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Effects of Food Sources and Culture Conditions on Feeding Behaviour and Growth Rates of Nile Tilapia Fry (Oreochromis niloticus)

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I dedicate this work to my family……

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ABSTRACT

ABSTRACT

The importance of fish aquaculture in general as an alternative source of animal protein, is raised during this century after the significant reduction of natural marine resources. Such reduction has increased the interest of many countries as well as international agencies in worldwide food security, especially for developing countries. The success of any aquaculture is chiefly judged by its economic development, which always depends on the management of producing acceptable marketing size and weight of fish in short time. In the last few decades, many aquacultures failed in balancing between the financial input and the resultant income. That is due to many reasons; one of them is the high mortality in fish fry which resulted from either the difficulty of obtaining a suitable diet (live food) or from the quality of the living conditions of the cultured fry.

In the Middle East countries especially, and in Asia as general, certain fish species are becoming the most cultured ones because of their high consumption and because of their acceptable commercial price by local populations. However, in the last few years, such prices have increased, due to many reasons. These include, the increase of artificial diets items and the high mortalities of fish fry due to the lack of the food necessary to the fry immediately after its hatching. A viable example is Tilapia nilotica species (*Oreochromis niloticus*).

In the present study two types of experiments were designed to investigate the effect of different food sources on the growth rates of Nile tilapia fry (*Oreochromis niloticus*), and to evaluate the effect of some culture conditions on the feeding behaviour of the fry. In the first experiment, the effects of three types of food (*Chlorella vulgaris*, *Artemia franciscana*, and Artificial feed) and various combinations of the three types (*Chlorella* + *Artemia*; *Chlorella* + Artificial feed; *Artemia* + Artificial feed; and *Chlorella* + *Artemia* + Artificial

feed) on the growth, feed utilization and survival of Nile tilapia fry has been investigated, with average initial weight of 12 mg fry^{-1}. Tilapia fry were cultured in a closed system and fed with seven feed combinations. Triplicate groups of fry with 24 fry per 6 L tank were used for each treatment. The diet was offered 3 times a day, for 35 days. The results indicated that the maximum body weight and survival was achieved by fry fed on *Artemia*, followed by those fed on the combination of the three food types, then by *Artemia* + Artificial. On the other hand, the minimum growth and survival were observed from the fry fed only with *Chlorella* suspension. The fry fed only with Artificial feed showed moderate growth and good survival.

In the second experiments a series of trials were carried out to detect the response of tilapia fry to live food suspension of *Chlorella*, *Artemia*, and Artificial feed, and the smell of the filtrates of the *Chlorella, Artemia* and Rotifers cultures. Another trial was designed to study the response of tilapia fry to different environmental opaque colours (green, yellow, red, and blue). These experiments were carried out in a rectangular glass basin divided into six tracks using five longitudinal transepts. These tracks were used as swimming pools. At both ends of each track mobile doors were inserted to separate a small chamber.

In the food suspension experiment, the basin was filled with tilapia fish tanks water. The mobile doors were tightly fixed at both ends. The small chambers of two tracks were filled with *Chlorella* on one side and with experiment water on the opposite side. The chambers of another two tracks were filed with *Chlorella* on one side and *Artemia* on the opposite side, while the remaining two track ends were filled with *Chlorella* on one side and Artificial feed on the opposite side on the suspension experiment.

For the smell experiment the basin was filled with tap water and the Artificial feed replaced by Rotifers. Also, the doors of the chambers were raised about 0.5 cm in the beginning of the experiment. Five tilapia fry were introduced into the middle of each track at the beginning of the experiments. The movement of the fry towards one source of food/extract or another was recorded every 5 minutes for a total of 30 minutes. The same process was followed on the four colour experiments but with a little modification: the doors of the chambers were removed and sheets of opaque colours inserted in the end of champers. In each experiment, a single colour was examined against the other three, as well as against itself.

The results have shown that the tilapia fry favoured the suspensions of *Artemia* nauplii and Artificial feed more than the *Cholorella* suspensions. Furthemore the tilapia fry were attracted by the smell of *Artemia* and Rotifers filtrates more than the filtrate of the *Chlorella* culture. However, the chlorella filtrate was highly attractive to the fry compared with the blank conditions. Results from the color experiments showed that green is the most attractive color followed by yellow then blue, while red was the least attractive one.

In conclusion, *Artemia* nauplii was the best live food, followed by *Artemia* nauplii combined with Artificial feed, to accelerate the growth rate of the fry. Moreover, it is recommended to use algae extract which may act as a natural colouring agent as well as an odour stimulator.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Food sustainability, in general and quality in particular, are serious challenges facing not only human beings but also many other living organisms. This challenge can be greatly complicated when these organisms represent an essential human food source. In this regard, fish are considered an important food source for humans and also as essential and alternative resource of animal protein.

During the last few decades, the world population density has been increasing rapidly. Simultaneously, new techniques and tools have been adopted in order to increase the fishing activities as well as the fish yield to meet the increased market demands. However, the over-exploitation of many fish stocks has resulted in a sharp decline in fish populations and yields in many regions, and also to the degradation of aquatic habitats. Therefore, fisheries enhancement is now of prime importance in many parts of the world.

Aquaculture is also another important food production sector that can play a significant role in meeting the shortage in fish supply (Holt, 2000; Pillay, 2001). Furthermore, aquaculture plays a major role as a provider of employment. Global aquaculture has been expanding at an outstanding rate. In fact, aquaculture has been recognized as the fastest growing food production sector in the world over the past 30 years (Josupeit and Lem, 2000). It has been recorded that since 1984 until 1990 world aquaculture production increased at an average rate of about 14% annually (Hardy, 1999). As a result aquaculture production has increased from 16.8 million mt representing only 16.3% of total production in 1990, to 51.4 million mt representing 35.2% of total production in 2002 (Table 1.1).

Table 1.1. The contribution (million metric tons) of tilapia culture and total aquaculture to world fish production during 1990 to 2002. Source: FAO (2004). FAO Fishstat Plus.

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Fisheries	86.1	84.9	86.4	87.7	93.3	93.7	95.2	95.7	88.8	95.1	96.8	94.2	94.6
Tilapia culture	0.38	0.40	0.49	0.55	0.60	0.71	0.81	0.93	0.95	1.11	1.27	1.40	1.51
Total aquaculture	16.8	18.3	21.2	24.5	27.8	31.2	33.9	35.9	39.2	43.1	45.7	48.4	51.4
Total production	102.9	103.2	107.6	112.2	121.1	124.9	129.1	131.6	128	138.2	142.5	142.6	146

Feed represents over 50% of the operating costs in intensive aquaculture. Many researchers determined the nutrient requirements of many cultured fish and crustacean species with the aim of increasing the growth rate and yield and minimizing food costs (De Silva, 1985, 1992; Sehagal and Toor, 1991; El-Sayed, 1999). The two main sources of food for cultured fish species are: 1) Artificial feed and 2) Live food. Artifical diets must contain the necessary components (protein, carbohydrates, lipids, minerals and vitamins) that meet the nutritional requirements of the cultured aquatic animals (Pillay, 2001).

Live food is also another important food sources in aquaculture, especially during larval stages. Live food consists of aquatic plants and animals obtained from the environment, and it is generally rich in essential nutrients (Pillay, 2001). Today, there are three groups of live foods which are widely applied in industrial larvae culture of marine fish and crustaceans. They are: 1) large micro algae, 2) the rotifer *Brachionus plicatilis* and 3) the brine shrimp *Artemia* spp. (Sorgeloos and Leger, 1992).

In recent years, different formulations of supplements and substitutional products have been added to the list of live food diets (Sorgeloos and Leger, 1992). In aquaculture, live food can be vital for many fish species, especially for the larvae of marine fish, where the initial feeding should be microbiotic organisms (Rosenlund *et al*,. 1997). Furthermore, the knowledge of the feeding habits of cultured fish is crucial for the selection of fish species in successful aquaculture. In fact, omnivorous species are comparatively easy to feed, and the more attractive species for aquaculture are those which feed at low trophic levels (Pillay, 2001).

On the other hand, culture conditions including 1- abiotic factors (temperature, salinity, light, dissolved oxygen, and ammonia), and 2- biotic factors (stocking density and food type)) have an important role in aquaculture production because of their significant effects on the survival and production of fish species selected for aquaculture (Huang and Chiu, 1997; Ridha *et al.*, 1998; Frances *et al.*, 2000; Kumlu *et al.*, 2000; Powell *et al.*, 2002; Biswas *et al.*, 2002; Biswas and Takeuchi, 2002; El-Sayed, 2002; Lu *et al.*, 2002; Turker *et al.*, 2003a).

Tilapias are one of the most important aquaculture groups, as they rank third in terms of global production only after carps and salmonids (FAO, 2004). Tilapia are freshwater fishes, belong to family Cichlidae. They are native to Africa, but have been introduced into many tropical, subtropical and temperate regions of the world during the second half of the 20th century, mainly for farming as food fish (Pillay, 2001). Tilapia have many attributes that make them ideal candidates for aquaculture. These include:

1. fast growth,
2. tolerance to of a wide range of environmental conditions (such as temperature, salinity, low dissolved oxygen, etc.),
3. resistance to stress and disease,
4. ability to reproduce in captivity and having short generation time, and
5. feeding at low trophic levels and accepting Artificial feeds immediately after yolk-sac absorption.

In addition to these attributes, tilapia can be easily raised in a variety of aquaculture systems (Chervinski, 1982; Philippart and Ruwet, 1982; Macintosh and Little, 1995; Siddiqui and Al-Harbi, 1995; Pillay, 2001; El-Sayed and Kawanna, 2004).

The increasing importance of tilapias as an aquaculture candidate makes it necessary to understand their food preferences and feeding regimes in their natural habitats, in order to prepare suitable diets for them and adopt the appropriate feeding regimes under culture conditions. Tilapia are generally herbivorous/omnivorous (i.e. they are low on the aquatic food chain) (Bowen, 1982; Harbott, 1982). This characteristic is extremely important in regards to the economics of tilapia culture.

Tilapia nutrition has received considerable attention in recent years, particularly their nutrient requirements. However, the effects of culture conditions and live food quality and quantity on the growth, survival and feeding behaviour of Nile tilapia fry are not well-understood. Very little information is available on this subject. Therefore, the present study has been carried out to:

1. Evaluate the effect of different food sources on growth rates of Nile tilapia (*O. niloticus*) fry, and
2. Evaluate the effect of some culture conditions on feeding behaviour and growth rates of Nile tilapia (*O. niloticus*) fry.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1. LIVE FOOD

Fish stocks are declining in many fisheries as a result of over-fishing, habitat degradation, and pollution of natural waters. The rapid expansion of aquaculture could compensate for these declines by supplying consumers with alternate sources of fish products (Holt, 2000). A reliable and regular availability of fish larvae is one of the most important factors in the success of commercial aquaculture production. The shortage in larval productions remains the major problem facing fish farmers worldwide (Shiekh-Eldin *et al.*, 1997). This is mainly because larviculture in general and larval nutrition in particular, especially during the early larval stages, are major bottle-necks for the industrial up-scaling of the aquaculture of fish and shellfish (Sorgeloos and Leger, 1992; Sharma and Chakrabarti, 1999).

The production of marine fish larvae is dependent on the use of live feeds for initial feeding (Rosenlund *et al.*, 1997). Until recently, it was considered impossible to feed newly hatched marine fish species with a compound diet. The organogenesis of marine fish larvae is not completely achieved at hatching and histological studies have revealed that the anatomy of the digestive tract undergoes developmental changes over some weeks (Cahu and Infante, 2001). However, live foods may be a source of diseases or parasites to the larval rearing system, and are expensive and labour-intensive to produce (Blair *et al.*, 2003).

Live food for aquaculture consists of micro-plants and animals obtained from the environment. Live foods are generally rich in almost all the essential nutrients required by cultured organisms (Pillay, 2001). Today, three groups of live foods are widely applied in industrial larviculture of marine fish, mollusks and crustaceans. They are: 1) different species of algae in the size range of 2-20 μm; 2) the 50-200 μm rotifer *Brachionus plicatilis*; and 3) the 200-500 μm brine shrimp *Artemia* sp. In recent years, different formulations of supplements and substitution products have been added to this list (Sorgeloos and Leger, 1992).

2.1.1. PHYTOPLANKTON

Phytoplankton comprises the base of the food chain in the aquatic environment (Coutteau, 1996). Many aquatic animals rely on phytoplankton as a food source especially during their early stages of development (Leonardos and Lucas, 2000). It is no surprise, therefore, that micro-algae are an indispensable food source in the commercial rearing of many cultivated species, including all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of many phytoplankton filter feeders fish species (Coutteau, 1996). In their studies, Albentosa *et al.* (1996) and Camacho *et al.* (1998) found a good complement of live phytoplankton in diets for the seed of the little-neck clam *R. decussates*. Algae are furthermore used to produce mass quantities of zooplankton (rotifers, copepods, and brine shrimp) which in turn serve as food for the larval and early-juvenile stages of crustaceans and fish (Coutteau, 1996). Moreover, for the rearing of fish larvae according to the "green water technique", algae are used directly within the larval rearing tanks, where they are believed to play a role in stabilizing the water quality, nutrition

of the larvae, and microbial control (Coutteau, 1996; Cahu *et al.*, 1998). Planas and Cunha (1999) and Lazo *et al.* (2000) reported that the larvae from green water tanks showed higher survival and growth, and less gut contents than larvae reared in clear water. Diatoms and green algae are the two dominant groups of cultured microalgae. Algae species have been selected on the basis of their mass culture potential, cell size, digestibility, and overall food value, using trial and error techniques much more than any other scientific selection process (Sorgeloos and Leger, 1992).

Chlorella is an important source of live food in aquaculture. It can be grown with an organic carbon source, such as glucose or acetic acid, which significantly reduces the costs for lighting. *Chlorella* is also characterized by a short doubling time, which can be shortened to 3h by selecting the suitable *Chlorella* strain (Hagiwara *et al.*, 2001).

Little quantitative work has been carried out on feeding tilapia on a mono-algal population (Trewavas, 1983; Moriarty and Moriarty, 1973; Getachew, 1987; McDonald, 1987). Lu *et al.* (2003) suggested that tilapia fed solely on *Spirulina* sp. have a high flesh quality. Meanwhile, Olvera-Novoa *et al.* (1998) reported that *Spirulina* sp. can replace up to 40% of the fish meal protein in tilapia diets.

2.1.2. THE ROTIFER *Brachionus plicatilis*

The rotifer *Brachionus plicatilis* is widely used as a food for the cultivation of small-mouthed larval fish (Nichols *et al.*, 1996; Hagiwara *et al.*, 2001). The success of rotifers as a culture organism is manifold, including their planktonic nature, tolerance to a wide range of environmental conditions, and high reproduction rate. Moreover, their small size and slow swimming velocity make them a suitable prey for fish larvae that have just absorbed their

yolk sac but cannot yet ingest the larger *Artemia* nauplii (Dhert, 1996). Rotifers are mostly used as a starter diet in marine fish larviculture. Rotifer culture appears simple, with the use of microalgae (often *Chlorella* spp.) supplemented with bakers' yeast as food. However, many fish hatcheries have reported that they experience considerable problems in maintaining large cultures and producing sufficient quantities of rotifers that are needed to feed the hundreds of thousands to millions of larval fish they have in culture (Sorgeloos and Leger, 1992). In countries like Japan, the success of mass production of marine fish larvae is largely dependent on the availability of rotifers (Pillay, 2001).

The effect of rotifers on larval growth and survival is controversial, depending on cultured species, larval size, rotifer size and density (Blair *et al.*, 2003). For example, Blair *et al.* (2003) found that survival of larval haddock fed rotifer and *Artemia* nauplii was significantly high. On the contrary, Næss *et al.* (1995) reported poor growth and survival of halibut larvae fed *Artemia* and rotifer, *B. Plicatilis*.

Mixed feeding of live food and artificial feed (co-feeding) is a commonly used strategy to help wean larval fish onto nonliving or manufactured foods. It has been shown that such a strategy enhances larval growth and survival beyond that achieved by feeding either types of food alone (Woods, 2003). Co-feeding seems to serve two purposes: 1) it improves and stabilizes the nutritional condition of the larvae, and 2) it pre-conditions the larvae to accept the manufactured diet when live feed is withdrawn (Rosenlund *et al.*, 1997).

2.1.3. THE BRINE SHRIMP *Artemia* spp.

Artemia is used extensively in aquaculture, as a live food for many hatchery-reared fishes, particularly marine species (Sorgeloos and Leger, 1992; Barata *et al.*, 1996; Stappen,

1996; Pillay, 2001). Since no artificial diets have been developed to completely replace *Artemia* as a food source (Sorgeloos *et al.*, 2001), considerable progress has been made in the past decade to improve and increase the value of *Artemia* as a larval diet (Pillay, 2001).

Artemia nauplii are widly used in aquaculture for feeding fish larvae during the early stages. *Artemia* are convenient for feeding because they are readily available, may be grown to a larger size and its food could be enriched to manipulate their biochemical composition. Reared larvae are attracted to the swimming behaviour of *Artemia* and readily capture, eat and extract their nutrients (Pillay, 2001). Blair *et al.* (2003) found that the growth of larval haddock fed on *Artemia* nauplii were significantly higher than those fed enriched rotifers. It should be noted, however, that the nutritional quality and physical properties of *Artemia* nauplii vary, depending on the source and time of harvest of cysts (Kovalenko *et al.*, 2002). The optimization of the use of *Artemia* cysts as a larval food has also been realized by the commercial provision of high quality cyst products (Sorgeloos and Leger, 1992). However, *Artemia* production is labour-intensive and costly (Ritar *et al.*, 2002).

Artemia nauplii have also been considered as food for crustacean and mollusc larvae (Ritar *et al.*, 2002). Kovalenko *et al.* (2002) found that the growth of freshwater prawn *Macrobrachium rosenbergii* larvae fed with newly hatched live *Artemia* nauplii was significantly higher than that of larvae fed a micro-bound diet. *Artemia* nauplii and blue mussel (*Mytilus edulis*) meat have also been reported to be the most suitable food for the early larval stages of *Phyllosoma* sp. (Ritar *et al.*, 2002).

2.2. TILAPIA

Tilapias represent a large number of freshwater fish species within the family Cichlidae. They originated exclusively from Africa (excluding Madagascar), and from Palestine (Jordan Valley and coastal rivers) (Philippart and Ruwet, 1982). They are distributed all over Africa, except the northern Atlas Mountains and south-west Africa (McAndrew, 2000). Outside Africa, they are also widely distributed in the Americas and Asia (Philippart and Ruwet, 1982). They are mainly lacustrine fish that are well adapted to enclosed bodies of water (Shiau and Huang, 2001). Tilapias also inhabit a wide range of ecosystems (El-Sayed, 1999).

2.2.1. FEEDING HABITATS

Tilapia are generally herbivorous/omnivorous (i.e. they are low on the aquatic food chain). Feeding habits and dietary preferences of tilapias depend on tilapia species and size, time of the day, photoperiod, water depth and geographical location. During larval stages, tilapia feed initially on zooplankton, especially crustaceans (copepods) (Bowen, 1982; Harbott, 1982)). When Florida red tilapia fry were stocked in fertilized seawater pools containing different food resources, copepods were selectively ingested (Grover *et al.*, 1989).

The food of juvenile and adult tilapia consists of a considerable variety of aquatic vegetation, phytoplankton, zooplankton, periphyton and detritus of plant origin (Hepher & Pruginin, 1981), depending on tilapia species. Many research studies have been carried out on the feeding habits of different tilapias, in their natural habitats, under a variety of environmental conditions. Generally speaking, tilapia can be classified into one of the following broad categories according to their feeding patterns:

1. Tilapias of the genus *Oreochromis* are primarily microphagous, feeding mainly on phytoplankton, periphyton, and detritus. *O. niloticus*, *O. aureus* and *O. mossambicus* are examples of this genus. These species can efficiently ingest these food sources mentioned through "filter-feeding".
2. Tilapias of the genus *Sarotherodon* are also primarily phytoplankton feeders, but they are more selective. For example, Spataru (1976) found that the dinoflagellate *Peridinium cinctum* was the most abundant foods in the stomachs of *S. galilaeus* in Lake Kenneret, sometimes comprising >95% of the phytoplankton biomass.
3. Tilapias of the genus *Tilapia* are generally macrophyte feeders (Lowe McConnell, 1975). However, they can incidentally ingest algae, phytoplankton, zooplankton, bacteria, benthic invertebrates, insect larvae, fish and vertebrate eggs and detritus which are attached to the macrophytes they feed on. These attached materials are therefore an important food component for *Tilapia* species (Bowen, 1982).

2.2.2. TILAPIA CULTURE

Tilapia culture is believed to have originated more than 4000 year ago (Balarin and Hatton, 1979). However, other than illustrations from ancient Egyptians tombs, very little information is available on their culture during those early days (Chimits, 1957). Currently, more than 100 countries practice tilapia culture world wide (FAO, 2004). It is no surprise, therefore, that among cultured fishes of the world, tilapias ranks third in terms of production: only after carp and salmonids (FAO, 2004). The production of farmed tilapia has increased more than 390% during the past decade, to jump from 0.38 million mt in 1990, representing 2.28% of total aquaculture production, and reaching 1.51 million mt in 2002, representing

2.93% of total production. The average annual growth of tilapia production during that period approached 12.2% (FAO, 2004).

Commercial tilapia culture is currently restricted to about 10 species. Nile tilapia (*O. niloticus*) is by far the most important farmed tilapia species in the world. It represented more than 80% of total tilapia production during 1970-2002 (FAO, 2004). Nile tilapia also ranked 6th in terms of global fish production in 2002, after silver carp, grass carp, common carp, Crucian carp and bighead carp. Mozambique tilapia comes second, with a production of 54,146 mt in 2002, representing 3.6% of the production of total farmed tilapia. Meanwhile unidentified tilapias represented a significant proportion of the production. In 2002, that category amounted 227,741 mt, representing 18.7% of total tilapia production (FAO, 2004).

The value of farmed tilapia has also sharply increased during the past two decades. The value increased from about $US 154 million in 1984 to $US 1,800.7 million in 2002. As expected, the value of Nile tilapia represented between 60% to >70% of the total market value of farmed tilapia during the past decade (FAO, 2004).

2.2.3. TILAPIA NUTRITION

Nutrition is the most expensive component in the intensive aquaculture, since it represents over 50% of operating costs. Therefore, the production of good quality feed, in addition to adopting proper feeding management strategies is necessary for successful tilapia culture (De Silva, 1985; Sehagal & Toor, 1991; De Silva, 1992; El-Sayed, 1999). In this regard, tilapia nutrition has received great attention in recent years. Several publications have considered the protein, lipid, carbohydrates, vitamins and minerals requirements of different

tilapia species under various culture conditions. These requirements are summarized in the following review.

2.2.3.1. Protein and Amino Acid Requirements:

Protein is the most expensive component in artificial fish diets (Olvera-Novoa *et al.*, 2002). Traditionally, high-quality, but expensive, fishmeal (FM) has been used as the major protein source in fish diets. However, the periodic low availability of FM could affect aquaculture production (Mohsen and Lovell, 1990; Watanabe and Pongmaneerat, 1991; Anderson *et al.*, 1993).

Protein utilization has been the main focus of research on nutrient requirements of tilapia (Cruz and Laudencia, 1977; Davis and Stickney, 1978; Mazid *et al.*, 1979; Winfree and Stickney, 1981; Jauncey, 1982; De Silva and Perera, 1985; Siddiqui *et al.*, 1988, Shiau and Huang, 1990). The protein fraction should be optimally utilized for growth rather than serve as an energy source for fish (Shiau and Huang, 1989). El-Sayed and Teshima (1991) reviewed the dietary protein requirements of several species of tilapia, and reported that the requirements range from 20% and 56%, depending on tilapia species, size, sex, protein and energy sources and culture conditions (Table 2.1). Other studies have indicated the tilapia brood stock require about 30-40% dietary protein for optimum reproductive performance and egg hatchability in clear water (Santiago *et al.*, 1985; Wee and Tuan, 1988; De Silva and Radampola, 1990; Gunasekera *et al.*, 1996; El-Sayed et al., 2003). The protein requirements of farmed tilapia are summarized in table 2.1.

Fishes, like other animals, are known to require the same 10 indispensable or essential amino acids (EAA) in their diets for maximum growth and well-being (Wilson, 1989). Among the fish species, however, the requirements for individual EAA vary (Arginine,

Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine), often significantly (NRC, 1981; Wilson, 1991; NRC, 1993). The requirements for all the EAA are known only for a limited number of cultured fish species like rainbow trout, channel catfish and tilapia (Table 2.2) (NRC, 1993).

2.2.3.2. Lipids and Essential Fatty Acids

Dietary lipids play an important role in fish nutrition for provision of both Essential Fatty Acids (EFA) and energy (Sargent *et al.*, 1989). Dietary lipids are also carriers of fat-soluble vitamins, and provide other compounds such as polar lipid and sterols (Castell, 1979), which are important in membrane biochemistry and thus have direct impact on membrane-mediated processes, such as osmoregulation, and nutrient assimilation and transport. Lipids are energy rich and can be used to spare surplus dietary protein for growth (NRC, 1983). However, little information is available on the lipid nutrition of tilapia (Chou and Shiau, 1996). Lipid requirements of tilapia have not been well-studied. The requirements depend on lipid source, dietary protein and energy contents, and tilapia species and size. Generally speaking, tilapia require about 10-18% dietary lipids for maximum growth performance (Table 2.3).

The lipid fatty acid content is critical to prevent essential fatty acid (EFA) deficiency signs in fish (NRC, 1983). EFA are fatty acids that either cannot be biosynthesized or synthesized in inadequate amounts by animals for growth, maintenance and proper functioning of many physiological processes (Stickney *et al.*, 1982; Stickney and McGeachin, 1983).

Table 2.1. Protein requirements of cultured tilapia. (*c.f.* Al-Darmaki, 2003)

Species	Size (g)	Protein	Requirements	References
O. niloticus	Fry	FM	45%	El-Sayed and Teshima, 1992
	0.002-0.028	FM	28.3	De Silva and Perera, 1985
	0.09	-	45	Jovert *et al.*, 1993
	0.56	Casein /Gelatin	35	Teshima *et al.*, 1985
	0.80	FM	40	Siddiqui *et al.*,1988
	1.00	Casein	40	Kanazawa *et al.*, 1982
	1.29	Casein	40	Teshima *et al.*, 1982
	1.5-7.5	Casein/Gelatin	36	Lim (undated)
	3.50	Casein	30	Wang *et al.*, 1985
	6.1-16.5	FM	30	De Silva and Radampola, 1990
	24	FM+SBM+BM	27.5	Wee and Tuan, 1988
	34	Egg albumen	28-30	Yong *et al.*, 1989
	40	FM	30	Siddiqui *et al.*, 1988
O. mossambicus	Fry	FM	50	Jauncey and Ross, 1982
	0.50-1.00	FM	40	Jauncey, 1982
	1.00-2.50	FM+SBM+CM	29-38	Cruz and Laudencia, 1977
	6-30	FM	30-35	Jauncey and Ross, 1982
O aureus	0.30-0.50	SBM or FM	36	Davies and Stickney, 1978
	2.50	Casein/albumen	56	Winfree and Stickney, 1981
	7.5	Casein/albumen	34	Winfree and Stickney, 1981
T. zilli	1.35-1.80	Casein	35	Mazid *et al.*, 1979
	1.4	Casein /Gelatin	35	El-Sayed, 1987

FM: Fish Meal

BM: Bone Meal

SBM: Soybean Mean

CSM: Cotton Seed Meal

Table 2.2. Essential amino acids requirements of *O. mossambicus* and *O. niloticus* as a percent of dietary protein and diet (in parentheses) (*c.f.* Al-Darmaki, 2003)

Amino Acid	Species		
	O. mossambicus (Jackson and Capper, 1982)	*O. mossambicus* (Jauncey *et al.*, 1983)	*O. niloticus* (Santiago and Lovell, 1988)
Lysine	4.05 (1.62)	3.78 (1.51)	5.12 (1.43)
Arginine	3.80 (1.52)	2.82 (1.13)	4.20 (1.18)
Histidine		1.05 (0.42)	1.72 (0.48)
Threonine		2.93 (1.17)	3.75 (1.05)
Valine		2.20 (0.88)	2.80 (0.78)
Leucine		3.40 (1.35)	3.39 (0.95)
Isoleucine		2.01 (0.80)	3.11 (0.87)
Methionine	1.33 (0.53)	0.99 (0.40)	2.68 (0.75)
Phenylalanine		2.50 (1.00)	3.75 (1.05)
Ttryptophan		0.43 (0.17)	1.00 (0.28)

Table 2.3. Lipid requirements of tilapia.

Tilapia species	requirements (% of diet)	Reference
T. zillii	15	El-Sayed and Garling (1988)
O. aureus	10	Stickney and Wurts (1986)
O. aureus x *O. niloticus*	12	Jauncey and Ross (1982)
O. mossambicus x *O. niloticus*	18	De Silva *et al.* (1991)
O. niloticus x *O. aureus*	12	Chou and Shiau (1996)

The available information on fatty acid requirements of tilapia has been contradictory. Several studies have indicated that tilapia require *n-6* EFA rather than *n-3* EFA (Stickney *et al.*, 1982; Stickney and McGeachin, 1983). The growth of Nile tilapia fed on a diet containing fish oil (rich in *n-3* EFA) was significantly reduced as compared with one containing soybean oil or corn oil (rich in *n-6* EFA) (Takeuchi *et al.*, 1983). Similar results were reported on Nile tilapia broodstock, where fish fed with diets containing fish oil had significantly poor reproductive performance compared to those fed soybean oil diets (Santiago and Reyes, 1993).

On the other hand, many other studies indicated that tilapia may require both *n-3* EFA and *n-6* EFA. Stickney and McGeachin (1983) found that 10% soybean oil or 10% fish oil produced similar growth in blue tilapia. Furthermore, Chou and Shiau (1999) found that both *n-3* and *n-6* highly unsaturated fatty acids (HUFA) are required for maximum performance of tilapia hybrids (*O. niloticus* x *O. aureus*). More recently, El-Sayed *et al.* (2005) found that

Nile tilapia brood stock reared in brackish water required *n-3* HUFA for optimum spawning performance.

2.2.3.3. Carbohydrates

Carbohydrates are the least expensive dietary source (Wilson, 1991). In general, fish use dietary carbohydrates poorly (Shiau, 1997). Moreover, fish of low trophic level (herbivorous or omnivorous) tend to be more efficient at both the uptake and clearance of glucose compared with carnivorous species (Furuichi and Yone, 1981; Garcia-Riera and Hemre, 1996; Peres *et al.*, 1999). Also, different types of carbohydrate may not be used equally by fish. For example, tilapia grow better when fed on starch rather than on a glucose diet (Anderson *et al.*, 1984; Tung and Shiau, 1991). Hsieh and Shiau (2000) suggest that the physiological responses of tilapia during starvation are affected by pre-fasting diets containing different carbohydrates. El-Sayed (1987) found that *Tilapia zilli* utilizes up to 40% dietary carbohydrate without adverse effect on their performance.

2.2.3.4. Vitamins

Vitamins are organic compound that are required in minute amounts for normal growth, reproduction, health and general maintenance of fish metabolism (NRC, 1983). They are either not synthesized by organisms or synthesized at rates insufficient to meet the organisms' needs (Pillay, 2001). Considerable research has been conducted to determine the vitamin requirements of optimum tilapia diets (Jauncey and Ross, 1982; Roem *et al.*, 1990; El-Sayed and Teshima, 1991). Table 2.4 shows the vitamin requirements in tilapia diet.

Table 2.4. Vitamin requirements of tilapia. (O.n = *Oreochromis niloticus*; O.a = *O. aureus*)

Vitamin	Fish species	Requirement Mg kg^{-1}	Reference
Niacin	*O. n* x *O. a*	26 (fed glucose) 121 (fed dextrin)	Shiau and Suen (1992)
Pantothenic Acid	*O. a*	10	Soliman and Wilson (1992a)
Thiamin	*O. m* x *O. n*	2.5 (sea water, 32‰)	Lim and Leamaster (1991)
Riboflavin	*O. aureus*	6	Soliman and Wilson (1992b)
Pyridoxine	*O. aureus*	50 (ascorbic cid)	Shiau and Hsieh (2001)
Vitamin C	*O. niloticus*	420 (ascorbic cid)	Soliman *et al.* (1994)
D	*O. n* x *O. a*	374.8 IU	Shiau and Hwang (1993)
E	*O. n*	50-100 (5% lipid) 500 (10-15% lipid)	Satoh *et al.* (1987)
A	*O. n*	5000 IU	Saleh *et al.* (1995)

2.2.3.5. Minerals

Minerals are inorganic nutrients generally ingested as salts dissolved in food and water (Solomon *et al.*, 1993). They are required by all animals, either in their elemental form or incorporated into specific compounds, for various biological functions such as the formation of skeletal tissue, respiration, digestion and osmoregulation (Pillay, 2001). Fish require at least 22 minerals, 7 major minerals (calcium, phosphorus, potassium, sodium, chlorine, magnesium, and sulphur) and 15 trace elements (iron, zinc, copper, manganese, iodine, fluorine, cobalt, molybdenum, selenium, chromium, nickel, tin, silicon, vanadium, and arsenic) (NRC, 1983).

A number of studies have been conducted on the requirements of tilapia for certain minerals (Table 2.5).

Table 2.5. Mineral requirements of tilapia.

Mineral	Species	Requirement G kg^{-1}	Reference
Phosphorus	*O. niloticus*	4600	Haylor *et al.* (1988)
	O. niloticus x *O. aureus*	7000	Viola *et al.* (1986)
Potassium	*O. niloticus* x *O. aureus*	2100-3300	Shiau and Hsieh (2001)
Magnesium	*O. aureus*	500	Reigh *et al.* (1991)
Zinc	*O. niloticus*	30	Eid and Ghonim (1994)
	O. aureus	20	McClain and Gatlin (1988)
Manganese	*O. niloticus*	12	Watanabe *et al.* (1988)
Iron	*O. niloticus*	60	Kleemann *et al.* (2003)

2.2.4. TILAPIA LARVAL REARING

Despite the great potential of tilapia culture, shortage of fry production to meet the increased global demand remains one of the major constraints that hinder expansion in tilapia reproduction (Ridha and Cruz, 2000; El-Sayed, 2002). Also, the information regarding larval culture, especially the effect of stocking density and feeding regimes on fish performance, is limited, inconsistent and sometimes controversial (El-Sayed, 2002).

2.2.4.1. Factors Affecting Larval Rearing of Tilapia

a. Abiotic factors

I. Temperature

Water temperature is a controlling factor in the biochemical, molecular and metabolic processes of aquatic organisms (Brett, 1979). Water temperature also affects fish growth, respiration, feed intake, digestion, assimilation (Tuker *et al.*, 2003a) fecundity and spawning (Ridha *et al.*, 1998). Tilapia is strongly thermophilic, and feeding rates increase with increasing water temperature. For example, Bhikajee and Gobin (1997) found that the feeding rate of red tilapia hybrid *O. mossambicus* x *O. niloticus* x *O. aureus* increased from 3.70 % to 4.97% when ambient water temperature increased from 20°C to 32°C. Similarly, Mironova (1975) and Lauzanne (1978) observed an increase in daily feed consumption rate by increasing water temperature for *O. mossambicus* and *Sarotherodon galilaeus*. In addition, Tsai *et al.* (2003) found that water temperature has a differential influence on the development of central neurotransmitter systems according to the developmental period in tilapia (*Oreochromis mossambicus*).

The Nile tilapia *Orochromis niloticus* (*L.*) do not grow at temperatures below 16°C and do not survive at temperature below 10°C for more than a few days (Chervinski, 1982). Fish activity and feeding rate are reduced when the temperature falls below 20°C and stops completely at around 16°C. In addition, reproductive activities occur above 22°C (Chervinski, 1982). Studies on low temperature tolerance of Nile tilapia (Atwood *et al.*, 2003) showed that smaller fish are less tolerant to low temperatures than larger fish.

II. Water salinity

Water salinity and temperature are the most important physical (abiotic) factors influencing growth and survival of aquatic organisms, and the biological effects of these factors are complex and wide-ranging (Kinne, 1972 C.F. Ponce-Palafox, 1997; Kumlu *et al.*, 2000). Brett (1972) (C.F. Ponce-Palafox, 1997) stated that salinity imposed the greatest additional load on the metabolic requirements of an animal. The effect of water salinity on nutrient requirements of Nile tilapia is not fully understood. Moreover, the interactive effect of dietary nutrients and water salinity on reproductive performance of tilapia is also not clear. However, El-Sayed *et al.* (2003) indicated that spawning performance and larval growth of Nile tilapia were better in freshwater than at 7‰ and 14‰.

III. Light

Photoperiod is one of the main artificial Zeitgebers (i.e. cues or synchronizers) that may influence the daily rhythm of fish. In fishes, light has various effects on the circadian variation of some physiological activities (Muller, 1978; Peter *et al.*, 1978; Hurd *et al.*, 1998) like growth, locomotive activity, metabolic rates, body pigmentation, sexual maturation, reproduction and hormone activity (Hurd *et al.*, 1998; Boeuf and Le Bail, 1999; Biswas and Takeuchi, 2002; Biswas *et al.*, 2002; Simensen *et al.*, 2002; Trippel and Neil, 2002). Biswas and Takeuchi (2002) found that the fish conserve energy when raised under longer photoperiod cycles. However, studies on the effects of photoperiod on larval and juvenile fish (Barlow *et al.*, 1995) showed that photoperiod may positively affect larval stages, but not juvenile stages. From the results of Biswas *et al.*, (2002) study, it is suggested that young tilapia *O. niloticus* conserve more energy if they are exposed to longer photoperiods. El-

Sayed and Kawanna (2004) conducted a study on the farmed Nile tilapia *Oreochromis niloticus* to investigate the effects of photoperiod on growth, feed utilization efficiency and survival of fry and fingerlings. The fish were exposed to four photoperiod (light:dark, L:D) cycles (24L:0D, 18L:6D, 12L,12D and 6L:18D). The result revealed that Nile tilapia fry, but not fingerlings, reared in indoor, recirculating systems are significantly affected by photoperiod. The insignificant difference in fry performance between 24L:0D and 18L:6D groups suggest that a 18L:6D cycle be used in larval rearing, while shorter light phases are suggested for optimal growth, feed efficiency and survival of fish fingerlings, taking into consideration the cost of electricity.

V. Dissolved Oxygen (DO)

Low dissolved oxygen (DO) concentration limits production of many aquaculture species (Boyd and Watten, 1989) because it limits fish metabolism when the partial pressure (concentration) falls below the threshold level (Brett, 1979). For that, DO is classified as a limiting factor for growth (Fry, 1971; Brett, 1979). Although it does not act directly on growth as many toxins do, it limits the scope for aerobic metabolism (Fry, 1971). In general, aquatic organisms are oxygen regulators or oxygen conformers, depending upon their ability to regulate metabolism as a function of oxygen concentration (Vernberg, 1983). Tilapia survive routine dawn DO concentrations of less than 0.3 mg L^{-1}, considerably below the tolerance limits for most other cultured fish (Popma and Masser, 1999). In research studies of Popma and Masser (1999) Nile tilapia grew better when aerators were used to prevent morning DO concentrations from falling below 0.7 to 0.8 mg L^{-1} (compared with unaerated control ponds). Growth was not further improved if additional aeration kept DO

concentrations above 2.0 to 2.5 mg L^{-1}. Although tilapia can survive acute low DO concentrations for several hours, tilapia ponds should be managed to maintain DO concentrations above 1 mg L^{-1}. Metabolism, growth, and possibly disease resistance are depressed when DO falls below this level for prolonged periods.

IV. Ammonia

Ammonia is one of the most toxic compounds for fish under intensive culture conditions (Jeney *et al.*, 1992). The respiratory organs, liver, kidneys and nervous system of fish are damaged by ammonia (Nemcsók *et al.*, 1981). Studies on the effects of ammonia on juvenile silver perch (*Bidyanus bidyanus*) (Frances *et al.*, 2000) showed that changes in gill histopathology were observed in fish exposed to ammonia. Knowing the concentration of ammonia that limits growth and impairs respiration is useful for aquaculture management and may assist in the maximization of production (Colt and Armstrong, 1981). Rowland *et al.* (1995) found that slower growth of silver perch in ponds was associated with concentrations of un-ionised ammonia (UAN) up to 0.65 mg L^{-1}.

The effect of ammonia on tilapia performance is related to water pH and exposure period, in addition to ammonia concentration. Hargreaves and Kucuk (2001) found that brief daily exposure of *O. aureus* to up to 0.91 mg L^{-1} NH_3-N at pH 9 did not affect fish growth and feed utilization, while brief daily exposure to 1.81 mg L^{-1} NH_3-N reduced specific growth rate (SGR). At a concentration of 3.23 mg L^{-1} NH_3-N at pH 9.5, SGR was only 35% that of the control fish. These results suggested that sub-lethal ammonia exposure in aquaculture has little effect on tilapia performance.

b. Biotic Factors

1. Stocking density

A close relationship has been found between stocking density, and the growth and survival of fish, for example, a negative relationship was discovered for the fry of Africa catfish *Clarias gariepinus* (Burchell) (Haylor, 1991). However, the results of Miao (1990) on golden shiner *Notemigonus Crysoleucas* (Mitchill) showed that the stocking density was not a factor affecting growth and production. Fox and Flowers (1990) found also that the survival rate was not related to stocking density of juvenile walleye *Stizostedion vitreum* (Mitchill). Nevertheless, Papst *et al.* (1992) suggested that in intensive aquaculture the stocking density is an important factor that determines the economic viability of the production system. Furthermore, the size variation of fish may also be affected by stocking density. Wallace *et al.* (1988) showed that high density cultures reduced size variation in the early stage of Arctic charr *Salvelinus alphinus* (Linnaeus).

On the other hand, studies on the effects of stocking density on survival, growth, size variation, and production of Tilapia fry (Huang and Chiu, 1997) showed that there is no general agreement regarding the effects of stocking density on the fish. But a usually linear or curvilinear relationship is found between stocking density, and growth and survival rate of fish. Also, Macintosh and De Silva (1984) found that the relationship between the survival of *O. mossambicus* and *O. niloticus* female x *O. ureus* male fry and stocking density was not consistent. El-Sayed (2002) found that the optimum stocking density of Nile tilapia fry was 5 fry L^{-1}.

II. Food

Food composition and concentration have important influences on the performance of all organisms (Santer, 1994) such as larval cohorts (Powell *et al.*, 2002). The diet quality is directly proportional with its ability to support growth (Lu *et al.*, 2002). Jimmy *et al.* (2003) demonstrated that the life span of common sea urchin larvae *Echinus esculentus* and the survival at metamorphosis can be manipulated through diet quality and quantity.

Protein is the limiting growth factor for fish, since all fishes require the 10 EAA (as stated earlier) for building new tissue (Lu *et al.*, 2002). It was demonstrated also that dietary protein level influences onset of puberty, oocyte growth, spawning performance and egg quality in Nile tilapia *O. niloticus* (Gunasekera *et al.*, 1996). Dietary protein levels also affected the morphological measurements of silver perch. The relative gut length of fish fed lower amounts of protein varies among fish species and seems to be related to their feeding habitats. The relative gut lengths of fish fed lower protein diets were longer that those of fish fed higher protein diets (Yang *et al.*, 2002). Different protein sources were used which may influence larval quality due to variation in the composition of fatty acids and minerals (Gunasekera *et al.*, 1996).

Lipids are well utilized by most fish, but at high levels may reduce fish growth (Chou and Shiau, 1996). On the other hand, the principal signs of essential fatty acid (EFA) deficiency reported in studies with warm-water species are reduced growth rate, reduced feed efficiency and in some cases increased mortality (Alava and Kanazawa, 1996). This affects aquaculture since the composition of fatty acids accumulated in the muscle influences the taste and the texture of the fish meat (Guillou *et al.*, 1995).

Carbohydrates are one of the dietary components that profoundly affect feed utilization. Increases in starch levels above 10% of the dietary dry matter content result in reduced feed utilization in cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*) and salmon, all obligate carnivores. Also, as carnivorous fish fed with high starch diets seem to have a poor ability to take care of excess glucose it may be assumed that these fish are under constant metabolic stress, which may lead to suppressed immune functions (Hemre *et al.*, 2002). On another hand, the balance between lipid and starch affects protein uptake, yielding the best growth rate in *Chinook salmon* when carbohydrate and lipids occur in equal caloric quantities (Hemre *et al.*, 2002).

Minerals are required for the maintenance of the normal metabolic and physiological functions of an animal. The lack of an essential mineral in the diet may lead to several deficiency symptoms. The advantage of dietary mineral supplementation has been demonstrated by higher yield, better fish growth and more efficient feed utilization. Supplementation of macro-minerals has had significant effect as compared to supplementation of micro-minerals. The addition of phosphorus was found to significantly affect tilapia weight gain, feed conversion ratio, and protein efficiency ratio (Dato-Cajegas and Yakupitiyage, 1996). Vitamins also played an important role in improving the quality of hatchery-produced fry (Lavens and Sorgeloos, 2000). Further studies on food quality and the importance of live food introduction, together with artificial meals, for aquaculture fish (tilapia included) are being run in order to optimize the best feeding conditions.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The present study was conducted to evaluate the use of different live food sources for Nile tilapia (*Oreochromis niloticus*) fry, and the feeding behaviour of the fish. In this regard, two experiments were carried out. The first experiment considered the food preference of the fish fry, while the second experiment evaluated the feeding behaviour of the fry.

3.1. REARING FACILITY

The present work was conducted at the aquaculture laboratory of the Department of Aridland Agriculture, College of Food Systems, UAE University. A closed, recirculating culture system was used throughout the study. The system consisted of 18 L semi quadrate fibreglass tanks, with each tank connected to a central standpipe surrounded by an external pipe (sleeve) perforated at the bottom to allow self-cleaning of the tank (Fig. 3.1a and b). Dechlorinated tap water was used throughout the study. Water was supplied by nozzles, which were placed at certain angles to enhance water circulation in the tanks. Continuous water circulation was necessary for tank self-cleaning. Each tank was also provided with an air supply through air stone. Drainage water from all tanks was collected into a settling reservoir where faeces and other particulate wastes were removed by siphoning. Water was then passed through a series of tanks containing biological filtration media made up from many plastic tubes. After filtration, the water was pumped up into a head tank (1000 litre) using 1.5 horse power pump and passed through an ultraviolet light source (Aquafine, 0.67 Amp., model 1, MP-2-SL) for sterilization and microbial disinfections. Water was then

A

B

Fig. 3.1. A and B: The closed recirculating culture system used during the present study.

distributed to culture tanks by gravity at a flow rate 4 L min^{-1}. Water temperature was maintained at 27°C (±2°C) through a central thermostat.

3.2. FOOD QUALITY AND GROWTH RATE EXPERIMENTS (EXPERIMENT I)

3.2.1. FRY PRODUCTION

Swim-up Nile tilapia (*O. niloticus*) fry (Fig. 3.2) were produced from a brood stock kept in captivity in the aquaculture facility, College of Food Systems, UAE University. The original stock was obtained from Saudi Arabia. The fry were counted and weighed collectively, and the average initial weight (mg fry^{-1}) was calculated. The average initial weight of fry used in the food preference experiment was 12 mg fry^{-1}. The fry were then stocked in culture tanks filled with 6 L of water, at a density of 24 fry per tank (i.e. 4 fry $litre^{-1}$).

Water quality parameters, including dissolved oxygen (DO) ammonia (NH_4-N), pH, salinity and temperature were monitored before starting the experiment. The values of these parameters and the used instruments are shown in table 3.1.

Table 3.1. The value of water quality parameters and the measuring instrument utilized.

Parameter	Value	Measuring Instrument
Do	6.7± 1.4 mg L^{-1}	Oxygen meter, YSI, model 58
NH_4-N	0.053 ± 0.002 mg L^{-1}	Orion Aquafast, Germany
pH	8.1 ± 0.06	Ion meter, Jenway, UK
Salinity	.4 ‰	Conduktometer, Germany
Temperature	22 °C	Ion meter, Jenway, UK

3.2.2. EXPERIMENTAL FOOD TYPES

In the present experiment, the effects of three types of food on the growth, feed utilization and survival Nile tilapia fry has been investigated. These three types were: phytoplankton (*Chlorella vulgaris*), newely hatched (48-h hatched) *Artemia fransciscana*, and Artificial feed. These food sources were provided to the fish either solely or in combination (*Chlorella* + *Artemia*; *Chlorella* + Artificial feed; *Artemia* + Artificial feed; and *Chlorella* + *Artemia* + Artificial feed).

I. Phytoplankton (*Chlorella vulgaris*) Culture

Chlorella vulgaris cells were isolated from a water sample obtained from an outdoor concrete tank. The cells were collected by centrifuging the water at 1500 rpm, for 30 min and the decanting the supernatant. The concentrated cells were resuspended in distilled water and centrifuged again. This operation was repeated at least two times. The aggregate was then used in the establishment of the *Chlorella* culture.

Chlorella cells were cultured in the WC medium according to Wight's modification of Chu's medium n°10 (Hamza, 1993). The WC medium was prepared as shown in tables (3.2.a to 3.2.d). The medium was aerated in an outdoor illuminated system for about 24 h before adding the *Chlorella* cells. Every week the culture was sieved and transferred to larger jars containing new WC medium until enough quantity of *Chlorella* was produced. This operation was repeated every 2 weeks for maintenance of the stock (Fig. 3.3). The surrounded temperature was almost similar to that of the fish rearing system, since the outdoor system was established inside the fish lab.

Fig. 3.2. Nile tilapia fry seen under stereo microscope.

Fig. 3.3. *Chlorella vulgaris* culture.

Table 3.2. a. WC medium components (Hamza, 1993)

To make 1 litre of WC medium:	
Glass distilled water	1 litre
$CaCl_2H_2O$ *	1 mL
$MgSO_2.7H_2O$ *	1 mL
$NaHCO_3$ *	1 mL
K_2HPO_4 *	1 mL
$NaNO_3$ *	1 mL
$Na_2SiO_3.9H_2O$ *	1 mL
Trace metals.(II m Medium stock) **	1 mL
H_3BO_3 *	0.6 mL
Vitamins *	0.5 mL
Tris *	1 mL
Soil Extract ***	10 mL
Adjust the pH at 7.5	

* **Refer to Table 3.2.b.** ** **Refer to Table 3.2.c.** *** **Refer to Table 3.2.d.**

Table 3.2.b.

Stocks:	
$CaCl_2.2H2O$	3.676 g/100 mL dist. water
$MgSO_4.7H_2O$	3.697 g/100 mL dist. water
$NaHCO_3$	1 260 g/100 mL dist. water
$K2HPO_4$	1.141 g/100 mL dist. water
$NaNO_3$	8.501 g/100 mL dist. water
$Na2SiO_3.9H_2O$	2.842 g/100 mL dist. water
H_3BO_3	500 mg/100 mL dist. water

Vitamins Stock solution:

Primary stock:

- Biotin
  (dissolve biotin in a little (2ml) 0.1 N-NaOH first)
- B12 (cyanocobalamin)

Secondary stock:

Add 1.0 mL of biotin primary stock and 1.0 mL of cyanocobalamin primary stock to about 900 mL dist. water. Add 200 mg of thiamine HCL and bring to 1 litre. Dispense in 10 mL lots in screw cap test tubes and autoclave. Store in refrigerator and freeze after opening.

Tris Stock solution: (Hydroxymethyle Amino methane)

50 g/200 mL of dist. water. Add 20-30 mL of HCL to dissolve Tris at first (pH at 7.1-7.3), and bring to 200 mL.

N.B. Trizma can be used instead of Tris. In this case:

25 g/500 mL dist. water solution is prepared and 5 mL is added to 1 litre of the WC medium.

Table 3.2.c

Trace metals stocks:

a- Primary stock

$CuSO_4.5H_2O$	980 mg/100 mL dist. water
$ZnSO_4.7H_2O$	202 g/100 mL dist. water
$CoCl_2.6H_2O$	1.0 g/100 mL dist. water
$MnCl_2.4H_2O$	18.0 g/100 mL dist. water
$Na_2MoO_4.2H_2O$	630 mg/100 mL dist. water

b- Secondary stock

Dissolve 5.0 g of ferric sequestrene (FeNaEDTA) in about 900 mL of distilled water. Add 1 mL of each trace metal primary stock and bring to 1 litre.

Table 3.2.d

Soil Extract

The soil extract was a garden soil (or garden loam) extracted with unknown chemical composition. A satisfactory soil was the one containing small quantities of clay and humus. This soil should not have been fertilized with commercial fertilizers recently.

About 1 kg of garden loam was put in a large flask, 1 litre of distilled water was added inside the flask and it was autoclaved at 115-120° C, 17 lbs. for 60 minutes. Then it was stood overnight to permit sedimentation. The supernatant solution was filtered through a Whatman filter (2-3 times), until a clear solution was obtained. Then it was filtered through a HA Millipore filter (0.45 μm). Distilled water was added to make one litre. The amount most commonly used (10-20 mL) was dispensed into screw cap test tubes and autoclaved. The soil extract was preserved in the refrigerator until it was used (this prevented fungal growth in the stock) (Hamza, 1993).

II. *Artemia francescana* Culture

The cysts of *Artemia fransciscana* were incubated in filtered sea water (using GF/C filter paper and the salinity adjusted with distilled water to reach 28‰), for two days until they hatched. The newely hatched *Artemia* were fed to Nile tilapia fry.

One litre of filtered sea water was aerated (in a 2 L container) for about 24 h and then, 1 g of *Artemia* cysts (about 280,000 cysts) was added with small amounts of yeast. After 48 h, approximately 90% of *Artemia* cysts were hatched. At this time, the culture needed more yeast.

III. Artificial Feed Preparation

The test diet used in this experiment was prepared by weighing the dry ingredients (fish meal, and wheat flour) separately and mixing them together (Table 3.3). Then, the vitamin and mineral mixture was added with continuous mixing. Sun flower oil was added drop by drop, with continuous mixing. Warm tap water was added gradually until the diet was molded into small balls. After that, the diet was passed through a commercial meat grinder to form spaghetti-like threads, and spread on a metal plate and left to dry using cold air from an air condition, for 48 h. Finally, the dried diet was chopped into pellets, which were sieved through standard sieves to sepa ate pellets into the appropriate size (600μ) required for fish larval stage.

Table 3.3. Composition of the test diet.

Ingredient	%
Fish meal	50
Wheat flour	42
Sun flower oil	5
Vitamin and mineral mix	3
Total	100

3.2.3. ESTIMATION OF FOOD REQUIREMENTS OF NILE TILAPIA FRY

I. Phytoplankton (*Chlorella vulgaris)*

Chlorella culture was harvested at its sigmoid growth level, where the absorbance of the culture was 0.455 when the λ = 440 nm. About 90 mL from the culture was centrifuged at 1500 rpm for 30 min, and the supernatant was discarded. *Chlorella* was suspended in distilled water and centrifuged again. This operation was repeated at least two times. The aggregates were diluted to 1 L in distilled water (100% concentration) with an absorbance of 0.919 measured at a wave length of 440 nm. Various concentrations (75%, 50% and 25%) were prepared from the previous solution. The absorption was measured three times for all concentrations by spectrophotometer at 440 nm and the average was taken (the absorptions were: 100% = 0.919, 75% = 0.667, 50% = 0.461, and 25% = 0.267). Finally, the number of cells was counted three times in each concentration by heamocytometer and the average was taken (the number of cells L^{-1} for each concentration was: 100% = 1,100,000,000 cells L^{-1}; 75% = 750,000,000; 50% = 550,000,000; and 25% = 300,000,000).

In order to determine the amount of *Chlorella* cells eaten by fish fry, different concentrations of *Chlorella* density (100%, 75%, 50% and 25%) were used in triplicate. Three beakers were filled with 100 mL of each concentration and 3 Nile tilapia fry were added to each beaker. After 4 h the concentrations was measured by spectrophotometer (λ = 440 nm), then the difference between initial and final concentration was estimated to determine how many cells were consumed by each fish fry using a calibration curve prepared in advance based on the spectrophotometer absorbance reading (X axis) and the corresponding *Chlorella* cell counts (Y axis) (Fig. 3.4) This experiment showed that the best concentration was the highest concentration (100%) where the absorbance at λ = 440 nm equaled 0.919.

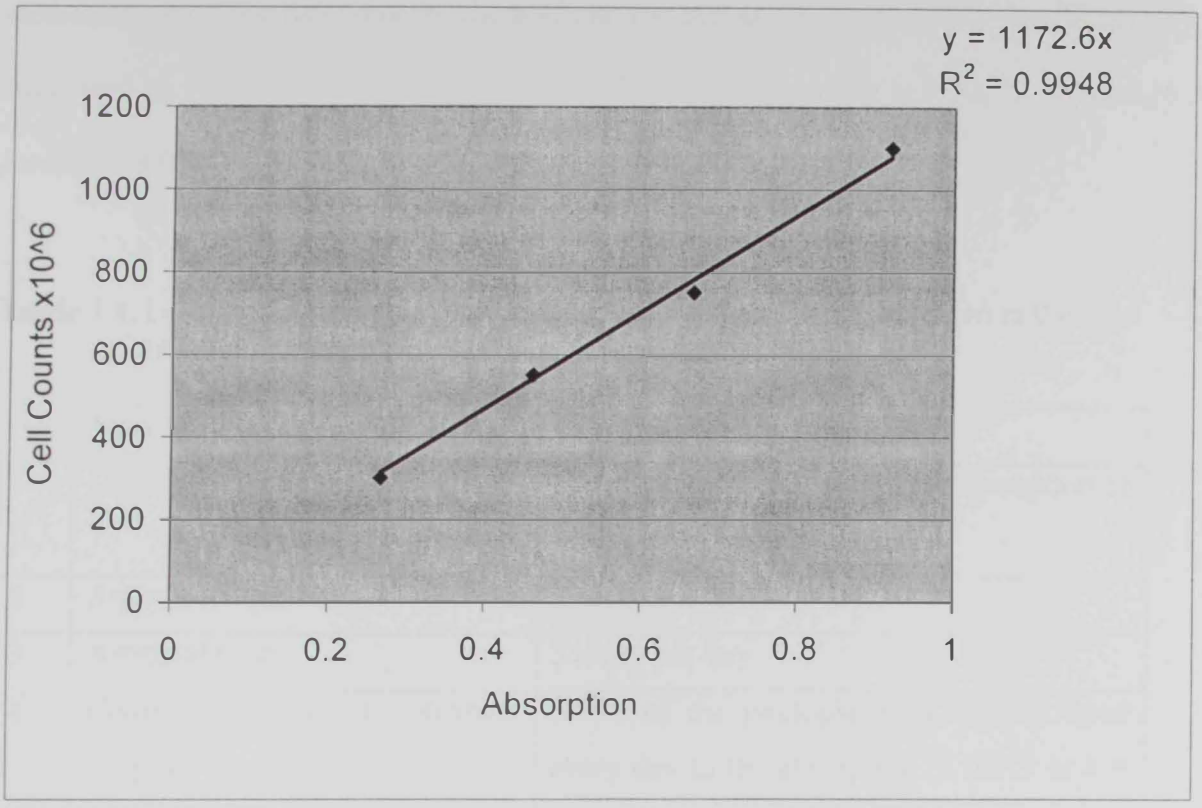

Fig. 3.4. Calibration curve for *Chlorella* cell counts.

II. *Artemia francescana*

The *Artemia* nauplii were counted in a sample of 1mL withdrawn from the well-mixed culture. In this test, 2 groups of 5 fry were independently introduced in two beakers (500 mL each) contained 400 mL of the *Artemia* culture. After 4 h, the *Artemia* nauplii were counted again in each beaker to calculate how many organisms were consumed by each fish fry. It was noticed that each fry consumed 28 *Artemia* per hour, on average.

3.2.4. THE FEEDING TRIALS

Tilapia fry were cultured in the closed system (described above) and fed with seven feed combinations (Table 3.4). Triplicate groups of fry (24 fry per 6 L tank) were used for

each treatment. The fish were fed the artificial test diet at 30% of their live weight per day as suggested by El-Sayed (2002). The diet was offered 3 times a day at 8:30, 12:30, and 16:30, for 34 days (from 27/3/2003 to 1/5/2003).

Table 3.4. Live food, feed combinations, and feeding regimes implemented in the food quality and growth.

No.	Food source	Regimes / Frequency
1	*Chlorella* cells	Density fixed every day to the absorption of 0.919 at λ = 440 nm.
2	*Artemia* nauplii	3 times per day.
3	Artificial feed	3 times per day.
4	*Chlorella* cells + *Artemia* nauplii	50 % of the phytoplankton density fixed every day to the absorption of 0.919 at λ = 440 nm, and 50 % of the zooplankton added 3 times per day.
5	*Chlorella* cells + Artificial feed	50 % of phytoplankton density fixed every day to the absorption of 0.919 at λ = 440 nm, and 50 % of the Artificial feed added 3 times per day.
6	*Artemia* nauplii + Artificial feed	50 % of zooplankton + 50 % of Artificial feed were added 3 times per day.
7	*Chlorella* cells + *Artemia* nauplii + Artificial feed	33% of the phytoplankton density fixed every day to the absorption of 0.919 at λ = 440 nm, 33 % of zooplankton + 33 % of Artificial feed were added 3 times per day.

3.2.5. DATA COLLECTION AND SAMPLE ANALYSIS

Fish mortality in each tank was recorded daily. Fish growth and vitality was also observed with attention given to the differences between the distinct diets. Water quality parameters including pH, salinity, O_2, NH_3, and water temperature were measured weekly in all tanks. Fish in each tank were weighed weekly, and the average larval weights were recorded.

At the end of the experiment, all fish in each aquarium were netted, counted, weighed and the average final weight (g fry^{-1}) was calculated. The fish were stored in labelled plastic bags and frozen for final body composition analysis.

3.2.5.1. Proximate Analysis

Proximate compositions, including dry matter, crude protein, lipid and ash contents were determined for the fish according to AOAC (1994), as follows.

I. Water content

Determination of water content of fish was accomplished by drying a pre-weighed sample at 60° C for 24 hours. The difference between the final and the initial weight represented the water content according to the following equation:

% Water = 100 (weight of water / weight of wet sample)

II. Protein

The protein content (on dry weight basis) was determined as total nitrogen content using the micro kjeldahl method. Protein content was calculated by multiplying total nitrogen by 6.25 according to the following equation:

% Protein = % nitrogen X 6.25

III. Lipids

Total lipids were determined using the Soxhlet method, and diethyl ether was used as an organic solvent according to the following equation:

% Lipid = 100 (weight of lipid / weight of dry sample)

IV. Ash

Because of the small number of fry obtained at the end of feeding trials, fry samples were insufficient for the determination of ash content. Therefore, ash content was calculated by subtracting % protein + lipid from 100 % (on dry weight basis) according to the following equation:

% Ash = 100 - (% protein + % lipid)

3.2.5.2. Calculation of Fish Growth Rate

- Average daily gain (ADG) = $(W_2 - W_1)/t$
- % Weight gain = 100 $((W_2 - W_1)/ W_1)$
- Specific growth rate (%SGR) = 100 $(\ln W_2 - \ln W_1)/t$

Where

W_1: Average initial weight (g)

W_2: Average final weight (g)

t: Time (days of experiment)

3.3. FEEDING BEHAVIOUR EXPERIMENTS (EXPERIMETN II)

A series of trials were carried out in experiment II to evaluate the effect of some culture conditions (food texture, smell, and colour) on the feeding behaviour of Nile tilapia (*O. niloticus*) fry. These trials were designed to detect the response of tilapia fry to live food suspension *Chlorella*, *Artemia* nuplii, artificial food, and the smell of the filtrates of the *Chlorella, Artemia* and rotifer cultures.

Another trial was designed to study the response of tilapia fry to different environmental colours. The colours used and their wavelengths were (blue: 452 nm, green: 495 nm, yellow: 565 nm, and red: 697 nm) (Hamza & Ruggiu, 2000). All trials were carried out at room temperature, illuminated by white cool light from topside.

3.3.1. EXPERIMENTAL BASIN

Experiment II was carried out in a rectangular glass basin (72 cm long, 48 cm wide and 10.5 cm high) (Fig 3.5). The basin was divided into six tracks (72 x 9 x 10.5 cm) using five longitudinal transepts. These tracks were used as swimming pools. At both ends of each track mobile doors were inserted to separate a small chamber (8 x 9 x 10.5 cm). These chambers were used to hold the different tested food suspensions as well as the culture media filtrates.

To evaluate the response of tilapia fry to different colours, opaque paper sheets of blue, green, yellow and red colours were trimmed to 9 cm length and 13 cm height and covered in plastic with laminating pouch films. The mobile doors were removed and the coloured sheets were inserted in the ends of tracks.

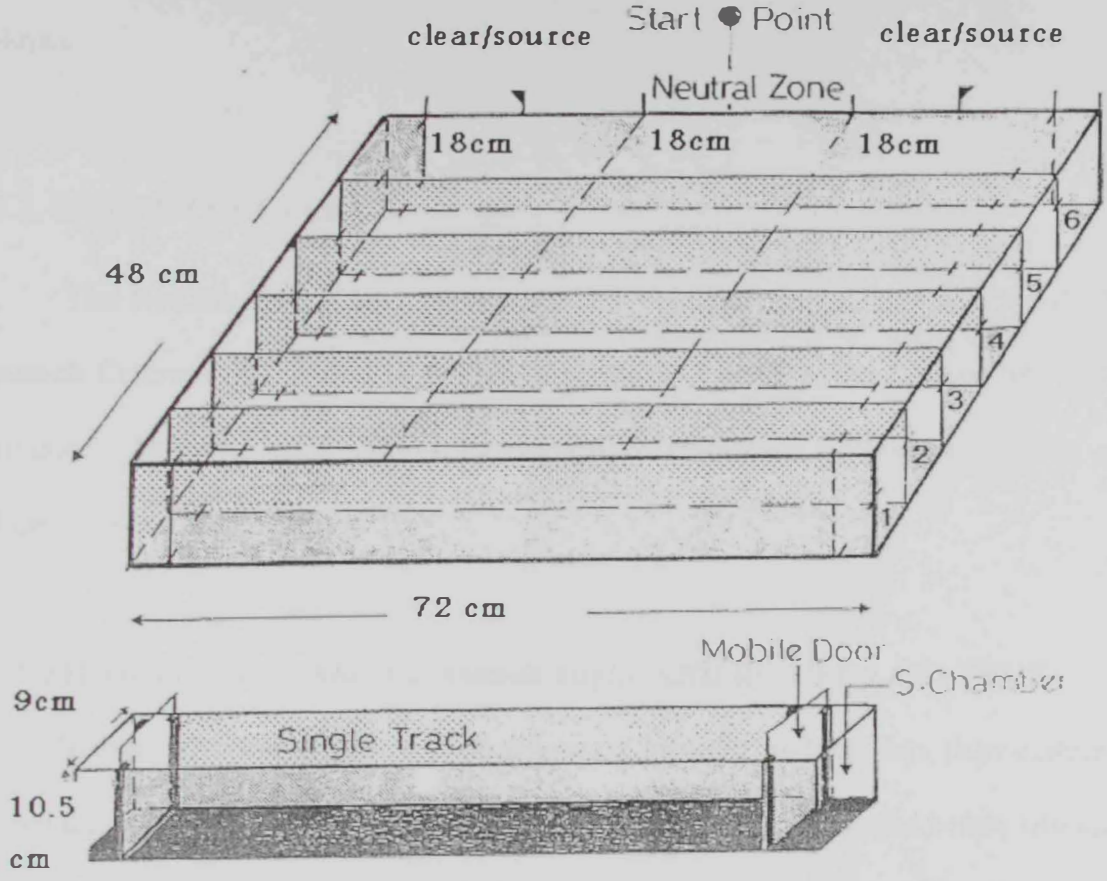

Fig 3.5. Diagram showing the rectangular glass basin manufactured for the feeding behaviour experiments (source/clear, and neutral zone definitions are related to the food suspension and smell trials).

The external side of the basin bottom was covered by a black sheet, while the outer borders were coated with aluminium foil sheets with the bright surface on the outside. The longitudinal transepts were also coated, in order to prevent any visual disturbances to the animals.

3.3.2. ROTIFER CULTURE

The Rotifers *Brachionus plaeatilis* were obtained from Umm Al Qewain Marine Research Centre, and cultured in the fish laboratory systems at the College of Food, UAE University. They were cultured in filtered sea water (salinity = 30-38‰) and fed on marine-cultured *Chlorella* sp.

3.3.3. FILTRATES OF *Chlorella, Artemia* nuplii, AND ROTIFER CULTURE

In order to obtain the filtrates of *Artemia, Chlorella*, and Rotifers, their cultures were filtered through GF/C filter paper. The *Chlorella* culture was centrifuged before filtration and the supernatant was filtered through GF/C filter paper.

3.3.4. LIVE FOOD SUSPENSION TRIAL

The basin was filled up to 7 cm height with water from the tilapia fish tanks. The mobile doors were tightly fixed at both ends. The small chambers of two tracks were filled with *Chlorella* (C) on one side and with experiment water (B) on the opposite side. The chambers of another two tracks were filed with *Chlorella* (C) on one side and *Artemia* (A) on the opposite side, while the remaining two tracks ends were filled with

Chlorella (C) on one side and Artificial feed (F) on the opposite side. The setting of one experiment could be represented as fig. 3.6.

The distance from each chamber door to the opposite fixed door was 56 cm, which is ideally divided into three consecutive zones. Five tilapia fry were introduced into the middle of each track at the beginning of the experiments. The movement of the fry towards one food source or another was recorded each 5 minutes for a total of 30 minutes. The setting of one experiment could be represented as in fig. 3.6.

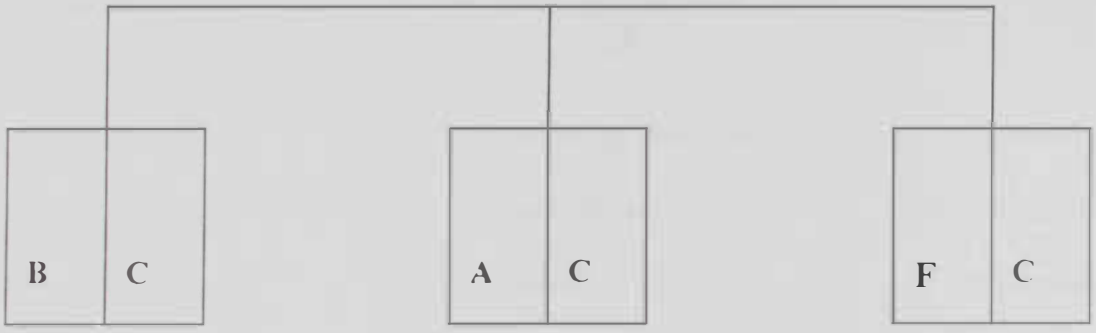

Fig. 3.6. Diagram representing the location of the suspended food at the experimental basin.

3.3.5. FOOD SMELL TRIAL

The basin was filled up to 7 cm height with tap water (kept at room temperature for about 24 hours with aeration). The mobile doors were tightly fixed (as in the previous section). Two tracks ends were filled with *Chlorella*-filtered culture medium (C) in one side and clean water (B) in the opposite side. The second two tracks ends were filled with *Chlorella* filtered-culture in one side and Rotifer–filtered culture medium(R) in the opposite side, where the remain two tracks ends were filled with *Chlorella*-filtered culture and

Artemia-filtered culture medium (A). The setting of one experiment could be represented as in fig. 3.7.

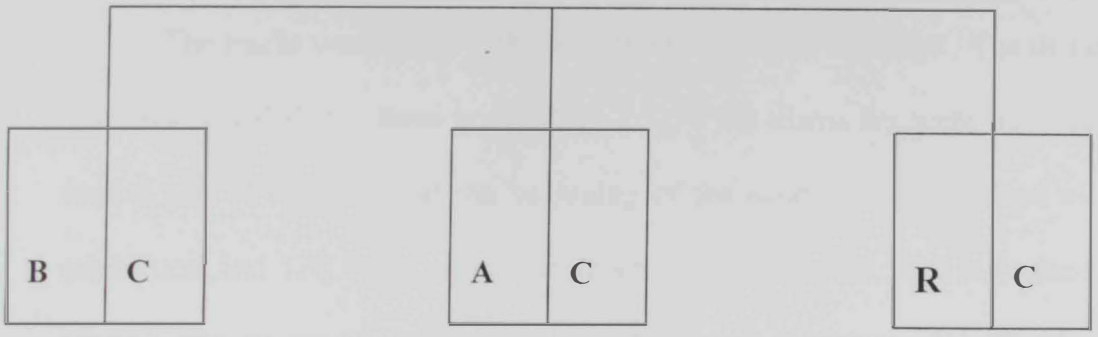

Fig. 3.7. Diagram representing the location of the food-filtrated at the experimental basin.

As in the previous trial five tilapia fry were introduced into the middle of the each track at the beginning of the trial. The mobile doors were raised up about 0.5 cm to allow the filtrate to diffuse, due to density difference. The movement of the fry towards one food filtrate or the other was recorded each 5 minutes for a total of 30 minutes.

3.3.5. COLOUR RESPONSE TRAILS

The attraction of tilapia fry to the four colours adopted for this experiment (green, yellow, red, and blue) was assessed in four consecutive experiments. In each experiment, a single colour was examined against the other three, as well as against itself. To do this, the mobile doors of all chambers were removed and substituted on one side of each track by a sheet of the same colour (single colour). At the other side, after removing the respective opposite doors, four additional sheets were inserted in the order blue (B), green (G), yellow (Y) and red (R). In another track, a sheet of the single colour faced the

blank opposite end door (no colour) (O). The setting of one experiment could be represented as in fig. 3.8 and 3.9 with the initials of the respective colours representing the coloured sheets which were facing each other at the ends of the tracks,.

The tracks were filled with water from the tilapia fry tanks. The distance in each track was divided into three zones (Fig. 3.5). Five tilapia fry were introduced into the central part of each track at the beginning of the experiments. The total of 30 fry per experiment and 120 fry in all colour experiments were used. The movement of the fry towards one colour or another was recorded every 5 minutes, for 30 minutes. Each colour experiment was repeated twice.

B	B
B	G
B	Y
B	R
O	B
O	O

Fig. 3.8. Diagram representing the colour positions at the experimental basin. The G, Y and R colours were substituted B as "single colours" in the other three experiments (Hamza & Ruggiu, 2000).

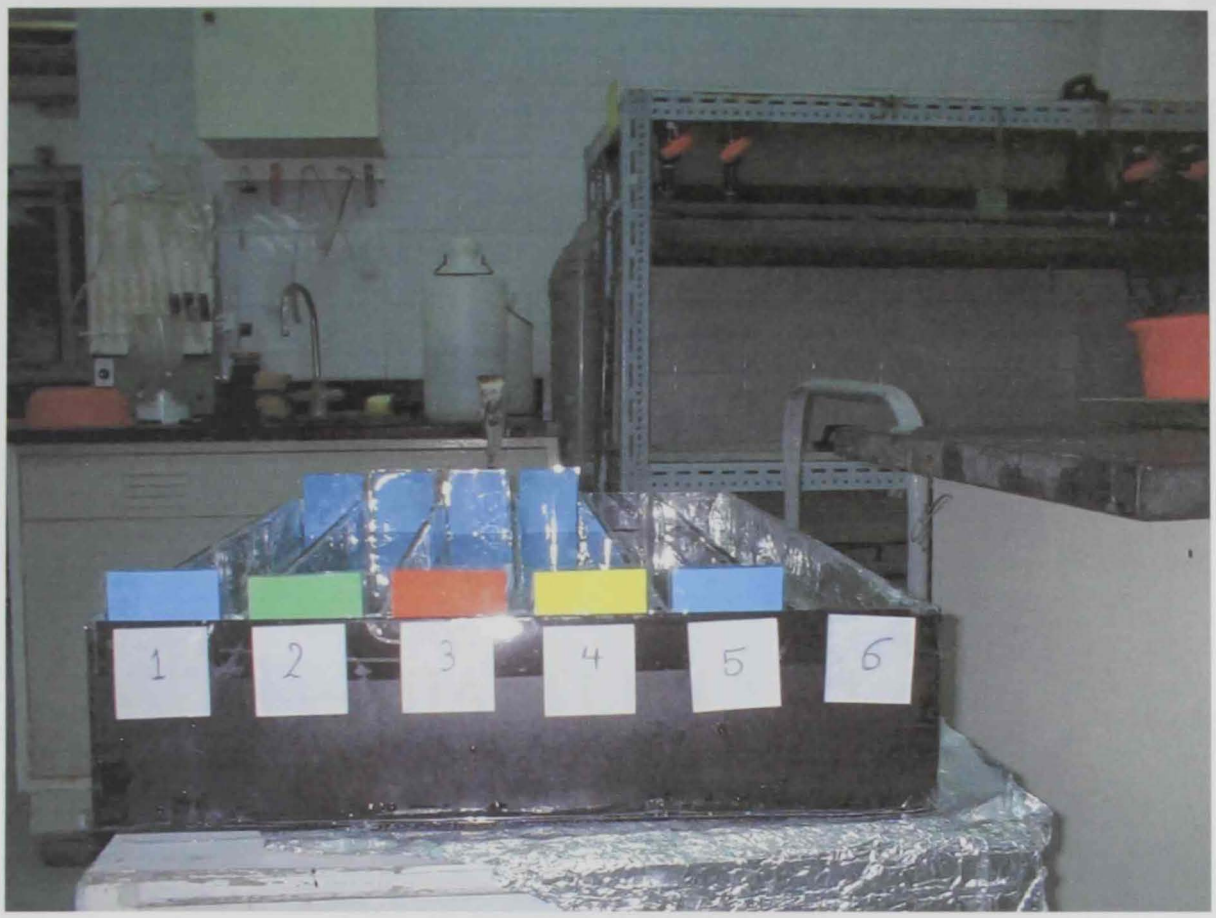

Fig. 3.9. A picture showing the coloured sheets in the experimental basin.

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

4.1. EFFECTS OF FOOD QUALITY ON GROWTH RATES OF NILE TILAPIA (EXPERIMENT I)

This experiment was conducted to evaluate the growth rates of Nile tilapia fry fed on different food types. As mentioned in the materials and methods, seven food types were tested in 21 aquariums, for up to 35 days, as shown in table 3.7. The results obtained have shown a general increase in the body weight of the fry during the experiment (Table 4.1) for the different food sources (Fig. 4.1).

As shown in fig. 4.1, the maximum body weight was obtained in the fry fed *Artemia*, followed by that fed on the combination of *Chlorella* + *Artemia* + Artificial feed, and then by *Artemia* + Artificial feed. On the other hand, the minimum growth was observed within the fry fed only with *Chlorella* cells; while, fish fry fed only with Artificial feed showed moderate growth rates.

The present results were analyzed statistically using One-way and Two-way ANOVA (Tables 4.2-4.4.). In table 4.2, the Two-way ANOVA analysis showed the significant effects of both time and food type on the body weight of Nile tilapia fry. The results of the One-way ANOVA (Table 4.3) indicated that food source had significantly affected fish performance ($P<0.05$). At the end of the feeding trials, all fish groups gained weight, except the two groups which were fed on *Chlorella* cells and on *Chlorella* cells + Artificial feed.

Table 4.1. Variations in the body weight (g fry^{-1}) of Nile tilapia fry fed different types of food for 35 days. (Chlo. = *Chlorella*, A.F. = Artificial feed, Art. = *Artemia*).

Feed type \ Days	0	7	20	28	35
Chlo.	0.012	0.014	0	0	0
Chlo.	0.012	0.013	0.011	0.011	0.019
Chlo.	0.012	0.012	0.011	0.023	0.050
Chlo.+Art.	0.012	0.016	0.03	0.028	0
Chlo.+Art.	0.012	0.019	0.032	0.047	0.050
Chlo.+Art.	0.012	0.017	0.028	0.039	0.034
Chlo.+A.F.	0.012	0.019	0.043	0	0
Chlo.+A.F.	0.012	0.016	0.047	0.055	0.054
Chlo.+A.F.	0.012	0.017	0.039	0.033	0.040
Art.	0.012	0.02	0.054	0.064	0.105
Art.	0.012	0.023	0.057	0.068	0.100
Art.	0.012	0.022	0.053	0.078	0.125
Art. +A.F.	0.012	0.025	0.068	0.071	0.104
Art. +A.F.	0.012	0.022	0.062	0.086	0.123
Art. +A.F.	0.012	0.019	0.057	0.064	0.081
A.F.	0.012	0.018	0.047	0.042	0.071
A.F.	0.012	0.015	0.045	0.053	0.063
A.F.	0.012	0.016	0.046	0.05	0.075
Chlo.+Art. +A.F	0.012	0.019	0.045	0.061	0.093
Chlo.+Art. +A.F	0.012	0.018	0.05	0.074	0.110
Chlo.+Art. +A.F	0.012	0.018	0.058	0.089	0.128

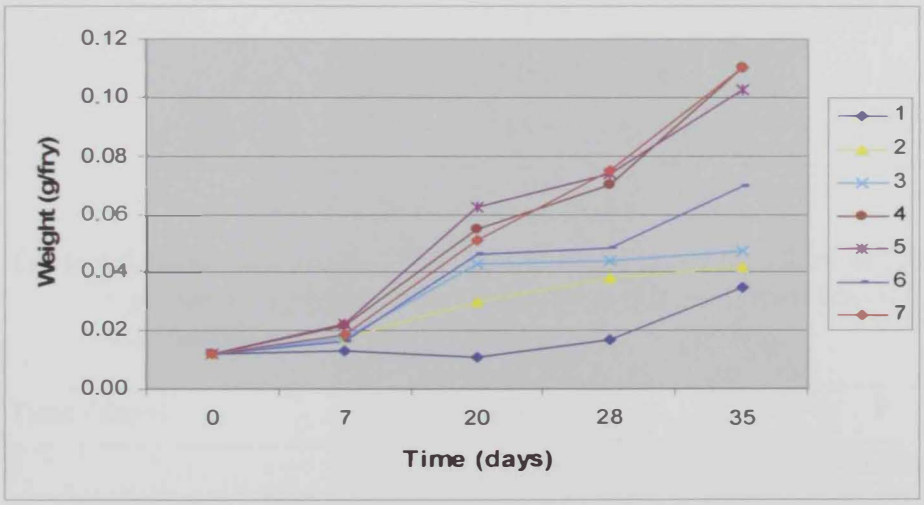

Fig. 4.1. Variations in fry body weight of Nile tilapia fry fed different types of food for 35 days. (1 = *Chlorella*, 2 = *Chlorella* + *Artemia* , 3 = *Chlorella* + Artificial feed, 4 = *Artemia*, 5 = *Artemia* + Artificial feed, 6 = Artificial feed,7 = *Chlorella* + *Artemia* + Artificial feed)

Table 4.2. Two-way ANOVA tested the effects of time and food type on body weight of Nile tilapia fry ($r^2 = 0.942$).

Analysis of variance	df	Mean-Square	F-ratio	p
Time (T)	4	0.012	144.687	0.000
Food Type (F)	6	0.003	37.449	0.000
T * F	24	0.001	8.099	0.000
Standard Error	65	0.000		

Table 4.3. Results of one way ANOVA analysis tested the effect of time on the weight of Nile tilapia fry after feeding with different food types. (NS = not significant at P = 0.05).

Treatments	F-ratio	P
Chlorella (Chlo.)	2.54	0.133 ^{NS}
(Chlo.) + *Artemia* (Art)	2.15	0.148 ^{NS}
(Chlo.) + Artificial (A.F.)	15.52	<0.01
(Art)	99.52	<0.001
(Art) + (A.F.)	34.61	<0.001
(A.F.)	119.07	<0.001
(Chlo) + (Art) + (A.F)	45.62	<0.001

Table 4.4. Results of one way ANOVA analysis tested the effect of different food types on the weight of Nile tilapia fry at different times (days) of feeding experiments.

Time (days)	F-ratio	P
7	10.36	<0.001
20	46.96	<0.001
28	10.70	<0.001
35	11.18	<0.001

Table 4.4 shows the results obtained from one way ANOVA that tested the effect of different food types on the weight of Nile tilapia fry at different times of feeding, where significant results for the treatments were obtained. Paired t-test analysis was carried out to compare the final fish weight fed different food types with the Artificial feed as a control group. The results showed that fish weights were significantly affected by food source ($P<0.05$), except the groups that were fed with *Chlorella* and *Artemia* + Artificial feed ($P>0.05$) (Table 4.5).

The increase in body weight of tilapia fry reflected other variations in the growth parameters such as: average daily gain (ADG), Percentage of weight gain (%W. gain) and specific growth rate (% SGR). These parameters have been calculated for the tilapia fry at the different treatments and the results are summarized in table 4.6. The results showed that the highest percent weight gain, ADG, and % SGR occurred in the fish fry fed diets containing *Artemia* (Fig.'s 4.2-4.4); while the lowest values were found within the fry fed only *Chlorella* cells.

The mortalities of tilapia fry fed the different food types during the experimental period are presented in table 4.7 and fig. 4.5. The results showed that the *Chlorella* treatment had the highest mortality rate, while the *Artemia* treatment and the combination of *Artemia* and Artificial feed had the lowest mortality.

The body composition analysis of tilapia fry showed that the fry fed only on *Chlorella* cells have the lowest moisture content and highest crude protein contents. On the other hand, fish fed on *Artemia* have the highest moisture and crude lipid contents and lowest crude protein contents (Table 4.8). The correlations between the growth parameters: final weight (after 35 days), average daily gain, specific growth rate, and body compositions are

shown in table 4.9. The results indicated that there are negative correlations between body protein content and final weight, average daily gain, and specific growth rate.

Table 4.5. Results of Paired t-test with final weight, where all treatments were compared with Artificial feed group which was used as the control group. (Chlo. = *Chlorella*, Art. = *Artemia*, A.F. = Artificial feed, * NS = not significant at P = 0.05).

Paired-variant	t	P
A.F – Chlo.	-2.832	0.066 ^{NS*}
A.F – Chlo. + Art.	-2.749	0.050
A.F - Chlo. + A.F.	-3.273	0.047
A.F – Art.	4.794	0.009
A.F – Art. + A.F.	2.610	0.059 ^{NS*}
A.F – Chlo. + Art. + A.F.	-3.800	0.019

Table 4.6. Results of Percent weight gain (%W. gain), average daily gain (ADG), and specific growth rate (% SGR) of Nile tilapia fry fed on different food types for 35 days. (Chlo. = *Chlorella*, Art. = *Artemia*, A.F. = Artificial feed).

Test Diet	I.W.	F.W.	%W. gain	ADG	% SGR
	g/fry	g/fry			
Chlo.	0.012	0.035	191.67	0.001	3.058
Chlo.+Art.	0.012	0.042	250.00	0.001	3.579
Chlo.+A.F.	0.012	0.047	291.67	0.001	3.901
Art.	0.012	0.110	816.667	0.003	6.330
Art.+A.F.	0.012	0.103	755.556	0.003	6.133
A.F.	0.012	0.070	480.556	0.002	5.025
Chlo.+Art.+A.F	0.012	0.110	819.444	0.003	6.339

I.W.: Initial Body Weight g fry^{-1}
F.W.: Final Body Weight g fry^{-1}
%W. gain: Percent Weight Gain
ADG: Average Daily
% SGR: Specific Growth Rate

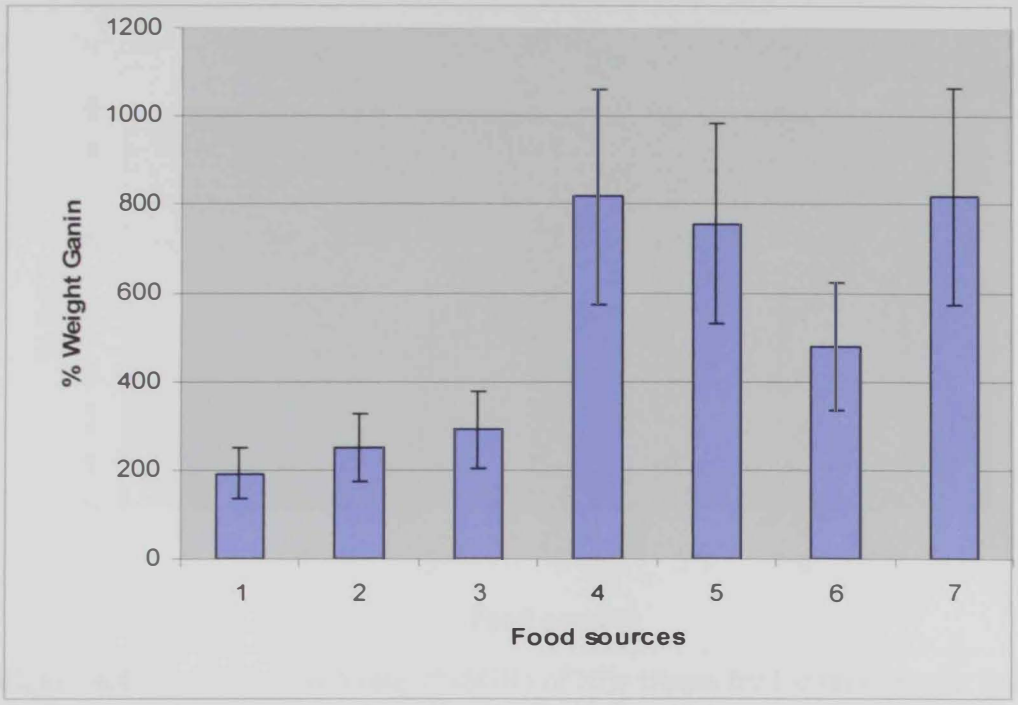

Figure 4.2. Percentage weight gain of Nile tilapia fry fed on different food types. (1 = *Chlorella*, 2 = *Chlorella* + *Artemia*, 3 = *Chlorella* + Artificial feed, 4 = *Artemia*, 5 = *Artemia* + Artificial feed, 6 = Artificial feed, 7 = *Chlorella* + *Artemia* + Artificial feed).

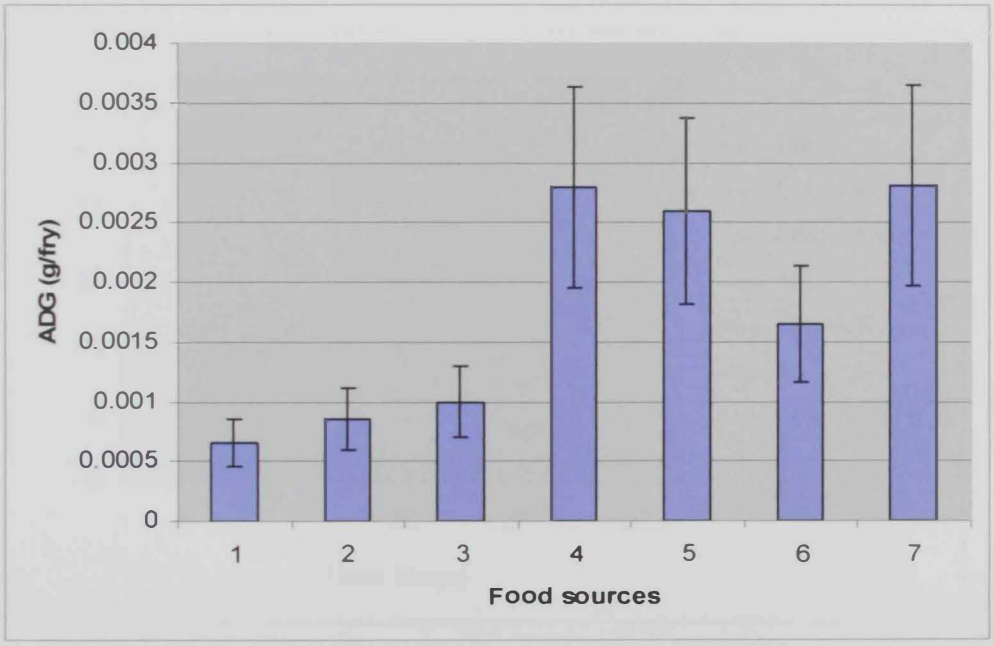

Figure 4.3. Average daily gain of Nile tilapia fry fed on different food types. (1 = *Chlorella*, 2 = *Chlorella* + *Artemia*, 3 = *Chlorella* + Artificial feed, 4 = *Artemia*, 5 = *Artemia* + Artificial feed, 6 = Artificial feed, 7 = *Chlorella* + *Artemia* + Artificial feed).

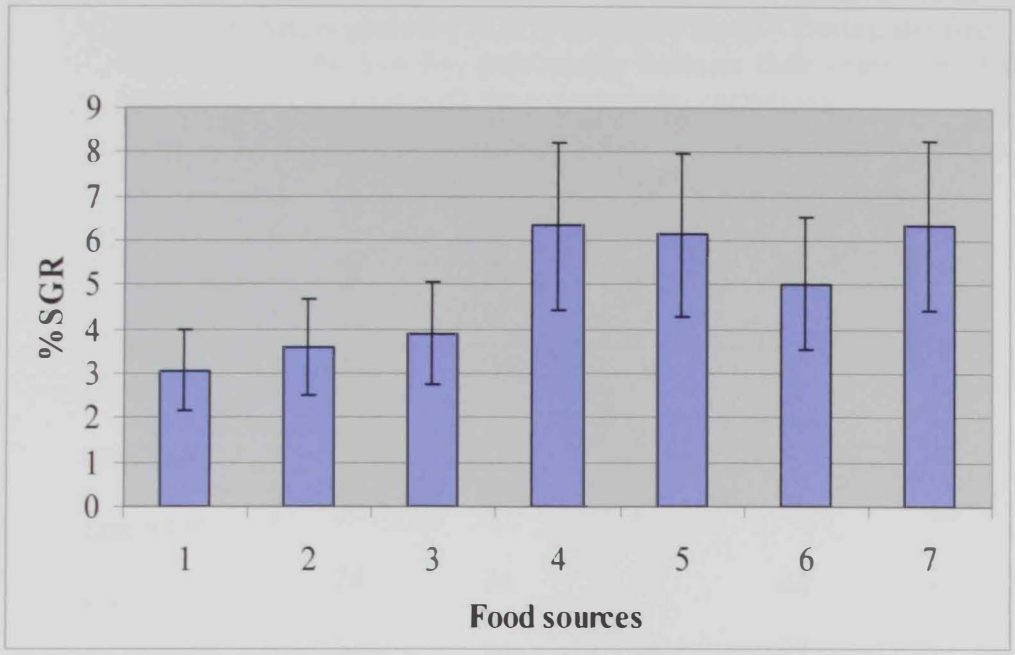

Figure 4.4. Specific growth rate (%SGR) of Nile tilapia fry fed on different food types. (1 = *Chlorella*, 2 = *Chlorella* + *Artemia*, 3 = *Chlorella* + Artificial feed, 4 = *Artemia*, 5 = *Artemia* + Artificial feed, 6 = Artificial feed, 7 = *Chlorella* + *Artemia* + Artificial feed).

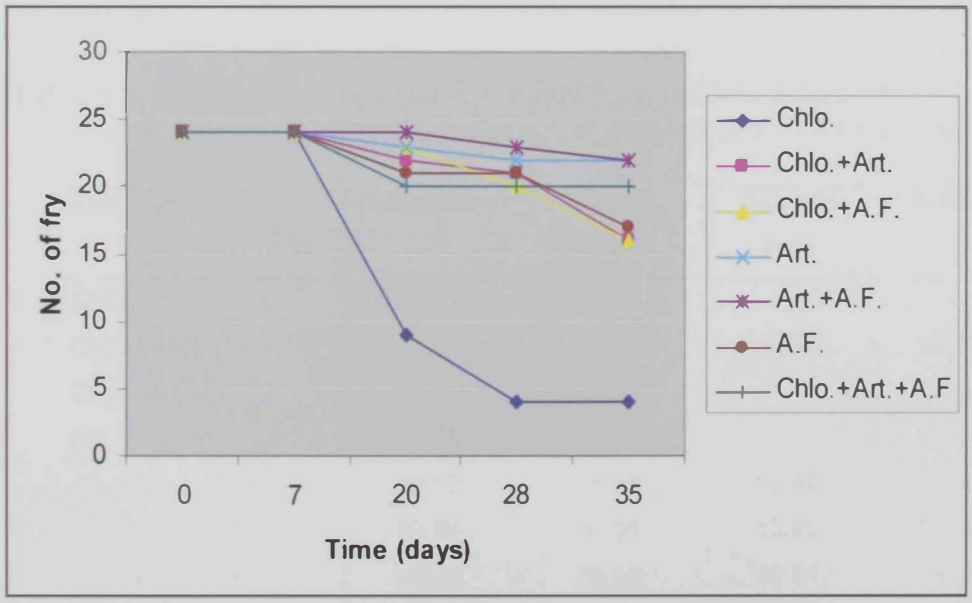

Fig. 4.5. Variations in fry numbers along the experimental period at the different feeding treatments. (Chlo. = *Chlorella*, Art. = *Artemia*, A.F. = Artificial feed).

Table 4.7. The average survival of Nile tilapia fry fed different food types. (Chlo. = *Chlorella*, Art. = *Artemia*, A.F. = Artificial feed).* During the first week, dead fish were replaced by live fry, presumably because their death may have been due to inability to acclimatize with the experimental conditions.

Test diet	Days				
	0	7*	20	28	35
Chlo.	24	24	9	4	4
Chlo.+Art.	24	24	22	21	16
Chlo.+A.F.	24	24	23	20	16
Art.	24	24	23	22	22
Art.+A.F.	24	24	24	23	22
A.F.	24	24	21	21	17
Chlo.+Art.+A.F	24	24	20	20	20

Table 4.8. Body composition (on dry weight basis) of Nile tilapia fry fed on different food types. (Chlo. = *Chlorella*, Art. = *Artemia*, A.F. = Artificial feed).

Test Diet	%Moisture	%Crude Protein	%Crude lipid	% Ash
Chlo.	79.41	89.26	-	-
Chlo.+Art.	81.25	73.55	9.36	17.10
Chlo.+A.F.	80.10	78.57	-	-
Art.	82.88	68.33	15.72	15.95
Art.+A.F.	80.52	68.78	12.42	18.80
A.F.	80.94	70.08	10.95	18.98
Chlo.+Art.+A.F	79.28	70.34	10.81	18.85

Table 4.9. Results of Pearson correlation matrix showing the correlation between the final weight, average daily Gain (ADG), specific growth rate (% SGR), and body composition of Nile tilapia fry fed on different food types.

	F.W.		%W. gain		ADG		%SGR	
	r^2	P	r^2	P	r^2	P	r^2	P
%Moisture	0.282	1.000	0.280	1.000	0.259	1.000	0.302	1.000
%Crude Protein	-0.775	1.000	-0.774	1.000	-0.749	1.000	-0.825	0.803
%Crude lipid	0.692	1.000	0.690	1.000	0.680	1.000	0.685	1.000
% Ash	0.055	1.000	0.053	1.000	0.130	1.000	0.120	1.000

F W Final Body Weight g $fish^{-1}$
%W gain Percent Weight Gain
ADG Average Daily Gain
% SGR Percent Specific Growth Rate

4.2. FEEDING BEHAVIOUR OF NILE TILAPIA FRY (EXPERIMENT II)

A number of experiments were designed to detect the response of tilapia fry to different types of living food organisms namely; *Chlorella*, *Artemia* nauplii and Artificial feed. The response of tilapia fry to the smell of the filtrate of *Chlorella, Artemia* and Rotifer cultures was also tested. Furthermore, other experiments were designed to study the response of tilapia fry to different environmental colours (i.e. red, green, yellow and blue colours). The wave lengths of these colours were previously detected by Hamza and Ruggiu (2000), as blue: 452 nm, green: 495 nm, yellow: 565 nm, and red: 697 nm.

4.2.1. LIVE FOOD SUSPENTION TRIAL

The numbers of tilapia fry attracted to the suspension of *Chlorella*, Artificial feed, *Artemia*, blank or stay at the neutral zone are given in tables 4.10-4.12. The time step (5 minutes) results have shown that the tilapia fry favoured by the suspensions of Artificial feed and *Artemia* nauplii more than the *Cholorella* suspensions (Fig.'s 4.6-4.8).

As shown in fig. 4.9, there are no differences in the attraction of tilapia fry toward both Artificial feed and *Artemia* suspensions during the whole experimental period.

Table 4.10. Time step attraction of tilapia fry toward *Chlorella*, Artificial feed, or stay in neutral zone. (a and b are replicates)

Time (min.)/ Suspension	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	1	4	4	0	0	0	1	1	2	0	0	2	15
Neutral zone	0	1	0	0	4	1	4	1	0	1	4	0	16
Artificial	4	0	1	5	1	4	0	3	3	4	1	3	29

Table 4.11. Time step attraction of tilapia fry toward *Chlorella*, *Artemia*, or stay in neutral zone. (a and b are replicates).

Time (min.)/ Suspension	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	4	0	5	0	3	0	1	2	1	0	3	0	19
Neutral zone	0	0	0	4	1	0	2	2	0	0	0	3	12
Artemia	1	5	0	1	1	5	2	1	4	5	2	2	29

Table 4.12. Time step attraction of tilapia fry toward *Chlorella*, blank, or stay in neutral zone. (a and b are replicates).

Time (min.)/ Suspension	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	1	0	1	0	2	2	1	0	1	2	3	1	14
Neutral zone	1	1	3	0	3	0	4	2	4	2	2	1	23
Blank	3	4	1	5	0	3	0	3	0	1	0	3	23

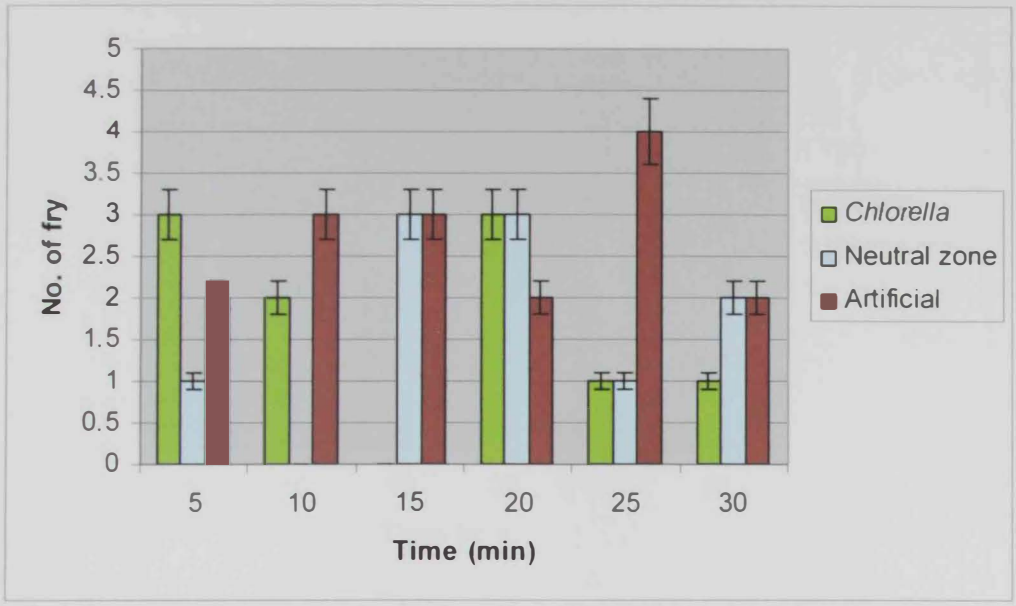

Fig. 4.6. Numbers of organisms (tilapia fry) attracted to *Chlorella*, Artificial feed, or stay on neutral zone every 5 min during the experimental period.

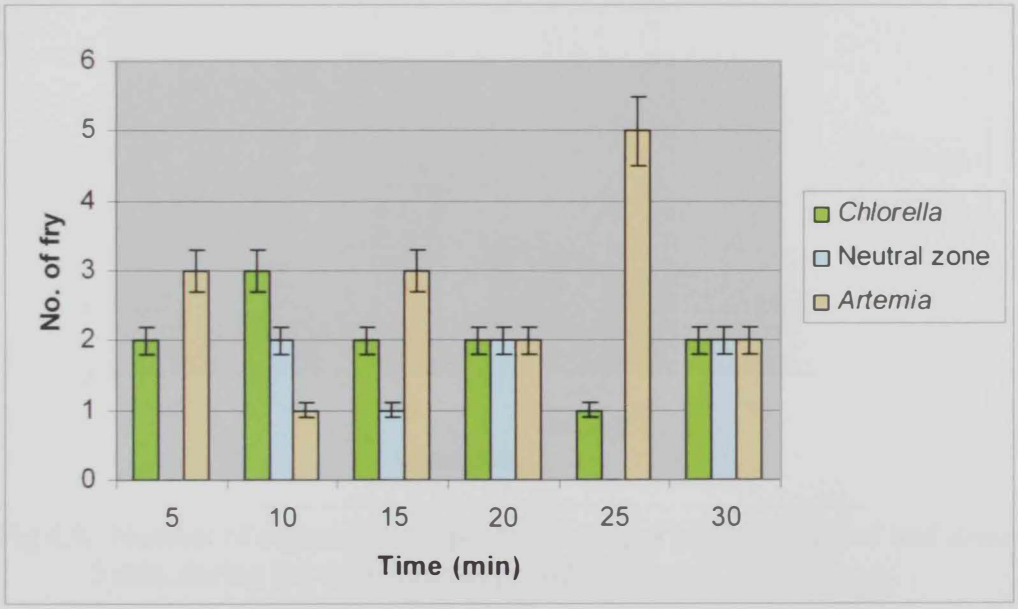

Fig. 4.7. Number of organisms (tilapia fry) attracted to *Chlorella*, *Artemia*, or stay on neutral zone every 5 min during the experimental period

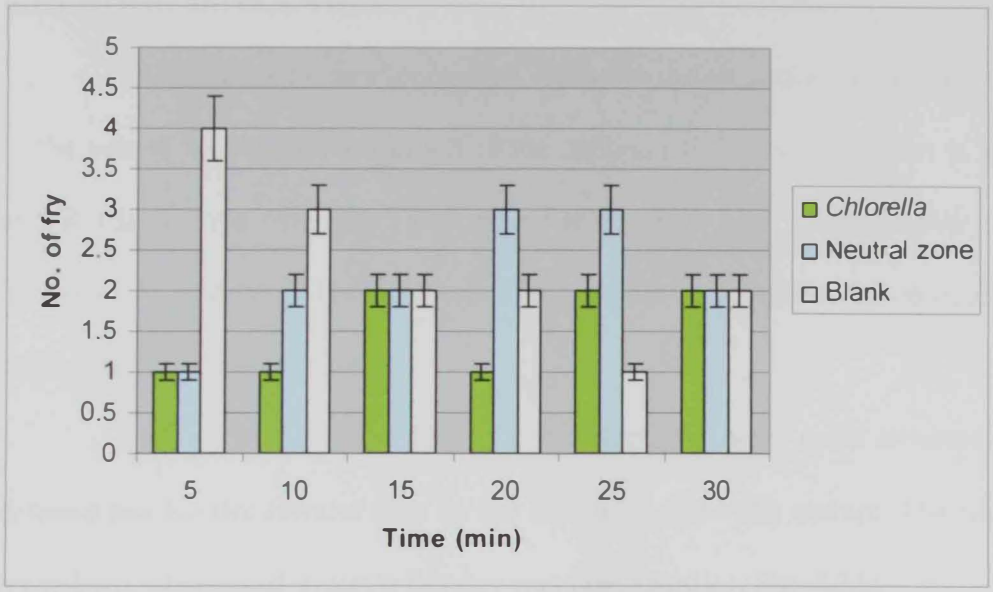

Fig. 4.8. Number of organisms (tilapia fry) attracted to *Chlorella,* blank, or stay on neutral zone every 5 min during the experimental period

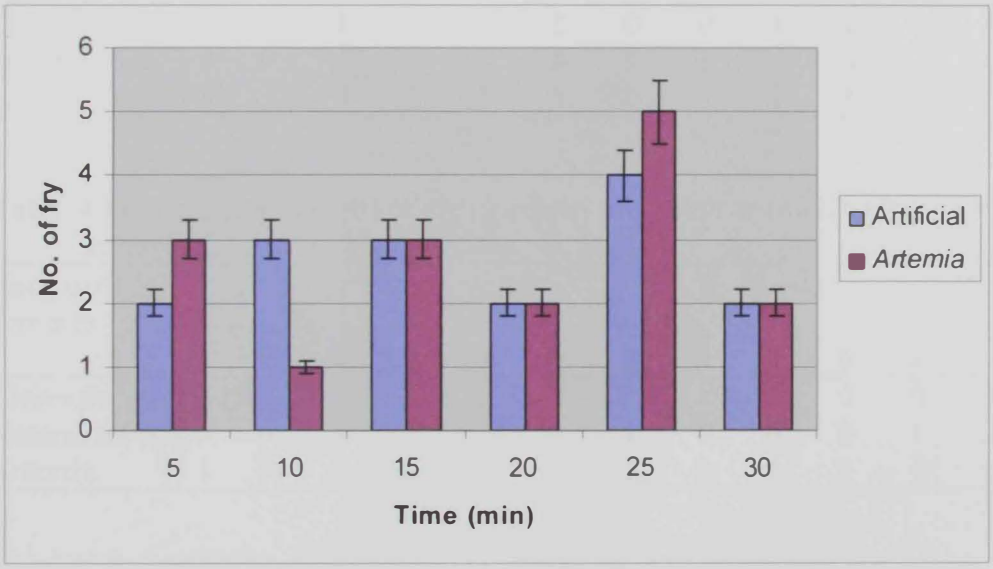

Fig 4.9. Number of organisms (tilapia fry) attracted to Artificial feed and *Artemia* every 5 min. during the experimental period

4.2.2. FOOD SMELL TRIAL

The results of these experiments represent the attraction and preference of tilapia fry to the culture media (filtrate) in which the different live food organisms (*Chlorella*, *Artemia* and Rotifers) were cultured. These observations have been recorded every 5 min during a period of 30 minutes. The absolute values of the recorded results in duplicates (a, b) are summarized in tables 4.13-4.15.

As shown in figures (4.10-4.12), the tilapia fry were more attracted by the smell of *Artemia* and Rotifer filtrates than by the filtrate of *Chlorella* culture. The response of tilapia fry to both rotifers and *Artemia* filtrates was fairly similar (Fig. 4.13).

Table 4.13. Time step records of the tilapia fry attraction towards *Chlorella* and Rotifer extracts, (a) and (b) are replicates.

Time (min.)\ Extracts	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	0	2	1	2	0	0	0	0	4	1	1	0	11
Neutral zone	0	0	4	0	0	0	5	2	0	0	1	0	12
Rotifer	5	3	0	3	5	5	0	3	1	4	3	5	37

Table 4.14. Time step records of the tilapia fry attraction towards *Chlorella* and *Artemia* extracts, (a) and (b) are replicates.

Time (min.)\ Extracts	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	2	1	1	2	1	2	0	1	0	0	0	3	13
Neutral zone	0	0	3	1	0	0	4	4	0	0	1	2	15
Artemia	3	4	1	2	4	3	1	0	5	5	4	0	32

Table 4.15. Time step records of the tilapia fry attraction towards *Chlorella* and blank extracts, (a) and (b) are replicates.

Time (min.)\ Extracts	5		10		15		20		25		30		total
	a	b	a	b	a	b	a	b	a	b	a	b	
Chlorella	0	5	2	5	1	5	3	5	3	5	1	5	40
Neutral zone	0	0	1	0	1	0	0	0	0	0	0	0	2
Blank	5	0	2	0	3	0	2	0	2	0	4	0	18

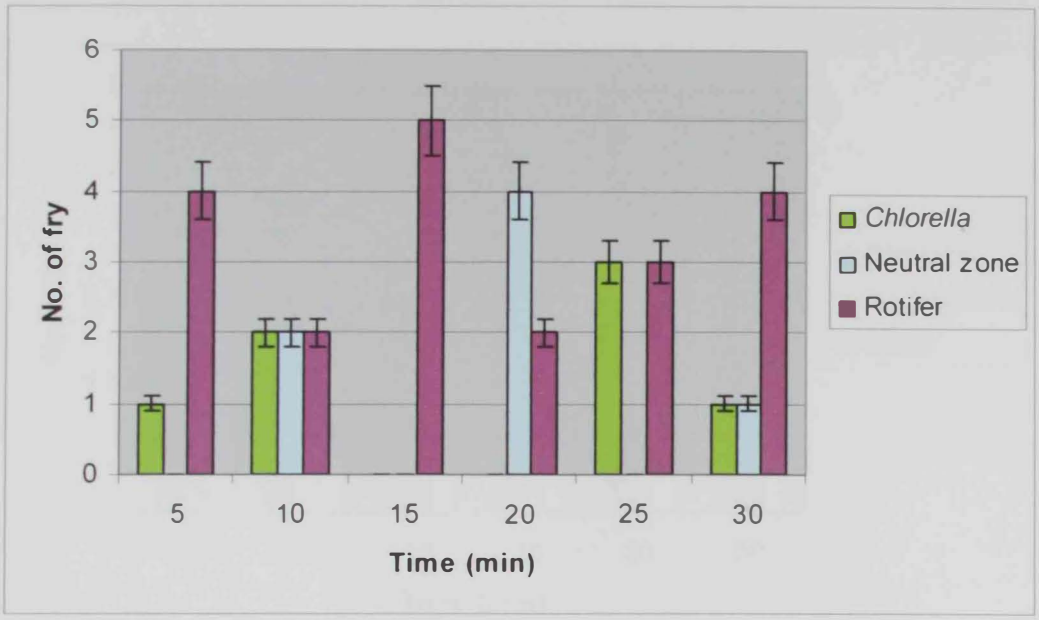

Fig. 4.10. Numbers of tilapia fry attracted by *Chlorella* or Rotifer extracts, or stays at neutral zone every 5 min during the experimental period.

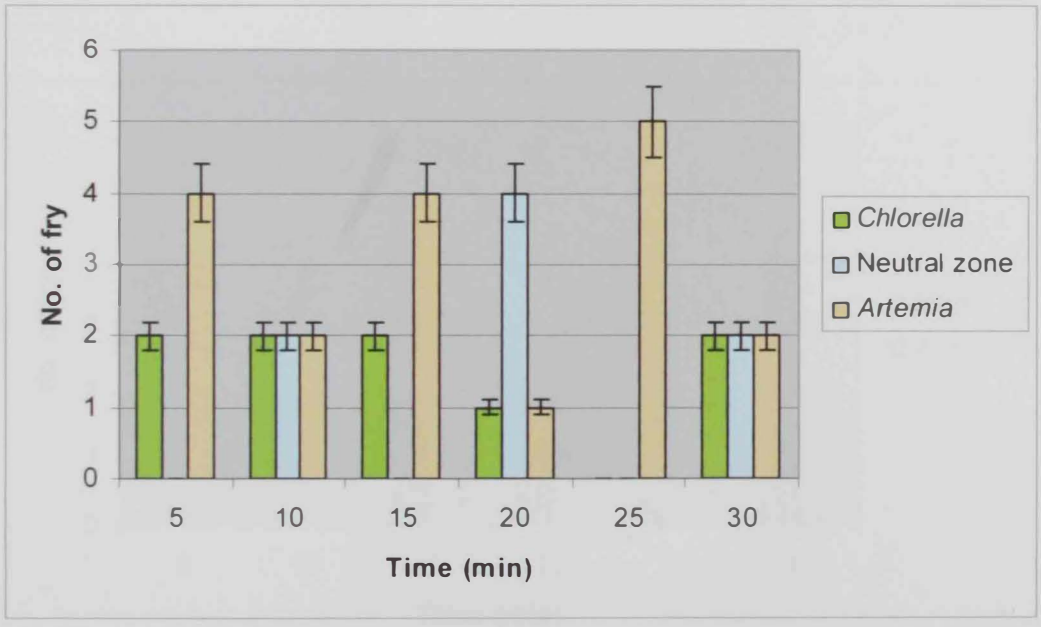

Fig. 4.11. Numbers of tilapia fry attracted by *Chlorella* or *Artemia* extracts, or stay at neutral zone every 5 min during the experimental period

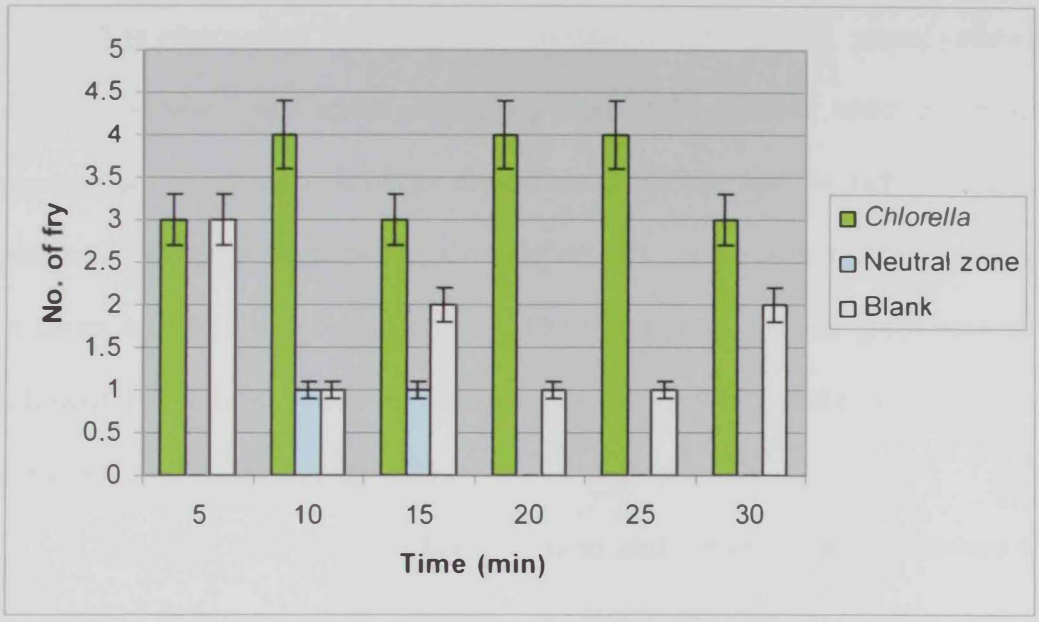

Fig. 4.12. Number of tilapia fry attracted by *Chlorella* extracts, blank, or stay at neutral zone every 5 min during the experimental period

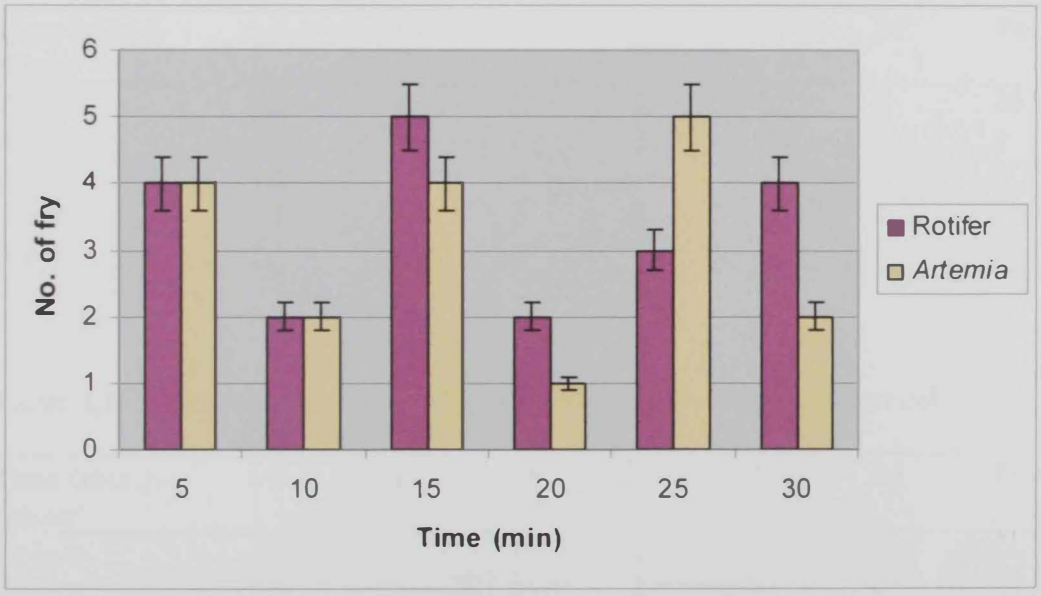

Fig. 4.13. Number of organisms (tilapia fry) attracted to extracts of Rotifer and *Artemia* every 5 minutes during the experimental period.

4.2.3. COLOUR RESPONSE TRAILS

The response of Nile tilapia fry to opaque colours (red, green, yellow and blue) in their environment was tested during a period of 30 minutes under a top light source to prevent any interference between the different colours and the light intensity. The results obtained for the fry response to individual colours compared with other colours are reported in tables 4.16-4.19 and figs. 4.14-4.17. The results revealed that green was most attractive, followed by yellow. The blue colour came in the third place, while red was the least attractive (Table 4.20 and fig. 4.18).

Although, both the calculated numbers and the graphical illustrations demonstrated that certain colours are more attractive to tilapia fry than others, the statistical analyses (Pearson Chi-square analysis ($p= 0.05$)) indicated that there was no significant differences ($P>0.05$) in the preference of the fry for the different colours (Table 4.21).

Table 4.16. The attraction of the tilapia fry to green versus the other colours.

Time (min.)\ colour	5	10	15	20	25	30	Total
Yellow	2	4	5	5	2	2	20
Blue	1	1	0	2	1	0	5
Red	4	0	0	1	0	0	5
Green	1	3	3	2	2	3	14
Blank	3	2	1	2	2	2	12

Table 4.17. The attraction of the tilapia fry to yellow versus the other colours.

Time (min.)\ colour	5	10	15	20	25	30	Total
Yellow	2	3	1	2	3	3	14
Blue	3	0	4	3	1	0	11
Red	0	1	5	0	0	2	8
Green	1	3	0	1	2	0	6
Blank	2	2	2	3	2	3	14

Table 4.18. The attraction of the tilapia fry to blue versus the other colours.

Time (min.)\ colour	5	10	15	20	25	30	Total
Yellow	1	1	0	0	1	1	4
Blue	1	2	2	3	2	1	11
Red	3	1	0	1	3	3	11
Green	5	0	3	0	3	2	13
Blank	3	2	2	1	2	2	12

Table 4.19. The attraction of the tilapia fry to red versus the other colours.

Time (min.)\ colour	5	10	15	20	25	30	Total
Yellow	5	0	0	2	0	2	9
Blue	5	4	4	0	2	4	19
Red	1	2	2	2	2	2	11
Green	4	2	1	5	5	4	21
Blank	2	2	3	3	2	3	15

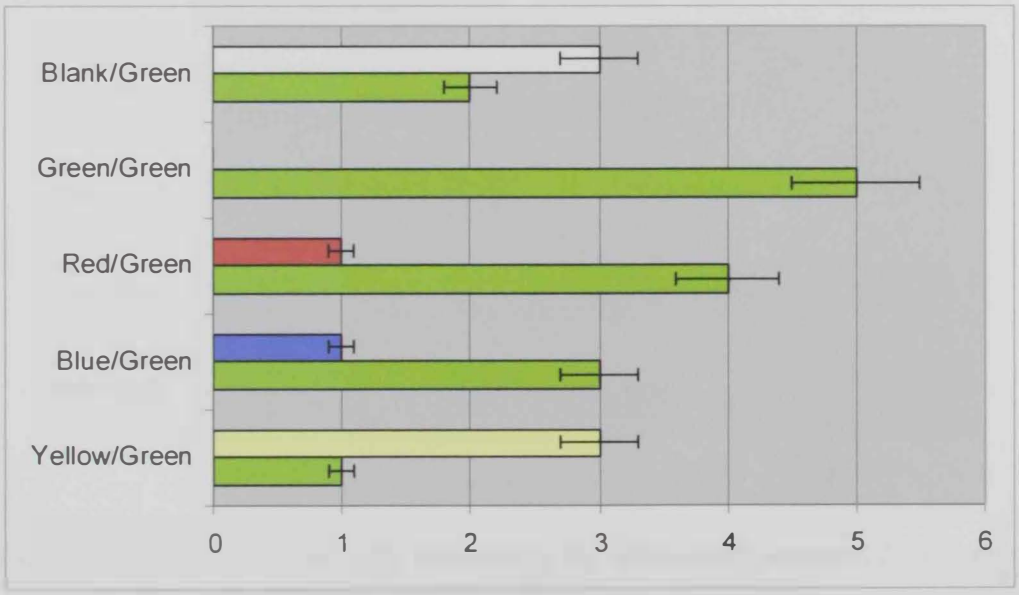

Fig 4.14. Numbers of tilapia fry attracted to the green colour compared with the other colours (i.e. yellow, blue, red, or blank).

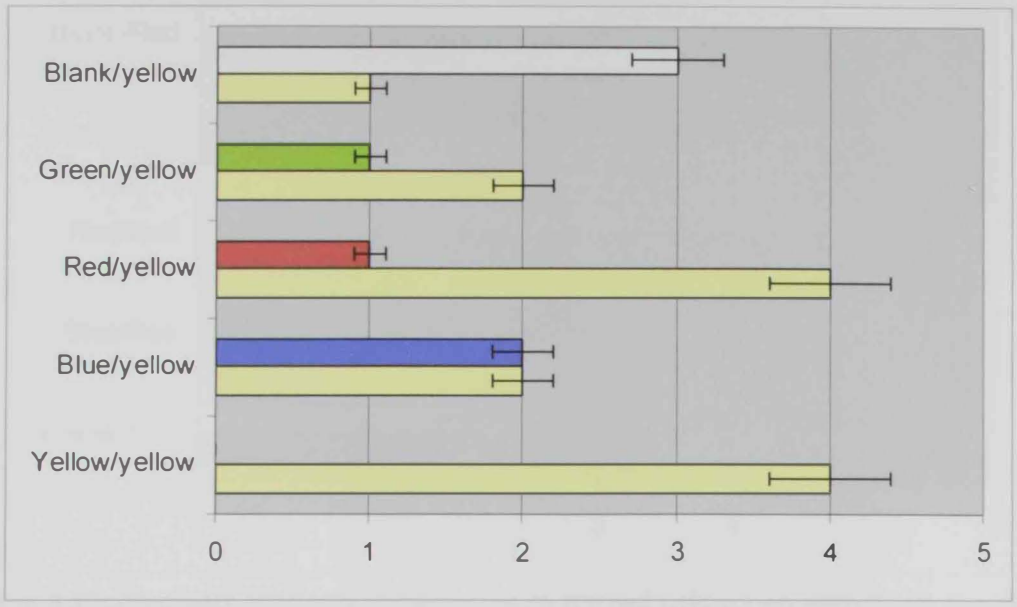

Fig 4.15. Numbers of tilapia fry attracted to the yellow colour compared with the other colours (i.e. green, blue, red, or blank).

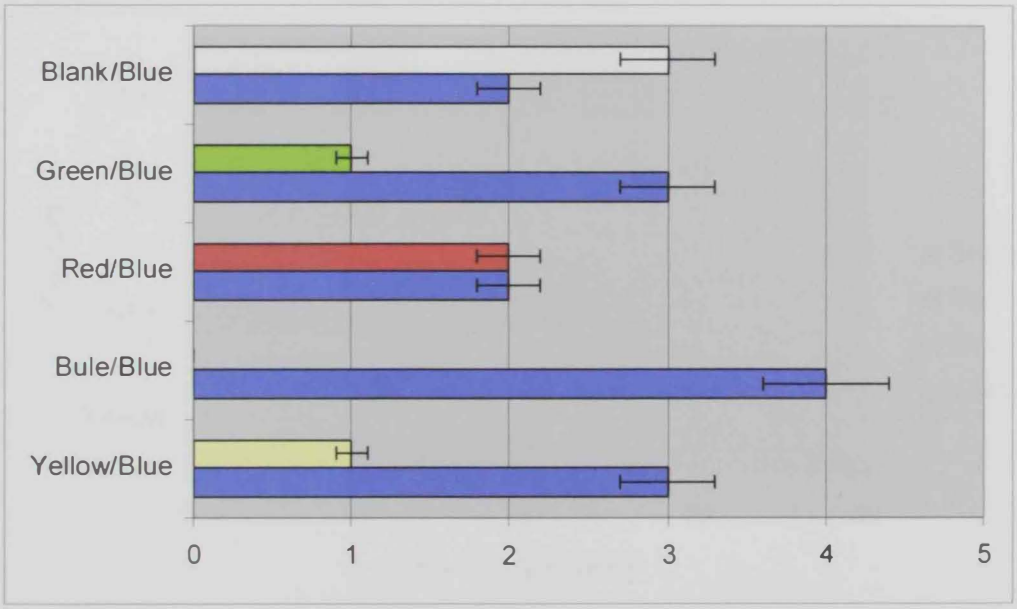

Fig 4.16. Numbers of tilapia fry attracted to the blue colour compared with the other colours (i.e. green, yellow, red, or blank).

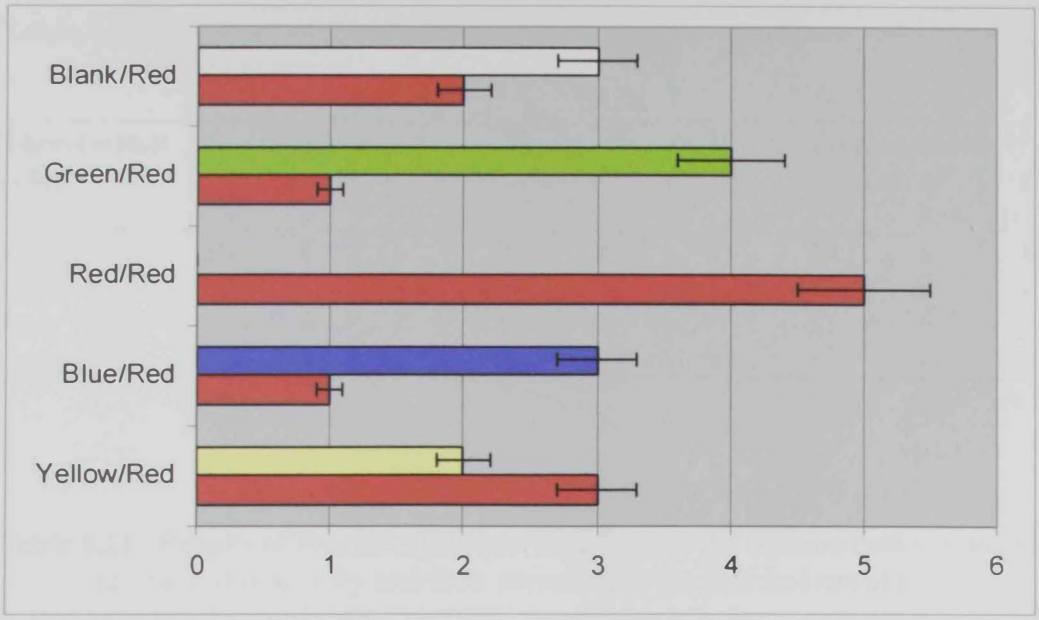

Fig 4.17. Numbers of tilapia fry attracted to the red colour compared with the other colours (i.e. green, yellow, blue, or blank).

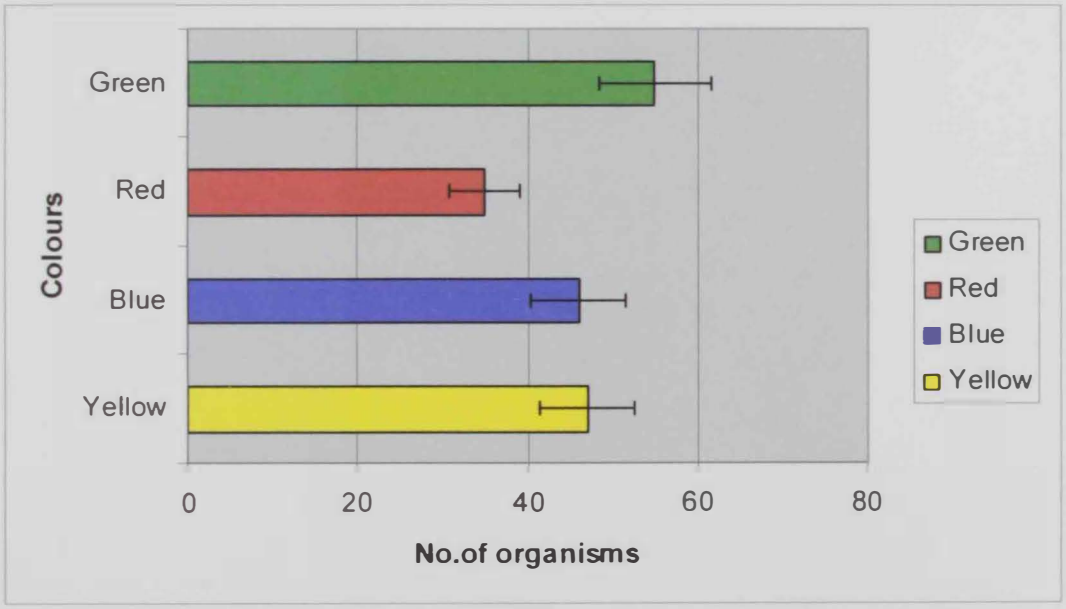

Fig 4.18. Total numbers of tilapia fry attracted to different colours in the different colour experiments (i.e. green, yellow, blue, and red).

Table 4.20. Summary of the attraction of the tilapia fry to different colours along the experimental period

Time (min.)\ colour	5	10	15	20	25	30	Total	Mean of total	Neutral zone
Yellow	10	8	6	9	6	8	47	8	1
Blue	10	7	10	8	6	5	46	8	1
Red	8	4	7	4	5	7	35	6	0
Green	11	8	7	8	12	9	55	9	1

Table 4.21. Results of Pearson Chi-square analysis of the relation between the total numbers of tilapia fry and their attraction to the four colours (i.e. green, yellow, blue, and red).

Statistical parameters	value	df	p
Pearson Chi-square	4.432	3.000	0.218

CHAPTER 5

DISCUSSION

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The contribution of Aquaculture to total global fisheries production was very low during 1950-1970, ranging from only 638,577 mt (3.2%) in 1950 to 3,525,872 (5.2%) in 1970. Global aquaculture production continued to grow 9.6% in 1980 and 16.3% in 1990. In the 1990s and early 2000s, this production grew at an outstanding rate to reach an annual rate of 32.1% in 2000, 34% in 2001 and 35.2% in 2002. The average annual compounded growth rate of aquaculture production was 9% per year during 1970-2000, compared with only 1.3% for capture fisheries (FAO, 2004). This means that aquaculture plays a crucial role in providing the consumers with alternative sources of fish products (Holt, 2000; Pillay, 2001).

The increasing potential of tilapia culture worldwide has made it necessary that more information be gathered on tilapia culture practices in general, and food selectivity and feeding regimes which can support fish growth and yields, in particular. However, available information on tilapia culture did not pay much attention on the effect of culture conditions and food quality on the feeding behaviour of these fishes, especially during their larval stages. This is mainly because of their high tolerance to a wide range of environmental conditions, such as temperature, salinity, water hardness, ammonia, low oxygen concentration, etc. (Chervinski, 1982; Philippart and Ruwet, 1982; El-Sayed and Kawanna, 2004). However, since human impact on natural habitats, where tilapia are living, is damaging different components within the food web on which tilapia fry feed, it becomes our obligation to pay more attention to studying the feeding behaviour of such valuable fishes, especially at the most critical stages; i.e. the larval stages.

The present study was conducted to fulfill the following objectives:

1) to evaluate the effect of different food sources on the growth rates of Nile tilapia fry (*Oreochromis niloticus*), and

2) to evaluate the effect of some culture conditions on feeding behaviour and growth rates of Nile tilapia fry (*Oreochromis niloticus*).

In the present study, the growth rates and feed conversion efficiency of Nile tilapia fry fed on different types of live food and Artificial feed have been compared. The obtained results have shown that the fry fed on *Artemia* and on a combination of *Chlorella*, *Artemia*, and Artificial feed have the highest growth rate compared with those fed on other food types (Fig. 4.1). This preference may have been due to *Artemia* having visual and chemical stimuli which attract the tilapia larvae. It has also been suggested that *Artemia* bodies contain substances exerting an influence on larval digestion and assimilation (Kolkovski *et al.*, 1997a). Similarly, *Artemia* has been considered as the most successful live food for larval rearing of many freshwater and marine fishes, crustaceans and molluscs (Fluechter, 1980; Blair *et al.*, 2003; Morales-Ventura *et al.*, 2004).

In support, Fluechter (1980) found that protein digestion enzymes in live *Artemia* nauplii were responsible for successful rearing of white fish (*Coregonus lavaretus*) larvae. Furthermore, Blair *et al.* (2003) evaluate micro-diets (artificial food) versus live foods (*Artemia* and Rotifers) on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*). They found that the growth rate among groups fed on micro-diets and on Rotifers was lower than that of larvae fed on *Artemia*.

In general, it has been found that live foods are considered more appropriate for several reasons such as that they are: (a) natural; (b) capable of eliciting behavioural

responses, including active swimming and prey capturing ability; (c) nutritionally adequate; (d) non-polluting to the medium, unlike dry feed; (e) highly digestible by the fish; and also that they (f) have a high rate of capture due to their movement (Morales-Ventura *et al.*, 2004). The present results support this argument and indicate that *Artemia* nauplii were the best food with the highest growth rate of Nile tilapia fry compared with other types of live food and Artificial feed. It has also been documented that a combination of live food and artificial diets (referred to as co-feeding) has enhanced larval performance of different fish species beyond that achieved by feeding either types of feeds alone (Kanazawa *et al.*, 1989; Holt, 1993; Abi-Ayad and Kestemont, 1994).

The varying response of larval fish to different food sources has been related mainly to the variations in the composition of these food sources, especially with respect to their protein, lipid and mineral contents and fatty acids and amino acid profiles (Gunasekera *et al.*, 1996). However, it has been reported that other important factors, such as fish species, size, and general biology and husbandry, also affect food and feeding preference, and in turn, determine the success of co-feeding (Rosenlund *et al.*, 1997). For example, when Rosenlund *et al.* (1997) compared the growth of halibut (*Hippoglossus hippoglossus*) larvae feed with dry food, *Artemia* and combination of both (co-feeding), they found that co-feeding resulted in the highest growth rates. Furthermore, Kolkovski *et al.* (1997b) found that the presence of *Artemia* increased dry micro-diet assimilation by 30-50% in European seabass (*Dicentrarchus labrax*) larvae.

In the present study, the group of fry which were fed with *Chlorella* showed the lowest growth rates. This poor performance may have been due to: a) the small size of chlorella cells, and/or b) the difficulty of tilapia fry to digest plant tissues. The few

publications that have investigated the effect of the size of live food organisms on tilapia fry indicated that the suitable size of prey for fish larvae varies according to the larval mouth size, and fish larvae generally select larger prey when they grow larger (Hagiwara *et al.*, 2001). The prey size may also affect the prey ingestion especially during early larval stages (Planas and Cunha, 1999). The growth rate of fish larvae may also be affected by the size of the feed particles (Olsen *et al.*, 1999). Turker *et al.* (2003b) found that Nile tilapia filtered more of the larger-sized particles from green algae and cyanobacteria cells and filaments grown in natural environments.

On the other hand, digestion of plant matter by fish is hindered by plant cell walls, which act as a barrier to the digestive enzymes. Preliminary research on adult *O. niloticus* has suggested that these fish lyse the cell walls of algae with gastric acid secreted to produce a pH in the stomach as low 1 (Bowen, 1976). Although no reports have been documented on the gastric acid secretion in larval tilapia, Lu *et al.* (2002) found that the gastric acid secretion of tilapia larvae did not show complete digestion of *Spirulina* filaments. Similarly, Olvera-Novoa *et al.* (1998) found that tilapia *Oreochromis mossambicus* fry were unable to efficiently digest the microalgae *Spirulina maxima* when it was used as a fish meal replacement.

The growth of Nile tilapia fry fed on a combination of *Chlorella* and Artificial feed in the present study was higher than that fed on *Chlorella* and *Artemia*. This may have been related to the high growth of the *Chlorella* cells inside the rearing tanks which may have reduced the fry vision to the point that they could not track the constant movement of the *Artemia* nauplii. Conversely, the artificial diets sink to the bottom where fry can consume them with less effort compared to that required to look for the mobile *Artemia* nauplii.

Although there is no literature supporting this interpretation, the obtained results are supported by the preference of tilapia fry for large size food. Furthermore, since the highest growth came from tilapia fry fed on solely *Artemia* compared to the other diets, especially the combined ones, it was clear that the fry prefered large-size food particles regardless of their nutritive value. It is also possible here to speculate on the possible energy that was spent for capturing motile prey compared with that spent by feeding on available, large-size food accumulated on the bottom, regardless of its nutritive value.

The present study showed that Nile tilapia fry fed on *Chlorella* had the highest mortalities rates (Table 4.7), while fry fed on *Artemia* and on the combination of *Artemia* and Artificial feed had the lowest mortality rate. Furthermore, the combination of the three types of tested food (*Chlorella*, *Artemia*, and Artificial feed) had the next lowest mortality rate. These results seem parallel with the results of the growth data. The effects of different food types on the survival of other fish species have also been documented. Næss *et al.* (1995) used different combinations of *Artemia* and zooplankton for the first time feeding of Atlantic halibut (*Hippoglossus hippoglossus*). They found that *Artemia* was more successful in supporting fish growth and survival during the early larval stage than other food sources. Furthermore, Ohs (1995) and Wan (1999) developed several semi-purified spray-dried diets and evaluated their effects on larval performance of striped bass (*Morone saxatilis*) and freshwater prawn (*Macrobrachium rosenbergii*) compared with *Artemia*. The larvae of both species were able to consume the dried diets, but the growth and survival were significantly less than that of *Artemia*-fed larvae. Similarly, Enz *et al.* (2000) studied the growth and survival of Lake Hallwil whitefish (*Coregonus* sp.) larvae reared on dry, artificial diets and live food. They found that the larvae fed live zooplankton (*Artemia* nauplii) suffered lower

mortalities than those fed dry diets. Also, the fry fed on *Chlorella* suspension showed the lowest survival rate, presumable due to the same reasons proposed above for the results obtained from present study.

The proximate analyses of the different food sources used in the present study have shown that *Artemia* nauplii had the highest percentage of moisture and lipid and the lowest percentage of crude protein. On the other hand, *Chlorella* cells had the highest percent of crude protein; while the combination of *Chlorella*, *Artemia* and Artificial feed had the lowest percentage of moisture (Table 4.7). Blair *et al.* (2003) suggested that the energy-dense lipids of *Artemia* may have contributed to the greater weight gain of haddock larvae fed on *Artemia*, compared to Rotifers and micro-diet. Watanabe *et al.* (1990) and Liao *et al.* (1990) reported that a 5% dietary supplement of *Spirulina* depressed the lipid content in the muscle of striped jack *Pseudocarnax dentex*. This may explain the increase in the body weight of the tilapia fry fed on *Artemia*, which supply the fry with high lipid concentrations compared to the other diets. In the meantime, Lu *et al.* (2003) studied the flesh quality of tilapia *Oreochromis niloticus* fed solely on raw *Spirulina*. They found that the *Spirulina*-fed tilapia had high protein content.

The statistical analyses of the growth parameters have indicated that there are negative correlations between final body protein content and many growth parameters, such as final body weight, average daily gain, and specific growth rate (Table 4.8). This may have been due to certain metabolic processes which appear in fry stages, when the body needs to build up enough lipids than protein. But that should depend on the food quality and its lipids contents, and on the metabolic capabilities of existing enzymes at such a very young age. From these results, it is possible to highlight the role of food quality in accelerating the

growth rate, especially at fry stage. That would be very important for certain fish species such as tilapia, where marketing size and/or weight is a crucial factor in the economic evaluation of its aquaculture.

This study put forward the proposal that the factors that stimulate the food selection of the Nile tilapia fry may have determined the feeding behaviour of tilapia fry in the present study. It has been reported that most fish larvae are visual feeders, and the feeding success at various developmental stages depends on the provision of suitable food and rearing environment, and on the visibility and adequate density of the prey (Hunter, 1980). The results of the present study showed that there are no differences in the attraction of tilapia fry toward both Artificial feed and *Artemia* suspensions during the whole experimental period (Fig. 4.8). It has also been found that the fry were less attracted to the *Chlorella* suspension (Fig. 4.5-4.7), presumably because they can see *Artemia* and Artificial feed particles, but cannot see *Chlorella* cells. Moreover, the feeding apparatus of Nile tilapia fry is not equipped with an efficient filtering system such as gill rackers, as found in some planktivorous fish (e.g. Herring). This may have reduced the ability of the fish to consume *Chlorella* cells. Clearly that proves the importance of food particle size as a limiting factor in controlling the feeding behaviour in tilapia fry.

It is well documented that fish can be attracted to bait by chemically mediated rheotaxis (Løkkeborg, 1998). Similarly, Kallayil *et al.* (2003) found that cod actively search when chemically stimulated by food odour even under non-visual conditions. The present study tested olfaction stimulation by exposing Nile tilapia fry to different culture filtrates and/or media of living organisms (i.e. *Artemia*, Rotifers and *Chlorella*). The obtained results have shown that the attraction of tilapia fry toward both *Artemia* and Rotifers filtrates was

similar (Fig. 4.12), and it was higher than their response to the *Chlorella* culture filtrate (Fig. 4.9 and 4.10). That could be due to the excretion of certain organic compounds able to attract fry. However, more research is needed to confirm this assumption. This finding does not also mean that the fry prefer *Artemia* and Rotifers more than *Chlorella*, but it means that, the odour of *Artemia* and Rotifers could be a good stimulator to attract fry toward food. Even so, the *Chlorella* species belongs to the green algae group (Chlorophyta) whose secretion within cultures may contain other organic compounds that could be associated with rich food areas in natural environments. In fact, when fry were tested to choose between the *Chlolrella* filtrate and blank condition, a highly significant attraction toward *Chlorella* filtrate compared with the blank condition was found (Fig. 4.11). The perception of the fry to such compounds could be of specific ecological significance, which may help in orienting the fish fry toward feeding grounds (Hamza and Ruggiu, 2000).

The third factor tested in the present study was the attraction of tilapia fry to opaque colours. The obtained results have shown that the green colour is the most attractive one followed by yellow and blue. On the other hand, the red colour was the least attractive to the tilapia fry (Fig. 4.14). Hamza and Ruggiu (2000) argued the attraction of *Daphnia galeata X hyalina* to green was due to the ecological significance of green areas being rich with food for the animal. This would confirm the previous interpretation of the attraction of the tilapia fry toward the filtrate of algae. Although that attraction was dependent on the olfaction sense organ, the attraction by colour could be referred to the vision sense. In support, Blaxter (1986) found that most first-feeding fish larvae are dependent upon vision for prey detection. In the present study, the active response toward the green colour can be translated into areas where food can be found, while the moderate attractions of tilapia fry to yellow and blue

colours can be translated into environmental references for orientation. Hamza and Ruggiu (2000) have also considered these colours as natural environmental colours which may represent the sun and the sky respectively. On the contrary, the red colour has interpreted as signal of a harmful wavelength or of light reflected by certain predators.

The repellent nature of red colour to *Daphnia* was also documented by Waterman (1961), and explained as harmful radiation. Although, the present study was dealing with fish fry which are animals much larger in size than *Daphnia*, the obtained results have confirmed the use of vision for feeding and food selection, based on colours' wavelengths. This means that colours can be used as feeding stimulators in Nile tilapia fry.

The statistical comparison between the preferences of tilapia fry between the selected colours has shown marginal significance. This may reduce the importance of colours as a feeding factor. However, such results could be biased due to the low number of observations included in this analysis. In fact, the absolute numbers represented in fig. 4.15 demonstrated the clear differences between the responses of tilapia fry toward the different colours. However, further work is needed to verify this dispute.

In conclusion, the present study achieved the objectives for which it was conducted. The results indicated that *Artemia* nauplii are the best live food for Nile tilapia fry, together with the Artificial feed. The results revealed also that phytoplankton could be used only as a supplementary food. However, the size of the organisms should be large enough to satisfy the feeding mechanism of tilapia fry. Although, algae could be the least favourable feed, their odour could be used as a feeding stimulator. It is also important to consider the food source that can provide the fish with the protein, lipids and other nutrients required for the optimum growth performance. Finally, feed/food colours and odour can be used as feeding stimulators

for fish, especially during larval stages. In this regard, algae extracts, which may act as a natural colouring agents and odour stimulators, are suggested as feeding stimulators

In tilapia aquaculture, where artificial diets are preferred by the farm managers, an artificial diet containing dried *Artemia* and/or *Artemia* cysts plus dried algae could be adopted during the larval stages. In addition, the incorporation of natural, dark green colour into the pellet diets may stimulate feeding behaviour and improve fish performance, especially during light phases.

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الملخص العربي

ازداد الوعي في القرن الحالي بأهمية الاستزراع السمكي، وذلك بعد الانخفاض الملاحظ في الموارد الطبيعية البحرية، حيث أدى هذا الانخفاض إلى زيادة اهتمام الكثير من الدول النامية والوكالات العالمية العاملة في مجال الأمن الغذائي بهذا الأمر. و يعتمد نجاح الاستزراع السمكي في المقام الأول على التقدم الاقتصادي، والذي بدوره يعتمد على إنتاج أنواع جيدة من الأسماك تصل لحجم التسويق في فترة زمنية قصيرة. الجدير بالذكر أنه في العهود الماضية فشلت الكثير من محاولات الاستزراع السمكي، وذلك بسبب عدم الموازنة بين النفقات المالية والأرباح العائدة منها، ويرجع هذا لعدة أسباب منها زيادة نسبة نفوق يرقات الأسماك، اختيار نوعية الغذاء المناسبة و العوامل البيئية التي تؤثر على حياة اليرقات.

و قد اشتهرت قارة آسيا وبالأخص منطقة الشرق الأوسط باستزراع أنواع معينة من الأسماك، وذلك بسبب الإقبال عليها من قبل السكان الأصليين نظرا لأسعارها المناسبة لهم. ولكن في الأونة الأخيرة ارتفعت أسعار هذه الأسماك نتيجة ازدياد تكلفة الغذاء الصناعي المقدم لها، بالإضافة لازدياد نسبة النفوق فيها بسبب عدم توفر الغذاء المناسب بعد عملية تفقيسها، وتعتبر أسماك البلطي مثالا على ذلك.

في الدراسة الحالية تم إجراء نوعين من التجارب، استهدفت التجربة الأولى تقييم تأثير المصادر المختلفة للغذاء على نمو يرقات سمك البلطي النيلي. بينما استهدف النوع الثاني من التجارب تقييم تأثير الظروف البيئية و السلوك الغذائي على نمو تلك اليرقات. في التجربة الأولى تمت دراسة تأثير ثلاثة أنواع من الأغذية و هي (1- حيوان الأرتيميا، 2- طحلب الكلوريلا، 3- الغذاء الصناعي) بالإضافة للخلائط بينهم مثل(1- طحلب الكلوريلا + حيوان الأرتيميا, 2- طحلب الكلوريلا + الغذاء الصناعي, 3- حيوان الأرتيميا + الغذاء الصناعي, 4- طحلب الكلوريلا + حيوان الأرتيميا + الغذاء الصناعي) على النمو و نسبة النفوق. حيث بدأت التجربة بمتوسط وزن 12 ملغ/يرقة. تمت التجربة في أحواض مغلقة وغذيت 21 مجموعة من اليرقات بالأنواع السبع للغذاء، بحيث تم استخدام كل نوع من الغذاء في تغذية اليرقات الموجودة في ثلاثة أحواض، و في كل حوض تم وضع 24 يرقة لسعة 6 لتر من الماء. و كانت التغذية بمعدل ثلاث مرات في اليوم لمدة 35 يوم. أشارت النتائج إلى أن تغذية اليرقات بحيوان الأرتيميا تعطي أعلى معدل نمو وأقل نسبة من النفوق، يليها خليط الاغذية الثلاث ثم خليط الارتيميا + الغذاء الصناعي . ومن ناحية أخرى أعطت نتائج التغذية بطحلب الكلوريلا أقل معدل نمو وأعلى نسبة نفوق لليرقات .

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رسالة مقدمة من الطالبة

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