Introduction
1. INTRODUCTION

The ornamental fish sector is a widespread and global component of international trade fisheries aquaculture and socio-economic development. Since 1985, the international trade in exports of ornamental fish which usually takes place in the majority of developing countries, followed on increasing trend with an average growth rate of approximately 14% per year. Successful rearing of larval stages of aquatic organisms is a challenge for aquarium hobbyists, as aim and a necessity for the success of the aquaculturists. All these specialists will agree that the primary problem in any type of larval rearing is that of food. Ideally, one would prefer to lead larvae their natural diet, which is characterized by a wide diversity of nutritious live organisms. Live feed is an essential food source for the fry of cultured species, especially those without a fully developed digestive system (Arulrasu and Munuswamy, 2009).

Ornamental aquaculture is a 5.6 billion dollar industry worldwide. Aquaculture produces more than 1500 species of ornamental fish available for the aquarists. In the market movement, the fish colour intensification determines their acceptability in the market, which is one of the main factors that determine the price. Among the same species and lineages, the most colorful fish appear healthier and more attractive for the consumer. The multiple color patterns found in the fish depend on multiple interactions between the pigments in the integumentary cells, known as cromatophores, and it is the result of the
combination between the different types of cromatophores (Odiorne, 1957; Fujii, 2000). The cromatophores are classified by their pigment colors.

The art of rearing and keeping fish in an aquarium is an age-old practice. At the dawn of the 21st century fish keeping is reflected in *ubiquitous aquaria* that feature as an integral part of modern interior decoration (Katia Oliver, 2001). Most of the fish are small in size, having attractive colours. Their movements are gentle and quiet without causing any sound. They have adaptability to live within a confined space in captivity. The aquarium fish are thus of a rapidly growing importance not only because of their aesthetic value but also of their immense commercial value in the export trade world over.

Brazil is one of the leading exporters of freshwater ornamental fishes, but also appears as a consistent supplier of marine species (Wood, 2001). Unlike freshwater aquaria species, where 90% of fish species are currently farmed, the majority of marine aquaria are stocked from wild caught species (Andrews, 1990). About 400 species of ornamental fishes belonging to 175 genera and 50 families are reported in Indian waters. But this figure is on the rise as more numbers of surveys are made in different coral locations of the country (Satheesh, 2002). Generally the ornamental fishes found in mangroves represent estuarine species along with inclusion of some marine species. Nyanti *et al.* (2012) reported that the fish population found in the river especially the larger-sized individuals are sporadic visitors to the area. During the rainy season, the increased flow of freshwater results in the appearance of freshwater species. The
mangrove dominated estuarine habitats plays very significant roles for essential ornamentation of estuarine and coastal fin fishes (Banani et al., 2012).

The growing interest in aquarium fish has resulted in steady increase in aquarium fish trade globally. Today, with a turn over of US$ 9 billion a year and an annual growth of 8% production of ornamental fish is an important business activity as well as one of the most popular hobbies in the world. It is thought that this sector can contribute to the economic development in under developed countries, especially in the tropics (Yanar et al., 2008).

One of the most attractive features of aquatic creatures is arguably their beautiful display of colours. Colour is one of the major factors that determines the price and marketability of several ornamental fishes from fresh and marine habitats traded across the world, USA, European countries and Japan are the largest market for ornamental fish but more than 65% of the exports done from Asia. It is encouraging news for developing countries that more than 60% of the total world trade gas to their economies. Although, India is still in a marginal position, its trade is developing rapidly.

Ornamental fish are acceptable to consumers if they have striking and vibrant colours. Colouration is one of the most important factors deciding the market value of the ornamental fish is controlled by the endocrine and nervous system. However, dietary sources of pigments also play a role in determining the fish colour. Fish are capable of producing some pigments but others must be supplied in the diet. Natural sources of pigments are available in the diets of most
fish colour enhancing diets may contain additional natural pigments to enhance colours of ornamental fish.

Differences in species, varieties, colours and patterns of ornamental fishes play an important role in attracting home owners to keep these fishes in their house. Ornamental fishes also derive the name as “life jewels” because of its ornamental values. That means it must beautiful and decorative as it helps to beatify our living environment. In other words, colouration of ornamental fish play the most important role.

Ornamental fish’s pigment is one of the most important quality criteria dictating their market value. Like other animals, fish are unable to perform denovo synthesis of carotenoids (dietary carotenoids) (Goodwin, 1984) and therefore rely on dietary supply to achieve their natural pigmentation. The characteristic pink colouration of the flesh is among the most important quality criteria for salmonid fishes (Sigurgisladottir et al., 1997) and it is important for their marketing (Moe, 1990). Currently, the carotenoids supplemented to fish feeds are either manufactured by chemical synthesis or obtained from organisms that biosynthesize carotenoids denovo. Plants, algae and certain fungai and bacteria can biosynthesize carotenoids.

Ornamental fishes are rapidly gaining importance now a days because of their aesthetic and immense commercial value in the export trade world over. Ornamental fishes are characterized by a wide diversity of colours. Colour patterns and success in the ornamental fish trade is very much dependent on the bouncy colour of the fish. Colour is one of the major factors which determinesthe
price of aquarium fish in the world market (Saxena, 1994). Attractive colouration determines the commercial value of ornamental fishes. Pigmentation in the skin is responsible for the colouration in the fish. Carotenoids are the primary source of the pigmentation on the skin of fishes. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chains.

Colouration and hence, pigmentation plays an important role in both ornamental and food fishes such as gold fish, koi, salmon, trout, sea bream and prawns. Normally, the ‘quality’ of the fish is based on the pigmentation. The colour of fish might also bring some other meanings for certain people. For example, red-coloured fishes such as koi and gold fish will be more attractive and higher priced as the Chinese, Taiwanese and Japanese consider them more auspicious.

People involved in the trade of ornamental fish are constantly exploring methods of enhancing skin colouration. In addition to enhancing colouration of the fish different pigments used in the diets are also reported to give better results of growth (Ezhil et al., 2008).

Fish are coloured in nature often show faded colouration under intensive culture conditions. Fish, like other animals do not synthesize carotenoid and depend on dietary carotenoid content for the colouration. Hence, a direct relationship between dietary carotenoids and pigmentation exists in them (Halten et al., 1997). The most used synthetic colouring matters are astaxanthin and canthaxanthin (Yesilayer et al., 2008).
Colour of aquarium fishes has an important role in preference of consumers. Insufficient colouration reduces economical value and not meet consumer demands both. Producers prefer natural carotenoids due to increase cost of feed with synthetic carotenoids. Ornamental fishes are characterized by a wide diversity of colours and colour patterns and success in the ornamental fish trade is very much dependent on the vibrant colour of the fish (Ramamoorthy et al., 2010).

Color patches in fish, reptiles, and amphibians are multilayer, multicomponent signals (Grether et al., 2004). the result of selective light scatter owing to variable refraction within the integument, and only one blue pigment has been described in fish (Bagnara et al., 2007).

Carotenoids are natural fat soluble pigments also known as lipochromes, produced in nature via suprenoid pathway shared with such diverse substances groups like the essential oils, phytohormones, steroids, cardiac glycosides and vitamins A, D, E and K. Among the various classes of natural pigments such as the arthocyanin, flavanoids, pyoroles, blood and bile pigments; the carotenoids are the most wide spread and structurally diverse pigmenting agents. Their total annual world wide production is estimated at over 100 million metric tonnes representing on output of over three metric tonnes per second (Latscha, 1991). To day, about 600 different natural carotenoids have been identified. They are responsible for many of the brilliant yellow to red colours in plants, animals as well as the variety of bluish, greenish, purplish, brownish and blackish colours seen in many fish and crustaceans (Latscha, 1990).
Gold fish (Carassius auratus) is the most popular variety of ornamental fish (NRE, 2002), besides body shape, fin shape and size an important characteristics affecting the market price of gold fish is body colour. To achieve consumer acceptance and optimal price, the gold fish must be pigmented to have orange red colour (Parippatananont et al., 1999). Marigold meal as a natural carotenoid source was effective on skin pigmentation of gold fish (Martinezet al., 2013).

Some eye catching examples are provided by the skin and flesh of some fish (eg. Astaxanthin in gold fish). The crustacean hypodermis and exoskeleton accounts for about 60 – 90% of animals pigment content astaxanthin. The main pigment of crustacean is a red C-40 dihydroxy diketo carotenoids. The bulk of pigments in the crustaceans epidermal tissue, however, are found in the ester form (Latscha, 1991).

Astaxanthin is ubiquitous in nature, especially in the marine environment and is probably best known for eliciting the pinkish red hue to their flesh of salmonids, as well as shrimp, lobsters and cray fish. Two keto carotenoids give a red pigmentation to salmonid muscle, astaxanthin and canthaxanthin. Astaxanthin is the dominant pigment of wild salmonids muscle and is retained more effectively than canthaxanthin (Foss et al., 1984 ; Choubert and Storebakken, 1989 ; Torrissen, 1989). Since the fish do not synthesize keto carotenoid pigments de novo, they must get them from their diet (Goodwin, 1951).

Fish in the nature have special skin and flesh colour. On the other hand, those from aquaculture are pale and grayish, which bring about reduction in the
domestic consumption and exploration. In the diets of fish for which pigmentation is important, synthetic and natural carotenoid sources are included in order to eliminate the problem. Asteraxanthin is one of the synthetic sources, is fairly effective on making fish colourful (No and Storebakken, 1991; Haard, 1992; Torrisen et al., 1989). One of the most attractive features of aquatic creatures is arguably their brilliant display of colours. The source of their colours comes from the foods in their natural environment. However, some producers use hormones and artificial colourants to attract consumers, increase their profit margin and to make the fish they produce more vivid and shiny. Nevertheless, the colours acquired through such methods are not stable and the fish lose their colour after a while (Kop and Durmaz, 2008). The effect of synthetic and natural pigments on the colour of the guppy fish supplementing for longer periods are needed to evaluate a possible role on growth (Mirzaee et al., 2012).

Appearance of an animal product, especially colour plays an important role on the marketing, colour, nutritional values, healthy appearances, freshness and sensory test components are the subconscious elements to choose the product. Choosing a products is effected by the educational condition, environment and customs as well (Diler, 1997; Meyers, 1994; No and Storebakken, 1991).

Carotenoid pigments are responsible for many examples of sexually attractive red, orange and yellow colouration in animals and play an important role in antioxidant and immune defenses. Because vertebrates cannot synthesize carotenoids, limited dietary availability may impose a trade off between
maintaining ornamental colouration and health. The yellow orange and red hues found in skin and flesh result from the deposition of pigments classified as carotenoids (Simpson et al., 1981). Carotenoids are also vital nutrients for healthy growth, metabolism and reproduction. However, carotenoid cannot be synthesized by most animals, including fishes and must be obtained from dietary sources (Hata and Hata, 1971; Torrissen et al., 1989; Storebakken and No, 1992). For example, several researchers confirmed the primary source of the pink to red colouration in wild trout muscle tissue was due to the consumption of crustaceans (Stevens, 1947; Goodwin, 1954; Fox, 1957).

The rapid changes in the western diet, particularly during the past 100 years, may be potent promoters of chronic diseases, such as atharoclerosis, hypertension, diabetes, many cancers and life style diseases (eg. Metabolic syndrome and obesity) causing great concern for health authorities world wide (Who, 2002). Astaxanthin (3,3'-dihydroxy-4, 4'-diketo-β, β-carotene - 4, 4'-dione) belongs to the family of xanthophylls which are the oxygenated derivatives of carotenoids (Higuera-Cia para et al., 2006). In the wild, over 98 percent of the carotenoid pigments deposited in salmon flesh are astaxanthin and the synthetic astaxanthin used in aquaculture is structurally identical to the forms found in nature (Springale and Nickell, 2000). In aquaculture astaxanthin is mainly associated with its function in the feed to produce the typical pink flesh colour in salmonid species as they are unable to produce astaxanthin de novo. The main type of astaxanthin used in feed is synthetic, although extracts from shrimp, yeast and algae have also been tested as possible natural sources of pigmentation with varied results (Springale and Nickell, 2000).
Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. There are over 600 known carotenoids, they are split into two classes. Xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen), carotenoids in general absorb blue light. They serve two key roles in plants and algae; they absorb light energy for use in photosynthesis and they protect chlorophyll from photo damage (Armstrong and Hearst, 1996). In humans, carotenoids such as beta carotene are a precursor to vitamin A.

Carotenoids are naturally occurring tetraterpenes of yellow to red colouration, widely occurring in nature in the form of isoprenoid polyene pigments synthesized by photosynthetic and some non-photosynthetic organisms (Goodwin, 1984 ; Britton et al., 1995). Carotenoids are relatively hydrophobic molecules that are typically associated with membranes. They are found to be covalently bound to chlorophyll binding proteins in photosynthetic systems (Armstrong, 1994). An important structural feature of these compounds is the presence of highly conjugated system of double bonds, which results in various cis and trans isomers. Carotenoids are not only essential for human health but are also considered as preventive and therapeutic agents. It was proved that consumption of these pigments decreases the risk of degenerative and cardiovascular diseases. Carotenoids play an important role as antioxidants due to their ability to quench singlet oxygen, and to scavenge free radicals (Britton et al., 1995 ; Gerster, 1993). The exploitation of carotenoid natural function led to valuable application in animal feeds, food and pharmaceutical technology.
(Astrong, 1997). It is well known that the pink to red colouration of the flesh of wild salmonids is due to carotenoid pigments, mainly astaxanthin of dietary origin (Khare et al., 1973; Kitahara, 1984; Schiedt et al., 1986; Scalia et al., 1989a). Pigmentation of farmed salmonids is of economic importance for the salmon industry (Torrissen et al., 1989). The natural pigmentation of farmed fish can be achieved principally by the inclusion in the diet of synthetic astaxanthin (Foss et al., 1987; Storebakken et al., 1987; Choubert and Storebakken, 1989) or with astaxanthin from crustacean sources (Saito and Regier, 1971; Spinelli and Mahnken, 1978; Arai et al., 1987).

The intermediates in the transformation of dietary carotenoids, such as echinenone and canthaxanthin, are often detected as accompanying minor carotenoids. Tunaxanthin is also a major carotenoids of fish. Other biologic functions or actions attributed to carotenoids are independent of the provitamin A activity and have been attributed to an antioxidant property of carotenoids through singlet oxygen quenching and deactivation of free radicals (Palozza and Krinsky, 1992; Burton, 1989; Krinsky, 1989).

Carotenoids are yellow to orange – red pigments composed of a polyene skeleton which usually consists of 40 carbon atoms and is either acyclic or terminated by one or two cyclic and groups. All carotenoids are derived from the same basic CHO isoprenoid skeleton by modification such as cyclization, substitution, elimination, addition and rearrangement. They are structurally related to retinol and β-carotene forms the main source of vitamin A for animals. Both in nature and chemical synthesize β-carotene can be considered as the basic
compound for many chemical reactions. It basically consists of 40 carbon atoms arranged in two β-carotene rings connected by a chain of conjugated double bonds representing the central light absorbing unit, the chromophore, which is responsible for the carotenoids typical colours. Among carotenoids, the bicyclic mono, di and poly forms of hydroxyl carotenoids and keto carotenoids are most important regarding animal nutrition.

It is important to use carotenoids in fish feeds. These given yellow, red and pink to the skin, flesh and eggs of fish. It is desired that fish products have some physical characters to effect choosing and to meet the demand of the customer in the market of such characteristics as colour. Fish in nature have the characteristic colour due to their preys (e.g. freshwater shrimp, gammarus, Artemia). Because of fish from aquaculture are pale due to the feeds used, this reduces the attractiveness of the products.

The production and trade of ornamental fish is a profitable alternative in the aquaculture activity. Inspite of economic importance of ornamental fishes, the nutritional information for these fishes is few or even no data is available. The feed of fish and their nutrient value is one of the most important factors in production cost and health of fish. Fish should be able to get enough nutrients from the food they can consume in two minutes, as long as the food provides for their nutritional needs (Feeding your fish –Guide, 1999). Feeding ornamental fish is more serious and difficult compare to pond fish culture due to limitation of space and lack of natural food. In order to make the aquarium similar to their habit: adjusting the temperature, light, salinity, oxygen, pH and hardness and
filling with plants, the fish may feel self-confident and take the food. In ornamental fish, a correct formulation of the diet improve the nutrient digestibility, supply the metabolic needs and reducing the maintenance cost and at the same time, the water pollution (Yohana and Wilson, 2011). In natural condition, fish can regulate and maintain their food intake and therefore their nutritional requirements, reducing the possibility of suffering nutritional deficiencies. Despite the economical importance of this sector, the nutritional information for ornamental fish is scarce and often few or even no data of the nutritional requirements is available (Chong et al., 2003). Dietary nutrients are a source of stored energy for fish digestion, absorption, growth, reproduction and other life processes of fish. The nutritional value of a dietary ingredients is in part dependent on its ability to supply energy. Properly formulated prepared feeds have a well balanced energy to protein ratio that is different in different fish.

Like all other animals, ornamental fish require their diet to provide them with a controlled, slow release source of energy that is able to fuel every metabolic process. The energy from any feed is influenced by a series of complex enzymes, each of which require the presence a range of vitamins or minerals to function properly (Halver and Ronald, 2002). Ornamental fish have basically the same nutritional requirements as other farmed fish. The efficiency of nutrient use by ornamental fish can contribute to the formulation of appropriate diets, as well as helping to decrease the elimination of nitrogen and phosphorus in excreta, thereby favoring the maintenance of the water quality and reducing environmental pollution caused by effluents (Zuanon and Saloro, 2011). The improvement of feed will based on the nutritional requirements of ornamental
fish facilitate an increase in activity, growth, reproduction and quantity and quality of the fish produced. In this respect a balance food considering exact requirement of fish with using live feed and supplementary of probiotic, prebiotic, enzymes and other new product will guarantee the successful ornamental fish culturing (Mehdi et al., 2012).

In addition to actual culture operations, tremendous growth has occurred in numbers of feed manufacturing facilities world wide as well as organizations involved in supplying feed ingredients or additives, algae for larval cultures, vaccines, antibiotics, disease diagnostic and other products that may be required for successful aquaculture. The fastest growing segment of the international feed industry is that of feed production for aquaculture. From 3 – 6 million metric tonnes in 1988, aquaculture feed manufacture is expected to increase to as much as 14 million metric tonnes by the year 2000.

Dietary carotenoids play significant part in the regulation of skin and muscle colour in fish (Ezhil et al., 2008). The colour enhancing diets should contain additional natural pigments to enhance the colour of ornamental fish (Lubzens et al., 2003). Carotenoids of Hibiscus rosa-sinensis, Rosa India, Ixora coccinea, Gossandra infundibuliformiss petals could induce pigmentation to make orange sword tail more colour (Baby Joseph et al., 2011).

Factors such as carotenoid source, diet composition, fish size, growth rate, duration of feeding, and metabolic turnover influence the dietary carotenoid uptake and muscle deposition (Torrissen et al., 1989; Storebakken and No, 1992). A high growth rate is closely related to a high feed intake (Austreng et al., 1987).
Carotenoids are contained in numerous food items (Holden et al., 1999) and comprise a major source of vitamin A precursors (Bauernfeind, 1981). The conspicuous colouration of many seafoods is due to carotenoid pigments (Shahidi et al., 1998), colouration is determined by the specific carotenoids used on the carotenoid composition.

Pigments are characterized by their colours. Carotenoids pigments are red and orange. Xanthophylls are yellow melanin pigments are black and brown. Phycocyanin is the blue pigment derived from blue green algae cells containing yellow pigments overlying those containing blue pigments can produce green hues. Fish are capable of producing some pigments, but others must be supplied in the diet. Fish are incapable of producing carotenoid and xanthophylls pigments. Therefore, those must be supplied in the diet. Colour enhancement through the use of carotenoids in feed has been confirmed by a number of authors (Fey and Meyers, 1980; Ako et al., 2000; Buttle et al., 2001; Kiessling et al., 2003; Alagappan et al., 2004). Ako et al. (2000) reported intense colouration of freshwater red velvet sward tails (Xiphophorus helleri), rainbow fish (Pseudomugil furcatus) and topaz cichlids (Cichlasoma myrmae) when fed containing carotenoids rich strain of Spirulina platensis and Haematococcuspluvialis. Wallat et al. (2005) used six different commercial diets and reported different resultant colouration of red oranda variety of gold fish (Carassiusauratus).

Salmonids are attractive for their pink colour obtained from a diet of crustacean and insects. Salmonids cannot synthesize carotenoid pigments, so
these pigments must be added in the feed during the production cycle (Storebakken and No, 1992). The carotenoid astaxanthin and canthaxanthin are commonly used in aquaculture as pigmentation sources for fish and shrimp. Commercialization of these synthetic products is widespread because they are standardized products chemically stable and their carotenoid concentration is high.

All animals, including fishes are unable to biosynthesis carotenoids de novo (Davis, 1985) and depend on dietary supply for these pigments. Salmonid fishes are distinguished from other fish species by their conspicuous muscle colouration (Goodwin, 1984), caused by deposition of relatively large amounts of carotenoids, viz. astaxanthin (Khare et al., 1973; Schiedt et al., 1986), obtained from their diet. Numerous fish species are known to absorb dietary carotenoids. Appreciable amounts of carotenoids are only found in the skin, eggs and liver in the majority of these fishes (Simpson et al., 1981; Goodwin, 1984; Matsuno and Hirao, 1989). Also in salmonid fishes, the bright red nuptial coloration is caused by deposition of carotenoid pigments (Ando, 1986). Carotenoids pigmentation is affected by dietary pigment source, dosage level, duration of feeding and by dietary composition. Among the dietary macronutrients, lipids appear to affect pigmentation appreciably. In nature, fish gain their colour through their natural diets such as algae because only plants and protests are able to synthesize carotenoids.

Colouration is controlled by the endocrine and nervous system, but dietary sources of pigment also play a role in determining colour in fishes. The
endocrine and nervous system both influence colouration in fish. The pituitary
gland secretes hormones that direct the production and storage of pigments
throughout the life of a fish.

Under intensive rearing conditions fish are fed exclusively on compound
foods which must therefore be supplemented with carotenoids. Astaxanthin (3,3’-
dihydroxy-4, 4’-diketo-β, β-carotene) and canthaxanthin (4, 4’-diketo-β, β-
carotene) are widely used as dietary supplements in diets for salmonids as a
method for inducing the typical pink colour in their flesh (Choubert and

Astaxanthin has many functions in crustaceans. It has been demonstrated
to be important in the reproductive performance of crustaceans (Pangantihon –
Kuhtmann et al., 1998 ; Perez-Velazquez et al., 2003) and in larval and post
larval development (Petit et al., 1997 ; Pan et al., 2001). It also appears to have
an important role as an antioxidant and to be involved in immunocompetence and
stress-tolerance (Meyers and Latscha, 1997 ; Linan-Cabello et al., 2002 ; Chien et
al., 2003).

In addition to pigmentation, carotenoids have also been proposed for a
role in a number of important functions in crustaceans such as a source of
provitamin A activity (Miki et al., 1982), increased stress tolerance (Torrissen,
1984 ; Chien et al., 2003) and in various development and differentiation
processes, as summarized by Linan – Cabello et al. (2004). The red muscle
colour of salmonid fishes is an important quality criterion for consumers
acceptance (Moe, 1990; Sigurgisladottir et al., 1997) and is a result of the deposition of 4(4’)-ketocarotenoids.

Astaxanthin is a natural pigment that belongs to the family of carotenoids (Lee and Min, 1990; Miki, 1991) and it is one of the major carotenoids supplied in aquaculture (Bjerkeng, 2008). In addition to its role in the colouration of aquatic animals, primarily in salmon and trout, this pigment possesses several important bioactivities, including antioxidation, enhancement of immune response and anticancer activities (Kobayashi et al., 1991; Tanaka et al., 1995; Guerin et al., 2003).

In fish, carotenoids have similar functions as those found in other animal species; they are vitamin A precursors (Schiedt et al., 1985; Guillau et al., 1992; Christiansen and Torrissen, 1994; White et al., 2003), markedly affect reproductive performance are potent antioxidants enhance immune system (Nakano et al., 1995; Amar et al., 2003) and affect liver structure (Segner et al., 1989; Page et al., 2005). Although some authors claim that the biological functions of carotenoids in fish are still speculative (Choubert et al., 2005).

Also carotenoids could enhance nutrient utilization and may ultimately result in improved growth (Amar et al., 2001). They also occur in yeast and moulds where they carry out a protective function against damage by light and oxygen. Carotenoids also play other important functions as pro vitamin-A, antioxidants, immunoregulators and they are mobilized from muscles to ovaries in salmonids which suggest a function in reproduction (Shahidi et al., 1998; Nakano et al., 1999; Bell et al., 2000). Carotenoids may improve resistance to
pathogens by increasing the production of antibodies or the proliferation of immune cells (Bendich, 1989; Jyanouchi et al., 1994; McGraw and Ardia, 2003), which itself may be necessary for immune functioning, vision and development (Stephensen, 2001; Blomhoff and Blomhoff, 2006; Palace and Werner, 2006). In addition to physiological functions, in many species, carotenoids provide external pigmentation that may be used to attract mates. Positive effects of astaxanthin on growth and disease resistance may be related to carotenoid antioxidant activity (Bjerkeng, 2000). The deteriorative effect of oxygen was found to be higher than that of ordinary day, light. Also carotenoid themselves may act as antioxidants in muscle food systems (Mortensen and Skibsted, 2000).

In many cases, the amount of pigment expressed is dependent on an individual’s foraging success and physiological efficiency (Grether, 2000; Grether et al., 2004). The behavior of pigment-containing cells is controlled by both the nervous and endocrine systems, with more rapid changes typically reflecting neural control (Fujii, 2000). The development of color patterns and the synthesis and deposition of associated pigments in fish are the product of complicated physiological processes. These physiological processes and the color pattern–related behaviors discussed here should inform those interested in the ways in which physiology mediates environmental and social influences on behavior (Price et al., 2008). The colouration of farm raised fish is inferior to their wild counterparts (Booth et al., 2004). A recent study reported that a light intensity of 20 – 50 lx could brighten the skin colour of clown fish (Yasir and Qin, 2009) and this colour could add to the commercial value in the aquarium.
trade (Hoff, 1996). Yasir and Qin (2009b) further demonstrated that a blue or green background could strengthen the orange colour, whereas a white background made fish less colour saturated but brighter. Effect of dietary carotenoids on skin colour and pigments of false clown fish, *Amphiphion ocellaris*, Cuvier study suggests that astaxanthin has the potential to enhance the red hue on clown fish skin and its withdrawal from the diet did not fade the red hue of the skin (Qin and Yasir, 2010).

Carotenoids are a widespread group of naturally occurring fat soluble pigments. They are especially abundant in yellow-orange fruits and vegetables. In plant cells, carotenoids are mainly present in lipid membranes or stored in plasma vacuoles (West and Poortvliet, 1993; Mangels *et al.*, 1993). Food carotenoids have been compiled in several tables and databases, generally including provitamin A carotenoids such as β-carotene and β-cryptoxanthin, as well as others without that provitamin activity, such as lycopene and lutein and others less studied in relation to human health such as phytoene or phytofluene (West and Poortvliet, 1993; Mangels *et al.*, 1993; O’Neill *et al.*, 2001; U.S. Department of Agriculture, 2005).

Carotenoids are known to contribute to the colourful appearance of eggs from several freshwater species. However, most marine pelagic fish eggs, including those of Atlantic halibut, which are almost fully transparent also have a slightly yellowish tinge. In mammals, about 50 of the more than that 500 carotenoids acts as vitamin A precursors (Olson, 1994). Some of the carotenoids
produced by plants also present some vitamin A activity. Among them, \( \beta \)-carotene exhibits the highest vitamin A activity.

Carotenoids are isoprenoid polyenes, a chemical group which includes canthaxanthin chemically described as \( \beta, \beta \)-carotene -4,4’ dione. Carotenoids play a major role in the biological colouration of animals. Fishes contain various kinds of carotenoids, the dominant of which is peculiar to the species concerned. Carotenoids commonly occurring in fishes with their colours are tunaxanthin (yellow), lutein (greenish yellow), betacarotene (orange), alpha, beta doradexanthins (yellow), zeaxanthin (yellow orange), canthaxanthin (orange – red), astaxanthin (red), echinenone (red) and tetraxanthin (yellow). Among these, dominant carotenoid is asteraxanthin which is common in red fishes. Lutein pigment is common of freshwater fishes, but also widely found in many marine species. Some carotenoids specific to certain groups of fishes, gold fish lack the ability to metabolize lutein and have limited ability to convert \( \beta \)-carotene to astaxanthin (Hata, 1975).

Carotenoids are biosynthesized by plants, algae and certain yeast and bacteria (Ong and Tee, 1992). Since farmed fish have no access to carotenoids from natural food, necessary carotenoids must be obtained from their diet to maintain their bright colouration. Astaxanthin is a carotenoid classified as a xanthophylls, which means, ‘yellow leaves’ (Winterhalter and Rouseff, 2002). Similarly, the addition of astaxanthin to the diet of gold fish, \textit{Carassius auratus} increased the red pigmentation density of the skin (Xu \textit{et al.}, 2006). Therefore a study of the role of carotenoids in changing fish pigmentation has become an
important aspect in ornamental fish diet. Carotenoids is a non enzyme low molecules that such as pyruvate and flavonoids microorganisms use them for oxidative damage. Using antioxidant enzymes such as super oxide dismutases and a system based on repair enzymes which remove or repair oxidatively damaged macromolecules (Crawford et al., 1994). Carotenoids which dissolve in fat give the skin the yellow and red colours. They also give the orange and green colours to the egg, skin and flesh of many fish (Fuji, 1969). Carotenoids are synthesized by all photosynthetic organisms (Giuliano et al. 2000). Plant material containing the most favourite of these alternative materials both effects nutritive quality and its being a good source of carotenoid (Ghiasvand and Shapouri, 2006).

More than 600 naturally occurring carotenoids are now known (Kull and Pfander, 1995; Pfander Key, 1987) Dietary carotenoids from fruits and vegetables in particular β, β – carotene, have been associated with a decreased risk of certain cancer forms and cardio vascular diseases (Gaziano and Hennekens, 1993; Copper et al., 1999). Carotenoid pigments are biosynthesized by higher plants, algae and certain yeast and bacteria, whereas all animals are believed to depend on the diet for carotenoid supply. Carotenoids are usually present in the integuments of fish, reptiles, amphibians, birds and invertebrates and are often basis for species – specific colouration (Fox, 1976). Dietary carotenoids and carotenoid protein complexes are the main source of fish skin and muscle pigmentation (Sales and Janssens,2003; Lovell, 2000; Chatzitotis et al., 2005); therefore to increase the skin and flesh colour in captivity, fish must
obtain an optimum level of carotenoids in their diet (Sinha and Amed, 2007). Fish skin colour is mainly attributed to the presence of chromatophores that contain pigments including melanins, pteridines, purines and carotenoids (Chatzitotis et al., 2005).

Carotenoids are pigments widely distributed in the animal kingdom, particularly in crustaceans. A number of experiments have shown the colourless appearance of arthropods lacking dietary carotenoids for a prolonged period (Lenel and Surle, 1961). Alterations of carotenoid pigmentation, ascribed to a deficiency of pigment in the diet, have been observed in adult individuals and in juveniles maintained on artificial diets (Howell and Matthews, 1991; Huner and Meyers, 1979; Menasveta et al., 1993; Morrissy, 1984). This could explain the progressive pigment depletion of Homarus americanus larval stages receiving a carotenoid free diet (Conklin et al., 1977; D’Abramo et al., 1983).

Carotenoid pigments are largely represented in both plant and animal kingdoms. Crustaceans, and all the animals, cannot synthesise these substances; however, they are able to metabolize certain carotenoids occurring in the diet. A number of works on Penaeus japonicus adult individuals have clearly demonstrated the absorption of different pigments from the diet (Yamada et al., 1990; Chien and Jeng, 1992; Negre et al., 1993); yet, the postlarva is also found to absorb dietary astaxanthin.

The carotenoids have an universal distribution occurring in most primitive bacteria and the blue-green algae up to the highly developed flowering plants and from unicellular organisms up to mammals. Some of the best known examples of
the natural occurrence of carotenoids are provided by the yellow orange colours of the flowers (e.g. Sunflower, marigold), the orange red colour of fruits (e.g. Tomato, Orange) and the orange roots of carrots from the latin name of which the name ‘carotene’ is derived. Carotenoids are commonly thought as plant pigments, but also occur widely in microorganisms and animals.

Pigmentation is one of the important quality attributes of the fish for consumer acceptability. Carotenoids are responsible for pigmentation of muscle in food fish and skin colour in ornamental fish (Gupta et al., 2007), when first imported tend to preserve their original pigments as long as they ingest pigment added feeds. Carrot is natural beta carotene source and red pepper is dark red colour due to its capxanthin in it content, being used for flesh pigmentation of salmonids given capsorobin in it (De La Mora et al., 2006; Torrissen et al., 1989; Gurocak, 1983). Both of which are cheaply available considering their high level of carotene. Effect of carrot and red pepper in diets on colouration of jewel cichlid experimental diets tested whether due to their vary features, they could have any positive effects as pigment sources in pigmentation of parrot jewel cichlid or not (Mirzaee et al., 2013).

Studies with microalgal biomass supplementation have shown that Chlorella vulgaris is as efficient as synthetic pigments in the pigmentation of rainbow trout Oncorhynchus mykiss (Gouveia et al., 1996), gilthead seabream Sparusaurata (Gouveia et al., 2002), koi carp Cyprinus carpio and gold fish Carassiusauratus (Gouveia et al., 2003; Gouveia and Rema, 2005).
As the pigmentation additive, spirulina was also found to improve the colour of tilapia. The feeding raw spirulina as uni-fed to tilapia *O. niloticus* resulted in slightly better evaluation of colour, texture and fatness than were obtained with commercial diets. The taste and smell evaluation were not different between the fish feed, the spirulina and those fed commercial diets (Lu *et al.*, 2002, 2003). The growth and nutrition quality of red tilapia being positively affected at all levels of *S. platensis* inclusion, thus clearly indicate the suitability of *S. platensis* for incorporation in the diet (Ruangsomboon *et al.*, 2010).

Microalgae sources have also been utilized for inducing pigmentation in fish. For example, *Haematococcus, Spirulina* have been used as a source of carotenoid pigments for rainbow trout, red tilapia (Boonyaratpatin and Unprasert, 1989; Choubert and Heinrich, 1993; Sommer *et al.*, 1992). Cyanobacteria is one of the microalgae, it is ubiquitous naturally, successfully mass cultured and serve as one of the biomaterials with high pigment content; phycocyanin, carotenoid, for use as a source of pigment for fish. Spirulina is a genus of cyanobacterium that common in freshwater aquatic habitats. Human pathogens (fungal and bacterial) are inhibited by phenolic extracts from Spirulina. Spirulina cells contained chlorophyll, phycoerythrin, phycocyanin and carotenoid. Spirulina is a source of phycobiliproteins that are used as fluorescent markers in biomedical research. In the case of ayu *Plecoglossus altivelis*, it has been claimed that fish grown on feed containing Spirulina are of better quality, with better flavour, firmer flesh and brighter skin colour (Mori *et al.*, 1987).
Sinha and Amed (2007) reported that the China rose (*Hibiscus rosasinensis*) petal is a potent natural carotenoid source of gold fish (*Carassius auratus*) to enhance its colour. Ezhil *et al.* (2008) reported that encouraging results on colouration of red sword tails (*Xiphophorous helleri*) fed diet containing marigold petal meal. In addition to enhancing colouration of the fish.

Shrimp waste is an important natural source for carotenoids, particularly astaxanthin and its esters (Sachindra *et al.*, 2005, 2006a). Methods have been developed for the recovery of carotenoids from shrimp waste using organic solvents and vegetable oils (Sachindra and Mahendrakar, 2005 ; Sachindra *et al.*, 2006b). However, carotenoids are highly unstable compounds and need to be protected by suitable storage conditions from excessive heat, exposure to light and oxygen to prevent their breakdown. In crustacean wastes, the processing and storage also affect the carotenoids. The mechanism of carotenoid degradation is hypothesized to be similar to lipid oxidation (Frankel, 1985) and the antioxidants, which inhibit lipid oxidation, also decrease the degradation of carotenoid. Goldman *et al.* (1983) suggested that free radicals produced by lipid oxidation might interact with carotenoid to intensity their oxidation. Use of antioxidants has been attempted for prevention of carotenoid related colour degradation in fish, meat and poultry (Green *et al.*, 1971 ; Chasteain *et al.*, 1982 ; Li *et al.*, 1998). The major cause for carotenoid degradation in foods is oxidation with rates dependent on contact with oxygen, light, heat and presence of pro-and antioxidants (Francis, 1985 ; Haard, 1988).
Colour is an important feature of carrot chips. The colour achieved is a combination of the natural colour arising from the pigment content of the carrots and the colour developed during the process of deep-frying. With control by the fermentation process, the pigment content of the raw carrots becomes crucial for the colour appearance of the chips. Achieving control of factors which influence pigment accumulation in carrots is therefore an important challenge.

In animal tissues, the carotenoid degradation probably involves both lipoxygenase catalysed enzymatic oxidation and non-enzymatic oxidation such as autoxidation and photosensitized oxidation (Frankel, 1985; German and Kinsell, 1985). In nature, the interaction of protein and carotenoids as occurring in crustaceans increases the pigment stability (Britton, 1996). However, when carotenoids are recovered from crustacean wastes, they are extracted as pigments solubilized in lipid. Thus it is necessary to protect the lipids from oxidation, which in turn can stabilize the pigments in it.

The freshwater, unicellular green alga Haematococcus pluvialis has been recognized for many years as an accumulator of the carotenoid astaxanthin (Goodwin and Jamirkan, 1954; Czygan, 1968; Kobayashi et al., 1991). The fledging freshwater ornamental fish industry in Hawaii has experienced the problem of faded colouration to fish, especially those grown in clear works. These fish are traditionally rejected by ornamental fish whole solars, leaving the growers with fish that can’t be sold at a very reduced price. For this reason, it was decided to investigate fish colouration. Fish cannot fully synthesize their own carotenoid colourings and these must therefore be included in their diet.
Considering the biopotential of carotenoids as narrated above, the present study was undertaken to investigate the nutrition induced carotenoid changes in the freshwater ornamental fishes gold fish (*Carassius auratus*) and gourami (*Trichogastertrichopterus*) with the following objectives:

1. To formulate and prepare the test diets supplemented with various sources of carotenoids.
2. To assess the efficiency of test diets on growth, colouration in the selected tissues of chosen ornamental fishes.
3. To find out solvent system dependent variation in carotenoids in the selected tissues of chosen ornamental fishes.
4. To analyse the qualitative and quantitative changes of carotenoid using TLC and Spectrophotometer.