.

Name of periods of embryogenesis	Duration (Hour)
Zygote Period	(0-0.25h)
Cleavage Period	(0.25-2.25h)
Blastula Period	(2.25-5.25h)
Gastrula Period	(5.25-10 h)
Segmentation Period	(10-24 h)
Pharyngula Period	(24-48 h)
Hatching Period	(48-72 h)

2.16 Statistical analysis

The data obtained on the growth of fish, FCR, survival rate, spawning rate and fertilization rate were statistically analyzed to see whether the influence of different treatments on the growth, yield and reproduction of fishes were significant or not. Significant differences between treatments were identified by Tukey Test and one-way ANOVA. This included significant results ($p<0.05$) were taken as rejection of the null hypothesis significance differences between the treatments. All the structured designs and data were analyzed using MS Excel 2007 and SPSS software.

CHAPTER 3: RESULTS

Chapter 3

Results

3.1 Water quality parameters

Different water quality parameters such as temperature (°C), DO (ppm), hydrogen ion concentration (pH), total dissolved solids (TDS), oxygen reduction potentiality (ORP), Conductivity (mS/cm) and Resistivity (MΩcm) were recorded throughout the experimental period. The mean values (±SEM) of water quality parameters of different treatments have been showed in table 5.

3.1.1 Water temperature (ºC)

The water temperature of experimental tanks varied from 25.20 to 30°C during the study period. The highest mean value (29.70±0.36ºC) of water temperature was in tanks of diet 4, the lowest mean value (28.93±0.27) was in tanks of diet 2 but there was no significant differences (P>0.05) among the diets.

3.1.2 Dissolved oxygen (DO)

Dissolved oxygen was recorded at 9:30 to 10:30 am. The highest mean value (3.91±0.29 ppm) of dissolved oxygen was in tanks of diet 2 and the lowest mean value (3.30±0.15) was in tanks of diet 1 but there was no significant differences (P>0.05) among the diets. Average dissolved oxygen concentrations under different diets are shown in table 5.

3.1.3 Hydrogen ion concentration (pH)

pH was recorded at 3:30 to 4:30 pm. The highest mean value (8.53±0.04) of pH was in tanks of diet 2 and the lowest mean value (8.10±0.06) was in tanks of diet 5. There were significant differences (p<0.05) of mean values of pH among different diets but there were no significant differences among diet 1, diet 2, diet 3, diet 4. Average hydrogen ion concentrations under different diets are shown in table 5.

Table 5: Water quality parameters (mean±SEM) recorded from different tanks of different diets.

Parameters	Diet					ANOVA
	D1	D2	D3	D4	D5	
Temperature(ºC)	29.02±0.36	28.93±0.27	29.70±0.36	29.43±0.49	29.52±0.48	NS
pH	8.50±0.09^{a}	8.53±0.04^{a}	8.38±0.12^{ab}	8.24±0.04^{ab}	8.10±0.06^{b}	*
TDS(ppm)	186±0.57^{a}	217.67±9.06^{b}	202.67±9.20^{ab}	184.33±1.20^{a}	186±1.52^{a}	*
ORP	43.06±2.28^{a}	23.56±4.15^{b}	43.40±8.40^{a}	50.90±1.62^{a}	67.03±3.31^{c}	*
DO(ppm)	3.30±0.15	3.91±0.29	3.87±0.20	3.76±0.17	3.74±0.12	NS
Conductivity	0.37±0.002^{a}	0.43±0.018^{b}	0.40±0.018^{ab}	0.36±0.002^{a}	0.37±0.003^{a}	*
Resistivity(MΩcm)	0.0027±0.00^{a}	0.0023±0.0001^{b}	0.0025±0.0001^{ab}	0.0027±0.00^{a}	0.0027±0.00^{a}	*

NS – Not significant (P>0.05)

*Significant (P<0.05)

Values are mean ± SEM of 3 tanks from each diet. Means in the line with different superscripts are significant at (P<0.05) and not significantly different at (P>0.05). Figures in the parenthesis indicate lowest and highest values.

3.1.4 Total dissolved solids in water (TDS)

Total dissolved solid in water was recorded at 9:30 to 10:30 am. From table 5 the highest mean value (217.67±9.06 ppm) of TDS was in tanks of diet 2 and the lowest mean value (184.33±1.20 ppm) was in tanks of diet 4. There were significant differences ($p<0.05$) among diets but there were no significant differences among diet 1, diet 2, diet 3 and diet 4.

3.1.5 Oxygen Reduction Potentiality in water (ORP)

Oxygen Reduction Potentiality in water was recorded at 3:30 to 4:30 pm. There were significant differences ($p<0.05$) of mean values of ORP among different diets but there were no significant differences among diet 1, diet 3 and diet 4. From table 5 the highest mean value (67.03±3.31) of ORP was in tanks of diet 5 and the lowest mean value (23.56±4.15) was in tanks of diet 2.

3.1.6 Conductivity (mS/cm)

Conductivity was recorded at 9:30 to 10:30 pm. The highest mean value (0.43±0.018 mS/cm) of conductivity was in tanks of diet 2 and the lowest mean value (0.37±0.002 mS/cm) was in tanks of diet 1. There were significant differences ($p<0.05$) of mean values of conductivity among different diets but there were no significant differences among diet 1, diet 4 and diet 5. Average conductivity under different diets is shown in table 5.

3.1.7 Resistivity (MΩcm)

Resistivity in water was recorded at 3:30 to 4:30 pm. There were significant differences ($p<0.05$) of mean values of resistivity among different diets but there were no significant differences among diet 1, diet 3, diet 4 and diet 5. From table 5 the highest mean value (0.0027±0.00 MΩcm) of resistivity was in tanks of diet 1, diet 4 and diet 5. The lowest mean value (0.0023±0.0001 MΩcm) was in tanks of diet 2.

3.2 Growth, yield, survivability and reproductive performance of Zebrafish fed with different natural and commercial feed.

3.2.1 Weight gain

Significantly (P<0.05) higher weight gain (0.118±0.005) was measured in zebrafish at 62 days fed with commercial pellet feed is shown in table 6. Lower weight gain (0.034±0.004) was determined in zebrafish at 62 days fed with dried tubifex feed. There are significantly differences in diet 5 with diet 1, diet 2, diet 3 and diet 4.

Table 6: Weight gain (g) (mean±SEM) of zebrafish fed with various diets during the experiment period.

Diet	Weight gain (g)	ANOVA
D1	0.034 ± 0.004^{a}(0.026-0.043)	
D2	0.075 ± 0.015^{a}(0.058-0.107)	
D3	0.072 ± 0.004^{a}(0.063-0.077)	*
D4	0.074 ± 0.008^{a}(0.057-0.085)	
D5	0.118 ± 0.005^{b} (0.108-0.128)	

* Significant (*P<0.05*)

Values are mean ± SEM of triplicate groups of 21 fish. Mean weight gain in the column with different superscripts are significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 11 shows the mean values of weight gain (g) for different diets graphically.

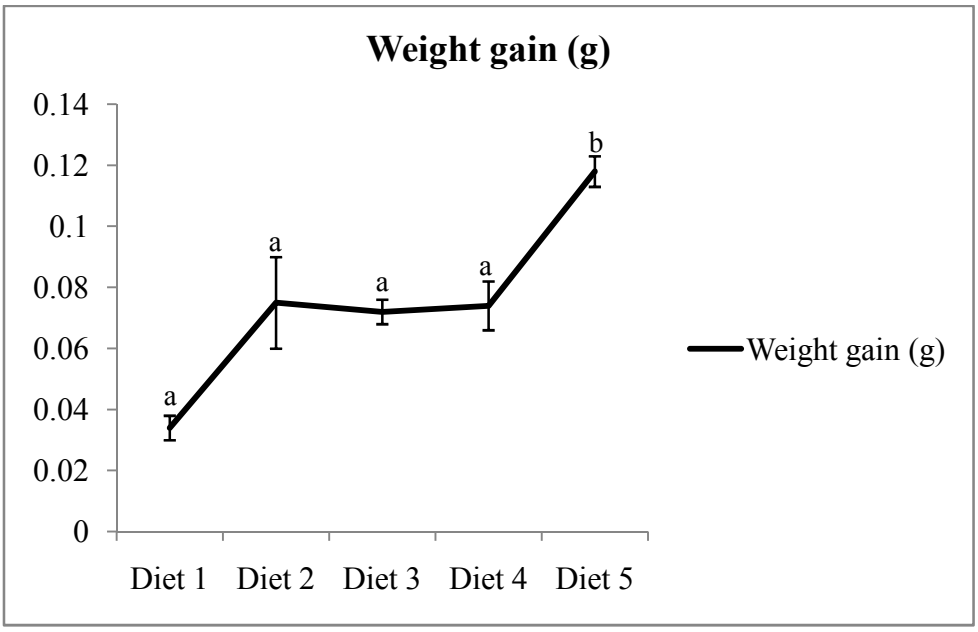

Fig. 11. Line diagram with different letters (a,b) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for weight gain (mean ± SEM) of different diets.

3.2.2 Specific growth rate (SGR)

Significantly (P<0.05) higher specific growth rate (1.510±0.115) was measured in zebrafish at 62 days fed with commercial pellet feed is shown in table 7. Lower specific growth rate (0.516±0.102) was determined in zebrafish at 62 days fed with dried tubifex feed. There are significantly differences in diet 5 with diet 1, diet 2, diet 3 and diet 4.

Table 7: Specific growth rate (%) (mean±SEM) of zebrafish fed with various diets during the experimental period.

Diet	Specific growth rate (%)	ANOVA
D1	0.516 ± 0.102^{a}(0.39-0.72)	
D2	1.253 ± 0.333^{ab}(0.89-1.92)	
D3	1.173 ± 0.112^{ab}(1.01-1.39)	*
D4	1.173 ± 0.033^{ab}(1.13-1.24)	
D5	1.510 ± 0.115^{b} (1.28-1.65)	

* Significant (*P<0.05*)

Values are mean ± SEM of triplicate groups of 21 fish. Mean specific growth rate in the column with different superscripts are significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 12 shows the mean values of specific growth rate (%) for different diets graphically.

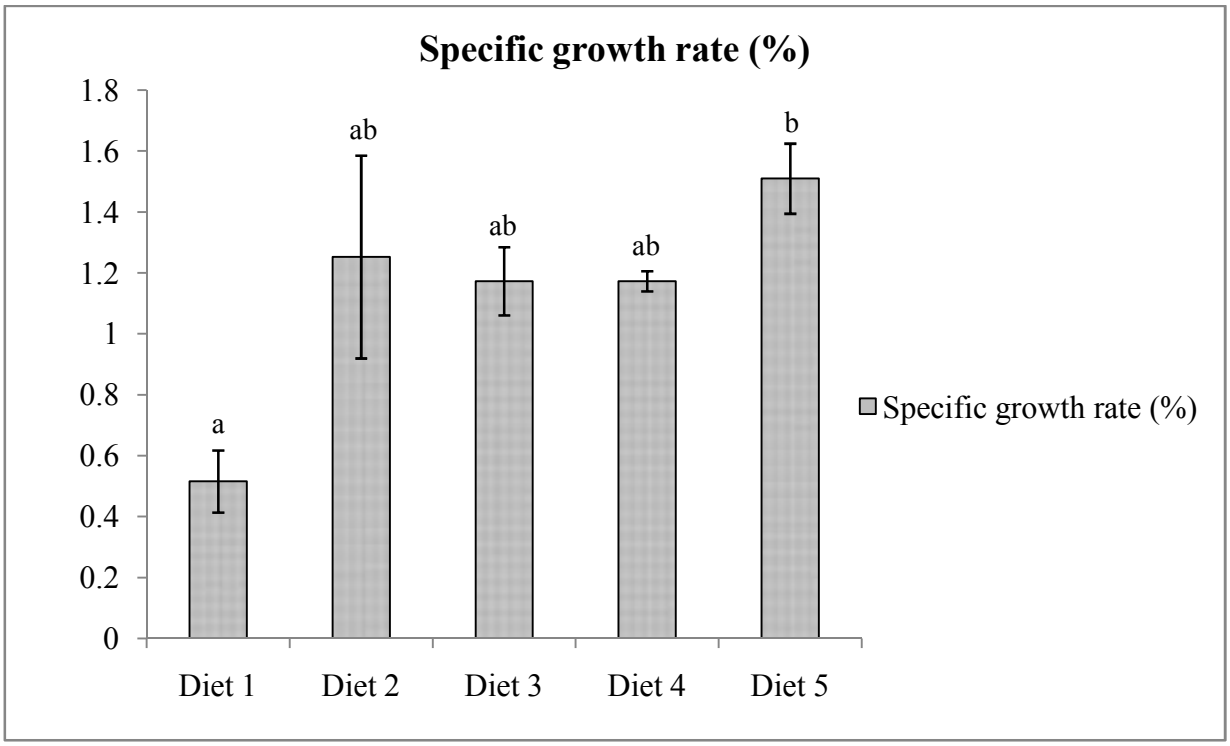

Fig. 12. Bar diagram with different letters (a,b) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for specific growth rate (mean ± SEM) of different diets.

3.2.3 Length gain (cm)

Significantly higher value (0.618 ± 0.033) of length gain was measured in zebrafish at 62 days fed with commercial pellet feed ($P<0.05$). Significantly lower value (0.199 ± 0.028) of length gain was determined in zebrafish at 62 days fed with dried tubifex feed ($P<0.05$).

Table 8: Length gain (cm) (mean ± SEM) of zebrafish was observed in all the diets at 62 days period.

Diet	Length gain (cm)	ANOVA
D1	0.199 ± 0.028^{a}(0.142-0.228)	
D2	0.443 ± 0.065^{bc}(0.357-0.571)	
D3	0.399 ± 0.053^{ab}(0.314-0.499)	*
D4	0.395 ± 0.037^{ab}(0.328-0.457)	
D5	0.618 ± 0.033^{c}(0.557-0.671)	

* Significant (*P<0.05*)

Values are mean ± SEM of triplicate groups of 21 fish. Mean length gain in the column with different superscripts are significantly ($P<0.05$) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 13 shows the mean values of length gain (cm) for different diets graphically.

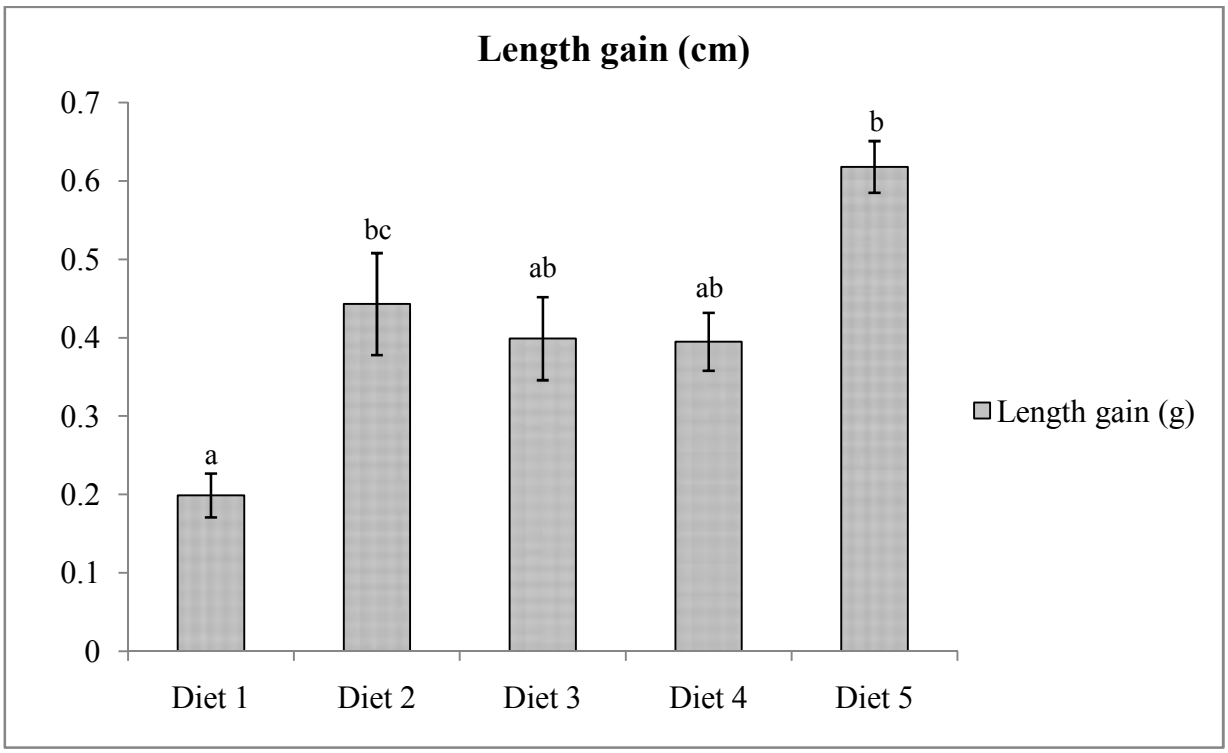

Fig. 13. Bar diagram with different letters (a,b,c) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for length gain (mean ± SEM) of different diets.

3.2.4 Gender weight gain

It is observed that gender weight gain of zebrafish more or less similar among diets. Higher value (1.196± 0.032) of gender weight gain was measured in zebrafish at 62 days fed with commercial pellet feed (P>0.05). Lower value (1.119± 0.032) of gender weight gain was determined in zebrafish at 62 days fed with dried tubifex feed (P>0.05). There was no significant difference (P>0.05) among different treatments when compared using ANOVA.

Table 9: Gender weight gain (g) (mean ± SEM) of zebrafish was observed in all the diets during the experimental period.

Diet	Gender weight gain ratio	ANOVA
D1	1.119±0.032 (1.13-1.24)	NS
D2	1.186±0.057 (1.11-1.30)	
D3	1.176±0.023 (1.13-1.20)	
D4	1.176±0.048 (1.11-1.27)	
D5	1.196±0.032 (1.16-1.26)	

NS – Not significant (P>0.05)

Values are mean ± SEM of triplicate groups of 21 fish. Mean gender weight gain in the column with different superscripts are not significantly (P>0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 14 shows the mean values of gender weight gain (g) for different diets graphically.

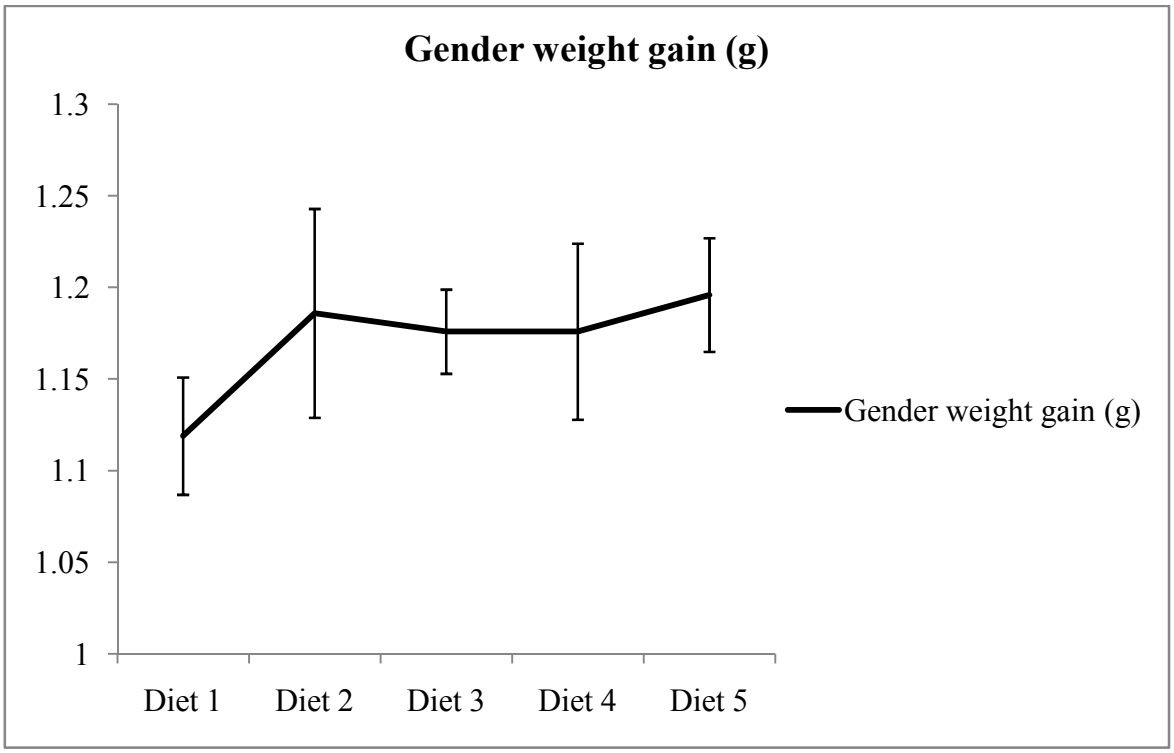

Fig. 14. Line diagram of gender weight gain (mean ± SEM) for different diets as determined by one-way ANOVA followed by Tukey HSD multiple comparison test.

3.2.5 Condition factor

It is observed from table 10 that condition factor of zebrafish from initial measurement more or less similar among diets. Higher value (1.983±0.012) of condition factor was measured in zebrafish at 62 days fed with Spirulina and commercial pellet feed(Diet 4) ($P>0.05$). Lower value (1.863±0.066) of condition factor was determined in zebrafish at 62 days fed with *Artemia* and commercial pellet feed (Diet3) ($P>0.05$).

Condition factor of zebrafish from mid measurement was significantly different. Significantly higher value (1.960±0.035) of condition factor was measured in zebrafish at 62 days fed with commercial pellet feed (Diet 5) ($P<0.05$). Significantly lower value (1.746±0.026) of condition factor was determined in zebrafish at 62 days fed with dried tubifex feed (Diet 1) ($P<0.05$).

And it was observed that condition factor of zebrafish from final measurement more or less similar among diets. Higher value (2.010±0.064) of condition factor was measured in zebrafish at 62 days fed with Spirulina and commercial pellet feed (Diet 4) ($P>0.05$). Lower value (1.856±0.066) of condition factor was determined in zebrafish at 62 days fed with commercial pellet feed (Diet 5) ($P>0.05$).

Table 10: Condition factor (mean ± SEM) of zebrafish was observed in all the diets for 0 days, 30 days and 62 days.

Condition factor	Diet					Level of significance (ANOVA)
	D1	D2	D3	D4	D5	
Initial (0 days)	1.956±0.092 (1.85-2.14)	1.87±.159 (1.56-2.09)	1.863±0.066 (1.75-1.98)	1.983±0.012 (1.96-2.00)	1.980±0.030 (1.92-2.02)	NS
Mid (30 days)	1.746±0.026[a] (1.72-1.80)	1.793±0.028[a] (1.76-1.85)	1.780±0.021[a] (1.1.78-1.85)	1.953±0.020[b] (1.92-1.99)	1.960±0.035[b] (1.91-2.03)	*
Final (62 days)	1.926±0.020 (1.89-1.96)	1.873±0.012 (1.85-1.89)	1.926±0.023 (1.89-1.97)	2.010±0.064 (1.91-2.13)	1.856±0.066 (1.78-1.90)	NS

NS – Not significant (P>0.05)

*Significant (P<0.05)

Values are mean ± SEM of triplicate groups of 21 fish from each diet. Means of Condition factors at different time periods in the line with different superscripts are significant at (P<0.05) and not significantly different at (P>0.05) as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 15 shows the mean values of condition factor for different diets for different time periods graphically.

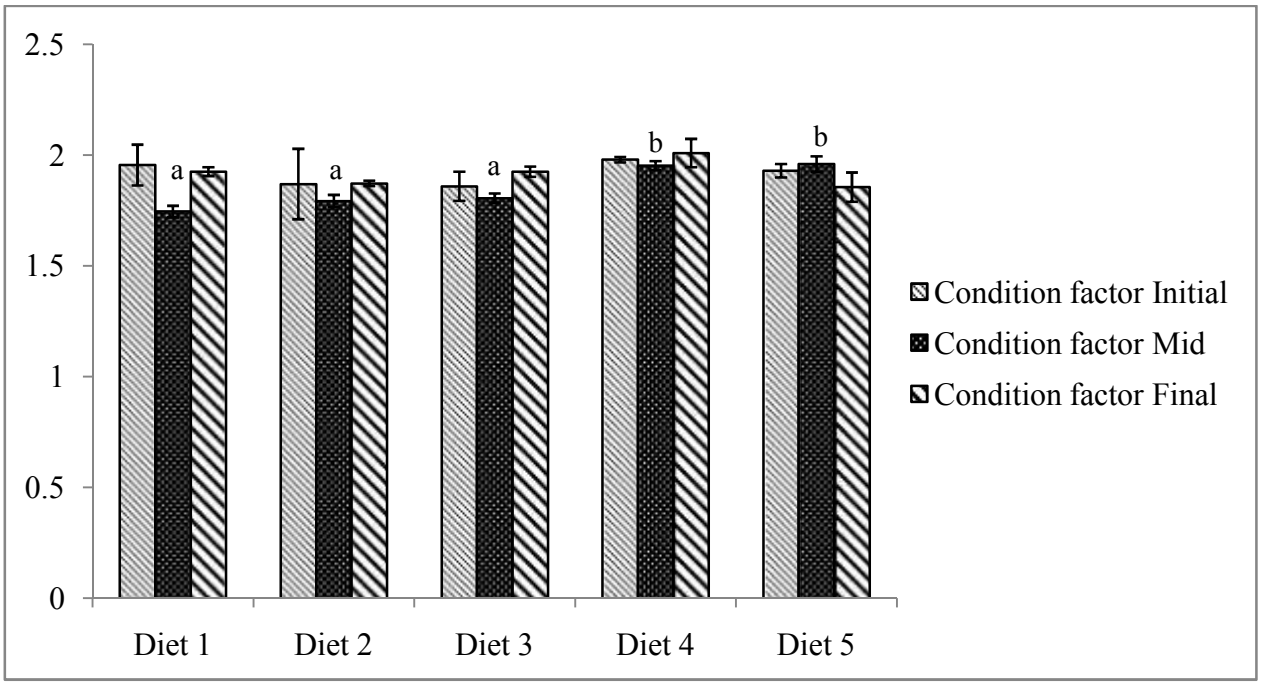

Fig. 15. Bars with different letters (a,b) are Statistically different at same time period determined by one-way ANOVA followed by Tukey HSD multiple comparison test for Condition factor (mean ± SEM) of different diets.

3.2.6 Food conversion ratio

Significantly higher value (3.190± 0.064) of food conversion ratio was measured in zebrafish at 62 days fed with dried tubifex feed (P<0.05) lower value (2.146± 0.042) of food conversion ratio was determined in zebrafish at 62 days fed with commercial pellet feed (P<0.05).

Table 11: Food conversion ratio (mean ± SEM) of zebrafish was observed in all the diets during the experimental period.

Diet	Food conversion ratio	ANOVA
D1	3.190 ± 0.064^{a}(3.07-3.29)	
D2	2.930 ± 0.173^{ab}(2.60-3.19)	
D3	2.733 ± 0.068^{b}(2.60-2.83)	*
D4	2.660 ± 0.043^{b}(2.59-2.74)	
D5	2.146 ± 0.042^{c}(2.09-2.23)	

* Significant (P<0.05)

Values are mean ± SEM of triplicate groups of 21 fish. Mean food conversion ratio in the column with different superscripts are significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 16 shows the mean values of food conversion ratio for different diets graphically.

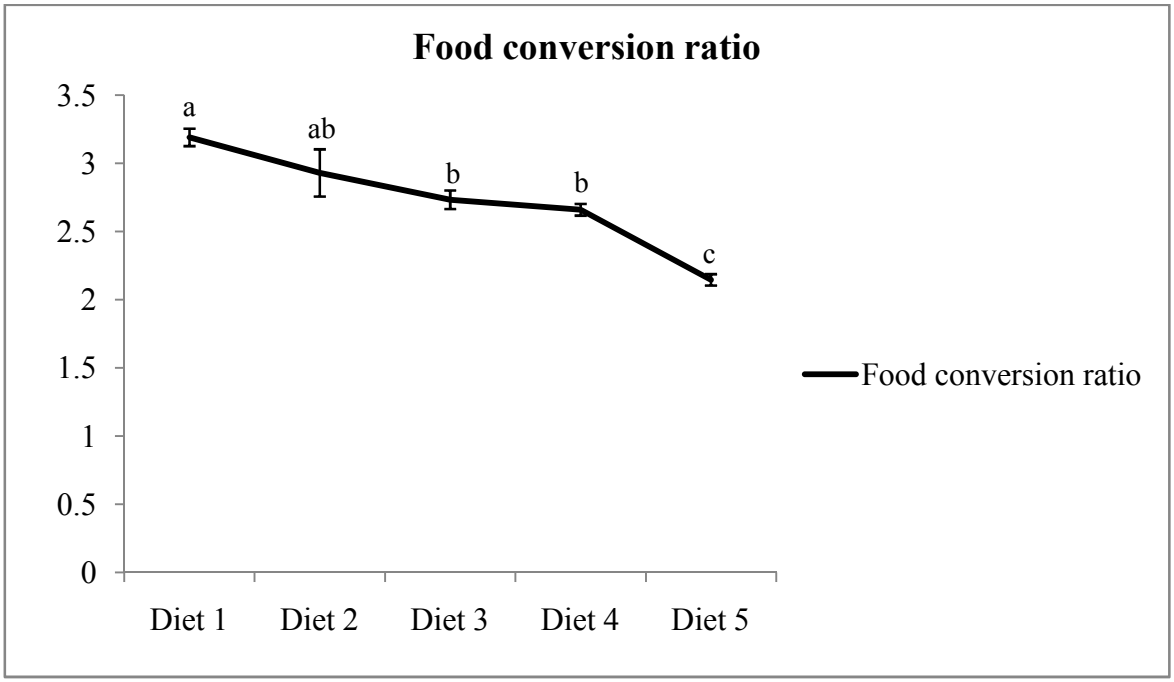

Fig. 16. Line diagram with different letters (a,b,c) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for food conversion ratio (mean ± SEM) of different diets.

3.2.7 Survival rate (%)

It is observed from table 12 that survival rate of zebrafish more or less similar among diets. Higher value (100.00± 0.000) of survival rate was measured in zebrafish at 62 days fed with Spirulina and commercial pellet feed(Diet 4) (P>0.05). Lower value (90.256± 1.015) of survival rate was determined in zebrafish at 62 days fed with dried tubifex feed (Diet1) (P>0.05). There was no significant difference (P>0.05) among different treatments when compared using ANOVA.

Table 12: Survival rate (%) (mean ± SEM) of zebrafish was observed in all the diets during the experimental period.

Diet	Survival rate (%)	ANOVA
D1	90.256± 1.015 (88.40-91.90)	NS
D2	92.150± 0.940 (90.27-93.13)	
D3	95.420± 2.864 (90.15-100.00)	
D4	100.00± 0.000 (100.00-100.00)	
D5	93.083± 4.351 (85.05-100.00)	

NS – Not significant (P>0.05)

Values are mean ± SEM of triplicate groups of 21 fish. Mean survival rate in the column with different superscripts are not significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 17 shows the mean values of survival rate (%) for different diets graphically.

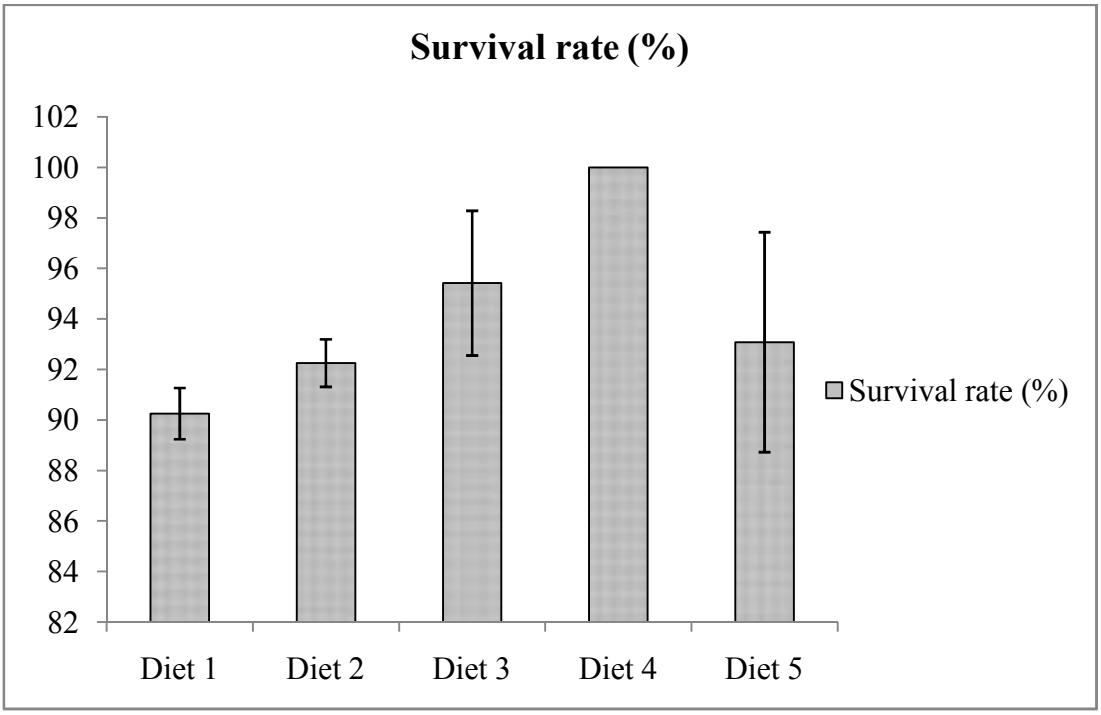

Fig. 17. Bar diagram of survival rate (mean ± SEM) for different diets as determined by one-way ANOVA followed by Tukey HSD multiple comparison test.

3.3 Reproductive evaluation

3.3.1 Spawning success (%)

Significantly higher value (95 ± 2.886) of spawning success was measured in zebrafish at 62 days fed with commercial pellet feed (P<0.05) and lower value (20.370 ± 0.973) of spawning success was determined in zebrafish at 62 days fed with dried tubifex feed (P<0.05).

Table 13: Spawning success(%) (mean ± SEM) of zebrafish observed in all the diets.

Diet	Spawning success (%)	ANOVA
D1	20.370 ± 0.973^{a}(18.90-22.21)	
D2	35.483 ± 3.870^{b}(30.12-43.00)	
D3	43.166 ± 3.655^{b}(37.50-50.00)	*
D4	66.320 ± 2.229^{c}(62.30-70.00)	
D5	95 ± 2.886^{d}(90.00-100.00)	

* Significant (*P<0.05*)

Values are mean ± SEM of triplicate groups of 21 fish. Mean spawning success in the column with different superscripts are significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

Figure 18 shows the mean values of spawning success (%) for different diets graphically.

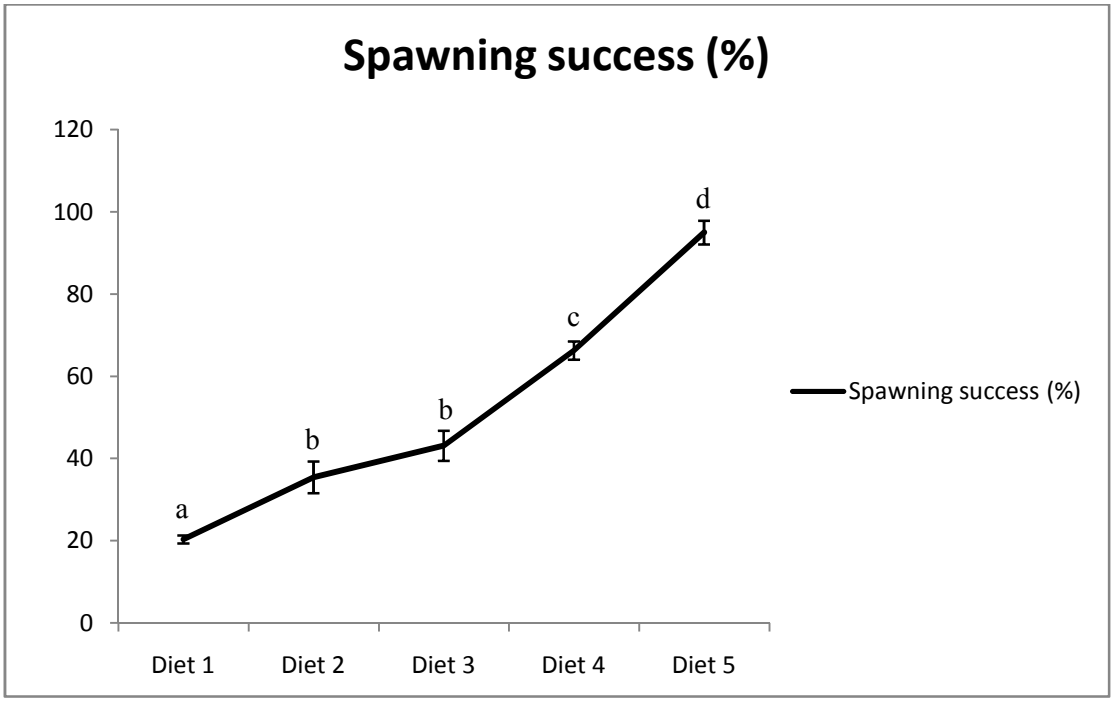

Fig. 18. Line diagram with different letters (a,b,c,d) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for spawning success (mean ± SEM) of different diets.

3.3.2 Fertilization rate

Significantly higher value (85.500 ± 2.542) of fertilization rate was measured in zebrafish at 62 days fed with commercial pellet feed (P<0.05) and significantly lower value (54.506 ± 3.473) of fertilization rate was determined in zebrafish at 62 days fed with dried tubifex feed (P<0.05).

Table 14: Fertilization rate (%) (mean ± SEM) of zebrafish observed in all the diets.

Diet	Fertilization rate (%)	ANOVA
D1	54.506 ± 3.473^{a} (48.15-60.11)	
D2	57.806 ± 3.023^{a} (52.00-62.17)	
D3	81.366 ± 2.436^{b} (78.00-86.10)	*
D4	83.066 ± 1.419^{b} (80.30-85)	
D5	85.500 ± 2.542^{b} (81.20-90.00)	

* Significant (P<0.05)

Values are mean ± SEM of triplicate groups of 21 fish. Mean fertilization rate in the column with different superscripts are significantly (P<0.05) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

The mean values of fertilization rate (%) for different diets are shown graphically.

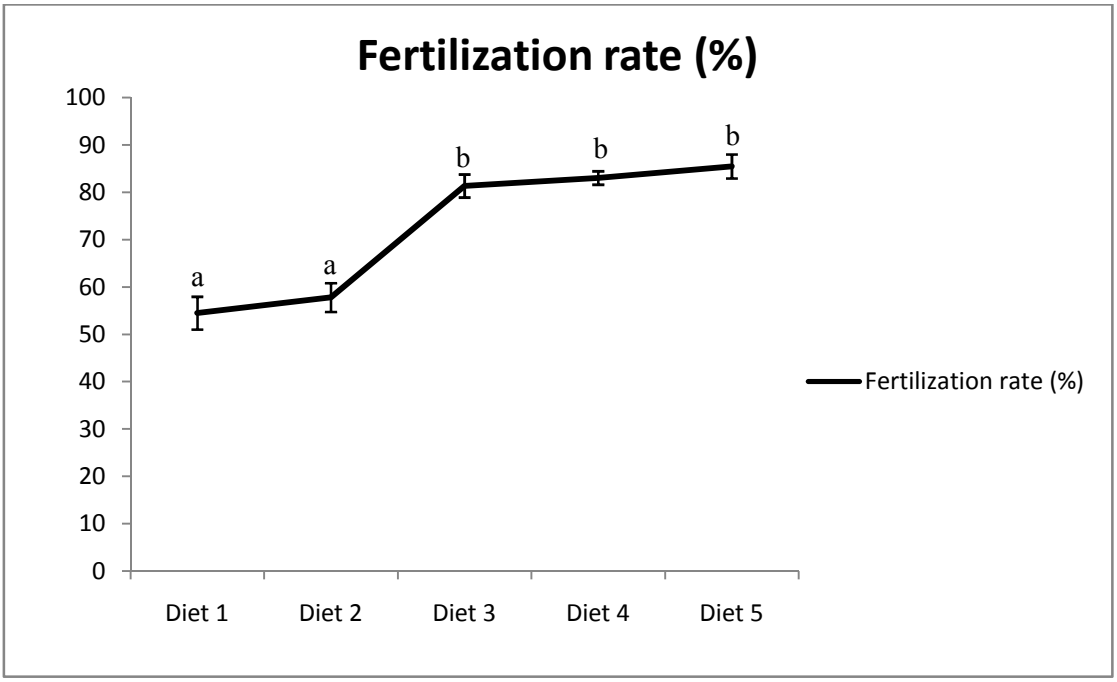

Fig. 19. Line diagram with different letters (a,b) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for fertilization rate (mean ± SEM) of different diets.

3.3.3 Hatching rate (%)

Significantly higher value (76.640 ± 3.118) of hatching rate was measured in zebrafish at 62 days fed with commercial pellet feed ($P<0.05$) and lower value (46 ± 3.617) of hatching rate was determined in zebrafish at 62 days fed with dried tubifex feed ($P<0.05$).

Table 15: Hatching rate (%) (mean ± SEM) of zebrafish observed in all the diets.

Diet	Hatching rate (%)	ANOVA
D1	46 ± 3.617^{a}(40.00-52.50)	
D2	47.900 ± 01.417^{a}(45.20-50.00)	
D3	68.030 ± 3.898^{b}(60.39-73.20)	*
D4	68.580 ± 1.901^{b}(65.01-71.50)	
D5	76.640 ± 3.118^{b}(70.50-80.64)	

* Significant ($P<0.05$)

Values are mean ± SEM of triplicate groups of 21 fish. Mean hatching rate in the column with different superscripts are significantly ($P<0.05$) different as determined by ANOVA and Tukey HSD multiple comparison test. Figures in the parenthesis indicate lowest and highest values.

The mean values of hatching rate (%) for different diets are shown graphically.

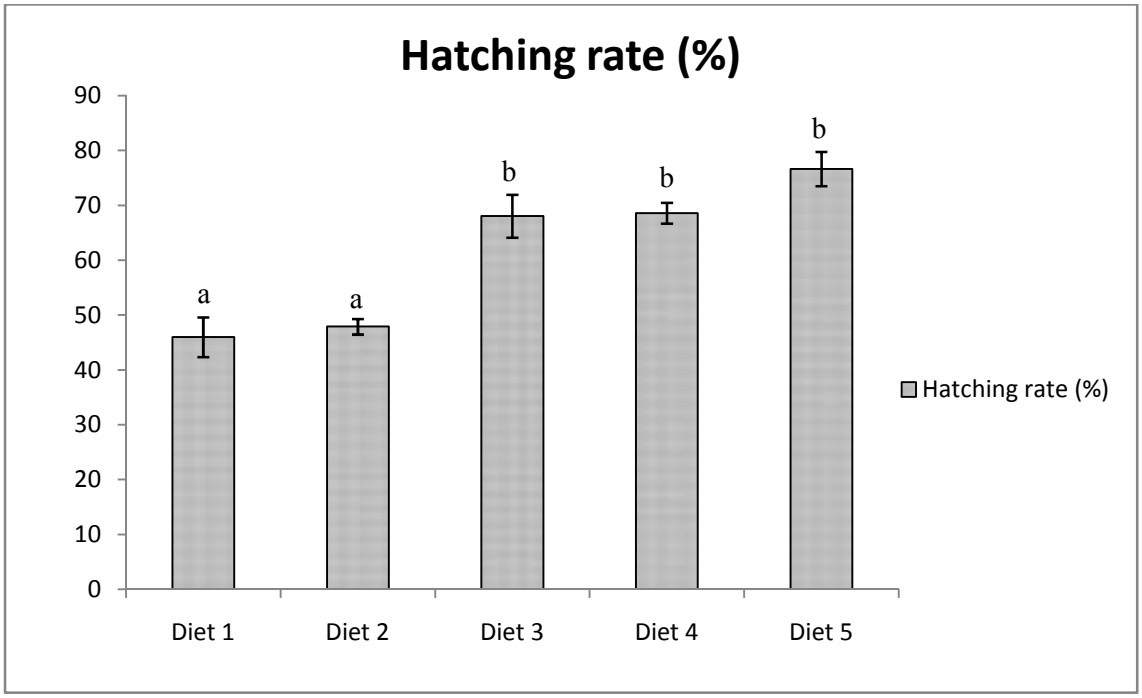

Fig. 20. Bar diagram with different letters (a,b) are significantly different as determined by one-way ANOVA followed by Tukey HSD multiple comparison test for hatching rate (mean ± SEM) of different diets.

3.3.4 Embryogenesis

Zebrafish have seven broad periods of embryogenesis. All the stages of embryogenesis were observed during the experiment for different diets. Significantly the highest value (85.500±2.542) of number of embryos was measured in zebrafish at cleavage period fed with commercial pellet feed ($P<0.05$) and the lowest value (54.506±3.473) of number of embryos was determined in zebrafish at cleavage period with dried tubifex feed ($P<0.05$).

At Gastrula period highest value (85.500±2.542) of number of embryos was measured in zebrafish fed with commercial pellet feed ($P<0.05$) and the lowest value (54.506±3.473) with dried tubifex feed ($P<0.05$).

Table 16: Periods of Embryogenesis (mean ± SEM) of zebrafish were observed for all the diets and results are showed.

Periods of Embryogenesis	Number of embryos of different Diets					ANOVA
	D1	D2	D3	D4	D5	
Cleavage Period	54.506±3.473[a]	57.806±3.023[a]	81.366±2.436[b]	83.066±1.419[b]	85.500±2.542[b]	*
Gastrula Period	54.506±3.473[a]	57.806±3.023[a]	79.081±1.313[b]	83.066±1.419[b]	85.500±2.542[b]	*
Segmentation Period	50.86±0.33[a]	51.79±0.40[a]	74.36±1.14[b]	77.47±0.57[bc]	80.70±0.59[c]	*
Pharyngula Period	47.61±0.39[a]	51.05±0.69[a]	71.15±0.17[b]	71.74±1.74[b]	78.40±0.74[c]	*
Hatching Period	46±3.617[a]	47.900±01.417[a]	68.030±3.898[b]	68.580±1.901[b]	76.640±3.118[b]	*

*Significant (P<0.05)

Values are mean ± SEM of triplicate groups of 21 fish from each diet. Means of embryos at different periods of embryogenesis in the line with different superscripts are significant at (P<0.05) as determined by ANOVA and Tukey HSD multiple comparison test.

Significantly the highest value (80.70±0.59) of number of embryos was measured in zebrafish at cleavage period fed with commercial pellet feed ($P<0.05$) and the lowest value (50.86±0.33) of number of embryos was determined in zebrafish at cleavage period with dried tubifex feed ($P<0.05$).

At Pharyngula period highest value (78.40±0.74) of number of embryos was measured in zebrafish fed with commercial pellet feed ($P<0.05$) and the lowest value (47.61±0.39) with dried tubifex feed ($P<0.05$).

At Hatching period highest value (76.640±3.118) of number of embryos was measured in zebrafish fed with commercial pellet feed ($P<0.05$) and the lowest value (46±3.617) with dried tubifex feed ($P<0.05$).

CHAPTER 4: DISCUSSIONS

Chapter 4

Discussions

Feed has been identified as an important factor for the growth and reproductive performance of fish species (Izquierdo *et al*., 2001). Very little published research has focused on clarifying the role of feed on zebrafish growth and reproduction, although there are estimated more than 1000 publications annually with zebrafish as the animal model (Kaushnik *et al*., 2011).

In this study, five different natural and commercial feed was used to identify a standard diet for zebrafish culture with a constant stocking density (21 fish/4L) as Gonzales (2012) did not identify one feed or feeding combination as superior to the other diets. To develop a standard diet, Kaushnik *et al*. (2011) initiated a present work of feeding zebrafish larvae with a formulated feed right from first feeding onward. Growth of zebrafish fed with the compound feed was very good, reaching a total length of 23±4mm in 9 weeks with a survival rate of (89±4)%. Carvalho *et al*. (2006) conducted an experiment with 4 diffrent diets (*artemia nauplii*, a commercial, a purified, a practical diet). The best overall larval performance was achieved in the group fed with *Artemia* nauplii (86% survival, 14.3 mm standard length and 46.1 mg wet weight). Gonzales (2012) also recommended *Artemia* as the sole food for rearing juvenile zebrafish.

The study was conducted with five different diets to observe growth, yield and reproductive performance of zebrafish. In case of weight and length gain this study shows that diet 5 (Commercial pellet feed) has significantly a higher value than other diets, whereas lowest weight and length gain was observed in diet 1 (Tubifex feed). The same thing can be stated for specific growth rate. As specific growth rate fed with diet 5 (1.510 ± 0.115) was the highest rate, the lowest specific growth rate was determined in diet 1 for tubifex and other diets were not significantly different from diet 5.

The experiment of Siccardi *et al*. (2009) shows that there were significant differences in general growth demographics (length/weight) after the 9-week feeding trial, no significant differences in overall health of *D. rerio* were observed for the different dietary treatments as determined by statistical analysis of condition factor. In our study, similar result is obtained except condition factor of 30 days time period. There are significantly differnece in diet 4 and diet 5 with diet 1, diet 2 and diet 3 during 30 days time period.

As female zebrafish grew more than 1 to 3 times greater in size than male zebrafish, the ratio of gender weight gain was not signifacantly different for different diets. Similar result was shown by the experiment of Gonzales (2012).

There was significant difference for food conversion ratio when compared among five diets. Significantly highest value (3.190±0.064) of food conversion ratio was measured in zebrafish fed with dried tubifex feed and the lowest value (2.146±0.042) of food conversion ratio was found in diet 5 (commercial pellet feed). Toguyeni *et al*. (1997) suggested that feeding behaviour have an effect on the efficiency of food conversion. May be pellet feed is very efficient for feeding.

Zebrafish is a very hardy in nature so survival rate is so higher (Spence *et al*., 2007). In this study it was observed that the highest survival rate was measured 100% in Diet 4(Spirulina+commercial pellet feed) and the lowest survival rate was (90.256±1.015) in diet 1(Dried tubifex) which is also higher. So there was no significant difference of survival rate among the diets. Similar result was obtained by Siccardi *et al*. (2009).

Wolter *et al*. (2010) stated that large females spawned more frequently and had significantly greater clutch sizes than small females. In this experiment, it has been observed that the highest weight, specific growth rate and length gain of zebrafish was obtained fed with diet 5 and the lowest was with diet 1. As a result, the highest value of spawning success (95±2.886) was for diet 5 and apparently the lowest value (20.370±0.973) was for diet 1. There was significant difference among diet 1, diet 2 diet 4 and diet 5. Diet 2 and diet 3 are significantly similar.

Wolter *et al*. (2010) also recommended that eggs from small fish, however, suffered from higher egg mortality than the eggs of large individuals. So fertilization rate also depends on the size of fish. The highest value of fertilization rate (85.500±2.542) was for diet 5 and apparently the lowest value (54.506±3.473) was for diet 1. There was significant difference in diet 1 and diet 2 with diet 3, diet 4 and diet 5. As well as hatching rate also influenced by feed and feeding regime. There was significant difference of hatching rate in diet 1 and diet 2 with diet 3, diet 4 and diet 5 as like as fertilization rate. Embryos from small-sized spawners also hatched later than offspring from eggs laid by large females (Wolter *et al*., 2010).

Data from all the tables of growth parameters and reproductive evaluation shows or clarify that commercially pellet feed is good as a supplementary fed with Spirulina or *Artemia* but best performances obtained when it was fed solely. In this study commercial pellet feed was labeled as diet 5. On the other hand fish fed with tubifex or *Artemia*

solely labeled as diet 1 and diet 2 respectively have poor growth, yield and reproductive performance. Whereas Goolish *et al*. (1999) and Meinelt *et al*. (1999) reported that feeding *Artemia* resulted in bigger larval zebrafish. Darrow and Harris (2004) stated that *Artemia* was the only diet that elicited a predator–prey feeding response and resulted in cleaner tanks.

Figueiredo *et al*. (2009, McEvoy *et al*. (1996) and Ozkizilcik and Chu (1994) recommended that *Artemia* can be enriched prior to being fed to zebrafish, thereby allowing modifications in the nutritional content fed to zebrafish as research identifies nutrient needs. Gonzales (2012) experimented on different feed for zebrafish growth performance and also recommended *Artemia* may be a better diet for rearing juvenile zebrafish but in this study fish fed solely *Artemia* did not perform well. May be there were problem in *Artemia* production or feeding behavior of *Artemia.* But the worst performance on growth, yield and reproduction was obtained from diet 1 (tubifex) so it is not recommended for the culture of zebrafish. As the best reproductive performance, weight gain, length gain, gender weight gain ratio, condition factor and food conversion ratio was obtained by fed with commercial pellet feed solely. And best survival rate was gained when commercial pellet feed was used as a supplementary feed with Spirulina. Numbers of embryos in different developmental stages of embryogenesis were also observed in the experiment. Significant difference was observed among diet 1 and diet 2 with diet 3, 4 and 5 in every period of embryogenesis.

Markovich *et al*. (2007) concluded that 7-moold zebrafish fed *Artemia* produced similar spawning results as those from zebrafish fed other commercially available diets. But this study shows that commercially available pellet feed (TetraBits Complete) has better output than *Artemia* or tubifex. So Commercially available pellet feed named "TetraBits Complete, Germany" is highly recommended for zebrafish culture for better growth, yield and reproductive performance.

CHAPTER 5: CONCLUSION

Chapter 5

Conclusion

Zebrafish (*Danio rerio*) is an important key laboratory model species to study for developmental studies like growth and reproductive performance. As the fish is very convenient for laboratory use so it was selected for the experiment. Different studies related to feeding regime did not find any specific standard diet for zebrafish culture. So the aim of the study was to develop a standard diet by observing the effects of several natural and commercially available feeds and different feeding regimes on the growth, yield and reproductive performance of zebrafish under laboratory condition. 21 zebrafish were stocked into each of 15 tanks with volume of 4 litre water. Three tanks were assigned to each of 5 feeding combinations for a period of 62 days. Fish were fed with each diet twice daily throughout the experiment. Diet 1 (Tubifex) and diet 2 (*Artemia*) showed significantly poor performance on mean weight gain, length gain, specific growth rate and survival rate whereas diet 3, diet 4 and diet 5 had higher growth performance than other diets. But to get the most fruitful growth rate diet 5 can be great to recommend where commercial pellet feed was fed with solely. In case of weight and length gain fish fed with diet 5 was significantly different than other diets but in case of survivability only fish fed with diet 4 showed 100% survivability where commercially pellet feed was used as a supplementary feed with Spirulina. Maximum reproductive performance was observed in diet 5 which was significantly different from other diets. Numbers of embryos in different developmental stages of embryogenesis were also observed in the experiment. Here, significant difference was observed among diet 1 and diet 2 with diet 3, 4 and 5 in every period of embryogenesis.

Some studies recommended that *Artemia* may be a better diet for zebrafish culture but in this study fish fed with solely *Artemia* did not perform well. May be there were problem in *Artemia* production or feeding behavior to *Artemia* by the fish individuals. The performance on growth, yield and reproduction of diet 1 (tubifex) was below the mark.

Through the experiment it can be said if commercial pellet feed is incorporated solely for the culture of zebrafish then maximum output of growth, yield and reproductive performance should be gained. So these findings can be recommended for the further research on zebrafish culture practice under laboratory condition.

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APPENDICES

APPENDICES

Weight gain (g)

Descriptives

Weight gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	.03400	.008544	.004933	.01278	.05522	.026	.043
Diet 2	3	.07567	.027209	.015709	.00808	.14326	.058	.107
Diet 3	3	.07200	.007810	.004509	.05260	.09140	.063	.077
Diet 4	3	.07433	.015144	.008743	.03671	.11195	.057	.085
Diet 5	3	.11800	.010000	.005774	.09316	.14284	.108	.128
Total	15	.07480	.030503	.007876	.05791	.09169	.026	.128

ANOVA

Weight gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	4	.003	11.028	.001
Within Groups	.002	10	.000		
Total	.013	14			

Multiple Comparisons

Dependent Variable: Weight gain

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.041667	.012668	.050	-.08336	.00003
	Diet 3	-.038000	.012668	.078	-.07969	.00369
	Diet 4	-.040333	.012668	.059	-.08203	.00136
	Diet 5	-.084000*	.012668	.000	-.12569	-.04231
Diet 2	Diet 1	.041667	.012668	.050	-.00003	.08336
	Diet 3	.003667	.012668	.998	-.03803	.04536
	Diet 4	.001333	.012668	1.000	-.04036	.04303
	Diet 5	-.042333*	.012668	.046	-.08403	-.00064
Diet 3	Diet 1	.038000	.012668	.078	-.00369	.07969
	Diet 2	-.003667	.012668	.998	-.04536	.03803
	Diet 4	-.002333	.012668	1.000	-.04403	.03936

Diet 4	Diet 5	-.046000*	.012668	.029	-.08769	-.00431
	Diet 1	.040333	.012668	.059	-.00136	.08203
	Diet 2	-.001333	.012668	1.000	-.04303	.04036
	Diet 3	.002333	.012668	1.000	-.03936	.04403
	Diet 5	-.043667*	.012668	.039	-.08536	-.00197
Diet 5	Diet 1	.084000*	.012668	.000	.04231	.12569
	Diet 2	.042333*	.012668	.046	.00064	.08403
	Diet 3	.046000*	.012668	.029	.00431	.08769
	Diet 4	.043667*	.012668	.039	.00197	.08536

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

Specific growth rate (%)

Descriptives

Specific growth rate (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	.5167	.17786	.10269	.0748	.9585	.39	.72
Diet 2	3	1.2533	.57813	.33378	-.1828	2.6895	.89	1.92
Diet 3	3	1.1733	.19553	.11289	.6876	1.6591	1.01	1.39
Diet 4	3	1.1733	.05859	.03383	1.0278	1.3189	1.13	1.24
Diet 5	3	1.5100	.20075	.11590	1.0113	2.0087	1.28	1.65
Total	15	1.1253	.42376	.10941	.8907	1.3600	.39	1.92

ANOVA

Specific growth rate (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.618	4	.405	4.517	.024
Within Groups	.896	10	.090		
Total	2.514	14			

Multiple Comparisons

Dependent Variable: Specific growth rate (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.73667	.24436	.077	-1.5409	.0675
	Diet 3	-.65667	.24436	.126	-1.4609	.1475
	Diet 4	-.65667	.24436	.126	-1.4609	.1475
	Diet 5	-.99333*	.24436	.015	-1.7975	-.1891
Diet 2	Diet 1	.73667	.24436	.077	-.0675	1.5409
	Diet 3	.08000	.24436	.997	-.7242	.8842
	Diet 4	.08000	.24436	.997	-.7242	.8842
	Diet 5	-.25667	.24436	.827	-1.0609	.5475
Diet 3	Diet 1	.65667	.24436	.126	-.1475	1.4609
	Diet 2	-.08000	.24436	.997	-.8842	.7242
	Diet 4	.00000	.24436	1.000	-.8042	.8042
	Diet 5	-.33667	.24436	.654	-1.1409	.4675
Diet 4	Diet 1	.65667	.24436	.126	-.1475	1.4609
	Diet 2	-.08000	.24436	.997	-.8842	.7242
	Diet 3	.00000	.24436	1.000	-.8042	.8042
	Diet 5	-.33667	.24436	.654	-1.1409	.4675
Diet 5	Diet 1	.99333*	.24436	.015	.1891	1.7975
	Diet 2	.25667	.24436	.827	-.5475	1.0609
	Diet 3	.33667	.24436	.654	-.4675	1.1409
	Diet 4	.33667	.24436	.654	-.4675	1.1409

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

Length gain (cm)

Descriptives

Length gain (cm)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	.19933	.049652	.028667	.07599	.32268	.142	.228
Diet 2	3	.44267	.113200	.065356	.16146	.72387	.357	.571
Diet 3	3	.39933	.093329	.053884	.16749	.63118	.314	.499
Diet 4	3	.39500	.064645	.037323	.23441	.55559	.328	.457
Diet 5	3	.61867	.057570	.033238	.47565	.76168	.557	.671
Total	15	.41100	.153747	.039697	.32586	.49614	.142	.671

ANOVA

Length gain (cm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.268	4	.067	10.639	.001
Within Groups	.063	10	.006		
Total	.331	14			

Multiple Comparisons

Dependent Variable: Length gain (cm)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.243333*	.064790	.024	-.45656	-.03010
	Diet 3	-.200000	.064790	.069	-.41323	.01323
	Diet 4	-.195667	.064790	.076	-.40890	.01756
	Diet 5	-.419333*	.064790	.001	-.63256	-.20610
Diet 2	Diet 1	.243333*	.064790	.024	.03010	.45656
	Diet 3	.043333	.064790	.959	-.16990	.25656
	Diet 4	.047667	.064790	.943	-.16556	.26090
	Diet 5	-.176000	.064790	.121	-.38923	.03723
Diet 3	Diet 1	.200000	.064790	.069	-.01323	.41323
	Diet 2	-.043333	.064790	.959	-.25656	.16990
	Diet 4	.004333	.064790	1.000	-.20890	.21756

Diet 4	Diet 5	-.219333*	.064790	.043	-.43256	-.00610
	Diet 1	.195667	.064790	.076	-.01756	.40890
	Diet 2	-.047667	.064790	.943	-.26090	.16556
	Diet 3	-.004333	.064790	1.000	-.21756	.20890
	Diet 5	-.223667*	.064790	.039	-.43690	-.01044
Diet 5	Diet 1	.419333*	.064790	.001	.20610	.63256
	Diet 2	.176000	.064790	.121	-.03723	.38923
	Diet 3	.219333*	.064790	.043	.00610	.43256
	Diet 4	.223667*	.064790	.039	.01044	.43690

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

Gender weight gain

Descriptives

Gender weight gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	1.1900	.05568	.03215	1.0517	1.3283	1.13	1.24
Diet 2	3	1.1867	.10017	.05783	.9378	1.4355	1.11	1.30
Diet 3	3	1.1767	.04041	.02333	1.0763	1.2771	1.13	1.20
Diet 4	3	1.1767	.08327	.04807	.9698	1.3835	1.11	1.27
Diet 5	3	1.1967	.05508	.03180	1.0599	1.3335	1.16	1.26
Total	15	1.1853	.05998	.01549	1.1521	1.2186	1.11	1.30

ANOVA

Gender weight gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	4	.000	.046	.995
Within Groups	.049	10	.005		
Total	.050	14			

Multiple Comparisons

Dependent Variable: Gender weight gain

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.00333	.05743	1.000	-.1857	.1923
	Diet 3	.01333	.05743	.999	-.1757	.2023
	Diet 4	.01333	.05743	.999	-.1757	.2023
	Diet 5	-.00667	.05743	1.000	-.1957	.1823
Diet 2	Diet 1	-.00333	.05743	1.000	-.1923	.1857
	Diet 3	.01000	.05743	1.000	-.1790	.1990
	Diet 4	.01000	.05743	1.000	-.1790	.1990
	Diet 5	-.01000	.05743	1.000	-.1990	.1790
Diet 3	Diet 1	-.01333	.05743	.999	-.2023	.1757
	Diet 2	-.01000	.05743	1.000	-.1990	.1790
	Diet 4	.00000	.05743	1.000	-.1890	.1890
	Diet 5	-.02000	.05743	.996	-.2090	.1690
Diet 4	Diet 1	-.01333	.05743	.999	-.2023	.1757
	Diet 2	-.01000	.05743	1.000	-.1990	.1790
	Diet 3	.00000	.05743	1.000	-.1890	.1890
	Diet 5	-.02000	.05743	.996	-.2090	.1690
Diet 5	Diet 1	.00667	.05743	1.000	-.1823	.1957
	Diet 2	.01000	.05743	1.000	-.1790	.1990
	Diet 3	.02000	.05743	.996	-.1690	.2090
	Diet 4	.02000	.05743	.996	-.1690	.2090

Condition factor (%) (Initial)

Descriptives

Condition factor (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	1.9567	.15948	.09207	1.5605	2.3528	1.85	2.14
Diet 2	3	1.8700	.27622	.15948	1.1838	2.5562	1.56	2.09
Diet 3	3	1.8633	.11504	.06642	1.5776	2.1491	1.75	1.98
Diet 4	3	1.9833	.02082	.01202	1.9316	2.0350	1.96	2.00
Diet 5	3	1.9800	.05292	.03055	1.8486	2.1114	1.92	2.02
Total	15	1.9307	.14109	.03643	1.8525	2.0088	1.56	2.14

ANOVA

Condition factor (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.042	4	.011	.447	.772
Within Groups	.236	10	.024		
Total	.279	14			

Multiple Comparisons

Dependent Variable: Condition factor (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.08667	.12554	.954	-.3265	.4998
	Diet 3	.09333	.12554	.941	-.3198	.5065
	Diet 4	-.02667	.12554	.999	-.4398	.3865
	Diet 5	-.02333	.12554	1.000	-.4365	.3898
Diet 2	Diet 1	-.08667	.12554	.954	-.4998	.3265
	Diet 3	.00667	.12554	1.000	-.4065	.4198
	Diet 4	-.11333	.12554	.889	-.5265	.2998

Diet 3	Diet 5	-.11000	.12554	.899	-.5232	.3032
	Diet 1	-.09333	.12554	.941	-.5065	.3198
	Diet 2	-.00667	.12554	1.000	-.4198	.4065
	Diet 4	-.12000	.12554	.868	-.5332	.2932
	Diet 5	-.11667	.12554	.879	-.5298	.2965
Diet 4	Diet 1	.02667	.12554	.999	-.3865	.4398
	Diet 2	.11333	.12554	.889	-.2998	.5265
	Diet 3	.12000	.12554	.868	-.2932	.5332
	Diet 5	.00333	.12554	1.000	-.4098	.4165
Diet 5	Diet 1	.02333	.12554	1.000	-.3898	.4365
	Diet 2	.11000	.12554	.899	-.3032	.5232
	Diet 3	.11667	.12554	.879	-.2965	.5298
	Diet 4	-.00333	.12554	1.000	-.4165	.4098

Condition factor (%) (Mid)

Descriptives

Condition factor (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	1.7467	.04619	.02667	1.6319	1.8614	1.72	1.80
Diet 2	3	1.7933	.04933	.02848	1.6708	1.9159	1.76	1.85
Diet 3	3	1.8067	.03786	.02186	1.7126	1.9007	1.78	1.85
Diet 4	3	1.9533	.03512	.02028	1.8661	2.0406	1.92	1.99
Diet 5	3	1.9633	.06110	.03528	1.8116	2.1151	1.91	2.03
Total	15	1.8527	.09989	.02579	1.7973	1.9080	1.72	2.03

ANOVA

Condition factor (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.118	4	.029	13.422	.000
Within Groups	.022	10	.002		
Total	.140	14			

Multiple Comparisons

Dependent Variable: Condition factor (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.04667	.03824	.741	-.1725	.0792
	Diet 3	-.06000	.03824	.546	-.1858	.0658
	Diet 4	-.20667*	.03824	.002	-.3325	-.0808
	Diet 5	-.21667*	.03824	.002	-.3425	-.0908
Diet 2	Diet 1	.04667	.03824	.741	-.0792	.1725
	Diet 3	-.01333	.03824	.996	-.1392	.1125
	Diet 4	-.16000*	.03824	.013	-.2858	-.0342
	Diet 5	-.17000*	.03824	.009	-.2958	-.0442
Diet 3	Diet 1	.06000	.03824	.546	-.0658	.1858
	Diet 2	.01333	.03824	.996	-.1125	.1392
	Diet 4	-.14667*	.03824	.022	-.2725	-.0208
	Diet 5	-.15667*	.03824	.014	-.2825	-.0308
Diet 4	Diet 1	.20667*	.03824	.002	.0808	.3325
	Diet 2	.16000*	.03824	.013	.0342	.2858
	Diet 3	.14667*	.03824	.022	.0208	.2725
	Diet 5	-.01000	.03824	.999	-.1358	.1158
Diet 5	Diet 1	.21667*	.03824	.002	.0908	.3425
	Diet 2	.17000*	.03824	.009	.0442	.2958
	Diet 3	.15667*	.03824	.014	.0308	.2825
	Diet 4	.01000	.03824	.999	-.1158	.1358

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

Condition factor (%) (Final)

Descriptives

Condition factor (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	1.9267	.03512	.02028	1.8394	2.0139	1.89	1.96
Diet 2	3	1.8733	.02082	.01202	1.8216	1.9250	1.85	1.89
Diet 3	3	1.9267	.04041	.02333	1.8263	2.0271	1.89	1.97
Diet 4	3	2.0100	.11136	.06429	1.7334	2.2866	1.91	2.13
Diet 5	3	1.8567	.06658	.03844	1.6913	2.0221	1.78	1.90
Total	15	1.9187	.07717	.01993	1.8759	1.9614	1.78	2.13

ANOVA

Condition factor (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.043	4	.011	2.676	.094
Within Groups	.040	10	.004		
Total	.083	14			

Multiple Comparisons

Dependent Variable: Condition factor (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.05333	.05181	.836	-.1172	.2238
	Diet 3	.00000	.05181	1.000	-.1705	.1705
	Diet 4	-.08333	.05181	.524	-.2538	.0872
	Diet 5	.07000	.05181	.669	-.1005	.2405
Diet 2	Diet 1	-.05333	.05181	.836	-.2238	.1172
	Diet 3	-.05333	.05181	.836	-.2238	.1172
	Diet 4	-.13667	.05181	.136	-.3072	.0338
	Diet 5	.01667	.05181	.997	-.1538	.1872
Diet 3	Diet 1	.00000	.05181	1.000	-.1705	.1705
	Diet 2	.05333	.05181	.836	-.1172	.2238
	Diet 4	-.08333	.05181	.524	-.2538	.0872
	Diet 5	.07000	.05181	.669	-.1005	.2405
Diet 4	Diet 1	.08333	.05181	.524	-.0872	.2538

	Diet 2	.13667	.05181	.136	-.0338	.3072
	Diet 3	.08333	.05181	.524	-.0872	.2538
	Diet 5	.15333	.05181	.083	-.0172	.3238
	Diet 1	-.07000	.05181	.669	-.2405	.1005
Diet 5	Diet 2	-.01667	.05181	.997	-.1872	.1538
	Diet 3	-.07000	.05181	.669	-.2405	.1005
	Diet 4	-.15333	.05181	.083	-.3238	.0172

Food conversion ratio

Descriptives

Food conversion ratio

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	3.1900	.11136	.06429	2.9134	3.4666	3.07	3.29
Diet 2	3	2.9300	.30116	.17388	2.1819	3.6781	2.60	3.19
Diet 3	3	2.7333	.11930	.06888	2.4370	3.0297	2.60	2.83
Diet 4	3	2.6600	.07550	.04359	2.4725	2.8475	2.59	2.74
Diet 5	3	2.1467	.07371	.04256	1.9636	2.3298	2.09	2.23
Total	15	2.7320	.38240	.09874	2.5202	2.9438	2.09	3.29

ANOVA

Food conversion ratio

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.790	4	.448	17.420	.000
Within Groups	.257	10	.026		
Total	2.047	14			

Multiple Comparisons

Dependent Variable: Food conversion ratio

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.26000	.13088	.337	-.1707	.6907
	Diet 3	.45667*	.13088	.037	.0259	.8874
	Diet 4	.53000*	.13088	.015	.0993	.9607
	Diet 5	1.04333*	.13088	.000	.6126	1.4741
Diet 2	Diet 1	-.26000	.13088	.337	-.6907	.1707
	Diet 3	.19667	.13088	.583	-.2341	.6274
	Diet 4	.27000	.13088	.306	-.1607	.7007
	Diet 5	.78333*	.13088	.001	.3526	1.2141
Diet 3	Diet 1	-.45667*	.13088	.037	-.8874	-.0259
	Diet 2	-.19667	.13088	.583	-.6274	.2341
	Diet 4	.07333	.13088	.978	-.3574	.5041
	Diet 5	.58667*	.13088	.008	.1559	1.0174
Diet 4	Diet 1	-.53000*	.13088	.015	-.9607	-.0993
	Diet 2	-.27000	.13088	.306	-.7007	.1607
	Diet 3	-.07333	.13088	.978	-.5041	.3574
	Diet 5	.51333*	.13088	.019	.0826	.9441
Diet 5	Diet 1	-1.04333*	.13088	.000	-1.4741	-.6126
	Diet 2	-.78333*	.13088	.001	-1.2141	-.3526
	Diet 3	-.58667*	.13088	.008	-1.0174	-.1559
	Diet 4	-.51333*	.13088	.019	-.9441	-.0826

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

Survival rate (%)

Descriptives

Survival rate (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	90.2567	1.75973	1.01598	85.8853	94.6281	88.40	91.90
Diet 2	3	92.1500	1.62862	.94028	88.1043	96.1957	90.27	93.13
Diet 3	3	95.4200	4.96112	2.86430	83.0959	107.7441	90.15	100.00
Diet 4	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
Diet 5	3	93.0833	7.53730	4.35166	74.3597	111.8070	85.05	100.00
Total	15	94.1820	4.94775	1.27750	91.4420	96.9220	85.05	100.00

ANOVA

Survival rate (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	168.378	4	42.095	2.414	.118
Within Groups	174.345	10	17.435		
Total	342.723	14			

Multiple Comparisons

Dependent Variable: Survival rate (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-1.89333	3.40925	.979	-13.1135	9.3268
	Diet 3	-5.16333	3.40925	.576	-16.3835	6.0568
	Diet 4	-9.74333	3.40925	.097	-20.9635	1.4768
	Diet 5	-2.82667	3.40925	.916	-14.0468	8.3935
Diet 2	Diet 1	1.89333	3.40925	.979	-9.3268	13.1135
	Diet 3	-3.27000	3.40925	.867	-14.4901	7.9501
	Diet 4	-7.85000	3.40925	.221	-19.0701	3.3701
	Diet 5	-.93333	3.40925	.999	-12.1535	10.2868
Diet 3	Diet 1	5.16333	3.40925	.576	-6.0568	16.3835
	Diet 2	3.27000	3.40925	.867	-7.9501	14.4901
	Diet 4	-4.58000	3.40925	.673	-15.8001	6.6401

Diet 4	Diet 5	2.33667	3.40925	.955	-8.8835	13.5568
	Diet 1	9.74333	3.40925	.097	-1.4768	20.9635
	Diet 2	7.85000	3.40925	.221	-3.3701	19.0701
	Diet 3	4.58000	3.40925	.673	-6.6401	15.8001
	Diet 5	6.91667	3.40925	.320	-4.3035	18.1368
Diet 5	Diet 1	2.82667	3.40925	.916	-8.3935	14.0468
	Diet 2	.93333	3.40925	.999	-10.2868	12.1535
	Diet 3	-2.33667	3.40925	.955	-13.5568	8.8835
	Diet 4	-6.91667	3.40925	.320	-18.1368	4.3035

Spawning success (%)

Descriptives

Spawning success (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	20.3700	1.68573	.97326	16.1824	24.5576	18.90	22.21
Diet 2	3	35.4833	6.70457	3.87088	18.8283	52.1384	30.12	43.00
Diet 3	3	43.1667	6.33114	3.65529	27.4392	58.8941	37.50	50.00
Diet 4	3	66.3200	3.86124	2.22929	56.7281	75.9119	62.30	70.00
Diet 5	3	95.0000	5.00000	2.88675	82.5793	107.4207	90.00	100.00
Total	15	52.0680	27.34899	7.06148	36.9226	67.2134	18.90	100.00

ANOVA

Spawning success (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10215.973	4	2553.993	99.933	.000
Within Groups	255.571	10	25.557		
Total	10471.544	14			

Multiple Comparisons

Dependent Variable: Spawning success (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-15.11333*	4.12772	.028	-28.6980	-1.5287
	Diet 3	-22.79667*	4.12772	.002	-36.3813	-9.2120
	Diet 4	-45.95000*	4.12772	.000	-59.5347	-32.3653
	Diet 5	-74.63000*	4.12772	.000	-88.2147	-61.0453
Diet 2	Diet 1	15.11333*	4.12772	.028	1.5287	28.6980
	Diet 3	-7.68333	4.12772	.394	-21.2680	5.9013
	Diet 4	-30.83667*	4.12772	.000	-44.4213	-17.2520
	Diet 5	-59.51667*	4.12772	.000	-73.1013	-45.9320
Diet 3	Diet 1	22.79667*	4.12772	.002	9.2120	36.3813
	Diet 2	7.68333	4.12772	.394	-5.9013	21.2680
	Diet 4	-23.15333*	4.12772	.002	-36.7380	-9.5687
	Diet 5	-51.83333*	4.12772	.000	-65.4180	-38.2487
Diet 4	Diet 1	45.95000*	4.12772	.000	32.3653	59.5347
	Diet 2	30.83667*	4.12772	.000	17.2520	44.4213
	Diet 3	23.15333*	4.12772	.002	9.5687	36.7380
	Diet 5	-28.68000*	4.12772	.000	-42.2647	-15.0953
Diet 5	Diet 1	74.63000*	4.12772	.000	61.0453	88.2147
	Diet 2	59.51667*	4.12772	.000	45.9320	73.1013
	Diet 3	51.83333*	4.12772	.000	38.2487	65.4180
	Diet 4	28.68000*	4.12772	.000	15.0953	42.2647

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

Fertilization rate (%)

Descriptives

Fertilization rate (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	54.5067	6.01548	3.47304	39.5634	69.4500	48.15	60.11
Diet 2	3	57.8067	5.23638	3.02322	44.7988	70.8145	52.00	62.17
Diet 3	3	81.3667	4.21940	2.43607	70.8851	91.8482	78.00	86.10
Diet 4	3	83.0667	2.45832	1.41931	76.9599	89.1735	80.30	85.00
Diet 5	3	85.5000	4.40341	2.54231	74.5613	96.4387	81.20	90.00
Total	15	72.4493	14.41830	3.72279	64.4647	80.4339	48.15	90.00

ANOVA

Fertilization rate (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2696.740	4	674.185	31.550	.000
Within Groups	213.685	10	21.368		
Total	2910.425	14			

Multiple Comparisons

Dependent Variable: Fertilization rate (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-3.30000	3.77434	.900	-15.7217	9.1217
	Diet 3	-26.86000*	3.77434	.000	-39.2817	-14.4383
	Diet 4	-28.56000*	3.77434	.000	-40.9817	-16.1383
	Diet 5	-30.99333*	3.77434	.000	-43.4150	-18.5717
Diet 2	Diet 1	3.30000	3.77434	.900	-9.1217	15.7217
	Diet 3	-23.56000*	3.77434	.001	-35.9817	-11.1383
	Diet 4	-25.26000*	3.77434	.000	-37.6817	-12.8383
	Diet 5	-27.69333*	3.77434	.000	-40.1150	-15.2717
Diet 3	Diet 1	26.86000*	3.77434	.000	14.4383	39.2817
	Diet 2	23.56000*	3.77434	.001	11.1383	35.9817
	Diet 4	-1.70000	3.77434	.990	-14.1217	10.7217
	Diet 5	-4.13333	3.77434	.805	-16.5550	8.2883
Diet 4	Diet 1	28.56000*	3.77434	.000	16.1383	40.9817

	Diet 2	25.26000*	3.77434	.000	12.8383	37.6817
	Diet 3	1.70000	3.77434	.990	-10.7217	14.1217
	Diet 5	-2.43333	3.77434	.964	-14.8550	9.9883
	Diet 1	30.99333*	3.77434	.000	18.5717	43.4150
Diet 5	Diet 2	27.69333*	3.77434	.000	15.2717	40.1150
	Diet 3	4.13333	3.77434	.805	-8.2883	16.5550
	Diet 4	2.43333	3.77434	.964	-9.9883	14.8550

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

Hatching rate (%)

Descriptives

Hatching rate (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	46.0000	6.26498	3.61709	30.4369	61.5631	40.00	52.50
Diet 2	3	47.9000	2.45561	1.41774	41.7999	54.0001	45.20	50.00
Diet 3	3	68.0300	6.75275	3.89870	51.2552	84.8048	60.39	73.20
Diet 4	3	68.5800	3.29346	1.90148	60.3986	76.7614	65.01	71.50
Diet 5	3	76.6467	5.40209	3.11890	63.2271	90.0662	70.50	80.64
Total	15	61.4313	13.37360	3.45305	54.0253	68.8374	40.00	80.64

ANOVA

Hatching rate (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2242.126	4	560.531	21.409	.000
Within Groups	261.818	10	26.182		
Total	2503.944	14			

Multiple Comparisons

Dependent Variable: Hatching rate (%)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-1.90000	4.17786	.990	-15.6497	11.8497
	Diet 3	-22.03000*	4.17786	.003	-35.7797	-8.2803
	Diet 4	-22.58000*	4.17786	.002	-36.3297	-8.8303
	Diet 5	-30.64667*	4.17786	.000	-44.3964	-16.8970
Diet 2	Diet 1	1.90000	4.17786	.990	-11.8497	15.6497
	Diet 3	-20.13000*	4.17786	.005	-33.8797	-6.3803
	Diet 4	-20.68000*	4.17786	.004	-34.4297	-6.9303
	Diet 5	-28.74667*	4.17786	.000	-42.4964	-14.9970
Diet 3	Diet 1	22.03000*	4.17786	.003	8.2803	35.7797
	Diet 2	20.13000*	4.17786	.005	6.3803	33.8797
	Diet 4	-.55000	4.17786	1.000	-14.2997	13.1997
	Diet 5	-8.61667	4.17786	.306	-22.3664	5.1330
Diet 4	Diet 1	22.58000*	4.17786	.002	8.8303	36.3297
	Diet 2	20.68000*	4.17786	.004	6.9303	34.4297
	Diet 3	.55000	4.17786	1.000	-13.1997	14.2997
	Diet 5	-8.06667	4.17786	.362	-21.8164	5.6830
Diet 5	Diet 1	30.64667*	4.17786	.000	16.8970	44.3964
	Diet 2	28.74667*	4.17786	.000	14.9970	42.4964
	Diet 3	8.61667	4.17786	.306	-5.1330	22.3664
	Diet 4	8.06667	4.17786	.362	-5.6830	21.8164

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

Temperature (ºC)

Descriptives

Temperature(°C)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	29.0233	.63058	.36407	27.4569	30.5898	28.62	29.75
Diet 2	3	28.9367	.48045	.27739	27.7432	30.1302	28.42	29.37
Diet 3	3	28.7000	.62450	.36056	27.1487	30.2513	28.20	29.40
Diet 4	3	28.4333	.86217	.49777	26.2916	30.5751	27.50	29.20
Diet 5	3	28.5267	.84388	.48721	26.4304	30.6230	27.58	29.20
Total	15	28.7240	.63944	.16510	28.3699	29.0781	27.50	29.75

ANOVA

Temperature(°C)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.776	4	.194	.392	.810
Within Groups	4.948	10	.495		
Total	5.724	14			

Multiple Comparisons

Dependent Variable: Temperature(°C)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.08667	.57433	1.000	-1.8035	1.9768
	Diet 3	.32333	.57433	.978	-1.5668	2.2135
	Diet 4	.59000	.57433	.837	-1.3002	2.4802
	Diet 5	.49667	.57433	.903	-1.3935	2.3868
Diet 2	Diet 1	-.08667	.57433	1.000	-1.9768	1.8035
	Diet 3	.23667	.57433	.993	-1.6535	2.1268
	Diet 4	.50333	.57433	.899	-1.3868	2.3935
	Diet 5	.41000	.57433	.949	-1.4802	2.3002
Diet 3	Diet 1	-.32333	.57433	.978	-2.2135	1.5668
	Diet 2	-.23667	.57433	.993	-2.1268	1.6535

Diet 4	Diet 4	.26667	.57433	.989	-1.6235	2.1568
	Diet 5	.17333	.57433	.998	-1.7168	2.0635
	Diet 1	-.59000	.57433	.837	-2.4802	1.3002
	Diet 2	-.50333	.57433	.899	-2.3935	1.3868
	Diet 3	-.26667	.57433	.989	-2.1568	1.6235
	Diet 5	-.09333	.57433	1.000	-1.9835	1.7968
Diet 5	Diet 1	-.49667	.57433	.903	-2.3868	1.3935
	Diet 2	-.41000	.57433	.949	-2.3002	1.4802
	Diet 3	-.17333	.57433	.998	-2.0635	1.7168
	Diet 4	.09333	.57433	1.000	-1.7968	1.9835

pH

Descriptives

pH

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	8.5067	.17214	.09939	8.0790	8.9343	8.37	8.70
Diet 2	3	8.5367	.07767	.04485	8.3437	8.7296	8.45	8.60
Diet 3	3	8.3833	.20984	.12115	7.8621	8.9046	8.22	8.62
Diet 4	3	8.2433	.08083	.04667	8.0425	8.4441	8.17	8.33
Diet 5	3	8.1067	.11930	.06888	7.8103	8.4030	7.97	8.19
Total	15	8.3553	.20594	.05317	8.2413	8.4694	7.97	8.70

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.393	4	.098	4.888	.019
Within Groups	.201	10	.020		
Total	.594	14			

Multiple Comparisons

Dependent Variable: pH

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.03000	.11574	.999	-.4109	.3509
	Diet 3	.12333	.11574	.820	-.2576	.5042
	Diet 4	.26333	.11574	.229	-.1176	.6442
	Diet 5	.40000*	.11574	.039	.0191	.7809
Diet 2	Diet 1	.03000	.11574	.999	-.3509	.4109
	Diet 3	.15333	.11574	.683	-.2276	.5342
	Diet 4	.29333	.11574	.158	-.0876	.6742
	Diet 5	.43000*	.11574	.026	.0491	.8109
Diet 3	Diet 1	-.12333	.11574	.820	-.5042	.2576
	Diet 2	-.15333	.11574	.683	-.5342	.2276
	Diet 4	.14000	.11574	.747	-.2409	.5209
	Diet 5	.27667	.11574	.195	-.1042	.6576
Diet 4	Diet 1	-.26333	.11574	.229	-.6442	.1176
	Diet 2	-.29333	.11574	.158	-.6742	.0876
	Diet 3	-.14000	.11574	.747	-.5209	.2409
	Diet 5	.13667	.11574	.762	-.2442	.5176
Diet 5	Diet 1	-.40000*	.11574	.039	-.7809	-.0191
	Diet 2	-.43000*	.11574	.026	-.8109	-.0491
	Diet 3	-.27667	.11574	.195	-.6576	.1042
	Diet 4	-.13667	.11574	.762	-.5176	.2442

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

TDS (ppm)

Descriptives

TDS(ppm)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	186.00	1.000	.577	183.52	188.48	185	187
Diet 2	3	217.67	15.695	9.062	178.68	256.66	200	230
Diet 3	3	202.67	15.948	9.207	163.05	242.28	192	221
Diet 4	3	184.33	2.082	1.202	179.16	189.50	182	186
Diet 5	3	186.00	2.646	1.528	179.43	192.57	183	188
Total	15	195.33	15.967	4.123	186.49	204.18	182	230

ANOVA

TDS(ppm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2543.333	4	635.833	6.197	.009
Within Groups	1026.000	10	102.600		
Total	3569.333	14			

Multiple Comparisons

Dependent Variable: TDS(ppm)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-31.667*	8.270	.022	-58.89	-4.45
	Diet 3	-16.667	8.270	.325	-43.89	10.55
	Diet 4	1.667	8.270	1.000	-25.55	28.89
	Diet 5	.000	8.270	1.000	-27.22	27.22
Diet 2	Diet 1	31.667*	8.270	.022	4.45	58.89
	Diet 3	15.000	8.270	.417	-12.22	42.22
	Diet 4	33.333*	8.270	.016	6.11	60.55
	Diet 5	31.667*	8.270	.022	4.45	58.89
Diet 3	Diet 1	16.667	8.270	.325	-10.55	43.89

	Diet 2	-15.000	8.270	.417	-42.22	12.22
	Diet 4	18.333	8.270	.249	-8.89	45.55
	Diet 5	16.667	8.270	.325	-10.55	43.89
	Diet 1	-1.667	8.270	1.000	-28.89	25.55
Diet 4	Diet 2	-33.333*	8.270	.016	-60.55	-6.11
	Diet 3	-18.333	8.270	.249	-45.55	8.89
	Diet 5	-1.667	8.270	1.000	-28.89	25.55
	Diet 1	.000	8.270	1.000	-27.22	27.22
Diet 5	Diet 2	-31.667*	8.270	.022	-58.89	-4.45
	Diet 3	-16.667	8.270	.325	-43.89	10.55
	Diet 4	1.667	8.270	1.000	-25.55	28.89

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

Oxygen reduction potentiality in water (ORP)

Descriptives

ORP

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	43.067	3.9552	2.2835	33.241	52.892	38.5	45.4
Diet 2	3	23.567	7.2037	4.1591	5.672	41.462	15.3	28.5
Diet 3	3	43.400	8.4071	4.8539	22.516	64.284	34.0	50.2
Diet 4	3	50.900	2.8160	1.6258	43.905	57.895	48.5	54.0
Diet 5	3	67.033	5.7492	3.3193	52.752	81.315	62.5	73.5
Total	15	45.593	15.3786	3.9707	37.077	54.110	15.3	73.5

ANOVA

ORP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2952.609	4	738.152	20.596	.000
Within Groups	358.400	10	35.840		
Total	3311.009	14			

Multiple Comparisons

Dependent Variable: ORP

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	19.5000*	4.8881	.017	3.413	35.587
	Diet 3	-.3333	4.8881	1.000	-16.420	15.754
	Diet 4	-7.8333	4.8881	.527	-23.920	8.254
	Diet 5	-23.9667*	4.8881	.004	-40.054	-7.880
Diet 2	Diet 1	-19.5000*	4.8881	.017	-35.587	-3.413
	Diet 3	-19.8333*	4.8881	.015	-35.920	-3.746
	Diet 4	-27.3333*	4.8881	.002	-43.420	-11.246
	Diet 5	-43.4667*	4.8881	.000	-59.554	-27.380
Diet 3	Diet 1	.3333	4.8881	1.000	-15.754	16.420
	Diet 2	19.8333*	4.8881	.015	3.746	35.920
	Diet 4	-7.5000	4.8881	.565	-23.587	8.587
	Diet 5	-23.6333*	4.8881	.005	-39.720	-7.546
Diet 4	Diet 1	7.8333	4.8881	.527	-8.254	23.920
	Diet 2	27.3333*	4.8881	.002	11.246	43.420
	Diet 3	7.5000	4.8881	.565	-8.587	23.587
	Diet 5	-16.1333*	4.8881	.049	-32.220	-.046
Diet 5	Diet 1	23.9667*	4.8881	.004	7.880	40.054
	Diet 2	43.4667*	4.8881	.000	27.380	59.554
	Diet 3	23.6333*	4.8881	.005	7.546	39.720
	Diet 4	16.1333*	4.8881	.049	.046	32.220

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

Dissolved oxygen (ppm)

Descriptives

DO (ppm)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	3.3067	.26858	.15506	2.6395	3.9738	3.00	3.50
Diet 2	3	3.9167	.51791	.29902	2.6301	5.2032	3.32	4.25
Diet 3	3	3.8767	.35303	.20382	2.9997	4.7537	3.50	4.20
Diet 4	3	3.7667	.30551	.17638	3.0078	4.5256	3.50	4.10
Diet 5	3	3.7467	.22030	.12719	3.1994	4.2939	3.60	4.00
Total	15	3.7227	.37076	.09573	3.5173	3.9280	3.00	4.25

ANOVA

DO (ppm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.711	4	.178	1.464	.284
Within Groups	1.214	10	.121		
Total	1.924	14			

Multiple Comparisons

Dependent Variable: DO (ppm)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.61000	.28446	.274	-1.5462	.3262
	Diet 3	-.57000	.28446	.330	-1.5062	.3662
	Diet 4	-.46000	.28446	.520	-1.3962	.4762
	Diet 5	-.44000	.28446	.558	-1.3762	.4962
Diet 2	Diet 1	.61000	.28446	.274	-.3262	1.5462
	Diet 3	.04000	.28446	1.000	-.8962	.9762
	Diet 4	.15000	.28446	.982	-.7862	1.0862
	Diet 5	.17000	.28446	.972	-.7662	1.1062
Diet 3	Diet 1	.57000	.28446	.330	-.3662	1.5062
	Diet 2	-.04000	.28446	1.000	-.9762	.8962
	Diet 4	.11000	.28446	.994	-.8262	1.0462
	Diet 5	.13000	.28446	.990	-.8062	1.0662
Diet 4	Diet 1	.46000	.28446	.520	-.4762	1.3962
	Diet 2	-.15000	.28446	.982	-1.0862	.7862

	Diet 3	-.11000	.28446	.994	-1.0462	.8262
	Diet 5	.02000	.28446	1.000	-.9162	.9562
	Diet 1	.44000	.28446	.558	-.4962	1.3762
	Diet 2	-.17000	.28446	.972	-1.1062	.7662
Diet 5	Diet 3	-.13000	.28446	.990	-1.0662	.8062
	Diet 4	-.02000	.28446	1.000	-.9562	.9162

Conductivity (mS/cm)

Descriptives

Conductivity (mS/cm)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	.37167	.004163	.002404	.36132	.38201	.367	.375
Diet 2	3	.43600	.031749	.018330	.35713	.51487	.400	.460
Diet 3	3	.40567	.032470	.018747	.32501	.48633	.384	.443
Diet 4	3	.36867	.004509	.002603	.35747	.37987	.364	.373
Diet 5	3	.37200	.006083	.003512	.35689	.38711	.365	.376
Total	15	.39080	.032398	.008365	.37286	.40874	.364	.460

ANOVA

Conductivity (mS/cm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	4	.003	6.095	.009
Within Groups	.004	10	.000		
Total	.015	14			

Multiple Comparisons

Dependent Variable: Conductivity (mS/cm)

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.064333*	.016880	.022	-.11989	-.00878
	Diet 3	-.034000	.016880	.326	-.08955	.02155
	Diet 4	.003000	.016880	1.000	-.05255	.05855
	Diet 5	-.000333	.016880	1.000	-.05589	.05522
Diet 2	Diet 1	.064333*	.016880	.022	.00878	.11989
	Diet 3	.030333	.016880	.425	-.02522	.08589
	Diet 4	.067333*	.016880	.017	.01178	.12289
	Diet 5	.064000*	.016880	.023	.00845	.11955
Diet 3	Diet 1	.034000	.016880	.326	-.02155	.08955
	Diet 2	-.030333	.016880	.425	-.08589	.02522
	Diet 4	.037000	.016880	.257	-.01855	.09255
	Diet 5	.033667	.016880	.334	-.02189	.08922
Diet 4	Diet 1	-.003000	.016880	1.000	-.05855	.05255
	Diet 2	-.067333*	.016880	.017	-.12289	-.01178
	Diet 3	-.037000	.016880	.257	-.09255	.01855
	Diet 5	-.003333	.016880	1.000	-.05889	.05222
Diet 5	Diet 1	.000333	.016880	1.000	-.05522	.05589
	Diet 2	-.064000*	.016880	.023	-.11955	-.00845
	Diet 3	-.033667	.016880	.334	-.08922	.02189
	Diet 4	.003333	.016880	1.000	-.05222	.05889

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

Resistivity (MΩcm)

Descriptives

Resistivity (

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	.002700	0E-7	0E-7	.002700	.002700	.0027	.0027
Diet 2	3	.002300	.0001732	.0001000	.001870	.002730	.0022	.0025
Diet 3	3	.002500	.0001732	.0001000	.002070	.002930	.0023	.0026
Diet 4	3	.002700	0E-7	0E-7	.002700	.002700	.0027	.0027
Diet 5	3	.002700	0E-7	0E-7	.002700	.002700	.0027	.0027
Total	15	.002580	.0001897	.0000490	.002475	.002685	.0022	.0027

ANOVA

Resistivity (

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	8.000	.004
Within Groups	.000	10	.000		
Total	.000	14			

Multiple Comparisons

Dependent Variable: Resistivity (

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	.0004000*	.0000894	.008	.000106	.000694
	Diet 3	.0002000	.0000894	.242	-.000094	.000494
	Diet 4	0E-7	.0000894	1.000	-.000294	.000294
	Diet 5	0E-7	.0000894	1.000	-.000294	.000294
Diet 2	Diet 1	-.0004000*	.0000894	.008	-.000694	-.000106
	Diet 3	-.0002000	.0000894	.242	-.000494	.000094
	Diet 4	-.0004000*	.0000894	.008	-.000694	-.000106
	Diet 5	-.0004000*	.0000894	.008	-.000694	-.000106
Diet 3	Diet 1	-.0002000	.0000894	.242	-.000494	.000094
	Diet 2	.0002000	.0000894	.242	-.000094	.000494
	Diet 4	-.0002000	.0000894	.242	-.000494	.000094
	Diet 5	-.0002000	.0000894	.242	-.000494	.000094
Diet 4	Diet 1	0E-7	.0000894	1.000	-.000294	.000294

	Diet 2	.0004000*	.0000894	.008	.000106	.000694
	Diet 3	.0002000	.0000894	.242	-.000094	.000494
	Diet 5	0E-7	.0000894	1.000	-.000294	.000294
	Diet 1	0E-7	.0000894	1.000	-.000294	.000294
Diet 5	Diet 2	.0004000*	.0000894	.008	.000106	.000694
	Diet 3	.0002000	.0000894	.242	-.000094	.000494
	Diet 4	0E-7	.0000894	1.000	-.000294	.000294

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

Cleavage period

Descriptives

Cleavage period

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	54.5067	6.01548	3.47304	39.5634	69.4500	48.15	60.11
Diet 2	3	57.8067	5.23638	3.02322	44.7988	70.8145	52.00	62.17
Diet 3	3	81.3667	4.21940	2.43607	70.8851	91.8482	78.00	86.10
Diet 4	3	83.0667	2.45832	1.41931	76.9599	89.1735	80.30	85.00
Diet 5	3	85.5000	4.40341	2.54231	74.5613	96.4387	81.20	90.00
Total	15	72.4493	14.41830	3.72279	64.4647	80.4339	48.15	90.00

ANOVA

Cleavage period

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2696.740	4	674.185	31.550	.000
Within Groups	213.685	10	21.368		
Total	2910.425	14			

Multiple Comparisons

Dependent Variable: Cleavage period

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-3.30000	3.77434	.900	-15.7217	9.1217
	Diet 3	-26.86000*	3.77434	.000	-39.2817	-14.4383
	Diet 4	-28.56000*	3.77434	.000	-40.9817	-16.1383
	Diet 5	-30.99333*	3.77434	.000	-43.4150	-18.5717
Diet 2	Diet 1	3.30000	3.77434	.900	-9.1217	15.7217
	Diet 3	-23.56000*	3.77434	.001	-35.9817	-11.1383
	Diet 4	-25.26000*	3.77434	.000	-37.6817	-12.8383
	Diet 5	-27.69333*	3.77434	.000	-40.1150	-15.2717
Diet 3	Diet 1	26.86000*	3.77434	.000	14.4383	39.2817
	Diet 2	23.56000*	3.77434	.001	11.1383	35.9817
	Diet 4	-1.70000	3.77434	.990	-14.1217	10.7217
	Diet 5	-4.13333	3.77434	.805	-16.5550	8.2883
Diet 4	Diet 1	28.56000*	3.77434	.000	16.1383	40.9817
	Diet 2	25.26000*	3.77434	.000	12.8383	37.6817
	Diet 3	1.70000	3.77434	.990	-10.7217	14.1217
	Diet 5	-2.43333	3.77434	.964	-14.8550	9.9883
Diet 5	Diet 1	30.99333*	3.77434	.000	18.5717	43.4150
	Diet 2	27.69333*	3.77434	.000	15.2717	40.1150
	Diet 3	4.13333	3.77434	.805	-8.2883	16.5550
	Diet 4	2.43333	3.77434	.964	-9.9883	14.8550

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

Gastrula period

Descriptives

Gastrula period

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	54.5067	6.01548	3.47304	39.5634	69.4500	48.15	60.11
Diet 2	3	57.8067	5.23638	3.02322	44.7988	70.8145	52.00	62.17
Diet 3	3	79.0819	4.21940	1.31359	70.8851	91.8482	78.00	80.16
Diet 4	3	83.0667	2.45832	1.41931	76.9599	89.1735	80.30	85.00
Diet 5	3	85.5000	4.40341	2.54231	74.5613	96.4387	81.20	90.00
Total	15	72.4493	14.41830	3.72279	64.4647	80.4339	48.15	90.00

ANOVA

Gastrula period

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2696.740	4	674.185	31.550	.000
Within Groups	213.685	10	21.368		
Total	2910.425	14			

Multiple Comparisons

Dependent Variable: Gastrula period

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-3.30000	3.77434	.900	-15.7217	9.1217
	Diet 3	-26.86000*	3.77434	.000	-39.2817	-14.4383
	Diet 4	-28.56000*	3.77434	.000	-40.9817	-16.1383
	Diet 5	-30.99333*	3.77434	.000	-43.4150	-18.5717
Diet 2	Diet 1	3.30000	3.77434	.900	-9.1217	15.7217
	Diet 3	-23.56000*	3.77434	.001	-35.9817	-11.1383
	Diet 4	-25.26000*	3.77434	.000	-37.6817	-12.8383
	Diet 5	-27.69333*	3.77434	.000	-40.1150	-15.2717
Diet 3	Diet 1	26.86000*	3.77434	.000	14.4383	39.2817
	Diet 2	23.56000*	3.77434	.001	11.1383	35.9817
	Diet 4	-1.70000	3.77434	.990	-14.1217	10.7217
	Diet 5	-4.13333	3.77434	.805	-16.5550	8.2883
Diet 4	Diet 1	28.56000*	3.77434	.000	16.1383	40.9817
	Diet 2	25.26000*	3.77434	.000	12.8383	37.6817
	Diet 3	1.70000	3.77434	.990	-10.7217	14.1217
	Diet 5	-2.43333	3.77434	.964	-14.8550	9.9883
Diet 5	Diet 1	30.99333*	3.77434	.000	18.5717	43.4150
	Diet 2	27.69333*	3.77434	.000	15.2717	40.1150
	Diet 3	4.13333	3.77434	.805	-8.2883	16.5550
	Diet 4	2.43333	3.77434	.964	-9.9883	14.8550

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

Segmentation period

Descriptives

Segmentation period

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	2	50.8650	.47376	.33500	46.6084	55.1216	50.53	51.20
Diet 2	2	51.7950	.57276	.40500	46.6490	56.9410	51.39	52.20
Diet 3	2	74.3650	1.61927	1.14500	59.8164	88.9136	73.22	75.51
Diet 4	2	77.4750	.81317	.57500	70.1689	84.7811	76.90	78.05
Diet 5	2	80.7050	.84146	.59500	73.1448	88.2652	80.11	81.30
Total	10	67.0410	13.70798	4.33484	57.2349	76.8471	50.53	81.30

ANOVA

Segmentation period

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1686.635	4	421.659	463.989	.000
Within Groups	4.544	5	.909		
Total	1691.179	9			

Multiple Comparisons

Dependent Variable: Segmentation period

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-.93000	.95329	.856	-4.7541	2.8941
	Diet 3	-23.50000*	.95329	.000	-27.3241	-19.6759
	Diet 4	-26.61000*	.95329	.000	-30.4341	-22.7859
	Diet 5	-29.84000*	.95329	.000	-33.6641	-26.0159
Diet 2	Diet 1	.93000	.95329	.856	-2.8941	4.7541
	Diet 3	-22.57000*	.95329	.000	-26.3941	-18.7459
	Diet 4	-25.68000*	.95329	.000	-29.5041	-21.8559
	Diet 5	-28.91000*	.95329	.000	-32.7341	-25.0859
Diet 3	Diet 1	23.50000*	.95329	.000	19.6759	27.3241
	Diet 2	22.57000*	.95329	.000	18.7459	26.3941
	Diet 4	-3.11000	.95329	.104	-6.9341	.7141

Diet 4	Diet 5	-6.34000*	.95329	.006	-10.1641	-2.5159
	Diet 1	26.61000*	.95329	.000	22.7859	30.4341
	Diet 2	25.68000*	.95329	.000	21.8559	29.5041
	Diet 3	3.11000	.95329	.104	-.7141	6.9341
	Diet 5	-3.23000	.95329	.091	-7.0541	.5941
Diet 5	Diet 1	29.84000*	.95329	.000	26.0159	33.6641
	Diet 2	28.91000*	.95329	.000	25.0859	32.7341
	Diet 3	6.34000*	.95329	.006	2.5159	10.1641
	Diet 4	3.23000	.95329	.091	-.5941	7.0541

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

Pharyngula period

Descriptives

Pharyngula period

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	2	47.6150	.55861	.39500	42.5960	52.6340	47.22	48.01
Diet 2	2	51.6150	.98288	.69500	42.7842	60.4458	50.92	52.31
Diet 3	2	71.1550	.24749	.17500	68.9314	73.3786	70.98	71.33
Diet 4	2	71.7450	2.46780	1.74500	49.5727	93.9173	70.00	73.49
Diet 5	2	78.4050	1.05359	.74500	68.9389	87.8711	77.66	79.15
Total	10	64.1070	12.86470	4.06817	54.9041	73.3099	47.22	79.15

ANOVA

Pharyngula period

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1480.965	4	370.241	216.783	.000
Within Groups	8.539	5	1.708		
Total	1489.504	9			

Multiple Comparisons

Dependent Variable: Pharyngula period

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-4.00000	1.30686	.127	-9.2425	1.2425
	Diet 3	-23.54000*	1.30686	.000	-28.7825	-18.2975
	Diet 4	-24.13000*	1.30686	.000	-29.3725	-18.8875
	Diet 5	-30.79000*	1.30686	.000	-36.0325	-25.5475
Diet 2	Diet 1	4.00000	1.30686	.127	-1.2425	9.2425
	Diet 3	-19.54000*	1.30686	.000	-24.7825	-14.2975
	Diet 4	-20.13000*	1.30686	.000	-25.3725	-14.8875
	Diet 5	-26.79000*	1.30686	.000	-32.0325	-21.5475
Diet 3	Diet 1	23.54000*	1.30686	.000	18.2975	28.7825
	Diet 2	19.54000*	1.30686	.000	14.2975	24.7825
	Diet 4	-.59000	1.30686	.989	-5.8325	4.6525
	Diet 5	-7.25000*	1.30686	.014	-12.4925	-2.0075
Diet 4	Diet 1	24.13000*	1.30686	.000	18.8875	29.3725
	Diet 2	20.13000*	1.30686	.000	14.8875	25.3725
	Diet 3	.59000	1.30686	.989	-4.6525	5.8325
	Diet 5	-6.66000*	1.30686	.019	-11.9025	-1.4175
Diet 5	Diet 1	30.79000*	1.30686	.000	25.5475	36.0325
	Diet 2	26.79000*	1.30686	.000	21.5475	32.0325
	Diet 3	7.25000*	1.30686	.014	2.0075	12.4925
	Diet 4	6.66000*	1.30686	.019	1.4175	11.9025

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

Hatching period

Descriptives

Hatching period

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Diet 1	3	46.0000	6.26498	3.61709	30.4369	61.5631	40.00	52.50
Diet 2	3	47.9000	2.45561	1.41774	41.7999	54.0001	45.20	50.00
Diet 3	3	68.0300	6.75275	3.89870	51.2552	84.8048	60.39	73.20
Diet 4	3	68.5800	3.29346	1.90148	60.3986	76.7614	65.01	71.50
Diet 5	3	76.6467	5.40209	3.11890	63.2271	90.0662	70.50	80.64
Total	15	61.4313	13.37360	3.45305	54.0253	68.8374	40.00	80.64

ANOVA

Hatching period

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2242.126	4	560.531	21.409	.000
Within Groups	261.818	10	26.182		
Total	2503.944	14			

Multiple Comparisons

Dependent Variable: Hatching period

Tukey HSD

(I) Diet	(J) Diet	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diet 1	Diet 2	-1.90000	4.17786	.990	-15.6497	11.8497
	Diet 3	-22.03000*	4.17786	.003	-35.7797	-8.2803
	Diet 4	-22.58000*	4.17786	.002	-36.3297	-8.8303
	Diet 5	-30.64667*	4.17786	.000	-44.3964	-16.8970
Diet 2	Diet 1	1.90000	4.17786	.990	-11.8497	15.6497
	Diet 3	-20.13000*	4.17786	.005	-33.8797	-6.3803
	Diet 4	-20.68000*	4.17786	.004	-34.4297	-6.9303
	Diet 5	-28.74667*	4.17786	.000	-42.4964	-14.9970
Diet 3	Diet 1	22.03000*	4.17786	.003	8.2803	35.7797
	Diet 2	20.13000*	4.17786	.005	6.3803	33.8797
	Diet 4	-.55000	4.17786	1.000	-14.2997	13.1997
	Diet 5	-8.61667	4.17786	.306	-22.3664	5.1330
Diet 4	Diet 1	22.58000*	4.17786	.002	8.8303	36.3297
	Diet 2	20.68000*	4.17786	.004	6.9303	34.4297
	Diet 3	.55000	4.17786	1.000	-13.1997	14.2997
	Diet 5	-8.06667	4.17786	.362	-21.8164	5.6830
Diet 5	Diet 1	30.64667*	4.17786	.000	16.8970	44.3964
	Diet 2	28.74667*	4.17786	.000	14.9970	42.4964
	Diet 3	8.61667	4.17786	.306	-5.1330	22.3664
	Diet 4	8.06667	4.17786	.362	-5.6830	21.8164

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