Effect of Dietary Azolla on Colouration and Growth Performance of an Ornamental Fish- Red Platy *(Xiphophorus maculatus)*

ltkoVh eNyh & yky lyVh ¼thQkQkjl edyVl½ d jxk ,o of) in'ku ij vkgkjh; ,tkyk dk iHkko

SANJEEV KUMAR

Thesis

Master of Fisheries Science

(Aquaculture)

2016

DEPARTMENT OF AQUACULTURE
COLLEGE OF FISHERIES
MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, UDAIPUR 313001

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

COLLEGE OF FISHERIES, UDAIPUR

CERTIFICATE – I

Dated: 2016

This is to certify that **Sanjeev Kumar** has successfully completed the Comprehensive Examination held on …/…./2016 as required under the regulations for **Post Graduate Studies.**

(Dr. O. P. Sharma)
Professor & Head
Department of Aquaculture
College of Fisheries,Udaipur (Raj.)

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

COLLEGE OF FISHERIES, UDAIPUR

CERTIFICATE – II

Dated: 2016

This is to certify that this thesis entitled **"Effect of Dietary *Azolla* on Colouration and Growth Performance of an Ornamental Fish- Red Platy *(Xiphophorus maculatus)"*** submitted for the degree of **Master of Fisheries Science** in the subject of **Aquaculture**, embodies bonafide research work carried out by **Ssnjeev Kumar** under my guidance and supervision and that no part of this thesis has been submitted for any degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on.....2016.

(Dr. O. P. Sharma)
Professor & Head
Department of Aquaculture
College of Fisheries, Udaipur (Raj.)

(Dr. S.K. Sharma)
Major Advisor
Professor
College of Fisheries, Udaipur (Raj.)

(Dr.O. P. Sharma)
Dean
College of Fisheries, Udaipur (Raj.)

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

COLLEGE OF FISHERIES, UDAIPUR

CERTIFICATE – III

Dated: 2016

This is to certify that this thesis entitled **"Effect of Dietary *Azolla* on Colouration and Growth Performance of an Ornamental Fish- Red Platy *(Xiphophorus maculatus)*"** submitted by **Sanjeev Kumar** to the Maharana Pratap University of Agriculture and Technology, Udaipur in partial fulfillment of the requirements for the degree of **Master of Fisheries Science** in the subject of **Aquaculture,** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory; we therefore, recommend that the thesis be approved.

Dr. S.K. Sharma
Major Advisor

Dr. B. K. Sharma
Advisor

Dr. B. Upadhyay
Advisor

Dr. D. P. Singh
DRI Nominee

Approved

(Dr. R.A. Kaushik)
Director, Resident Instructions
Maharana Pratap University of Agriculture and Technology, Udaipur

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

COLLEGE OF FISHERIES, UDAIPUR

CERTIFICATE – IV

Dated:.....2016

This is to certify that **Sanjeev Kumar** student of Department of Aquaculture, College of Fisheries, Udaipur has made all corrections / modifications in the thesis entitled **"Effect of Dietary *Azolla* on Colouration and Growth Performance of an Ornamental Fish- Red Platy *(Xiphophorus maculatus)*"** which were suggested by the external examiner and the advisory committee in the oral examination held on .....2016.The final copies of the thesis duly bound and corrected were submitted on .....2016 and are enclosed herewith for approval.

(Dr. S.K. Sharma)
Major Advisor

Enclosed: One original and two copies of bound thesis. Forwarded to the Director, Resident Instruction, Maharana Pratap University of Agriculture and Technology, Udaipur.

(**Dr. O. P. Sharma)**
Dean
College of Fisheries, Udaipur (Raj)

(Dr. O. P. Sharma)
Professor & Head
Department of Aquaculture

ACKNOWLEDGEMENT

First of all, I bow down my head to the Almighty, without whose blessing completion of this work could not be a reality.

I take it to be my proud privilege to express my sincere and deep sense of gratitude to my major advisor ***Dr. S.K. Sharma,*** *Prof.,& Head, Department of Aquatic environment, College of Fisheries, Udaipur for his stimulating guidance, constructive suggestions, keen and sustained interest and incessant encouragement bestowed during the entire period of investigation as well as critically going through the manuscript.*

I am highly indebted to ***Dr. O.P. Sharma,*** *Prof. and Head, Dept. of Aquaculture, COF, for consistently offering most affable help and valuable suggestions at every juncture of this study. I am highly thankful to* ***Dr. O. P. Sharma,*** *Dean, COF, Udaipur, for providing adequate facilities for carrying out the present study.*

I am gratified to record sincere thanks to the member of the advisory committee ***Dr. B.K. Sharma,*** *Prof.,& Head, Department of HPHT, COF, the DRI Nominee,* ***Dr. D. P. Singh,*** *Asst. Prof., Department of Soil Science, RCA, Udaipur and* ***Dr. B. Upadhyay,*** *Prof., Department of Statistics, RCA, Udaipur for their generous gesture and valuable suggestion in planning and execution of this study.*

Words can hardly register the sincere and heartfelt feeling, which I have for my teacher ***Dr. S. K. Sharma*** *I also wish to convey my sincere thanks to the faculty/ staff members of CTAE and COF specially* ***Dr. Ganesh Rajamani, Dr. Deepak Raj Poorohith, Dr. R.C. Varma,*** *and* ***Dr. B.K. Sharma*** *for their kind co-operation and timely help.*

I record my sincere thanks to my seniors ***Manoj Kumar Meena, Sharad R. Sounar****,* ***Varun Mishra, Md. Aman Hassan, Tarachand Kumawat (****Scientist, CMFRI****), Minal Wagde, Sarvesh Kumar,* *classmates* ***Mr. Rohitash Yadav, Miss. Bhuneshwari Usandi, Praveen Kumar, Raj Priyanka Rathod, Hanuman Ram Goliya*** *and friends* ***Mr. Avinash Kumar Gupta, Prashant, Rajeev Kumar, Babul Raja, Kaushal*** *and* ***Sheetal*** *for their utmost cooperation sacrifice and encouragement during the course of investigation.*

The words in lexicon would be exiguous to my adoration and grateful veneration towards my beloved father ***Sri. Yogendra Prasad Sah****, mother* ***Smt. Shobha Devi****, uncle* ***Er. Krishna Kumar Kaushal*** *and* ***Mr. Pawan Kumar*** *for their unfathomable love, encouragement and appreciation at every juncture of my educational career. I am fortunate enough to have a loving younger brother* ***Gautam*** *and sister* ***Pinki*** *for constant encouragement and help.*

Place: Udaipur

Date: **(Mr. SANJEEV KUMAR)**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1. INTRODUCTION

Considering the rich biodiversity of fish and shell fish species in our country, it is necessary to bring more species of promise into aquaculture practices. Ornamental fish culture classified under urban aquarium is one of the emerging avenues for diversification in aquaculture. Ornamental fishes are now a days rapidly gaining importance because of their aesthetic value and commercial importance in the export trade world over. Keeping colourful and fancy fishes popularly called ornamental fishes, aquarium fishes, or live jewels is one of the oldest and most popular hobbies in the world. The growing interest in aquarium fishes has resulted in the steady increase in aquarium fish trade globally. Existence of vibrant colours is one of the major factors which determine the price of the ornamental fish in the world market (Saxena, 1994). Besides this, body shape, behavior pattern and environmental adaptations are other important factors (Chapman, 2000).

The collection and keeping of good looking ornamental fish is of primary importance for the home aquarium. Ornamental fish keeping is a very popular hobby known for more than 1000 years (Santhanan et al, 1990). The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally (Silas *et al.*, 2011). It can be made profitable as other hobbies. Japan, Malaysia, Singapore, Taiwan, USA, Czech Republic, Indonesia and India have made the ornamental fish culture and trade as a flourishing business.

The ornamental fish trade with a turnover of US $ 6 Billion and an annual growth rate of 8 percent offers lot of scope for development (NABARD, 2013). The entire industry, including accessories and fish feed is estimated to be worth US $ 14 Billion. The top exporting country (with percentage contribution to global trade) is Singapore (19.8%) followed by Czech Republic (7.8%), Japan (7.4%), Malaysia (7.3%), Indonesia (5.3%), Israel (4.3%), Thailand (3.9%) and Sri Lanka (2.9%). India’s share in the global aquarium fish trade is only 0.008 per cent in terms of money, amounting rupees 23 millions. However, domestic market of ornamental fish trade also nears about rupees ten crores. The largest importer of ornamental fish is the USA followed by Europe and Japan. The emerging markets are China and South Africa.

As stated above, attractive colour is one of the major factors determining the price of the aquarium fish in the world market (Saxena, 1994). In fishes acquiring different body colours and hues is greatly dependent on the food. A direct relationship exists between dietary carotenoid and body pigmentation in fishes.

Fishes use colors primarily for signaling during courtship, mating or other intrasexual communications, and for threatening displays, camouflage, and protection from predators by scaring them or warning that the animal or some part of it is poisonous (Theis *et al.*, 2012).

Body color, together with the physics of light penetration, well-developed parental and territorial behavior patterns and remarkable feeding specializations have promoted sympatric speciation and adaptive radiation to proceed in just a few hundred thousand years in cichlid fish species in the great lakes of east Africa especially in the lake Tanganyika (Fryer & Iles 1972; Turner 1994; Barlow 2000; Kocher 2004; Seehausen & Schluter; Terai *et al*., 2006.

Under intensive aquarium rearing, ornamental fishes are fed exclusively on formulated feeds for better growth and colouration. In the past decade, the nutritional requirements of various fish species have been studied and technological advances in feed manufacturing have been pursued (Wang *et al.*, 1999).

As ornamental fishes are characterized by having a wide diversity of colours and colour patterns, success in the ornamental fish trade is very much dependent on the vibrant color of the fish. Ornamental fish often show faded or degraded body colouration especially when the fishes are kept under captivity for long duration and under starvation and stressful conditions. Fish, like other animals cannot synthesize carotenoids, and they rely on dietary supply of these pigments to achieve their natural skin colouration. A direct relationship between dietary carotenoids and pigmentation exists in these fishes (Halten *et al*., 1995).

The carotenoids are also vital nutrients for healthy growth, metabolism, and reproduction as well as colour (Miki, 1991). In culture system, ornamental fishes are normally fed with live feed, which is often difficult to obtain and can act as the transmitter of diseases (parasitic, bacterial and viral).

As fishes cannot synthesize their own coloring pigments *de novo*, the coloruing agents synthesized by some plants, algae and microorganisms are required

to be added in their diets to maintain their colour. This has facilitated the creation of the desired pigmentation pattern over the fish skin making it more attractive. Increased interest in fish pigmentation has arisen through the desire to create beautifully coloured ornamental fish species where emphasis is given to achieving high levels of skin pigmentation (Paripatanamont et al., 1999).

When global fish meal production is unable to fulfill the demand and its prices become more expensive, nutritionists seek less expensive plant protein sources such as soybean meal, Akiyama, (1991).

It is established now that in aquarium fish feeds must be supplemented with carotenoids for better colouration and as vital nutrient. Various synthetic carotenoids *e.g.* β-carotene, canthaxanthin, zeaxanthin, astaxanthin and natural sources (yeast, bacteria, algae, higher plants, and crustacean meal) have been used as dietary supplement to enhance the pigmentation of fish and crustacean (Shahidi *et al.*, 1998). Plant sources have also been used as dietary supplement for inducing colouration in fish.

However, detail studies on colouration enrichment in indigenous ornamental fish are lacking. Plant sources have been utilized for inducing pigmentation in fish.

There are various absolute factors involved in ornamental fish culture principal being density and water hardness (James, 1998; Vasudhevan, 2008). If enhancement of coloration can be achieved by administrating pigment enriched feed, it will definitely improve the quality and price of the fish. However, detailed studies on this are lacking. The blue green alga *spirulina* has been used as a source of carotenoid pigment for rainbow trout and fancy carp (Kottelm, 2001; Tan and Lim, 2004) while marigold petal meal was used for the tiger barb (Boonyarapatin, 1977).

The smallest flowering plant *Azolla* is one such non-conventional protein feed with carotenoids. It is an aquatic, free floating fern belongs to the family *Azollaceae* is a good source of protein and it contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese etc, apart from appreciable quantities of vitamin A precursor beta-carotene and vitamin B_{12}. It is also found to contain probiotics and biopolymers (Pillai *et al.,* 2002). Thus, *Azolla* appears to be a potential source of nutrients and pigment for preparation of dietary supplement (Hossiny *et al.,* 2008).

Previous research on the pigmentation of fish has focused mainly on the inclusion of dietary carotenoids but is relatively silent on the roles of other dietary pigments as natural colour enhancer.

To alleviate this problem the present study was conducted to study the effect of dietary *Azolla* on colouration and growth performance of an ornamental fish (*Xiphophorus maculates* Günther, 1866) commercially called Red platy. The fish is easily available in the tropical environment, easy to handle and keep in aquariums. The fish is always in good demand amongst the aquarium hobbyists.

The present study was aimed to achieve following objectives:

1. To assess the effect of dietary *Azolla* on skin colouration of red platy.
2. To determine the efficacy of *Azolla* mix diet on growth performance of red platy.

2. REVIEW OF LITERATURE

Different natural sources have been used by various researchers in augmenting growth and colouration of fishes by incorporating them as basic ingredient in supplementary diet. Few of the reviews on colouration and growth performance in ornamental fishes are enlisted here under.

Almazan *et al.* (1986) conducted a study where *Azolla pinnata* was fed to Nile tilapia (*Oreochromis niloticus*) fingerlings and adult males. Fingerlings were fed Azolla in fresh, powder and pellet form, replacing the complete control diet mix from 10 percent to 90 percent. VanHove and Lopez (1987) noted that the crude protein content of Azolla might vary from 13.0 to 34.5%.

Storebakken *et al.* (1987) and Bjerkeng *et al.* (1990) investigated the increase in carotenoid content in skin, fins and muscle of *Xiphophorus maculates* in relation to dietary carotenoid content of Azolla. Diets demonstrate that, the fish has capacity to utilize it efficiently.

Carotenoids have a number of double bond so they possess an ability to increase immunity by quenching singlet oxygen of free radical and other oxidative oxygen species. Thus can be used as antioxidant in a very limit way. Carotenoids, therefore, can increase the ability of immune system by boosting up host defense function found in animals and human that consumed carotenoids (Bendich, 1989).

El-Sayed (1992 and 2008) reported extremely poor performance for *O. niloticus* fingerlings and adults fed diet based on *A. pinnata*. This author incorporated dried Azolla powder at 25, 50, 75 and 100 percent replacement of fishmeal protein in a fishmeal-based control diet in a 70-day trial. Fresh Azolla as a total diet was also used as a control. Growth and feed utilization efficiency of fish fed with the control diet was significantly higher compared to fish fed with Azolla-supplemented diets. The performance of fish was inversely related to the increasing dietary incorporation of Azolla.

Lovell (1992) tried commercial feed ingredients such as yellow corn, corn gluten meal and *alfaalfa* as sources of carotenoids such as Zeaxanthin and Lutein in ornamental fishes. Mathew and Gopakumar (1992) used red colour extracted from Sandalwood (*Pterocarpus santalinus*) in fish feed which was found to increase the

acceptability of feed in tilapia (*Tilapia mossambica*). It imparted a pink colour to the whole fish as well as to the fish flesh. The fish fed with coloured feed showed increased feed intake and growth. Joseph *et al.* (1994) reported the increased FCR due to higher feeding of Azolla in *Etroplus suratensis.*

It is well known that carotenoids have an unsubtituted end groups alpha-carotene, beta-carotene and cryptoxanthin precursor of vitamin A in animals. Furthermore, cryptoxanthin is coverted into retinol in *Salmonidae* fish. Gross and Budowski (1996) reported that astaxanthin, canthaxanthin and isozeaxanthin were pre-cursors for vitamin A in both guppies (*Lebistes reticulatus*) and platies (*Xiphophorus variatus*).

On a commercial scale, an inclusion rate of 30 ppm astaxanthin is used to supplement live and flaked foods resulting in a significant colour improvement in most species of tetras, cichlids, gouramis, goldfish, koi and danios (Ako and Tamaru, 1999).

Kim *et al*. (1999) found the nuptical colour of the bitterling (*Rhodeus uyekii*) improved by feeding an artificial diet containing different carotenoids. The diets were supplemented with various carotenoids *viz*., astaxanthin, lutein and β-carotene. They found that the amount of total carotenoids in the group of fish fed by supplementary carotenoids were relatively higher than control group of fish. The group of fish fed by astaxanthin supplementation diet also showed the higher growth.

In goldfish, *Carassius auratus*, the optimum level of astaxanthin for intense colouration was found to be 36-37 mg/kg diets and the supplementation significantly improved the survival rate (Paripatnanont et al., 1999).

Pariapatananont *et al.* (1999) have observed the effect of astaxanthin on the pigmentation of gold fish (*Carassius auratus*) by feeding a series of diet containing astaxanthin 0, 25, 50, 75, and 100 mg/kg for 4 weeks. Skin pigmentation was measured by visual assessment against a colour chart and by counting chromatophores produced in the dermis. Both methods showed that astaxanthin 36-37 mg/kg was the optimum does for stimulating fish colour. The survival rate of fish fed diets with astaxanthin was significantly higher than fish fed diet without astaxanthin. However, astaxanthin had no significant effect on live weight gain.

In red velvet sword tail (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasoma myrnaae*) the intensity of colouration significantly improved when fed the diet containing 1.5–2 % of a carotenoid rich stain of *Spirullina platenasis* and 1 % of *Haematococcus pulvialis* for three weeks (Aok, 2000).

Ako *et al*. (2000) have found colour enhancement in ornamental fishes by feeding top coated algae in the diet. A cichlid *Cichlasoma myrnae* became significantly more intensively coloured when fed a diet containing 1.5-2.0 % of a carotenoid rich strain of *Spirulina platensis* and 1% of a specially grown *Haematococcus pluvialis* for three weeks, though colour enhancement was apparent after only a week. Further, they found that colour intensity was diminished when fish were stressed. Colouration appeared only in males in species where only the males were normally coloured. In rosy barb and topaz cichlids colour enhancement was environment sensitive. It was concluded that ornamental fishes are good models for colour enhancement through diet.

The skin colour could be changed in fish by altering surrounding conditions, light intensity and dietary conditions (Waagbe, 2002). The predominant carotenoids present into aquatic organisms are specially lutein (greenish-yellow), zea-xanthin (yellow-orange) and α-carotene (light yellow). He found that the blue green algae, spirulina as feed greatly influence in colour development of fish. Probably the experimental plankton feed in absence of this essential colour enhancing algae failed to produce the natural red colour in cherry barbs.

Fiogbé *et al*., (2004) obtained quite favorable results with Azolla-based diets fed to juvenile *Oreochromis niloticus* grown in a re-circulating system. Six diets were formulated with almost isonitrogenous levels of protein (27.25-27.52 percent DM) but different levels of dry Azolla meal (0, 15, 20, 30, 40 and 45 percent). All diets with incorporated Azolla meal showed weight gain. The Azolla-free diet and the diet containing 15 percent Azolla produced the same growth performance. The least expensive diet, which contained 45 percent Azolla, also showed growth and was thought to be potentially useful as a complementary diet for tilapia raised in fertilized ponds. These authors noted that mixing Azolla with some agricultural by-products such as rice bran; the use of fermentable by-products such as yeasts or the addition of purified enzymes; might improve ingestion and digestibility.

It is found that azolla meal could replace sesame meal on a digestible protein and digestible amino acid basis up to 200g/kg diet for better egg mass out put and FCR in laying hen (Khatun et al., (1999). Kannaiyan and Kumar (2005) noted higher egg yield and saved feed cost 20 per cent and with supplementing 100gm fresh Azolla per bird per day.

Gouveia and Rema (2005) investigated the effect of different carotenoid source/ concentration and temperature on goldfish (*Carassius auratus*) skin pigmentation. The effect of carotenoid source (natural microalgae *Chlorella vulgaris* and Synthetic carophyl pink) and carotenoid concentration (45, 80, and 12 mg pigment kg^{-1} diet) was tested. The effect of water temperature on skin pigmentation was also studied. The best carotenoid concentrations were achieved with astaxanthin diet. There was a tendency to an overall improvement of colour parameters (L and b) in fish fed diet in high level of *C. vulgaris.* The result indicated that the best temperature range to maximize skin pigmentation was 26-30°C.

Natural esterified forms of astaxanthin such as Naturose are known to increase skin redness and total carotenoid content in red porgy (Chatzifotis *et al.*, 2005). No other colour change was recorded until the end of the experiment. Maximum colouring of fish can be compared to the degree 3 of the scale and it was caused by astaxanthin and β-carotene.

Higuera *et al.* (2006) studied Astaxanthin and found it as a carotenoid widely used in salmonid and crustacean aquaculture to provide the pink colour characteristic of that species. Additionally, astaxanthin also plays a key role as an intermediary in reproductive processes. Authors also focused on common sources of natural astaxanthin which includes the green algae *Haematococcus pluvialis*, the red yeast, *Phaffia rhodozyma*, as well as crustacean byproducts. Astaxanthin not only possesses an unusual antioxidant activity which has caused a surge in thenutraceutical market for the encapsulated product but also health benefits such as cardiovascular disease prevention, immunesystem boosting, bioactivity against *Helicobacter pylori* and cataract prevention, have been associated with astaxanthin consumption. Research on the health benefits of astaxanthin is very recent and has mostly been performed *in vitro* or at thepre-clinical level with humans. Their paper reviews the available evidence regarding astaxanthin chemistry and itspotential beneficial effects in humans.

James *et al*. (2006) found that Red Sword tail (*Xiphophorus helleri)* fed with 8% Spirulina enhanced the lymphocyte and monocytes population among leucocytes which in turn increased the resistance. Gupta *et al*. (2007) experimented some naturally available carotenoid rich ingredients such as micro algal pigments, yeast extract, marigold, capsicum etc. and discussed their utility for enhancement of pigmentation in fishes. They recommended that 125 ppm carotene gave excellent pigmentation and higher doses 200-300 ppm further improved pigmentation.

Harpaz and Padowicz (2007) examined the effects of adding carotenoids from *Oleoresin paprika* to fish feeds for ornamental dwarf cichlid (*Microgeophagus ramirezi*) and the results indicate that addition of 60 mg *Oleoresin paprika* per kg diet is sufficient to obtain good coloration in *M. ramirezi*.

Kour (2007) studied the effect of herbs: Sarpgandha (*Rauwolfia serpentine*) (Bentch) at the rate of 6-30 mg/ kg body weight per day on koi carp and found improvement in fish body colour at 6-24 mg/kg body weight.

The crude protein level was generally lower and the studies were carried out with advanced fry, fingerling or adults. El-Sayed (2008) noted that young Nile tilapia utilized Azolla more efficiently than adults.

Ezhil *et al.* (2008) studied effect of marigold as a carotenoid source on pigment and growth of red sword tail (X*iphophorus hellerii*) and found increased growth rate after rearing of 60 days and fed with marigold petal at the rate of 15 gm/100 g of feed.

Sithara and Kamalaveni (2008) reported that Azolla as a protein supplement enhanced the bioenergetics parameters like feeding rate and growth in Tilapia.Vasudhevan (2008) found that 30% Spirulina diet significantly enhanced the feed consumption, feeding rate, specific growth rate and feed conversion rate in *Carassius auratus*.

Kop *et al.* (2010) examined influence of carrot (*Daucus carota*) and red pepper (*Capsicum annum*) as natural pigment material on colouration of cichlid (*Heros severum* previously known as *Cichlasoma severum* Heckel, 1840). Carotenoid amount in the fish samples by fed with red pepper and carrot diets were 5.25 ± 0.90 and 5.60 ± 0.29 mg g^{-1} respectively. Consequently a significant difference was found between individuals fed by natural pigment material and those by unpigmented feeds

($P < 0.05$). It was demonstrated that natural pigment substances have an impact on coloration of cichlid and the groups did not exhibit any distinctions in feed conversion and growth rates.

Wassef *et al*. (2010) investigated the effects of either red bell-pepper (*Capsicum annum*) meal or carrot (*Daucus carota*) meal as a natural dietary carotenoid source, on growth and skin coloration of gilthead seabream (*Sparus aurata*). Results suggested that gilthead seabream can effectively bio-absorb natural carotenoid pigments (mainly capsansin and capsorbin) in red-pepper but not in carrot (mainly beta -and alpha -carotene).

Dharmaraj and Dhevendaran (2011) studied the application of microbial carotenoids as a source of colouration and growth of ornamental fish *Xiphophorus hellerii*. The results indicated that the fishes fed with carotenoid enriched feed showed faster recovery of carotenoids in the skin of the fishes when compared to the control feed supplemented fishes and the results were found to be significant ($p < 0.01$). After 28 days of feeding trials, the growth parameters were found to be statistically significant ($p < 0.01$) when different diets were compared. The study proved that microbial carotenoid not only improves the pigmentation, but also promotes the growth of the ornamental fishes effectively.

Joseph *et al.* (2011) studied effect of four botanical additives (*H. rosa-sinensis, Rosa indica, Ixoracoccinea* and *Crossandra infundibuliformis*) on the growth and body colouration of red sword tail (*Xiphophorus hellerii*, Heckel). Three fold increases in growth was observed in *H. rosa-sinensis* fed fishes followed by *R. indica*. Furthermore, the similar weight gain has been observed with rest of the two flower petals (*I. coccinea* and *C. infundibuliformis*). The percentage of colour pigments obtained in adult fish was maximum with *I. coccinea* followed by *R. indica, H. rosa-sinensis* and *C. infundibuliformis*. Consequently a significant difference was found between individuals fed by natural pigment material and those by un-pigmented feeds ($p \leq 0.05$). It was demonstrated that natural pigment substances have a notable impact on colouration of the fish.

Ylmaz and Ergun (2011) examined the effect of two concentrations (20 g/kg and 50 g/kg) of red pepper (*Capsicum annum*) meal on the skin coloration of juvenile blue streak hap (*Labidochromiscaeruleus*). Results show that red pepper meal can be

used as an alternative natural carotenoid source in blue streak hap diets and that 50 g/kg is an appropriate dietary level to ensure good pigmentation, suitable growth, and feed utilization.

Guroy *et al*. (2012) used Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheusacei*. Results indicated that Spirulina meal has the potential to enhance the growth, reproductive performance and colouration on yellow tail cichlid.

Wagde (2013) used natural β-carotene sources *i.e.* Carrot and Spinach to supply 20, 25 and 30 mg of β-carotene added per 100 g in fish diet and found increase in colouration and growth rate in swordtail (*Xiphophorus hellerii*).

3. MATERIAL AND METHODS

As stated earlier, the present research programme was designed to investigate the effect of dietary *Azolla* on colouration and growth performance of an ornamental fish (*Xiphophorus maculatus* Günther, 1866) representing live bearers. The experiment for the purpose was conducted in indoor glass aquaria with partial water replacement at every third day and aeration of at least 20 hours a day. The duration of experiment was 8 weeks. The detail of material and methods adopted are given here under.

3.1 CLIMATE

Udaipur is located between latitude 24°35′N to 24°58′N and longitude 73°41′E to 73°68′E. It has an average elevation of 598.00 m (1,962 ft) above MSL. Udaipur experiences a sub-tropical climate with an average annual rainfall ranging from 65 to 76 cm and relative humidity of 75 to 95 percent during monsoon period. The maximum temperatures ranges between 35 ℃ to 46℃ on most of the days during summer season and in winter minimum temperatures remain around 5–10 ℃.

A description of materials and methods adopted for the present study is given below:

3.2 EXPERIMENTAL DESIGN

3.2.1 Experimental Fish and Their Conditioning

Experimental ornamental fish red platy (*Xiphophorus maculatus*) was obtained from a local ornamental fish dealer. The fishes were selected for their prominent body colour and size from a well maintained homogeneous stock. The test fishes were having weight range between 0.50 ± 0.08 g and the length ranged between 2.8± 0.3cm.

The fishes were kept in FRP tank in the Wet Laboratory of Department of Aquaculture, College of Fisheries, Udaipur for the conditioning period of 7 days before the experimentation.

3.2.2 Experimental Aquaria

The experiment was conducted in glass aquaria (size- 2x1x1) containing 50 litres of pre-oxygenated bore well water. In all 18 glass aquaria were used for this

purpose with replicates, in which 15 aquaria were used for experimental diet and 3 for control diet. All the aquaria were kept in indoors away from direct sunlight to prevent algal growth.

Water in aquarium was aerated for 3 days for stabilization before the commencement of the experiment. Thereafter, 10 homogeneous individuals of red platy (*Xiphophorus maculatus*) irrespective of their gender were introduced in each glass aquaria. These fishes were acclimatized for 8 days in respective aquarium tank and fed with controlled diet (no added *Azolla* source) at the rate 3 per cent of their body weight. The initial weight and colour intensity of fishes in each tank were recorded after acclimatization.

3.3 EXPERIMENTAL FEED

3.3.1 Preparation of experimental diet

The experimental feed (Basal diet) was prepared with wheat flour, rice bran, tapioca, soybean meal, groundnut oil cake and vitamin mixture. The diet was prepared by following Ramammoorthy *et al*. (2010). The basic ingredients of experimental diet were oven dried, powdered, ground and then sieved through fine meshed sieve (No. 36) before mixing.

The fresh *Azolla* as a natural carotenoid source, was procured from the organic farm of KVK Badgaon. It was washed thoroughly, cleaned, air dried at 60^0C for 48 hours, ground and powdered and sieved. The finely powdered *Azolla* was stored in refrigerator to avoid oxidation of carotenoids.

The experimental diet was prepared using five different levels of *Azolla* mixed in basal diet (BD) as shown in Table 1. The natural carotenoid source *Azolla* @ 10% (T_1), 20% (T_2), 30% (T_3), 40% (T_4) 50% (T_5), and 0% (control), was added on dry weight basis in the basal diet replacing soybean meal, fish meal and groundnut oil cake in equal quantities. The experimental diet was thus formulated mixed with small amount of vegetable oil and then with little amount of water to make a dough and its pellets were made by a hand operated extruder machine with the pore size of 1.0 mm. The diet thus prepared, was dried in the hot air oven at 55^0C and stored in air tight glass stopper bottles in refrigerator at 4^0C. In the experimental set-up there was 1 control (devoid of natural carotenoid) and 5 different experimental feeds containing natural carotenoid source. There were three replications for each experimental diet.

Thus there were 15 experimental tanks with 5 experimental feed levels and one control of 3 tanks.

3.3.1 Feeding

The experimental fishes were fed @3% of their body weight once a day at 0900 hour. Before beginning the experiment, total body length (mm) and weight (g) of the fish in each aquarium were measured. Thereafter, the feed quantity will be readjusted at every seven days interval according to increase in mean fish weight in each aquarium.

. The experiment was carried out for 60 days. The observation for growth parameter as well as colour enhancement of the fishes were measured initially and then at every 7 days interval.

Table 1. Formulation of experimental diets (Ingredients in gm/100gm of diet)

S. No.	**Treatments indicating final ingredients of the diet in gram**						
1.	**Ingredients**	**Control**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
2.	Groundnut oil cake*	15.0	13.5	12.0	10.5	9.0	7.5
3.	Fish meal*	15.0	13.5	12.0	10.5	9.0	7.5
4.	Soya bean meal*	17.0	15.3	13.6	11.9	10.2	8.5
5.	Wheat flour	14.5	14.5	14.5	14.5	14.5	14.5
6.	Tapioca flour	14.0	14.0	14.0	14.0	14.0	14.0
7.	Rice bran	14.5	14.5	14.5	14.5	14.5	14.5
8.	Vegetable oil	5.0	5.0	5.0	5.0	5.0	5.0
9.	Vitamins & mineral mixture	5.0	5.0	5.0	5.0	5.0	5.0
10.	*Azolla***	0.0	4.7	9.4	14.1	18.8	23.5
	% Increase in *Azolla*	-	10	20	30	40	50

*These ingredients were replaced with *Azolla*.

**Natural carotenoid source.

3.4 ANALYTICAL METHODOLOGY FOR WATER QUALITY PARAMETERS

Water quality parameter *viz.*, air and water temperature, pH, dissolve oxygen, free carbon dioxide, total alkalinity and total hardness were treated initially and at every week following APHA (1989) and Trivedi et al. (1987).

3.4.1 Air temperature

Air temperature in the vicinity of experimental aquaria was measured using centigrade thermometer having minimum graduation of 0.1°C.

3.4.2 Water temperature

The temperature of water was also noted directly up to single decimal place in °C by immersing the thermometer into the water of experimental aquaria.

3.4.3 pH

The hydrogen ion concentration of experimental waters in the aquaria was determined with the help of a standardized pen type digital pH meter (make- HANA pH 600).

3.4.5 Dissolved oxygen

The dissolved oxygen of water was estimated by modified Winkler's method. In this procedure, oxygen combines with manganous hydroxide to form higher hydroxide, which on acidification liberates iodine equivalent to that of oxygen fixed. This iodine is titrated by standard sodium thiosulphate solution using starch as an indicator.

Following steps were adopted for oxygen estimation:

(i) Water sample was collected in 250 ml glass bottle without bubbling of air

(ii) 1 ml of each manganus sulphate (Winkler A) and alkaline – iodine solution (Winkler B) were dispensed, one after the other, right at the bottom of the bottle using separate pipettes. Then stopper was replaced.

(iii) The bottle was shaken upside down at least 6 times so as to maximize the brown precipitate which was then allowed to settle.

(iv) The precipitate was dissolved by adding 1ml of concentrated sulphuric acid and the stoppered bottle was again shaken.

(v) 50 ml of aliquot was taken in a conical flask and then titrated against sodium thiosulphate (0.025 N) till the colour changed to pale straw/ pale yellow.

(vi) After that, 2-4 drops of starch indicator were added and titrated further till the blue colour disappeared for the first time to a colourless end point.

(vii) The total amount of titrant used was noted and the dissolved oxygen content was calculated as:

$$\text{Dissolve oxygen (mg/l)} = \frac{8 \text{ X } 1000 \text{ X } N}{V_1} \text{ X } V_2$$

Where,

N = Normality of the titrant (0.025 N)

V_1 = Volume of sample (ml)

V_2 = Volume of titrant used (ml)

(1 ml of solution thiosulphate solution is equivalent to 0.2 mg oxygen).

3.4.6 Free carbon dioxide:

It is the normal practice to distinguish free carbon dioxide as the concentration of CO_2+ H_2CO_3 which was estimated by titrating the sample with standard alkali titrant to pH 8.3 as per the method described below:

(i) 50 ml of water sample was taken in a flask and two drops of phenolphthalein indicator were added.

(ii) If the colour turns pink, free CO_2 was absent. If the sample remains colourless, it was titrated against 0.05 N NaOH. At the end point a pink colour appears.

(iii) The reading was noted and free CO_2 was calculated by the following formula.

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{\text{ml of titrant NaOH used X } N}{\text{ml of sample}} \text{ X } 1000$$

3.4.7 Total alkalinity

Alkalinity of the water used in the experiment was measured by titrating 50 ml of sample with standard solution of hydrochloric acid (0.02 N). Carbonate alkalinity was determined to the first end point (pH 8.3) using phenolphthalein indicator and

bicarbonate alkalinity was determined to the second end point (pH 4.5) using methyl orange indicator.

Following steps were followed:

(i) 50 ml of water sample was taken in a conical flask and two drops of phenolphthalein indicator were added.

(ii) If a slight pink colour appears, titrated with hydrochloric acid titrant to a colourless end point and the reading was noted as 'P' (ml of titrant used for phenolphthalein alkalinity).

(iii) Now two drops of methyl orange were added in the same flask and continued to titrate further till the colour changes from yellow to orange.

(iv) This reading was noted as 't' (total volume of the titrant used for both the titrations).

Total alkalinity was calculated using following formula:

$$\text{Total alkalinity (ml/l)} = \frac{\text{ml of titrant used 't'}}{\text{ml of sample}} \text{ X } 1000$$

Where,

t = Total volume of titrant used for both the titrations

3.4.10 Total hardness

Eriochrome black T forms wine-red complex compound with metal ions (Ca++ and Mg ++). The di-sodium salt of EDTA extracts the metal ions from the dye-metal ion complex as colourless chelate complexes leaving a blue coloured aqueous solution of the dye.

Hardness of experimental water was measured in the following way:

(i) 50 ml of water sample was taken in a conical flask.

(ii) Thereafter, 1 ml of ammonia buffer solution and 5 drops of indicator solution were added to this.

(iii) The colour of the sample turns wine- red.

(iv) It was then titrated with EDTA solution, until a clear blue colour appears.

(v) Then, the reading was noted and hardness was calculated using following formula:

$$\text{Total hardness (mg/l)} = \frac{\text{ml of titrant used}}{\text{ml of sample}} \text{ X } 1000$$

3.5 PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS

The experimental diets were analyzed for the proximate composition viz., moisture, crude protein, fat, carbohydrate and ash contents as per standard methods of AOAC (1980). The details of methods adopted for the purpose are as follows:

3.5.1 Estimation of Moisture:

A known amount of sample was taken in a weighed porcelain crucible and kept in a preheated oven at 60 ± 2°C for at least 24 hours. The crucible then transferred directly to a dessicator, cooled and weighed immediately. The moisture i.e. weight loss was reported in per cent as per the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \text{ X } 100$$

3.5.2 Estimation of Crude Protein by Microkjeldhal Method:

Nessler's reagent in an alkaline aqueous solution of potassium mercuri-iodide ($KIHgI_2$). It reacts with NH_3 (or NH - Salts) to give reddish – brown colour or precipitate in the presence of sodium leaving behind a clear, non turbid coloured solution. The colour developed, remains stable up to 15 hours at room temperature (20 – 40°C) and its intensity is proportional to the initial concentration of NH_3 Nitrogen. It is therefore, possible to calorimetically assay the initial concentration of NH_3 nitrogen by Nesslerization. The colour can be read at the wavelength of 440-650 nm.

I) Digestion:

a) The dried and powdered fish diets were used for the purpose. Then 0.1g of sample was taken in a dried Kjeldhal's flask.

b) Then added 2 ml of concentrate H_2SO_4 and it was digested on heater for at least 1.30 hrs (a short funnel may be used as a reflux).

c) To this added 0.5 ml of hydrogen peroxide (30 per cent H_2O_2) with alternate heating and cooling till the colour disappears. It was heated further until H_2O_2 fumes escaped completely.

d) The contents of kjeldhal's flask were transferred to 100 ml volumetric flask and the volume was made up.

II) Colour development:

a) 5 ml of aliquot was taken in 50 ml volumetric flask and added 2 ml of 10 per cent solution of NaOH and 1 ml of 10 percent sodium silicate, successively. In this solution 1.6 ml Nessler's reagent was added and finally volume was made to 50ml with distilled water. Thereafter, it was allowed for 10 minutes for full colour development.

b) A control was run with distilled water with the same procedure.

c) The spectrophotometer was adjusted using control and absorbance (O.D.) of sample was recorded at 540 nm.

d) A standard curve between NH_3-N and absorbance values was plotted using 25 ppm NH_3 solution.

e) The N – content of sample was determined using the standard curve. The crude protein content was calculated by multiplying the N-content with a conversion factor 6.25 (for fish / animal protein).

3.5.3 Estimation of Fat

Procedure: The fat in organic matter is soluble in organic solvent like petroleum ether, benzene, carbon tetrachloride etc. this fat content can be extracted using the organic solvent in a soxhlet apparatus. The difference in weight of sample is directly ascertained as total organic solvent fat content in the given sample. A sachet of filter paper (Whatman No. 40) was made and its weight was recorded. In this sachet one gm of dried and powered sample was taken.

The sample was then extracted with ether at 60-80 °C in soxhlet apparatus. The extraction was continued for at least six hours at condensation rate of 20-30 drops per min. After extraction, sample was dried for 30 minutes at 100°C, cooled and final weight was recorded. The difference in weight of sample before and after extraction indicated the total organic solvent soluble lipids. The fat content of the sample was expressed in g per 100 g of dried sample.

3.5.4 Estimation of Nitrogen free extract

The amount of carbohydrate in the sample was calculated by difference method. The sum of crude protein, fat, ash and moisture was subtracted from 100 as given below:

Per cent of Nitrogen free extract = 100 – (Crude Protein + Fat + Ash + Moisture in per cent).

3.6.5 Estimation of Ash

5 g dried and powdered sample was weighed in silicon crucible and incinerated in a furnace preheated to 550°C for four hours. The crucible containing fully burnt material was transferred to a dessicator cooled and weighed. The difference between initial and final weight was noted up to milligram using a balance. The ash was reported in per cent as follows:

$$\text{Ash (per cent)} = \frac{\text{Ash weight}}{\text{Sample Weight}} \text{ X } 100$$

3.7 GROWTH PARAMETERS

The growth parameter *viz*. net weight gain, specific growth rate, food conversion ratio, gross conversion efficiency were analyzed according to Garg *et al*. (2002).

3.7.1 Net weight gain (g) (NWG)

The weight gain of red platy was obtained initially and at every seven days interval. The net weight gain was calculated as:

Net weight gain (g) = Final weight (g) – Initial weight (g)

$$\text{Net weight gain in percent} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{ X } 100$$

3.7.2 Specific growth rate (SGR)

The specific growth rate was calculated by the following formula

$$\text{SGR} = \frac{\text{Log } W_2 - \text{Log } W_1}{D} \text{ X } 100$$

Where,

W_1 = Initial weight of the fish in g

W_2 = Final weight of the fish in g

D = Duration of study in days

3.7.3 Food conversion ratio (FCR)

The food conservation ration was calculated by the following formula.

$$FCR = \frac{\text{Food given (g)}}{\text{Weight gain (g)}}$$

3.7.4 Gross conversion efficiency (GCE)

The gross conservation efficiency was calculated by the following formula.

$$GCE = \frac{\text{Weight gain (g)}}{\text{Food given (g)}}$$

3.8 COLOUR PARAMETERS

Colour intensity of the fishes was analyzed every week following Raymond (1992) by using colour difference meter equipment (Hunter colour lab, USA). The instrument measures colour parameters in terms of CIE L* (Luminosity), a* (red-purple to bluish green), b*(yellow to blue), h° (hue angle) and C* (Chroma).

The lightness coefficient (CIE L*), ranges from black = 0 to white = 100, a* and b* are the values on the horizontal and vertical axis respectively. (+ a*) indicates a hue of red-purple and (- a*) indicate a hue of bluish green. On vertical axis, (+ b*) indicate yellow and (- b*) indicates blue colour (Plate 1).

a* and b* are merely co-ordinates that indirectly reflects hue and chroma but are difficult to interpret separately. These co-ordinates are not independent variables (Francis 1980). Chroma (C*) indicates degree of departure from grey toward pure chromatic colour and hue (h°) indicates red, orange, yellow, green etc. a colour from the spectrum.

The proper quantification of tristimulus colorimetric data is based upon trigonometric function (Hunter, 1942).

A colour wheel subtends 360° with red-purple traditionally placed at an angle of 0°, yellow at an angle of 90°, bluish-green at an angle of 180° and blue at an angle of 270°.

The values of a* and b* decides the hue angle (h°) of red-platy. For example in the first quadrant *i.e* between +a and +b as the hue angle (h°) in increases the yellow colour increases and as the value of hue angle (h°) decreases the red colour increases.

3.9 PIGMENT ANALYSIS

The pigment analysis in *Azolla* was carried out following (Sdashivam and Manikam, 1991). For this purpose pigment of *Azolla* meal was extracted in 80 % acetone and after centrifuging the supernent was filtered and its absorbance was read on the wavelength 470 nm, 645 nm and 662 nm. The data thus collected were used to calculate the pigment using the following formulas:

$$Chlorophyll\ \ 'a' = 11.75\, A_{662} - 2.35\, A_{662}$$

$$Chlorophyll\ \ 'b' = 18.61\, A_{645} - 3.960\, A_{662}$$

$$\text{Carotene} = \frac{1000\ A_{470} - 2.270\ C_a - 81.4\, C_b}{227}$$

$$Xanthophyll = Total\ carotenoid - carotene$$

$$\text{Total carotenoid} = \frac{(A_{max}/0.25) - Ether\ \ supernatent}{Sample\ weight}$$

Where,

C_a = Chlorophyll 'a'

C_b = Chlorophyll 'b'

A_{max} = Maximum absorption

3.10 STATISTICAL ANALYSIS

The data obtained for growth parameter and colouration characteristics of red platy maintained on *Azolla* fortified diets were statistically analyzed by applying complete randomized design (CRD) and analysis of variance (ANOVA) for checking the significance ($P < 0.05$) level of treatments

ANOVA:

Source of variation	**d.f**	**Sum of squares**	**Mean sum of squares**	**F. Cal.**	**F. Tab.**
Between treatment	(t-1)	S.S.T	$\frac{S.S.T.}{d.f.} = T.M.S$	$\frac{T.M.S}{E.M.S.}$	
Within treatments	t (n-1)	E.S.S	$\frac{S.S.T.}{d.f.} = E.M.S.$		
Total	tn-1	TSS	MSS		

Where,

t =Number of treatment

n = Number of replication

If F calculated > F tabulated at 5 per cent level of significance, the results of experiments is to be considered significant. The average values of experimental results were also analyzed to draw a standard deviation from the mean.

4. EXPERIMENTAL RESULTS

The experimental results of water quality parameters carried out during the experimental period are presented in (Table 4.1), proximate composition of *Azolla* in (Table 4.2 and Figure 4.1), pigment composition of *Azolla* in (Table 4.3 and Figure 4.2). Proximate composition of experimental fish diets are depicted in (Table 4.4 and Figure 4.3). Fish growth parameters with the effect of *Azolla* mixed diet are given in (Tables 4.5 to 4.9 and Figures 4.4 to 4.7.). The results pertaining to colouration of fishes are depicted in (Tables 4.10 to 4.15and Figures 4.6 to 4.11).

4.1 THE WATER QUALITY PARAMETER

The bore well water was used as source during the whole experimental period of 8 weeks. The physico-chemical parameter of water such as air and water temperature (°C), pH, dissolve oxygen (mg/l), total hardness (mg/l)**,** total alkalinity (mg/l), and free carbon dioxide (mg/l), were recorded as reference water quality. The average values of these water quality parameters are presented in and described hereunder.

The experiment was commenced during the pre summer season and the average air temperature during the experimental period was recorded between 28.50°C to 39.40°C. There was considerable fluctuation in water temperature during the experimentation period in source water. The highest water temperature of source water was recorded as 26.90°C whereas the minimum temperature was 21.13°C. The pH of source water was in the range of 8.47 to 9.00 and thus it bears mild alkaline nature during the whole experimental period.

During the experimental period narrow fluctuation in the dissolved oxygen was recorded and the values ranged between 5.20 to 6.93 mg/l. The total alkalinity was ranged between 342.76 to 391.21 mg/l in source water (Table 4.1). As depicted in results (Table 4.1), the free carbon dioxide was absent in all the treatments during the experimental period of 8 weeks.

4.2 PROXIMATE AND PIGMENT COMPOSITION OF *AZOLLA* FISH DIET

4.2.1 *Azolla* meal

The proximate analysis of *Azolla* used in present research work was analyzed for its proximate composition and treatment composition and the results shown in Table 4.2 and Figure 4.1. The result indicates that the *Azolla* contains 94.24 % moistire on wet weight basis whereas, in air dried *Azolla* the moisture content was 1.2 %, carbohydrate 46.54 %, protein 22.9 %, fat 4.12 % and 25.24 % ash. The analysis of pigments present in *Azolla* indicated a fairly good amount of total carotenoid i.e. 493.65 µg/g. Further, analysis of pigments indicated 2.42 µg/g of carotene, 491.22 µg/g of xanthophylls, 8.36 µg/g of chlorophyll 'a' and 8.12 µg/g of chlorophyll 'b' in Table 4.3 and Figure 4.2.

4.2.2 Proximate composition of fish diets

The proximate composition of the basal diet (control) and *Azolla* mixed diets used as the experimental diets are presented in (Table 4.4 and Figure 4.3).

The result indicates marginal difference in protein contents in experimental diets. The percentage of protein in the basal or control diet is higher than that of the rest of the treatments. Among the treatments, T_1 contains highest protein (26.59 %) whereas; treatment T_5 contains lowest protein (23.95 %). It was noted that the protein contents in diets were gradually decreased as the per cent composition of *Azolla* was increased in different treatments replacing equal quantity of protein sources.

The nitrogen free extract composition of the diets indicated highest values in T_5 (43.20 %) whereas, it was minimum (37.96 %) in control diet. The fat content was maximum (14.24 %) in the control diet. The minimum percentage of fat was found in the treatment T_5 (9.18 %). The ash content was higher in T_5 (21.40 %) whereas, it was minimum (16.56 %) in the control diet. As compared to control diet, rests of the treatments have higher percentage of ash and nitrogen free extract, which was gradually increased from T_1 to T_5. Similarly it was also noted that the protein, fat and moisture contents were gradually decreased from T_1 to T_5. The moisture content in all the diet varied from control (4.24 %), T_1 (3.94 %), T_2 (3.63 %), T_3 (3.33 %), T_4 (3.04 %) and in T_5 (2.72 %).

4.2.3 Carotenoids in fish diet

The analysis of carotenoids in different treatments indicated that the total carotenoid contents 23.24 µg/g in T_1, 46.02 µg/g inT_2, 69.26 µg/g in T_3, 92.64 µg/g in T_4 and 116.28 µg/g in T_5 . No carotenoid contents were recorded in control.

4.3 GROWTH PARAMETERS

4.3.1 Net Weight Gain (NWG)

The net weight gain in *Xephophorus maculatus* fed with experimental diet during every week and in total experimentalis given in Table 4.5 and Figure 4.4.

The result indicates that a minimum weight gain was recorded in fishes fed with control diet (0.68 g). The maximum weight gain (1.40 g) was found 241.38 per cent in weight in T_3 during 8 weeks of feeding trial. There was a higher net weight gain in fishes fed with 10, 20 and 30 per cent *Azolla* mixed diet in treatments T_1, T_2 and T_3 i.e. (0.85, 1.00 and 1.40 g) respectively. However, thereafter there was a lesser increase in the net weight gain in T_4 (1.25g) and in T_5 (1.01g) which were fed with 40 and 50 per cent *Azolla* mixed diet. The maximum weight gain was recorded in T_3 (1.40 g) and minimum in control (0.68 g).

The statistical analysis of the results (Appendix I-A) of net weight gain indicate significant results ($P < 0.05$). The critical difference (CD = 0.156) between *Azolla* mixed diet and the control fish was significant. However, a non- significant difference was noted between net weight gain of T_1 and T_2.

4.3.2 Specific Growth Rate (SGR)

The Specific growth rate of *Xephophorus maculatus* fed with experimental diet is given in Table 4.6 and Figure 4.5.

The results indicate that the minimum specific growth rate was recorded in fishes fed with control diet T_6 (0.624). Whereas, the maximum specific growth rate (0.990) was found in T_3. There was a gradual increase in specific growth rate in fishes fed with 10, 20 and 30 per cent *Azolla* mixed diet i.e. in T_1 (0.700), T_2 (0.847) and T_3 (0.990) respectively. However, a lesser increase in the specific growth rate was noted in fishes fed with 40 and 50 per cent *Azolla* mixed diet i.e. in T_4 (0.895) and T_5 (0.857) respectively.

The statistical analysis of the results (Appendix I-B) of specific growth rate indicates significant results ($P < 0.05$). However, the critical difference (CD = 0.057) indicates a significant difference between *Azolla* mixed diet and the control fishes and also within the treatments except T_4 and T_5.

4.3.3 Food Conversion Ratio (FCR)

The food conversion ratio of red-platy fed with experimental diet is given in Table 4.7 and Figure 4.6.

The results indicate that the poorest food conversion ratio was recorded in fishes fed with control diet with maximum values of 2.40 and the best food conversion ratio was found in T_3 with minimum values of 1.65 in 30 per cent *Azolla* mixed diet. There was a better food conversion ratio in fishes fed with 10, 20 and 30 per cent *Azolla* mixed diet in the treatments T_1 (2.11), T_2 (1.82) and T_3 (1.65) respectively. Relatively poor food conversion ratio was found in fishes fed with increased per cent of *Azolla* mixed diets 40 % and 50 % in the treatments T_4 (1.72) and T_5 (1.75) respectively.

The statistical analysis of the results (Appendix I-C) of food conversion ratio indicate significant results ($P < 0.05$). However, the critical difference (CD = 0.382) indicates a significant difference between FCR of fishes fed with *Azolla* mixed diet and the control diet except T_1. However, there was also a significant difference within the treatments.

4.3.4 Gross Conversion Efficiency (GCE)

The gross conversion efficiency of red-platy fed with experimental diet is given in Table 4.8 and Figure 4.7.

The results indicate that the minimum gross conversion efficiency was noted in fishes fed with control diet (0.417) and the maximum gross conversion efficiency (0.607) was found in fishes fed with *Azolla* mixed diet in the treatment T_3. There was higher gross conversion efficiency in fishes fed with *Azolla* mixed diet 10, 20 and 30 per cent i.e. in T_1, T_2 and T_3 i.e. 0.473, 0.550 and 0.607 respectively. Relatively, lower gross conversion efficiency was found in fishes fed with 40 and 50 per cent *Azolla* mixed diets i.e. 0.581 and 0.570 respectively.

The statistical analysis of the results (Appendix I-D) of gross conversion efficiency indicate significant results ($P < 0.05$). However, the critical difference (CD = 0.078) indicates significant difference between fishes fed with *Azolla* mixed diet and the control diet. However, there was a non- significant difference between the treatments T_2 and T_3, T_3 and T_4, and T_4 and T_5.

4.4 EFFECT OF *AZOLLA* MIXED DIET ON COLOURATION OF RED PLATY (*XIPHOPHORUS MACULATUS)*

The effect of carotenoid present in *Azolla*, fed in diet on colouration of red-platy was stated in terms of CIE L *a*b* scale and described hereunder on Colour Co-ordinate Luminosity (CIE L*), Green- red axis (CIE a*), Yellow- blue axis (CIE b*), Hue angle (h^0) and Chroma C*.

4.4.1 CIE L* Colour Co-ordinate (Luminosity)

The data in CIE L* (Luminosity) of red-platy fed with *Azolla* mixed diet during the experimental period are presented in Table 4.10 and analysis of variance is given in (Appendix II-A).

The data presented in Table 4.10 that CIE L* (Luminosity) of red-platy are varied. It was maximum in T_5 (12.82) where the fishes were fed with 50 per cent *Azolla* mixed diet and the minimum Luminosity was found in control (9.99), where the fishes were fed with control diet (without *Azolla*). The average value of Luminosity CIE L* of all the treatments indicate that it was gradually increased from T_1 (10.40) to T_5 (12.82).

The average value of Luminosity in increasing order of all the treatments are arranged in following manner:

Control (10.23) < T_1 (10.40) < T_2 (10.59) < T_3 (11.36) < T_4 (12.10) < T_5 (12.82).

The statistical analysis of variance of Luminosity (Appendix II-A) indicate significant results (p 0.05). The ANOVA shows that Luminosity was critically different (CD= 0.719) between control and all the treatments. However a non-significant difference was observed between T_1 and T_2 and rest of the treatments are significant with each other.

4.4.2 CIE a* Colour Co-ordinate [Green (-a) to Red (+a) Axis]

The data in CIE a* colour co-ordinate of red-platy fed with *Azolla* mixed diet during the experimental period are presented in Table 4.11 and the analysis of variance is given in (Appendix II-B).

As depicted from the data presented in Table 4.11 that CIE a* colour co-ordinate of red-platy is varied. It was recorded maximum in T_5 (5.98), where the fishes were fed with 50 per cent *Azolla* mixed diet and the minimum CIE a* was found in control (4.26), where the fishes were fed with control diet i.e. without *Azolla* mixed diet. The average value of CIE a* colour co-ordinate within the treatments indicate that it was maximum in T_5 (5.98) and minimum in T_1 (4.46) *Azolla* mixed diets.

The average value of CIE a* colour co-ordinate of red-platy in increasing order of all the treatments can be arranged in following manner:

Control (4.26) < T_1 (4.45) < T_2 (4.63) < T_3 (4.90) < T_4 (5.01) < T_5 (5.98)

The statistical analysis of CIE a* colour co-ordinate of red-platy indicates significant results at $P < 0.5$ %. The ANOVA (Appendix II-A) shows that there is a critical difference (CD= 0.327) between the treatments with respect control. However, within the treatments a non- significant difference can be noticed between T_1 to T_4.

4.4.3 CIE b* Colour Co-ordinate [Blue (-b) – Yellow (+b) Axis]

The data in CIE b* colour co-ordinate of red-platy fed with *Azolla* mixed diet during the experimental period are presented in Table 4.12 and their analysis of variance is given in (Appendix II-C).

It is clear from the data presented in Table 12 that CIE b* colour co-ordinate of red-platy is varied. It was found maximum in T_5 (7.04) where the fishes were fed with 50 per cent *Azolla* mixed diet and the minimum CIE b* value was found in T_5 (4.22) where the fishes were fed control diet.

The average value of CIE b* colour co-ordinate of red-platy in increasing order of all the treatments are arranged in following manner:

Control (4.22) < T_1 (4.48) < T_2 <. (4.78) < T_3 (5.47) < T_4 (5.81) < T_5 (7.04)

The statistical analysis of variance of CIE b* colour co-ordinate of red-platy indicate significant results (P ≤ 0.05). The ANOVA shows that there is a significant critical difference (CD= 0.369) between all treatments except T_1 and T_2.

4.4.4 CIE h° (Hue Angle)

The data in CIE h° (hue angle) of red-platy fed with *Azolla* mixed diet during the experimental period are presented in Table 4.13 and Figure 4.8 to 4.113 and their analysis of variance is given in (Appendix II-D)

It is clear from the data presented in Table 4.13, Figure 4.6 to 4.11 that CIE h° (hue angle) of red-platy is varied. It was found maximum in T_5 (49.60) where the fishes were fed with 50 per cent Azoll mixed diet and the minimum CIE h° was found in control (43.99) where the fishes were fed with control diet.

The average value of CIE h° of red-platy in increasing order of all the treatments are arranged in following manner:

Control (43.33) < T_1 (43.99) < T_2 (45.16) < T_3 (47.80) < T_4 (49.02) < T_5 (49.58).

The statistical analysis of variance of CIE h° of red-platy indicates significant results (P < 0.05). The ANOVA shows that there was a non- significant difference between T_1 & T_2, and T_4 & T_5 except T_3 & T_4.

4.4.5 CIE C* (Chroma)

The data pertaining to colour saturation i.e. CIE C* (Chroma) of red-platy fed with *Azolla* mixed diet during the experimental period are presented in Table 4.14 and their analysis of variance is given in (Appendix II-E)

As depicts from the data presented in Table 4.14 the CIE C* (Chroma) of experimental red-platy is varied. It was maximum in T_5 (9.28) where the fishes were fed with 50 per cent *Azolla* mixed diet and the minimum value of CIE C* (chroma) was found in control (6.00).

The average value of CIE C* (Chroma) of red-platy inincreasing order of all the treatments can be arranged in following manner:

Control (6.00) < T_1 (6.33) < T_2 (6.71) < T_3 (7.34) < T_4 (7.70) < T_5 (9.28).

The statistical analysis of CIE C* (Chroma) colour of red-platy indicates significant results at $P < 0.5$ %. However, the ANOVA shows that a critical difference (CD= 0.495) between treatments T_1 to T_5 as compared to control and a non-significant difference within the treatments except T_4 & T_5.

5. DISCUSSION

The results obtained in the present research exhibit varied growth rate and colour enhancement in red-platy fed with different level of *Azolla* mixed diets (Tables 4.4 to 4.15). The water quality in experimental phase (Table 4.1) was congenial during the period of 8 weeks. The results obtained during this study are discussed here under.

5.1 WATER QUALITY

As an important abiotic environmental factor, the water quality determines the growth of fish to a great extent. The water temperature influences the rate of chemical transformation (Jhingran, 1988) in fishes. The rate of digestion and frequency of food exchange in the digestive tract of fishes are mainly determined by ambient temperature (Janeck, 1976). During the experimental period (pre summer season) the maximum water temperature was 26.90°C and the minimum 21.13°C. This has shown a moderate metabolic rate of fish as evidenced by their growth (Table 4.1). However, it is also important to determine the growth of fishes in different climatic conditions and Rajasthan is a good example of such studies where temperature varies from below 5°C in the winter season to above 40°C in the summer.

The hydrogen ion concentration of natural water is an important environmental factor, the variation of which is linked with life processes of animals and plants inhibiting them. The pH of water undergoes wide fluctuation. The diurnal change in pH being most alkaline in afternoon and most acidic just before the day breaks. George (1969) has shown that the pH of pond water varied between 7.3 and 8.4. Swingle (1957) stated that waters having a pH range of 6.5 - 9.0 are most critical for fish culture. Lagler (1972) has also suggested that quality of water having pH range of 7.0 - 8.5 is favorable for fishes. During the present study the pH of water in glass aquaria ranged between 8.5 to 9.0 which is alkaline and was found suitable for fish growth. Kour (2003) found a pH range of 8.3 - 8.8, fairly congenial for aquarium experiment of fish nutrition using Bala (*Sida cordifolia* Linn.) supplemented diets.

The dissolved oxygen in water greatly determines growth and health of fishes residing therein. The lowest limit of dissolved oxygen for good fish production in pond has been suggested to be 5 mg/l (Dandraff and Dean, 1967). However,

Smitherman and Boyd (1974) considered minimum dissolved oxygen level at 2 mg/l for proper health and growth of fish in normal pond condition. In the present study the average dissolved oxygen in experimental waters ranged between 5.2 to 6.9 mg/l. However, the continuous aeration of water, removal of faecal matter as well as renewal of experimental water has largely helped in maintaining good dissolved oxygen levels during the whole experimental period.

The free CO_2 in all the treatments were absent during the experimental. Free CO_2 at the concentration of 15 mg/l is detrimental for fish growth (Swingle, 1967).

The total alkalinity of water, which is caused by the cations combined with either carbonate, or bicarbonates, is an important factor for pond productivity. Alikunhi (1957) has stated that in highly productive water, the alkalinity is ought to be over 100 ppm. Benerjee (1967) found water having total alkalinity above 90 mg/l to be productive. Higher alkalinity has a greater complement to most of the ions than waters with low alkalinity (Moyale, 1946). The total alkalinity of experimental water in the present study ranged from 342.7 to 391.2 mg/l which is in agreement with the earlier findings.

The total hardness of bore well water used for the experiment indicates that water was hard with a water hardness of 450-580 mg/l (Table 4.1). Swingle (1967) has suggested a hardness from 15 ppm or above as satisfactory for growth of fish and do not require addition of lime.

5.2 PROXIMATE AND PIGMENT COMPOSITION OF *AZOLLA*

The proximate composition of *Azolla* used in the present study is shown in Table 4.2 and Figure 4.1. The water fern *Azolla*, which grows in association with the blue green alga *Anabaena azollae*, a nitrogen fixing organism, is perhaps the most promising from the point of view of ease of cultivation, productivity and nutritive value (Lumpkin and Plucknette, 1982; VanHove and Lopez, 1982). Aquatic plant, free floating fern *Azolla* which belongs to the family *Azollaceae* is a good source of protein and it contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese etc, apart from appreciable quantities of vitamin 'A' precursor beta-carotene and vitamin B_{12} (Pillai *et al.*, 2002). These authors have also reported that *Azolla* contain probiotics and biopolymers. The use of *Azolla* as a feed resource for fish, swine and poultry had been tested with

favourable results (Castillo *et al.*, 1981; Alcantara and Querubin, 1985). In the present study the crude protein (CP) content in *Azolla* was 22.9% which indicated that *Azolla* could be used as a potential natural protein source in fish and livestock feeds. The CP value estimated in the present research work are almost similar to the results (23.49 %) obtained by Cherryl et al.(2014), Balaji *et al.* (2009), Kumar *et al*. (2012) and Singh and Subudhi (1978). However, Parthasarathy *et al.* (2001b) and Basak *et al.* (2002) reported slightly higher values of CP *i.e.* 26.62 and 25.78 per cent respectively in *Azolla* meal.

From this study it was revealed that the ether extract of *Azolla* meal was 4.12%, which was in agreement with Singh and Subudhi (1978), Basak *et al*. (2002), Titus and Periera (2006), Balaji *et al*. (2009) who found it in the range of 3.7 to 4.3 per cent.

The total ash content of *Azolla* obtained in this study was 25.24 percent, which is slightly higher than the ash content obtained (24.26 %) by Cherryl et el. (2014), Subudhi (1978), Parthasarathy *et al.* (2001b), Basak *et al*. (2002), Alalade and Iyayi (2006), Titus and Periera (2006), Balaji *et al*. (2009) and slightly lesser than that reported by Kumar *et al.* (2012).

The *Azolla* (*Azolla pinnata)* used in the present study has indicated 493.65 µg/g of total carotenoids contents. Further, it was revealed that the *Azolla* contains 2.42 µg/g of carotene, 491.22 µg/g of xanthophylls, 8.36 µg/g of chlorophyll 'a' and 8.12 µg/g of chlorophyll 'b'. This indicated the presence of the maximum amount of xanthophylls out of the total carotenoid in *Azolla* used. Lejeune et al. (2000) reported the range of carotenoid contents from 298 ± 10.03 to 424 ± 7.1 µg/g in *Azolla pinnata* var *pinnata* and 326 ± 11.2 µg/g to 488 ± 10.6 µg/g in *Azolla pinnata* var. *imbricata* while evaluating carotene contents and its variations during drying and storage among six *Azolla* strains produced in two culture conditions. Further, these authors found 60 % less carotene content during a storage duration of 120 days. These authors also pointed that the carotenoid contents of *Azolla* are comparable to the 451 and 556 µg/g of carotenoids in the dry matter obtained from carrot and spinach respectively. These two plants are generally considered as rich in carotenoids.

5.2 PROXIMATE COMPOSITION OF FISH DIETS

The proximate composition of experimental diet fed to *Xiphophorus maculatus* has been shown in Table 4.4 and Figure 4.3. The results indicate gradual differences between the basal *i.e.* control and *Azolla* mixed diets. The results indicate that the nitrogen free extract varied from (37.96 per cent) in control diet to a maximum of (43.20 per cent) in 50 % *Azolla* mixed diet T_5. The lipid value was minimum (9.18 per cent) in 50 % *Azolla* mixed diet T_5 to a maximum of (14.24 per cent) in control diet. The protein content varied between (23.95 per cent) in control T_5 to a maximum of (27 per cent) in control diet.

The ash content varied from minimum (16.56 per cent) in control diet to a maximum of (21.40 per cent) in 50 % *Azolla* mixed diet T_5. The moisture content of diet varied between (2.72 per cent) to (4.24 per cent). The experimental diet in the present study was prepared following Ramamoorthy *et al*. (2010) who used *Azolla* as a protein as well as carotenoid source in the experimental diets. The basal diet was prepared using soya bean, Fish meal, Groundnut oil cake, Wheat flour, Tapioca flour, Rice bran and Vitamins. However, the authors did not report proximate composition of the prepared experimental diet. Instead the individual proximate composition of feed ingredient was reported. The protein content with different ingredients varied from 2.3 to 70.5 per cent, carbohydrate 3.5 to 62.7 per cent, lipid value from 1.3 to 76.5 per cent of different feed ingredients. The diet incorporated with Carrot (*Daucus carota* 6.03 ± 0.572 mg/g/weight gave best survival and growth in *Amphiprion ocellaris* (Cuveir 1880) an ornamental marine fish.

Deka (2007) reported better growth of *Cirrhinus mrigala* fed with the diet containing (24.9 per cent) protein, (9.8 per cent) fat and (42 per cent) carbohydrate. The results of proximate composition in present study are comparable to Dhangar (2004) and Rathore (2005). Kour (2007) used Sarpgandha as colour enhancer mixed in a basal diet formulated with Rice bran and Groundnut oil cake containing 40.93 per cent carbohydrate, 25.02 per cent protein, 14.25 per cent fat, 13.4 per cent ash and 6.4 per cent moisture. Further, the energy values (392.05 kcal/100 g) could not reflect any significant impact on the fish growth in terms of total weight gain in 60 days. Minal (2013) while studying effect of spinach and carrot on pigmentation of swordtail (*Xephophorus hellerii*) used a basal diet composed of 20 per cent protein, 14.1 per

cent fat, 46.89 per cent carbohydrate and 18.60 per cent ash with three levels of β-carotene at 20, 25 and 30 mg/100g of diet.

5.3 EFFECT OF *AZOLLA* MIXED DIET ON GROWTH PERFORMANCE OF RED-PLATY

The result of growth parameters of experimental fishes are presented in Table 4.5 to 4.9 and Figure 4.4 to 4.7. The results indicate that a better growth performance was noticed in fishes fed with *Azolla* mixed diet (10%, 20% and 30%) upto a certain extent *i.e.*, from T_1 to T_3 and there after their growth was decreased in T_4 and T_5, where the fishes were fed with 40% and 50% *Azolla* mixed diet respectively. Whereas, the minimum growth was recorded in fishes fed with control diet. The same diets indicated better specific growth rate (SGR) in *Azolla* mixed diets from T_1 to T_3. The feed conversion ratio (FCR) was maximum in control compared to rest of the treatments which were fed with *Azolla* mixed diet. The result also reveal better gross conversion efficiency (GCE) of *Xiphophorus maculatus* when they were fed with 30 % *Azolla* mixed diet. As such the result in the present study reveals better growth performance and food conversion ratio when fishes were fed with 30 % *Azolla* mixed diet replacing the equal quantity of protein suppliments in basal diet.

The results in present investigation were also found in accordance to several researchers who found herbal ingredients as growth promoter in supplementary diet of fish. Kumar (2000) used the herb Ashwagandha (*Withania somnifera* L. Dunal), Kumar (2000) used Safed musli *(Asparagus adscendens* Roxb.), Rajkumar (2002) found Kali musli (*Circuligo orchioides Gaertn*.) and mulethi (*Glycyrrhiza glabra* Linn.), Kour (2003) investigated Bala (*Sida cordifolia* Linn.) and Singh (2003) used Makhana (*Euryale ferox* Salisb) and Kour (2007) successfully used Satavari (*Asparagus racemous* Wild) and Ashwagandha (*Withania somnifera* L. Dunal) as herbal growth promoter in fish diet. The dietary *Azolla* supplementation was found to have positive effect on the growth of fish in the present study. A fairly good amount of carotenoids (493.65 µg/g) was found in the *Azolla* meal used in the feeding trails. Its variations replacing equal quantity of protein source in the basal diet also revealed variation in growth parameters of experimental fish. The present study showed that various amount of dietary *Azolla* increased the growth of fish. Several authors used

other natural carotenoid sources as dietery supplement and found increased growth in fish. The results of the present study agrees with the study of Ezhil *et al.* (2008) who reported that feeding with marigold petal 15 g/100 g increased the growth rate of Red Swordtail (*Xiphophorus helleri)* reared for 60 days. Sinha and Asimi (2007) studied the growth rate of fishes in the group fed with the China rose petal feed was the highest in terms of weight, with an increased value of carotenoid in skin (4.0 microgram/g). However, Bell *et al.* (2000) reported insignificant effect of dietary supplement of - Astaxanthin 70 mg/kg on the growth of Atlantic salmon (*Salmo salar*) reared for 22 weeks.

In a study on effect of various natural carotenoids on the fish growth and colouration Ramamoorthy *et al*., (2010) found that the synthetic Astaxanthin was more effective than red pepper and marigold flower although they contained equal amounts of carotenoids. In their study the *Azolla* was absorbed better at a certain dose amount (14.1 g) *i.e.,* 30 % and then as the dose of *Azolla* increased (18.8 g and 23.5 g) *i.e.* 40 % and 50 % respectively their absorption got decreased. The results of increasing growth of *Xephophorus maculatus* during the present study are in agreement to Minal (2013) who found increased growth performance in *Xephophorus hellerii* using carrot and spinach as β–carotene in diet.

5.4 EFFECT OF *AZOLLA* MIXED DIET ON COLOURATION OF RED-PLATY

The results pertaining to the effect of diet as carotenoid source on colouration of red-platy are presented in Table 4.10 to 4.14 and figure 4.6 to 4.11. From the comparative colour characteristics of red-platy fed with *Azolla* mixed diet containing 23.24, 46.54, 69.24, 92.68 and 116.27 µg/g carotenoids *i.e.* in T_1, T_2, T_3, T_4 and T_5 respectively increased in colour intensity in red platy. The results when compared to the control fishes, revealed that the luminosity was increased in the fishes fed with *Azolla* mixed diets and the maximum luminosity was observed in the fishes fed with 50 per cent *Azolla* mixed diet.

The maximum of yellow colour intensity (b* value) was seen in fishes fed with *Azolla* mixed diets. The results indicated that as the concentration of *Azolla* increased in the diet, the yellow colour intensity also increased from T_1 to T_5 where

fishes fed were with 10 to 50 per cent *Azolla* mixed diet (Table 4.12). The present study thus indicated that at the yellow colour intensity is increased up to T_5.

The comparative colour characteristics of hue angle (h°) *i.e.* the angle between the hypotenuse and zero degree (0°) on the a* (red-purple/Bluish-green) axis, indicates a positive value (+) in all the treatments including control. However, the maximum of hue angle (h°) was found in T_5 followed by T_4 (Table 4.13 and figure 4.6 to 4.11. The results indicated that as the concentration of *Azolla* increased in the diet the hue angle (h^0) also increased and the minimum angle formed by control diet fed fishes whereas, maximum angle was formed by the fishes fed *Azolla* mixed diet.

The study further revealed that all the *Azolla* treated fishes have shown an increasing hue angle (h^0) on the x-axis whereas, the control fishes have shown less red-purple on the x-axis with lower hue angle. The results are also supported by Minal (2013) who found more of orange colour compared to control using carrot and spinach as β-carotene source in fish diet higher the hue angle compared to control while studying colouration in *Xephophorus hellerii*. The β-carotene is reported to enhance orange colour with increasing hue angle.

The saturation of colour as indicated by C* (Chroma) in Table 4.14 reveals that the maximum colour saturation was observed in the T_5 where the fishes fed with 50 per cent *Azolla* mixed diet and minimum saturation were found in the fishes were fed with control diet.

Colouration in fishes is influenced by several external and internal factors including dilation of blood capillaries, reflective irridocytes, chromatophores, genetic and environmental factors. An array of natural and artificial colour enhancing compounds and fish diets have also been tried by several researchers. The skin colour in fishes could be changed up to a certain extent by altering surrounding conditions, light intensity and dietary conditions (Waagbe, 2002). It was found that blue green algae and spirulina as feed greatly influenced colour development of fish. It was further, reported that in the absence of this colour enhancing algae, fishes maintained on the diet of plankton (control diet) failed to produce the natural red colour in cherry barbs. The use of natural microalgae *Chlorella vulgaries*, *Haematococcus pluvialis* and Cyanobacteria – *Arthrospira maxima* and *Spirulina platensis* have also been reported to be carotenoid source for enhancing skin pigmentation and prominent

colouration in ornamental fishes (Ako *et al.*, 2000; Gouveia *et al*., 2003 and Gouveia and Rema, 2005).

Colour enhancing agent as feed additive from plant origin have been successfully tried in ornamental fishes. Pariapatananont *et al.* (1999) have observed the effect of astaxanthin on the pigmentation of gold fish (*Carassius auratus*) and measured a significant increase in skin pigmentation and survival of fish maintained on diets containing astaxanthin 36-37 mg/kg. Mathew and Gopakumar (1992) found enhancement of pink colour in the fishes and flesh of Tilapia by feeding red colour extracted from sandalwood. Hancz *et al.* (2003) have evaluated colour intensity enhancement by paprika as feed additive in gold fish and koi carp using a computer – assisted image analysis. Here the 'r' value had a tendency to increase due to paprika feeding but significant difference was achieved only after four weeks of feeding.

Gupta *et al.* (2007) experimented some naturally available carotenoid rich ingredients such as micro algal pigments, yeast extract, marigold, capsicum etc. and discussed their utility for enhancement of pigmentation in fishes. They recommended 125 ppm carotene which gave excellent pigmentation and higher doses (200-300) ppm further improved pigmentation. However in the present investigation the higher doses of *Azolla* in diet gave better red-purple and yellow colouration in *Xiphophorus maculatus*.

Kour (2007) evaluated efficiency of Sarpgandha (*Rauwolfia serpentine* Benth) in fish diet for enhancement of colouration in Koi carp. The maximum colour enhancement in plain and spotted koi carp was noticed with Sarpgandha feeding @ 0.06 g/kg body weight/day. Ramamaorthy *et al.* (2010) estimated prominent composition and carotenoid content of natural carotenoid sources and their impact on marine ornamental fish *Amphiprion ocellaris* (Cuveir, 1880) for colour enhancement. It was found that pigmentation in fish was the highest in diets added with certain amount of *Azolla i.e.* 30 per cent (14.1 g/100g of diet).

Kop *et al.* (2010) examined influence of carrot (*Daucus carota*) and red pepper (*Capsicum annum*) as natural pigment material on colouration of cichlid *Cichlasoma severum* (Heckel, 1840). Carotenoid amount in the fish samples fed with red pepper and carrot diets were noticed to be 5.25 ± 0.90 and 5.60 ± 0.29 mg g^{-1}, respectively. Consequently a significant difference was found between individuals fed

on natural pigment material in comparision to those on unpigmented feeds ($P < 0.05$). Dharmaraj and Dhevendaran (2011) studied the application of microbial carotenoids as a source of colouration in *Xiphophorus hellerii* and obtained microbial carotenoids to improve the pigmentation pattern in the fishes effectively. The author's indicated that the fishes administrated carotenoid enriched feed showed faster recovery of carotenoids in the skin of the fishes when compared to the control ($P \square 0.01$).

Ylmaz and Ergun (2011) while examining the effect of two red pepper (*Capsicum annum*) meal on the skin coloration of juvenile blue streak hap (*Labidochromis caeruleus*) found it to be appropriate as an alternate natural carotenoid source (50 g/kg) to ensure good pigmentation, suitable growth, and feed utilization.

The carotenoid content microalgae and Spirulina are well utilized to enhance the skin colouration in fishes. Guroy *et al.* (2012) used *Spirulina* as a natural carotenoid source on pigmentation of yellow tail cichlid *Pseudotropheus acei*. Results indicated that *Spirulina* meal has the potential to enhance the coloration on yellow tail cichlid. Kouba *et al.* (2013) determined the effects of increasing the levels of dietary carotenoid-rich microalgae biomass on the skin colouration of angel fish (*Pterophyllum scalare*).

There is a general tendency amongst aquatic animals to preferentially accumulate xanthophylls rather than carotene pigments. Xanthophylls are one of the types of carotene which include; Astaxanthin (red), canthaxanthin (pink), zeaxanthine (orange) and lutein (yellow). Ako *et al*. (2000) has reported that the colour intensity in cichilid, *Cichlasoma myrnae* was diminished when fish were stressed during a feeding trial with top coated algae in the diet. The gold fish *Carassius auratus* belonging to Cyprinidae family is able to biosynthesize astaxanthin from lutein sources (Hata and Hata, 1975). As carotenoid degenerate over time in the same way as vitamins, the fish colours can fade. The rate of colour change was generally fastest during the first three week of trial out of which lutein fed at the level of 3 per cent in the diet resulted in fastest and most significant colour enhancers. However, in the present investigation the skin colouration of *Xiphophorus maculatus* has indicated an increasing pattern with prolonged feeding of *Azolla* mixed diets. From these observations, it can be inferred that for maintaining appropriate colour in ornamental fish, suitable pigment enhancing agent should invariably form part of fish diet on a prolonged basis.

Table 4.5 Weight gain and net weight gain (NWG) of *Xephophorus maculatus* fed with *Azolla* mixed diet during the experimental period

Treatments	Initial weight(g)	Fish Weight Gain (g)								Total NWG
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	
T_1	0.58	0.07	0.11	0.13	0.14	0.13	0.11	0.09	0.07	0.85
T_2	0.51	0.08	0.12	0.15	0.17	0.15	0.12	0.11	0.10	1.00
T_3	0.58	0.08	0.17	0.20	0.22	0.21	0.19	0.18	0.15	1.40
T_4	0.58	0.08	0.17	0.18	0.20	0.19	0.17	0.15	0.12	1.25
T_5	0.50	0.08	0.12	0.13	0.16	0.15	0.14	0.13	0.10	1.01
Control	0.55	0.06	0.10	0.12	0.13	0.11	0.08	0.05	0.03	0.68
CD	---	0.019	0.016	0.028	0.025	0.027	0.028	0.143	0.044	0.156
CV		13.95	6.88	10.32	8.41	9.81	10.45	67.71	26.11	8.31
SEm±		0.006	0.005	0.009	0.008	0.009	0.009	0.046	0.014	0.050

Table 4.6 Specific growth rate (SGR) of *Xephophorus maculatus* fed with *Azolla* mixed diet during the experimental period

Treatments	Specific Growth Rate (SGR) g/day in per cent								Average SGR
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	
T_1	0.707	0.970	0.980	0.906	0.737	0.562	0.425	0.311	0.700
T_2	0.904	1.235	1.174	1.107	0.836	0.596	0.500	0.422	0.847
T_3	0.802	1.422	1.339	1.201	0.963	1.124	0.607	0.464	0.990
T_4	0.802	1.422	1.218	1.121	0.905	0.711	0.566	0.418	0.895
T_5	0.921	1.167	1.057	1.094	0.875	0.719	0.600	0.425	0.857
Control	0.642	0.942	0.969	0.903	0.673	0.447	0.264	0.153	0.624
CD	0.080	0.115	0.109	0.087	0.068	0.064	0.041	0.030	0.057
CV	5.63	5.40	5.44	4.61	4.62	5.19	4.65	4.69	3.88
SEm±	0.026	0.037	0.035	0.028	0.022	0.021	0.013	0.010	0.018

Table 4.7 Food conversion ratio (FCR) of *Xephophorus maculatus* fed with *Azolla* mixed diet during the experimental period

Treatments	Food Conversion Ratio (FCR)								
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average FCR
T_1	1.95	1.45	1.44	1.55	1.87	2.42	3.17	4.29	2.11
T_2	1.55	1.26	1.22	1.28	1.67	2.29	2.71	3.19	1.82
T_3	1.73	1.03	1.08	1.19	1.46	1.93	2.25	2.91	1.65
T_4	1.73	1.03	1.18	1.27	1.55	1.94	2.41	3.22	1.72
T_5	1.52	1.23	1.34	1.30	1.60	1.92	2.28	3.17	1.75
Control	2.14	1.49	1.45	1.55	2.04	3.02	5.04	8.61	2.40
CD CV SEm±	0.359 11.30 0.117	0.164 7.44 0.053	0.245 10.66 0.080	0.176 7.28 0.057	0.194 6.41 0.063	0.614 15.87 0.199	7.251 171.07 2.353	1.853 23.81 0.601	0.382 11.33 0.124

Table 4.8 Gross conversion efficiency (GCE) of *Xephophorus maculatus* fed with *Azolla* mixed diet during the experimental period

Treatments	Gross Conversion Efficiency (GCE)								
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average GCE
T_1	0.513	0.689	0.696	0.647	0.534	0.412	0.315	0.233	0.473
T_2	0.646	0.794	0.821	0.778	0.600	0.436	0.369	0.313	0.550
T_3	0.577	0.975	0.925	0.838	0.685	0.517	0.444	0.343	0.607
T_4	0.577	0.975	0.849	0.787	0.646	0.516	0.415	0.311	0.581
T_5	0.657	0.816	0.746	0.770	0.627	0.521	0.439	0.315	0.570
Control	0.468	0.671	0.688	0.645	0.490	0.331	0.198	0.116	0.417
CD CV SEm±	0.068 6.54 0.022	0.055 3.72 0.018	0.112 7.94 0.036	0.078 5.96 0.025	0.050 4.70 0.016	0.088 9.99 0.029	0.471 77.04 0.153	0.126 25.96 0.041	0.078 8.08 0.025

Table 4.9 Growth parameters of *Xephophorus maculatus* fed with *Azolla* mixed diet during the experimental period of 8 weeks

Treatments	Initial individual weight of fish (g)	Net weight gain (g)	Specific growth rate (SGR)	Food conversion ratio (FCR)	Gross conversion efficiency (GCE)
T_1	0.58	0.85	0.700	2.11	0.473
T_2	0.51	1.00	0.847	1.82	0.550
T_3	0.58	1.40	0.990	1.65	0.607
T_4	0.58	1.25	0.895	1.72	0.581
T_5	0.50	1.01	0.857	1.75	0.570
Control	0.55	0.68	0.624	2.40	0.417
CD CV SEm±	---	33.474 9.83 10.864	0.057 3.88 0.018	0.382 11.33 0.124	0.078 8.08 0.025

Table 4.10 Colour parameter: L*value of experimental *Xephophorus maculatus* fed with *Azolla* mixed diets

Treatments	Carotenoids in diets (µg/g)	Observations									
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average
T_1	23.24	9.01	9.19	9.39	10.12	10.49	10.87	11.19	11.53	11.82	10.40
T_2	46.54	9.11	9.32	9.54	10.12	10.64	11.08	11.52	11.91	12.08	10.59
T_3	69.24	10.11	10.21	10.45	10.84	11.39	11.79	12.11	12.51	12.82	11.36
T_4	92.68	11.34	11.51	11.65	11.75	11.92	12.31	12.65	12.84	12.93	12.10
T_5	116.27	12.35	12.45	12.57	12.67	12.81	12.92	13.01	13.16	13.43	12.82
Control	Nil	8.19	8.49	8.89	9.11	10.36	10.91	11.08	11.31	11.55	9.99
CD CV SEm±	---		---	---	---	---	---	---	---	---	0.719 3.59 0.233

Table 4.11 Colour parameter: a* value of experimental *Xephophorus maculatus* fed with *Azolla* mixed diets

Treatments	Carotenoids in diets (µg/g)	Observations									
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average
T_1	23.24	3.6	3.7	3.9	4.0	4.5	4.7	4.8	5.1	5.8	4.46
T_2	46.54	3.7	3.9	4.0	4.1	4.6	5.0	5.2	5.3	5.9	4.63
T_3	69.24	4.0	4.3	4.4	4.5	4.7	5.0	5.5	5.7	6.0	4.90
T_4	92.68	4.1	4.4	4.5	4.7	4.8	5.1	5.5	5.9	6.1	5.01
T_5	116.27	5.0	5.2	5.4	5.6	6.0	6.4	6.6	6.7	6.9	5.98
Control	Nil	3.5	3.6	3.7	3.8	4.0	4.5	4.7	4.9	5.6	4.26
CD **CV** **SEm±**	---	---	---	---	---	---	---	---	---	---	0.327 3.77 0.106

Table 4.12 Colour parameter: b* value of experimental *Xephophorus maculatus* fed with *Azolla* mixed diets

Treatments	Carotenoids in diets (µg/g)	Observations									
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average
T_1	23.24	2.9	3.1	3.4	3.8	4.4	4.7	5.1	5.9	7.0	4.48
T_2	46.54	3.1	3.4	3.7	4.0	4.6	5.1	5.7	6.2	7.2	4.78
T_3	69.24	4.0	4.4	4.6	4.8	5.1	5.7	6.4	6.9	7.3	5.47
T_4	92.68	4.4	4.8	5.0	5.3	5.5	6.0	6.6	7.2	7.5	5.81
T_5	116.27	5.6	5.9	6.2	6.5	7.0	7.5	7.9	8.2	8.6	7.04
Control	Nil	2.8	3.0	3.2	3.5	3.9	4.5	4.9	5.6	6.6	4.22
CD CV SEm±	---	---	---	---	---	---	---	---	---	---	0.369 3.92 0.120

Table 4.13 Colour parameter: Hue angle (h⁰) of experimental *Xephophorus maculatus* fed with *Azolla* mixed diets

Treatments	Carotenoids in diets (µg/g)	Observations									
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average
T_1	23.24	38.85	39.95	41.08	43.53	44.36	45.00	46.73	49.15	50.35	44.33
T_2	46.54	39.95	41.08	42.77	44.3	45.00	45.56	47.62	49.47	50.67	45.16
T_3	69.24	45.00	45.65	46.27	46.84	47.34	48.74	49.32	50.44	50.58	47.80
T_4	92.68	47.02	47.49	48.01	48.43	48.88	49.63	50.19	50.66	50.87	49.02
T_5	116.27	48.24	48.60	48.94	49.52	49.39	49.52	50.12	50.75	51.29	49.60
Control	Nil	38.65	39.8	40.85	42.64	44.27	45.00	46.19	48.81	49.68	43.99
CD CV SEm±	---	---	---	---	---	---	---	---	---	---	2.890 3.48 0.938

Table 4.14 Colour parameter (C^*): value of experimental *Xephophorus maculatus* fed with *Azolla* mixed diets

Treatments	Carotenoids in diets (µg/g)	Observations									
		Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Average
T_1	23.24	4.62	4.83	5.17	5.52	6.30	6.65	7.00	7.80	9.09	6.33
T_2	46.54	4.83	5.17	5.49	5.73	6.50	7.14	7.71	8.57	9.3	6.72
T_3	69.24	5.65	6.15	6.36	6.58	6.93	7.58	8.43	8.94	9.45	7.34
T_4	92.68	6.01	6.59	6.73	7.08	7.30	7.95	8.59	9.30	9.66	7.69
T_5	116.27	7.86	7.86	8.22	8.58	9.22	9.86	10.29	10.59	11.02	9.28
Control	Nil	4.48	4.68	4.89	5.16	5.58	6.36	6.79	7.44	8.65	6.00
CD CV SEm±	---	---	---	---	---	---	---	---	---	---	0.495 3.85 0.161

Table 4.4 Proximate composition and carotenoid content in experimental diets fed to *Xephophorus maculatus*

	***Azolla* mixed diets**					**Basal diet**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**Control**
Protein (%)	26.59	26.18	24.67	24.26	23.95	27.00
Fat (%)	13.23	12.25	11.20	10.20	9.18	14.24
Nitrogen free extract (%)	38.37	39.49	41.34	42.07	43.20	37.96
Ash (%)	17.53	18.45	19.46	20.43	21.40	16.56
Moisture (%)	3.94	3.63	3.33	3.04	2.72	4.24
Carotenoids (µg/g)	23.24	46.54	69.24	92.68	116.27	Nil

Table 4.1 Ranges of water quality parameters during the experimental period of 8 weeks

Parameters	Control	T_1	T_2	T_3	T_4	T_5
Temperature (ºC)	**21.27- 26.63** (23.41 ± 5.36)	**21.43-26.90** (23.45 ± 5.47)	**21.13-26.83** (23.38 ± 5.70)	**21.47-26.90** (23.44 ± 5.53)	**21.50-26.63** (23.37 ±5.13)	**21.73-26.77** (23.58 ± 5.04)
pH	**8.50-9.0** (8.81 ± 0.50)	**8.47-9.0** (8.77 ± 0.53)	**8.50-9.0** (8.74 ± 0.50)	**8.60-8.97** (8.79 ± 0.37)	**8.60-8.93** 8.77 ± 0.33	**8.63-8.93** (8.78 ± 0.30)
DO (mg/l)	**5.87-6.80** (6.47 ± 0.93)	**5.47-6.67** (6.17 ± 1.20)	**5.60-7.07** (6.33 ± 1.47)	**5.47-6.80** (6.13 ± 1.33)	**5.20-6.93** (6.13 ± 1.73)	**5.20-6.80** (6.30 ± 1.60)
Total Hardness (mg/l)	**366.67-486.67** (440.00 ± 120.00)	**373.33-488.33** (425.42 ± 115.00)	**386.67-486.67** (426.67 ± 100.00)	**381.67- 483.33** (434.58 ± 102.26)	**373.33-476.67** (420.83 ± 103.34)	**373.33-483.33** (434.17 ± 110.00)
Total Akalinity (mg/l)	**345.25-388.45** (366.58 ± 43.20)	**360.35-388.56** (374.45 ± 28.21)	**355.51-379.63** (367.07 ± 24.12)	**349.58-382.45** (366.01 ± 32.87)	**356.56-391-21** (373.88 ± 34.65)	**342.76-376.55** (364.65 ± 33.79)

6. SUMMARY AND RECOMMENDATION

Since, healthy and brightly coloured varieties of ornamental fishes have relatively higher demand in aquarium trade; there is immense scope in growing healthy and colourful aquarium fishes. In this regard attempts have been made in formulation of fish diet with several compositions for promoting fish growth as well as fish colouration. Certain non conventional protein sources such as aquatic weeds (*Azolla*), plant and plant product may be used as experimentation. These can be utilized in fish feed with replacing some protein rich ingredients to minimized the expenditure on fish diet. Few of such plants *viz.* Marigold petals, Rose petals, Capsicum, Beet root, Sweet potato, Carrot, Spinach etc. were found suitable to enhance fish colour and growth.

The present investigation deals with the effect of dietary *Azolla* as a source of protein as well as carotenoids which enhances the growth and body skin colouration of an ornamental fish *viz.* Red platy (*Xiphophorus maculatus*) representing live bearer fish.

The thesis comprises 6 chapters, including 116 tables, 13 figures, elaborating introduction of the contest, reviews, materials and methods, results, discussion and recommendations.

The first chapter of the thesis highlights the present scenario of Indian fisheries. Further, the trade, scope of ornamental fish culture and the need for improving its health and colouration have been delt in brief. This chapter justifies the study of impact of *Azolla* mixed diets as colour and growth enhancer. The chapter also depicts objectives of the present research work -

i. To assess the effect of dietary *Azolla* on skin colouration of red platy.
ii. To determine the efficacy of *Azolla* mix diet on growth performance of red platy.

The second chapter of the thesis deals with an extensive review of literature including, role of herbal growth promoter, dietary formulation from plant origin on ornamental and commercial fishes. The latest reviews dealing with the proximate composition of *Azolla* and their impacts on the fishes and livestock on colouration and growth in the ornamental fishes have also been presented in this chapter.

The materials and methods used in the present investigation are presented in chapter three. The fishes were acclimatized in FRP tank; later the experiments were conducted in glass aquaria of size 2x1x1 feet. In all 18 glass aquaria were used to study the impacts of *Azolla* mixed diets on growth and colouration of test fishes. The water quality was maintained by aeration, removal of faecal matter and partial replacement of water. The experimental diets were prepared by replacing protein rich ingredients such as groundnut oil cake, soybean meal and fish meal in equal quantity by different levels of *Azolla* in the basal diet to supply 10, 20, 30, 40 and 50 per cent Azolla meal in treatments T_1, T_2, T_3, T_4 and T_5 respectively. The fishes were fed at the rate of 3 per cent of their body weight every day and the ration were readjusted according to the increase in fish body weight at every week. The observations for increase in fish body weight and colour enhancement were conducted every week.

During this period, the analysis of experimental water and proximate composition of *Azolla* as well as diets were conducted. During the experimental period various growth parameter of fish were recorded.

The study of colour intensity in fish in each experimental trial was analyzed by using colour difference meter equipment (Hunter Colour Lab, USA).

Chapter four of this thesis comprises results of the experiments conducted on red platy using Azolla mixed diet and basal diet for the control fishes. Various experimental results explained in this chapter are inferred from following experiments:

(i) Effect of *Azolla* mixed diets on growth of red platy.

(ii) Analysis of Proximate composition of fish diet as well as *Azolla* and also analyzed the pigment composition of *Azolla*.

(iii) Effect of *Azolla* mixed diets on colouration of experimental fish.

Besides these, results pertaining to water quality of experimental waters have also been explained.

The water quality during the experimental period was congenial for the rearing and growth of fishes. However, considerable fluctuation in water temperature was observed 21.13 to 26.90°C. The pH of experimental water was in the range 8.47 to 8.97. A considerable fluctuation in dissolved oxygen was recorded and the value ranged between 5.20 to 7.07 mg/l. The free carbon dioxide in all the treatments was

absent. The total alkalinity of experimental water ranged between 342.76 to 388.56 mg/l and total hardness between 366.67 to 488.33 mg/l.

The proximate composition of *Azolla*, *Azolla* mixed diets and control diet used in present study was carried out. The protein content in *Azolla* was 22.9 %, fat 4.12 %, nitrogen free extract 46.54 %, ash 25.24 % and moisture 1.2 %. The protein content in control diet was 27 %, fat 14.24 %, nitrogen free extract 37.96 %, ash 16.56 % and moisture 4.24 %. The protein per cent in *Azolla* mixed diet was maximum (26.59 %) in 10 per cent *Azolla* mixed diet (T_1) and minimum (23.95 %) in 50 per cent *Azolla* mixed diet (T_5), fat was maximum (13.23 %) in T_1 and minimum (9.18 %) in T_5, nitrogen free extract was maximum (43.20 %) in T_5 and minimum (38.37 %) in T_1, ash was maximum (21.40 %) in T_5 and minimum (17.53 %) in T_1 and moisture was maximum in (3.94 %) in T_1 and minimum (2.72 %) in T_5. The carotenoids content in the *Azolla* mixed was maximum in T_5 (116.27 µg/g) and minimumin T_1 (23.24 µg/g).

The effect of *Azolla* mixed diet has shown a significant increase in weight gain in fishes compared to control. The maximum weight gain (1.40 g) was noted in the fishes fed with 30 per cent *Azolla* mixed diet i.e. in T_3 followed by (0.85 g) in the fishes fed with 10 per cent *Azolla* mixed diet i.e. in T_1. The minimum weight gain (0.68 g) was recorded in fishes fed with control diet. Among the five levels of *Azolla* mixed diet the maximum specific growth rate (0.895 %) were recorded in T_3 followed by (0.700 %) in T_1 and minimum (0.624 %) was recorded in fishes fed with control diet. The best value of food conversion ratio (FCR) was (1.65), also recorded in T_3 and minimum (2.40) was recorded in fishes fed with control diet. The highest gross conversion efficiency (0.607) was recorded in T_3 and minimum (0.417) were recorded in fishes fed with control diet. The statistical analysis of data revealed a significant results of weight gain, food conversion ratio, specific growth rate and other growth parameters in treated fish as compared to control. The study also revealed a significant critical difference between all the treatments mixed with *Azolla*. However, the increase in growth of test fishes was not appreciating in T_4 and T_5 compared to T_3.

The comparative pigment composition of *Azolla* mixed diet revealed 23.24 µg/g, 46.54 µg/g, 69.24 µg/g, 92.68 µg/g and 116.27 µg/g carotenoids in T_1, T_2, T_3, T_4, and T_5 respectively. The results also revealed that compared to the control fishes the colour intensity and prominence of red-purple colour was increased in the fishes

fed with *Azolla* mixed diet. The maximum average luminosity (L*) (12.82) was in the fishes fed with 50 per cent *Azolla* mixed diet (T_5). The maximum average a* value was found in T_5 (5.98) on the x-axis followed by T_4. The maximum of yellow colour intensity (b* value) was also seen in T_5 (7.04) and minimum(4.22) in fishes fed with control diet.

The comparative colour characteristics of hue angle (h°) on the a* (red-purple-bluish green) axis, indicates a positive value (+) *i.e.* red-purple in all the treatments including control. However, the maximum of hue angle (h°) was found in T_5 (9.60) followed by T_4 (49.02). These results revealed that the fishes fed with 50 per cent *Azolla* mixed diet that shows maximum intensity of yellow and red colour as compared to the rest of the treatments including control.

The saturation of colour as indicated by Chroma (C*) in which the maximum colour saturation was observed inT_5 (9.28) where the fishes were fed with 50 per cent *Azolla* mixed diet. Whereas, minimum saturation was found in control (6.00) where the fishes were fed with control diet.

From the results of growth parameters the treatments can be recommended in descending order as $T_3 > T_4 > T_5 > T_2 > T_1$ control. Similarly the order of treatments no the basis of colour performance in red platy can be written as - $T_5 > T_4 > T_5 > T_2 > T_1$ > control.

From these observations, it can be inferred that for maintaining appropriate colour in ornamental fish, suitable pigment enhancing agent should invariably form part of fish diet on a prolonged basis.

Conclusions and Recommendations

In view of the above discussion and results of the study it can be concluded that -

i. The diets replaced with different levels of *Azolla* powder were acceptable to the red platy as it significantly increased the fish growth.

ii. The carotenoids source (*Azolla*) used in the present study have maximum amount of xanthophylls 116.27 µg/g i.e. 99.50 % among the total carotenoids.

iii. The Azolla on diet significantly effective in enhancing the skin colour of red platy (*Xiphophorus maculatus*).

iv. Among the *Azolla* mixed diets, T_5 i.e. 50 per cent *Azolla* mixed diet has been found most effective to enhance red-purple in skin colour with greater saturation compared to the control diet, where 50 per cent *Azolla* mixed diet (i.e. 23.50 g Azolla meal per 100 g diet) has been found as the best natural source of carotenoids for developing better red colouration in red platy (*Xiphophorus maculatus*). On the basis of colouration, the ranking of treatments having different levels of Azolla mixed diet can be written in a descending order of T_5 (49.60) > T_4 (47.80) > T_3 (45.16), T_2 (44.33) and T_1 (43.99) to supply 116.27 µg/g, 92.68 µg/g, 69.24 µg/g, 46.54 µg/g and 23.24 µg/g per 100 g of diet respectively.

v. For future research it can be suggested to analyze more natural sources for study of pigmentation in ornamental fish skin.

LITERATURE CITED

Alikunhi, K.H. 1957. Fish culture in India. *Farm Bulletin of Indian Council of Agriculture Research*, **20**: 144.

Almazan, G. S., Pullin, R. S. V., Angeles, A. F., Manalo, T. A., Agbayani, R. A. and Trono, M.T.B., 1986. *Azolla pinnata* as a dietary component for Nile tilapia *Oreochromis niloticus*. In proceeding of the first Asian Fisheries Forum, Asian Fisheries Society, Manila. J.L. Maclean, L.B. Dizon & L.V. Hosillos, (Eds.) pp. 523-528.

Akiyama,D.M. 1991 Soybean meal utilization by marine shrimp. In Proceedings of the Aquaculture feed processing and Nutrition workshop, Thailand and Indonesia, September 19-25, D.M. Akiyama and R.K.H. Tan (eds.), Amercian soybean Assoc. Singapore. pp 207- 225.

Ako, H., Tamaru, C.S., Asano, L., Yuen, B. and Yamamoto, M. 2000. Achieving natural colouration in fish under culture. *UJNRTechnical Report* No.9, *Aquaculture Panel Symposium, Kihei, Hawai*, Nov. 10-11

A.O.A.C. 1980. Official methods of analysis. Association of Analytical Chemist Washington D.C., U.S.A.

A.P.H.A. (American Public Health Association) 1989. Standard methods for examination of water and waste water (12^{th} Ed.) American Water Works Association and Water Pollution Control Federation, A.P.H.A. Washington, D.C. pp.1452.

Balaji K, Jalaludeen A, Churchil RR, Peethambaran PA and Sethilkumar S (2009). Effect of dietary inclusion of azolla (*Azolla pinnata*) on production performance of broiler chicken. *Indian Journal of Poultry Science*, **44**: 195-198.

Benerjee, S.M. 1967. Water quality and soil condition of fish pond in some states of India in relation to fish production. *Indian Journal of Fisheries*, **14**(1&2): 115-144.

Bjerkeng, B., Storebakken, T. and Liaaen – Jensen, S. 1990. Response to carotenoids by rainbow trout in the sea; Resorption and metabolism of dietary astaxanthin and canthaxanthin. *Aquaculture* **91**: 153-162.

Boonyarapatin, M. and R.T. Lovell. 1977. Diet preparation for aquarium fish. Aquac. **12**: 53-62.

Choubert, G. 1979. Tentative utilization of *spirulina*algae as a source of carotenoid pigmentsa for rainbow trout. Aquac., **18**: 135-143.

Chapman F. A., 2000. Ornamental fish culture, freshwater. In: Encyclopedia of aquaculture. Stickney R. R. (Ed), John Wiley & Sons, Inc., New York. pp. 602-610.

Chatzifotis, S. et al. 2005. The effect of different carotenoid sources on skin coloration of cultured red porgy (Pagrus pagrus). In *Aquaculture Research*, vol. 36, no. 15, pp. 1517–1525. DOI: http://dx.doi.org/10.1111/j.1365-2109.2005.01374.x.

Dandraff and Dean. 1967. Dissolved oxygen criteria for the protection of fish. *American Fisheries Society Special Bulletin*, pp. 44.

Dharmaraj, S. and Dhevendaran, K. 2011. Application of microbial carotenoids as a source of colouration and growth of ornamental fish *Xiphophorus hellerii*. *World Journal of Fish and Marine Sciences,* **3** (2): 137-144.

El-Sayed, A.F.M. 1992. Effects of substituting fish meal with Azolla pinnata in practical diets for fingerling and adult Nile tilapia, *Oreochromis niloticus L. Aquaculture and Fisheries Management*, **23**: 167-173.

Ezhil. J., C. Jeyanthi and M. Narayanan, 2008. Marigold as a carotenoid source on pigment and growth of red swordtail *Xiphophorus helleri*. *Turkish Journal of Fisheries and Aquatic Sci*. **8**: 99-102.

Fiogbé, E.D., Micha, J.C. & Van Hove, C. 2004. Use of a natural aquatic fern, Azolla microphylla, as a main component in food for the omnivorous-phytoplanktonophagous tilapia, *Oreochromis niloticus L. Journal of Applied Ichthyology*, **20**: 517-520.

Garg, S.K., Bhatnagar, A., Kalla, A. and Johal, M.S. 2002. Experimental Ichthyology, CBS Publishers and Distributors 4596/1-4, Darya Ganj, New Delhi-110002 (India). pp. 129.

George, Michael, R. 1969. Seasonal trends in physico-chemical factors and plankton of freshwarter fish pond and their role in fish culture. *Hydrobiologia*, **33**(1): 145-160.

Goodwin, T.W. 1951. Carotenoids in fish. In: The biochemistry of fish. Biochem. Soc. Symp. **6:** 63-82.

Gouveia, L., Rema, P., Pereira, O. and Empis, J. 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. *Aquaculture Nutrition,* **9**(2): 123-129.

Gouveia, L., Rema, P. 2005. Effect of microlgal biomass concentration and temperature on ornamental gold fish (*Carassius auratus*) skin pigmentation. *Aquaculture Nutrition,* **11**: 19-23.

Gupta, S.K., Jha, A.K., Pal, A.K. and Venkateshwarlu, G. 2007. Use of natural crotenoids for pigmentation in fishes. *Natural Product Radiance,* **6**(1):46-49.

Guroy, B., Sahin, I., Mantoglu, S., Kayal, S. 2012. *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. *Aquaculture International,* **20**(5): 869-878.

Halten, B., Aas, G.H., Jorgensen, E.H., Storebakken, T. and Goswami, U.C. 1995. Pigmentation of 1, 2 and 3 years old Arctic charr, (*Salvelinus alpinus*) fed two different dietary astaxanthin concentrations. *Aquaculture,* **138**: 303-312.

Hata, M. and Hata, M. 1975. Carotenoid pigments in rainbow trout *Salmo gaidneri irideus, Tohoku J. Agric. Res.,* **26**: 35-40.

Harpaz, S., Padowicz, D. 2007. Color enhancement in the ornamental dwarf cichlid *Microgeophagus ramirezi* by addition of plant carotenoids to the fish diet. *Israeli Journal of Aquaculture* - Bamidgeh; 2007. **59**(4): 195-200.

Higuera, I. Félix-Valenzuela, L. and Goycoolea, F. M. 2006. Astaxanthin: A review of its chemistry and applications, *Critical Reviews in Food Science and Nutrition,* **46**(2): 185-196.

Hossiny H, Setoudeh M, Rokni H, Dehghanzadeh H and Cheraghcheshm M. 2008. Using of silage azolla in Guilan male calves nutrition. Proceedings of *ThirdNational Congress of Recycling and Reuse ofRenewable Organic Resources in Agriculture IslamicAzad University, Khorasgan branch (Isfshan)Agricultural* faculty, *Waste and waste water Researchcentre.*

Hunter, R.S. 1942. Photoelectric tristimulus colorimetry with three filters. NBS Circ. C 249, U.S. Dept. Commerce, Washington, D.C.

James, R., Sampath, K., Thangarathinam, R. and Vasudhevan, I. (2006). Effects of dietary Spirulina on growth, fertility, coloration and leucocytes count in red swordtail Xiphophorus helleri. *Israeli Journal of aquaculture, Bamidgeh* **58**:97-104.

Janeck, V.S. 1976. Jak dekv intenzifickaci rybnikarstvi (How far with fertilization in ponds). VURH, Vodnany, pp. 70.

Joseph, A., P. M. Sherief and T. James.1994. Effect of different dietary inclusion levels of *Azolla pinnata* on the growth, food conversion and muscle composition of *Etroplus suratensis* (Bloch). *Journal of Aquaculture in Tropics* **9**: 87-94.

Joseph, B., S. Sujath, J. Jemima Shalin and A. Palavesama. 2011. International Centre for Bioresources Management, Malankara Catholic College Mariagiri, Kaliyakkavilai: 629-153.

Kannaiyan, S., and Kumar, K. (2005). Azolla in Animal feeding. In Azolla Biofertiliser for Sustainable Rice Production, Chapter 21, Daya Publishing House. Delhi.

Khatun, A., Ali, M.A. and Dingle, L.G., (1999). Comparison of the nutritive value for laying hens of diets containing azolla (Azolla pinnata) based on formulation using digestible protein and digestible amino acid versus total protein and total amino acid. Anim. Feed. Sci.Tec., **81**:43-56.

Kim, Hwa Sun, Youhee Kim, Sung Hwoan Cho and Jae, Yoon Jo. 1999. Effect of dietary carotenoid on the nuptical colour of the bitterling (*Rhodeus uyekii*). *Journal of the Korean Fisheries Society,* **32**(3): 276-279.

Kop, A., Durmaz, Y., Hekimoglu, M. 2010. Effect of natural pigment sources on colouration of cichlid (*Cichlasoma severum* sp. Heckel, 1840). *Journal of Animal and Veterinary Advances*, **9**(3): 566-569.

Kottelat M. 2001. Fishes of Laos: WHT Publications Ltd, Colombo. Tan HH, Lim KKP (2004) Inland fishes from the Anambas and Natuna Islands, South China Sea, with description of a new species of *Betta*(Teleostoi: Osphronemidae): Raffles Bull. Zool. Suppl, **11**: 107-115.

Kour, Dalveer 2000. Study of effect of selected herbs on growth and colouration in certain freshwater ornamental fishes. Ph.D. (Limnology and Fisheries) Thesis submitted to the Maharana Pratap University of Agriculture and Technology, pp.

Kumar DS, Prasad RMV, Kishore KR and Rao ER (2012). Effect of azolla (*Azolla pinnata*) based concentrate mixture on nutrient utilization in buffalo bulls. *IndianJournal of Animal Research*, **46**: 268-271.

Lagler, K.F. 1972. Freshwater fishery biology, 2nd Ed. 12th print, W.M.C. Brown Company publishers, Iowa., pp. 134-185.

Lovell, R.I., 1992. Dietary enhancement of colour in ornamental fish. *Aquaculture Magazine*, **18**(5):77-79.

Lumpkin, T.A. and D.L. Plucknette, 1982. Azolla as acrop Tamany, Y., G. Samanta, N. Chakraborty and L. Mondal, production. Series No. 15, pp 230, Westview Press Boulder, Colorado, U.S.A.

Mathew, P.T. and Gopakumar, K. 1992. Effect of incorporation vegetable colour from red sandal on acceptability, growth and colour development of tilapia (*Tilapia mossambica*). *Fish Technology*, **29**(2): 124-126.

Miki, W., 1991. Biological functions and activities of animal carotenoids. Pure Appl. Chem. 63, 141–146.

Moyale, J.B. 1946. Some indices of lake productivity. *Trans American Fisheries Society*, **76**: 322-324.

Paripatanamont, T., Tangtrongpairoj, J., Sailasuta, A. &Chansue, N. 1999. Effect of astaxanthin on the pigmentation of goldfishCarassiusauratus. Journal of the World Aquaculture Society, 30, 454e460.

Pillai KP, Premalatha S and Rajamony S (2002). Azolla-A sustainable feed substitute for livestock. *LEISA INDIA*, **4**: 15-17.

NABARD, 2013. National Bank for Agriculture and Rural Development, Model Bankable Projects-fisheries-ornamental fish breeding, http://www.Nabard.org/modelbankable projects/fish-ornamental-fish.asp.

Ramamoorthy, K., Bhuvaneswari, S., Sankar, G. and Sakkaravarthi, K. 2010. Proximate composition and carotenoid content of natural carotenoid sources and its colour enhancement on marine ornamental fish *Amphiprion ocellaris* (Cuveir 1880). *World Journal of Fish and Marine Sciences* **2**(6): 545-550.

Raymond, G., 1992. Reporting of objective colour measurements. U.S. Department of Agricultural Research Service, Subtropical Horticulture Research station. *Horticulture Science,* **27**(12): 1254-1255.

Sadashivam. S. and Manikam A. 1991. Biochemical method for Agricultural sciences. Depart of Biochemistry, Tamil Nadu Agricultural University, Coimbatore. Wiley Eastern Ltd, New Delhi, Bangalore, Bombay, Kolkata, Chennai, Hyderabad, Pune, Lucknow, and Guwathi. pp. 181-185.

Saxena, A. 1994. Health: Colouration of fish. Proceedings of International Symposium on Aquatic Animal Health: Programme and Abstracts. University of California, School of Veterinary Medicine, Davis, CA, pp. 94.

Shahidi, F., Metusalach, B. and Brown, J.A. 1998. Carotenoid pigments in sea foods and aquaculture. *Critical Review of Food Science and Nutrition* **38**:1-67.

Silas, E.G. and Gopalakrishnan, A., Ramachandran, AnnaMercy,T.V., Sarkar, Kripan, Pushpangadan, K. R., Anil Kumar, P., Ram Mohan, M.K. and Anikuttan, K.K. 2011. Guidelines for green certification of freshwater ornamental fish. Marine Products Export Development Authority, Kochi. ISBN 978 81-910104-0-4.

Sithara,K. and K.Kamalaveni, 2008. Formulation of low-cost feed using azolla as a protein supplement and its influence on feed utilization in fishes. Current Biota. **2**(2): 212-217.

Singh PK and Subudhi BPR (1978). Utilization of azolla in poultry feed. *Indian Farming*, **27**: 37-39.

Smitherman, R.O. and Byod, C.E. 1974. Intensive management of water for fish production. In: M.C. Blount (Ed.), Water resources utilization and conservation in the environment. Fort Valley State College, Fort Valley, Georgia, pp. 152-166.

Storebakken, T., Foss, P., Schiedt. K., Avstreng, E., Liaaen-Jensen, S. and Manz, U. 1987. Carotenoids in diets for salmonids IV pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. *Aquaculture*. **65**: 279-292.

Swingle, H.S. 1967. Standardization of chemical analysis for water and pond muds. *FAO Fish Research*, **44**(4): 397-421.

Titus A and Pereira GN (2006). Azolla as a biofertilizer in coffee plantations. *Retrieved fromhttp://www.ineedcoffee.com/06/azolla/*

Trivedi, R.K., Goel, P.K. and Trisal, C.L. 1987. Practical Methods in Ecology and Environmental Science. Environmental Publishers, Karad (India). pp. 340.

VanHove, C. and Y. Lopez, 1987. Fisiologia de Azolla. In: Preston, T.R. and E. Murgueitio,. Trees and Shrub Workshop on the assessment of Azolla use in legumes as protein sources for livestock. In: Forage Tropical Latin America. Chicklayo: Peru.

Vasudhevan, I. 2008. Effect of selected growth promoting immunostimulants on growth, reproduction and blood parameters in gold fish, (LINNAEUS,1758). Ph.D. Thesis submitted to Manonmaniam Sundaranar University, Tirunelveli, TN, India.

Wagde, M. 2013. Effect of selected natural carotenoid sources on pigmentation, growth and reproductive performance of an ornamental fish- Red Swordtail *(Xiphophorus hellerii)* M.F.Sc Thesis submitted to the Maharana Pratap University of Agriculture and Technology, pp.87.

Wallat, G. K., Lazur, A. M., Chapman F. A., 2005 Carotenoids of different types and concentrations in commercial formulated fish diets affect color and its development in the skin of the red oranda variety of goldfish. *North American Journal of Aquaculture* **67**:42-51.

Wang TY, Tzeng CS, Shen SC (1999) Conservation and phytogeography of Taiwan paradise fish: *Macropodusoper cularis* Linnaeus. Acta Zool Taiwan.

Wassef, E.A., Chatzifotis, S., Sakr, E.M., Saleh, N.E.2010. Effect of carotenoid sources in diets for gilthead seabream, *Sparus aurata*, on growthand skin coloration. *Journal of Applied Aquaculture*, **22**(3): 216-229.

Ylmaz, S., Ergun, S. 2011. Effect of red pepper (*Capsicum annuum*) on pigmentation of blue streak hap (*Labidochromis caeruleus*). *Israeli Journal of Aquaculture, Bamidgeh,* **63**: 633-639.

.