EVALUATION OF PROTEIN REQUIREMENT OF ANGEL FISH *PTEROPHYLLUM SCALARE* (Lichtenstein) LARVAE USING DEFATTED CLAM MEAT AS THE CHIEF PROTEIN SOURCE

BY

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THESIS

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2001

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My Dear
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DECLARATION

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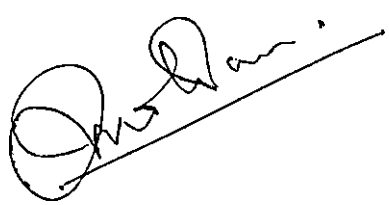

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INTRODUCTION

1. INTRODUCTION

Ornamental fishes are called the 'living jewels' due to their great variety of colour, shape and behavior. These are reasons for great excitement due to their incredible beauty, dazzling colouration and flowing fins.

Ornamental fish trade is flourishing in Asian countries. India has its stock of over 100 species of freshwater ornamental fishes. Most of the export of ornamental fishes from India depends on fish caught from the wild. The availability of fishes from the wild is fast reaching its limits due to over exploitation and pollution of natural water bodies. Hence breeding of ornamental fishes is gaining importance day by day.

Angel fish (*Pterophyllum scalare*) is one of the most beautiful and popular of the aquarium cichlids. These elegant graceful fish are available from two sources i.e. natural seed available from the freshwaters and the hatchery production. Live feeds like *Artemia* nauplii are most popular in the hatchery rearing of larvae. Formulated feeds have brought in very little success in larval rearing especially in the very early stage.

Feed management stands out to be one of the most decisive factors in ornamental fish larval rearing. The live feed *Artemia* nauplii is easily and readily accepted by the fish larvae than formulated feeds because of its mobility and acceptable size. It can also be easily and quickly hatched out as per demand. But experimental trials are being carried out with formulated feeds because these live feeds are costly and not easily available. Live feed culture methods for various species are available but there is the problem of uncertainty and failure of culture.

Quality of the artificial diets can be controlled during production. It can also be manufactured on a large scale, easily stored and distributed on a regular

basis. Artificial feeds can also be sterilized to prevent the risk of importing pathogens or parasites into the hatchery.

There are several ethological and technical considerations involved in larval feed preparation. The feed should have correct particle size, each particle should be water stable to restrict nutrient leaching and should have a low moisture content to promote stability during storage. Each feed particle must also have the complete composition of the feed as a whole. A few freshwater species have been successfully reared from first feeding exclusively on artificial diets.

A recent study on the effect of different diets on growth and survival of Angel fish fry showed that live feed and artificial feed based on clam meat did not differ significantly (Neelkanteswar, 1997). This study has proved that the formulated diet with a protein level of 29.7% was well accepted by Angel fish fry. But the optimum protein level was not worked out. Hence the present study has been taken up.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Larviculture today depends principally on live food. Artificial microfeeds are gradually gaining acceptance in the industry. A wholesome prepared food for the larvae is still unknown to science. The larvae require sufficient food to grow several fold during this period even though the gut characteristics are simple. Advances in biology and technology seek to break one of the last hurdles in the aquaculture cycle.

2.1 Early biology of fish related to feeding

2.1.1 Endogenous nutrition

Egg yolk provides the initial nourishment to the developing fish egg. The pattern of yolk absorption is similar among groups though the nutrient content of the yolk vary with species. Development of embryo in fish depends on a number of factors such as yolk composition, its digestion by syncytium or analogue tissue, the absorption and transport of yolk nutrients to developing tissues for somatic organization and metabolic demands for survival. Carbohydrates, lipids and proteins are consumed prior to hatching and the catabolization of the latter two also takes place after hatching (Heming and Buddington, 1988).

Growth during the endogenous nutrition period is also influenced by abiotic factors such as temperature regimes (Laurence, 1973), oxygen availability (Hamor and Garside, 1977),. salinity (Santerre, 1976), extreme pH (Nelson, 1982) and photoperiod (Hamor and Garside, 1975). Decrease in yolk absorption efficiency probably indicates increased homeostatic and maintenance cost within the physiologically tolerable range of these parameters (Hamor and Garside, 1977).

Perivitelline fluid and egg membranes are the other suspected non-yolk nutrient sources (Heming and Buddington, 1988). Some authors have described the possibility of assimilation of dissolved organic matter from the water after hatching (Amend and Fender, 1976; Lin and Arnold, 1982; Wiggins *et al.*, 1985). The feeding capabilities develop as and when the yolk reserves have been completely utilised and therefore larval survival ultimately depends on the availability of quality food in sufficient quantities (Rosenthal and Alderdice, 1976).

2.1.2 **Exogenous nutrition**

The most vital stage in the development of the larvae is the phase when the yolk has just been depleted and it turns to exogenous nutrients for further development. Salmon develops a mouth capable of accepting formulated feed only after the first three weeks during which phase it carries a large yolk sac (Sorgeloss and Leger, 1992). Newly hatched larvae of *Centropyge ferrugatus* measuring 1.28 to 1.3 mm in total length had a large ellipsoid yolk sac whose front tip clearly protruded beyond the snout of the larva (Hioki *et al.*, 1990). The rapid development of mouth facilitates a quick changeover from endogenous to exogenous nutrition. A linear relationship was found between mouth size and the total length of fish, from the initial exogenous feeding stage up to 20 to 30 mm in the cyprinids – *Aristichthys nobilis*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* (Dabrowski and Bardega, 1984). The gape of the oral opening at first feeding controls the size of food that can be accepted by the larvae (Shirota, 1970) and often it is less than 0.1 mm (Kohno *et al.*, 1988 and Glamuzina *et al.*, 1989). The increase of oesophagus diameter was related to the decrease of the yolk sac diameter, rather than to the increase in standard length in *Clupea harengus*, Ruegen spring herring (Busch, 1996).

The digestive functions were poorly developed immediately after hatching in *Clarias gariepinus*. However by 48 hours, when the yolk reserves were

depleted, the entire gastro intestinal tract excluding the stomach had formed into its final segments. After conducting an in-depth study of several fishes, Tanaka (1973) concluded that there were no pharyngeal teeth and hardly any taste buds at first feeding. The oesophagus had characteristic longitudinal folds and mucus cells. The intestine and rectum were the probable sites of digestion as indicated by the presence in the lining of columnar epithelial cells. On metamorphosis feeding patterns rapidly change accompanied by the demarcation of stomach and pyloric stomach. When the larval gut lengths were relatively small, the food passage time was short and in turn the time required for absorption was short. Prior to the development of gastric glands, fish relies on mechanical digestion, proper food selection and intestinal enzymes to compensate for the lack of gastric enzymes (Ferraris *et al.*, 1987). The presence of trypsin, chymotrypsin and intestinal enzymes such as amino peptidase, non-specific esterase and ATPase indicated the digestive capability of the fish at the start of exogenous feeding (Lauff and Hofer, 1984). In Cat fish (Verreth *et al.*, 1992) the pancreatic and intestinal hormones regulating the metabolism and digestive secretions were demonstrated within endocrine cells in 2 and 38-hour yolk sac embryos.

Larval fish may be divided into three groups according to their alimentary tract morphology and the enzymes secreted into the gut: those having a functional stomach before changing from endogenous to external food (salmonids); those having no functional stomach or gastric glands at the larval stage, but whose digestive organs differentiate during a complex metamorphosis (coregonids); and those who remain stomachless throughout the life, cyprinids (Dabrowski and Culver, 1991).

During the first days / weeks of fish life inescapable developmental changes occur in the structure and function of tissues and organs. The coordination of these morpho physiological events suggests the presence of an overriding factor that regulates the preprogrammed cells. There is evidence that several factors, intrinsic or genetic can be modified so that cells can be triggered

to differentiate by specific signal. The morphologic and enzymic differentiation occurring during exogenous feeding of larval fish can be distinguished into external (E- not programmed but triggered by external agent such as specific live food organisms), central (C - preprogrammed by a centrally released substance such as pituitary, thyroid and adrenal hormone) and local (L - preprogrammed by local timer in digestive tract; pancreatic enzymes expressed during fish metamorphosis) factors. The intestinal mucosa might be stimulated by continuous feeding behavior of the larvae and hence act as a tropic control of the absorptive cells. The hormones that control the development of intestinal enzyme expression and the onset of enterocyte differentiation are the central factors (Dabrowski, 1991a).

2.1.2.1 **Role of larval foods**

The first stages of larval rearing of many species largely depend upon expensive live zooplankton. Although most fish accepts artificial diets, they lead to low growth rate and high mortality when fed exclusively between hatching and metamorphosis of the fish. The digestive processes of the predator are supported and accelerated by the digestive enzymes which living prey contains. These exogenous enzymes represent up to 70% of the total proteolytic activity of the digestive tract in White fish, *Coregonus clupeaformis* (Lauff and Hofer, 1984). In larvae the activity of digestive enzymes is generally low but increases with age (Lauff and Hofer, 1984; Dabrowski, 1979).

In Roach larvae, the intestine amounts to only 45% of the body length compared to about 102% in adults. Intestinal length and gut passage time are positively correlated in larvae (Hofer, 1982). In Roach larvae, however, trypsin activity in the second half of the intestine amounts to 46% of total activity and it is reduced to 12% of the total activity in adults. This compensates for the short intestine by extending time at which enzymes may act on the food (Hofer and Nasiruddin, 1985). The larval diets must be nutritionally balanced, easily

digestible, palatable, and resistant to crumbling, water stable, buoyant and enhance pigmentation in ornamental fish (Boonyaratpalin and Lovell, 1977).

2.2 Nutrient demands in early stages

The nutritional requirements of fish embryos and eleuthero-embryos have not yet been defined. Nevertheless they would be expected to match the composition of the yolk that caters for the needs of the pre-feeding fish. Further, understanding of the nutrient needs of the early stages has been hampered by difficulty in changing the composition of the endogenous yolk reserve as a means to decipher the requirements (Heming and Buddington, 1988). The nutrient needs are demarcated specifically according to developmental stages based on the anatomical and physiological changes during ontogeny. The liver and related synthetase systems do not develop even after the appearance of the yolk syncytium (Takahashi *et al.*, 1978) and thus the later stages have narrower set of nutritional requirements than the pre-feeding fish (Heming and Buddington, 1988). Therefore it is not possible to compare the nutrient requirements of juvenile and adult fish.

Protein, which is the most abundant component among the nutrients contained in the egg, resides mainly in the yolk. It provides amino acids for tissue growth and energy through catabolic processes. The next major component is the lipid, which varies widely among the species in its content ranging from 0.1% of the egg weight in Plaice (*Pleuronectes platessa*) to 45% in mouth brooding cichlid, *Labeotrophus* (Balon, 1977). They mostly serve as the structural components of cell membrane or for energy production. Triglycerides and wax esters meet the increasing energy requirements after hatching. Yolk carbohydrates are present both in free state and as glycoproteins, though only available in relatively small quantities. Glycogen has been implicated as an energy source in the early embryonic stages and it is the primary carbohydrate in all fish eggs studied to date (Nakagawa, 1970; Terner, 1979; Cetta and Capuzzo,

1982; Vetter *et al.*, 1983). Carbohydrate has been attributed a nutritive role in initial cleavage because it is the nutrient utilised to the maximum between fertilization and hatching (Moroz and Luzhin, 1976).

Specific diets are required since the physiological capabilities are limited, be they live food organisms or formulated feeds. The lack of nutritionally competent ideal micro diets has brought in the use of the variety of live foods in larviculture. For the larval rearing, *Artemia* nauplii, rotifer and their enriched forms serve as successful diets for developing the fish. An understanding of the nutrient needs of the growing fish is required to develop an artificial diet, which acts as the missing link in the larviculture cycle. Current knowledge on nutrition has centred on the utilization of the yolk elements, survival and growth based on certain essential nutrients particularly essential fatty acids (EFA) derived from livefood or provided through micro diets since standard growth studies cannot be performed on early stages. The highly unsaturated fatty acids (HUFA) of the n-3 and n-6 series are crucial to survival and growth of first feeding larvae and this has been proved by several studies (Watanabe *et al.*, 1983b; Henderson and Sargent, 1985). Fyhn (1990) emphasized the importance of amino acids for the developing larvae. Free amino acid pool in the yolk was used up during development largely as a substrate in energy metabolism and was also employed at different rates for body protein synthesis (Ronnestad, 1992). The availability of dietary amino acids, their transport through intestinal mucosa and their availability at synthesis sites determine the body growth based on protein deposition. In vitamin requirement studies, enzymes, which use vitamins in a modified form as co-enzymes, could be used as status indicators (Dabrowski, 1991a).

2.2.1 **Lipids**

In the early stages of fish life lipids are indispensable. In marine fish embryos, lipids are the main source of energy even from the gastrula stage (Vetter

et al., 1983). The exponential loss in lipid reserves of larval fish on food deprivation underscores its primary role (Ehrlich, 1974; Tandler *et al.*, 1989). Triacylglycerols constitute the characteristic oil globule(s) in many eggs (Nakagawa and Tsuchiya, 1971). The fact that essential fatty acids have been attributed with several functions in the adult fish paves way to the importance of the role of lipids in larval nutrition (Cowey and Sargent, 1979; Watanabe, 1982; Kanazawa, 1985a).

For the normal growth and survival of the larval fish, it has been demonstrated that the n-3 highly unsaturated fatty acids (n-3 HUFA) are required. Mortality and deficiency signs such as under-developed swim bladder have been reported in larval fish, which were low in n-3 HUFA (Kanazawa, 1985b; Dhert *et al.*, 1991; Koven *et al.*, 1992). Due to low HUFA diets, incomplete metamorphosis of Summer flounder (*Paralichthys dentatus*) larvae has occurred (Bisbal and Bengston, 1991). One of the most essential fatty acid in fish, the eicosa pentaenoic acid (EPA, 20:5n-3) is a constituent of the cellular membranes of developing tissues (Kanazawa *et al.*, 1982). Rapidly growing larval fish takes up relatively large amount of exogenous EPA. Improved growth and survival were obtained by inclusion of phospholipids in larval feeds. It is proposed that the phospholipids were probably involved in the formation of new cell components though a precise physiological role was not demonstrated. The rate of biosynthesis of phospholipids in the larvae was probably insufficient to meet the developmental requirements (Kanazawa, 1985b).

2.2.1.1 **Enrichment of live food with PUFA**

Experiments were conducted to improve the dietary value of live foods such as rotifers, *Artemia* nauplii and *Moina* sp. by allowing them to feed on n-3 HUFA and fat-soluble vitamins by the direct method (Watanabe *et al.*, 1983b). Oka *et al.* (1980) found that linoleic acid (18:2n-6) had no EFA value whereas n-3 HUFA was effective in enhancing growth of the larvae of Ayu when

feeding experiments were conducted on this species. He used rotifers cultured on n-3 yeast rich in linolenic acid and n-3 HUFA as feed. Watanabe *et al.* (1989) after taking into consideration the activity and availability of n-3 HUFA and also on the basis of improved growth and survival obtained on larval Red sea bream, suggested that the minimum requirement of this species is about 0.4% wet weight basis of rotifer. Izquierdo *et al.* (1989) on the same fish at the *Artemia* eating stage established using fortified animals that the larvae require at least 3% n-3 HUFA in the *Artemia* nauplii on a dry matter basis. Tandler *et al.* (1989) in another study on *Pagrus major* revealed that there was a relative increase in n-3 fatty acids associated with food depreviation. Le Milinaire *et al.* (1984) reared the larva of Turbot (*Scophthalmus maximus*) with rotifers fed on artificial micro diets with or without n-3 HUFA and proved their importance as EFA. Walford *et al.* (1991) suggested that feeding Sea bass larvae with microcapsules along with rotifers was successful.

2.2.1.2 **Importance of poly unsaturated fatty acids**

Based on the chemical profile, Tocher and Sargent (1984) proposed the high n-3 PUFA requirement in early larval stages of marine fish. The concentration of docosa hexaenoic acid (DHA; 22:6n-3) was generally high in the eggs of marine species and fell quickly during development (Watanabe, 1993). The superiority of DHA over EPA in promoting growth and survival has been recently demonstrated in larval Red sea bream (Watanabe *et al.*, 1989; Takeuchi, *et al.*, 1991), Yellow tail (Watanabe, 1993) and Flounder (Kanazawa *et al.*, 1989). Feeding of DHA also led to increased resistance to stress in *Coryphaena hippurus* (Ako *et al.*, 1991). The importance of DHA may be seen from the fact that it is selectively retained in the polar lipids of developing larvae during starvation and when it is fed it is selectively incorporated into the larval glycerophospho lipids, which are essential components of biological membranes. Fluidity of the membrane in gills seems to depend less on the arachidonic acid content than on the DHA. DHA is the main component of the

glycerophospholipids in marine fish roe (Tocher and Sargent, 1984). It is also stored in the olfactory nerve and retina (Sargent *et al.*, 1993) and central nervous system of fish (Mourente and Tocher, 1992), enhancing the detection and capture of prey and the net energy gained by the larvae (Noakes and Godin, 1988). The DHA content in phosphoglyceride lipids of larvae was directly correlated with growth improvement in Gilt head sea bream (Rodriguez *et al.*, 1994).

One of the major prostaglandins in the gills of freshwater trout is reported to be a prostaglandin PGF_4 formed from DHA (Mai *et al.*, 1981). German *et al.* (1983) later demonstrated that this compound is a trihydroxylated derivative of DHA. Though the growth and survival of Summer flounder larvae were not affected by the elevation of dietary DHA levels, it slightly increased the pigmentation and number of fish that completed metamorphosis. In contrast. excessive levels of DHA significantly reduced total weight of Summer flounder larvae (Bisbal and Bengston, 1991).

Eicosapentaenoic acid (EPA), an important PUFA has not been paid due attention. Elevation of the dietary EPA effectively prevents low growth and survival rates (Watanabe *et al.*, 1989). The larvae of several species during starvation selectively retain arachidonic acid and it is regarded as the major source of prostaglandin (E_2 and F_2 alpha) although it is present in lower concentrations than EPA in fish tissues (Rainuzzo *et al.*, 1994). Arachidonic acid is present in high concentrations in the phosphatidyl inositols of marine fish (Bell *et al.*, 1984).

Present knowledge suggests that not only DHA but also other PUFAs are essential for marine fish larvae. A balanced dietary ratio of EPA/DHA may also be necessary to obtain optimum growth rates along with the importance of total content of DHA or n-3 PUFA (Izquierdo, 1996). Harel and Place (1999) have suggested that several fish species also require arachidonic acid and that diets should be carefully formulated with a species- specific dietary DHA: EPA: AA ratio. The level of DHA and the DHA: EPA ratio in *Dentex dentex* larvae given

rotifers and *Artemia* were significantly lower related to the corresponding values in the unfed larvae (Tulli and Tibaldi, 1997).

2.2.1.3 **Phospholipids**

Kanzawa *et al.* (1983a) examined the effects of supplemental growth of larval Red sea bream using the purified microparticulate diets. It was shown that phospholipids improved the growth and survival irrespective of age. In order to clarify which components of bonito-egg lecithin were most effective for the larval Ayu, Kanazawa *et al.* (1983b) has examined the effects of several phospholipid classes on the growth and survival of *Plecoglossus altivelis*. These compounds in addition to n-3 PUFA are believed to be indispensable for normal growth and survival. The requirement for the phospholipids may arise from a limited ability of the larval fish to synthesize it from ordinarily available precursors. High amounts of EPA in relation to DHA may create an imbalance in the structural composition of the phospholipids, which could affect the normal growth and the quality of marine larvae (Rainuzzo *et al.*, 1997).

2.2.2 **Amino acids**

Several research studies have revealed that the freely soluble amino acids (FAA) occur in high amounts in pelagic eggs of some marine species (Suzuki and Suyama, 1983; Fyhn and Serigstad, 1987; Fyhn, 1990; Ronnestad, 1992) and the variation observed among the species was related to egg size. In the newly spawned egg the FAA concentration represented about 50% of the total osmolality. Ronastad (1992) suggested that the FAA pool constituted 20 to 50% of the total amino acids in pelagic eggs. In the marine demersal egg this was only about 3% (Ronnestad, 1992) and about 5% in fresh water fishes (Dabrowski *et al.*, 1985b). The abundance of FAA in the pelagic eggs correlated its role in the process of oocyte hydration. The major amino acids detected were leucine, lysine, valine, isoleucine, alanine, serine and the related composition of these

FAA pool showed little variation (Ronnestad, 1992). The similar profiles have resulted from the hydrolysis of a common yolk protein - Phosvitin - corresponding to water uptake during swelling (Mc Pherson *et al.*, 1989; Thorsen and Fyhn, 1991). The absolute and relative composition of the yolk may change with development and a consistent reduction in the FAA pool occurs, the decrease applying to all individual FAAs. The decline in the larval FAA pool is the result of a metabolic turnover within the embryo, particularly the need for protein synthesis (Nakagawa and Tsuchiya, 1972). Larval protein synthesis has been described for Cod, *Gadus morhua* (Fyhn and Serigstad, 1987), Lemon sole, *Microstomus kitt* (Ronnestad *et al.*, 1992a), Turbot, (Ronnestad *et al.*, 1992b) and Atlantic halibut (Ronnestad *et al.*, 1993) in the yolk sac stage.

Ronnestad (1992) is of the view that body protein may be just a temporary storage molecule for amino acids removed from the free amino acid pool during egg stage to be utilised later for bio energetic purposes. When the larva turns to exogenous food, amino acids necessary for energy would be provided by the ingested food. It is not clear whether the primary intestine in the larval fish is able to digest food proteins in presence of the early proteolytic enzymes or whether they depend on FAA provided by the feed (Fyhn, 1990).

Some preliminary experiments in Flounder (*Paralichthys olivaceus*) was done using various protein sources which had an overall amino acid profile similar to the whole body of the larval fish. A comparison of the nutritive values of four micro particulate diets formulated with white fish meal, brown fish meal, bonito powder, yeast powder, crab meal, gluten meal and krill meal was also made with the live food control. The survival range for the test group was 36 - 45% and for live food control it was 52%. A drastic reduction of abnormal pigmentation was observed when the test micro diets were compared to live food (Kanazawa, 1988). Kanazawa *et al.* (1989) recorded better survival but slightly inferior growth compared to live food when the larvae of Red sea bream were fed microdiets based on sardine powder, squid powder, scallop powder and krill meal

containing a balanced proportion of amino acids. These studies revealed that the essential amino acid composition of the larval fish body closely match their dietary requirements.

As the larva starts exogenous feeding, spurt in activity demands lot of energy. The fish accepts its first food, probably tempted by chemo-attraction from the amino acids and other metabolites emanating from the prey organisms since the endogenous energy reserves are finite (Tanaka *et al.*, 1991). In the marine fish larvae, the FAA is predominantly used as metabolic fuel but they are also utilised for body protein synthesis. Amino acids are also important catabolic substrates after the onset of first feeding and may account for 60% or higher of the energy dissipation. *In vivo* studies have shown that there is higher absorption of FAA than peptides and protein bound amino acids from the larval gut in the early stages of marine fish larvae (Ronnestad *et al.*, 1999).

2.2.3 **Vitamins**

Studies on the vitamin needs in the early stages of fish have focused on vitamin-C. The minimum dietary requirements of ascorbic acid using stable phosphate derivatives has been determined for teleost species to be in the range of 10-20 mg /kg (Merchie *et al.*, 1996). Many workers noticed a rapid drop in the ascorbic acid content during development (Cowey *et al.*, 1985, Sato *et al.*, 1987 and Dabrowski, 1990). Since the fish is unable to synthesize the vitamin it is necessary to replace it during first feeding to ensure larval survival. As yolk reserves were limited in cyprinid larvae when compared to salmonids, ascorbic acid was particularly essential for development in the former (Dabrowski *et al.*, 1988). The body pool of ascorbate was used up rapidly due to the high growth rate and therefore structural malformations appeared easily. In cyprinids, vitamin C free diets led to retardation of growth, to lordosis and scoliosis and to increased mortality (Agrawal and Mahajan, 1980). Dabrowski (1990) fed White fish (*Coregonus lavaretus*) with a relatively high amount of vitamin-C (about 1500

micro gram ascorbate per gram dry matter fed) in order to maintain the body concentration of ascorbate during early ontogeny. It was revealed that a decrease in the body concentration of ascorbate was relatively slow in larvae fed on live food. Nakagawa et *al.* (2000) suggested that dietary supplementation with catechin as well as *Spirulina* improved vitamin-C metabolism in Red sea bream larvae. Merchie *et al.* (1996) in a study indicated that common carp larvae have a dietary requirement for ascorbic acid and the required level for maximum tissue storage is higher than that needed for survival and maximum growth. Based on growth response, a dietary requirement of about 45 mg ascorbyl polyphosphate equivalent kg^{-1} was suggested. Blom *et al.* (2000) based on the long possible life span of Angel fish in the aquarium, proposed a conservative dietary ascorbic acid requirement of 360mg/kg diet necessary to maintain maximum tissue storage of vitamin C.

Watanabe (1990) focussed on the importance of the vitamin E in salmonid larvae in his studies on the chemical composition of eggs. In both Chum salmon (*Oncorhynchus keta*) and Coho salmon (*O. Kisutch*), the vitamin-E content was depleted by almost one half during the developmental period of 40 days from hatching. Hence the role of vitamin E in larval survival cannot be underestimated.

2.3 **Hormones and larval quality**

The hormonal level in eggs / larvae may be a vital factor to determine the egg / larval quality. Recent studies suggest that hormones are passed on to eggs by brood fish. The fish larval growth, development, osmoregulation, stress response and other physiological functions, prior to the functional development of their own endocrine glands are controlled by the store of maternal hormones in eggs (Lam, 1994).

2.3.1 **Thyroid hormones**

The thyroid hormones thyroxine (T_4) and triiodo thyronine (T_3) are consistently present in teleost eggs and thus may play an important role in the egg development (Lam, 1994). T_4 concentrations were significantly greater than T_3 concentrations in most fresh water fishes, whereas T_3 concentrations were greater in seawater fishes (Tagawa *et al.*, 1990). The significance of this is not clear and seasonal variation in T_4 and T_3 levels are possible (Lam, 1994). During the course of development, thyroid hormones in eggs decreased markedly before hatching. Thyroxinogenesis (endogenous thyroid hormone production) appears to be turned on at around or before the time of yolk sac resorption (Tagawa and Hirano, 1990). A distinct surge of thyroid hormones were observed in 9 day old larvae indicating its major role in organ differentiation during early larval stages of *Catla catla* (Nayak *et al.*, 2000).

2.3.1.1 **Effect of treatments**

In several freshwater species: Tilapia, *Oreochromis mossambicus* (Reddy and Lam, 1992) *O. niloticus* (Nacario, 1983); Common carp, *Cyprinus carpio* (Lam and Sharma, 1985); Goldfish, *Carassius auratus* (Reddy and Lam, 1992) and Gauramy, *Colisa labia* (Reddy and Lam, 1987) larvae, treatment by immersion in T_4 or T_3 solution promoted growth. Fin differentiation and growth, yolk sac resorption, transition to free swimming stage, skin and scale formation, pigmentation, silvering and exophthalmia (in Black moor) were the developmental stimulating effects. Stimulation of heartbeat is a possible metabolic effect observed (Reddy and Lam, 1992), although this was not observed in Chum salmon (Dales and Hoar, 1954). Thus, the treatment with T_4 or T_3 had supplemented the level obtainable from the maternal store (Reddy and Lam, 1992), there by producing the enlarged growth and developmental effects.

An adverse effect on growth and development was noticed when excessive or prolonged treatment was given (Nacario, 1983). Even with early thyroxinogenesis, availability of iodine in these freshwater species may be limiting, thus allowing for efficacy of T_4/T_3 treatment. In marine species, iodine deficiency is not a problem (Lam, 1994). In such cases T_4/T_3 treatment of post larvae may bring in particular developmental events associated with the marked increase in T_4/T_3. They include transformed post larva of Milkfish, *Chanos chanos* from being long, slender and transparent to silvery, opaque and juvenile like (Lam *et al.*, 1985); accelerated metamorphosis in Flounder, *P. olivaceus* (Yamano *et al.*, 1991); promoted the transformation of post larvae of black 'telescopic eye' gold fish to the black exophthalmic form of the adult (Reddy and Lam, 1992); and accelerated appearance of black stripes on the body and change in habitat from pelagic to benthic in post larvae of Red sea bream, *Pagrus major* (Hirata *et al.*, 1989).

Injection to the mother fish is a more practical means of thyroid hormone administration to eggs / larvae. This method has been successful in Striped bass, *Morone saxatilis* (Brown and Bern, 1989). Mortality is the end result when overdose of thyroid hormones are administered which leads to thyrotoxicosis manifested in larvae by growth retardation and abnormal development (Lam and Sharma, 1985). Bengston *et al.* (2000) in his experiments indicated that thryoid hormone treated Summer flounder (*Paralichthys dentatus*) larvae do not exhibit significantly greater survival than untreated larvae. Administration of T_4 to *P. olivaceus* egg by immersion at a dose of 0.05×10^{-6} resulted in significant increase in growth rate in 0 to 10 age in days (Bao Baolong *et al.*, 1999).

2.3.2 **Other hormones**

Studies were conducted to find out the possible role of cortisol, the principal cortico steroid in early fish development (Idler and Truscott, 1972; Lam, 1994). In the newly fertilized eggs of the Flounder (*P. olivaceus*) cortisol has been

detected. When Seabass(*Lates calcarifer*) was subjected to salinity test, cortisol was found to promote survival (Sampathkumar *et al.*, 1993). Transient growth stimulation in the Brown trout, *Salmo trutta* was observed when the eggs of it were immersed in 15mg per ml recombinant trout GH (Le Bail *et al.*, 1991).

2.4 **Diets for larval fishes**

Research work in larval nutrition and feeding strategies stand out as the priority areas for the industrial up scaling of aquaculture of fin fishes and shell fishes. The cellular growth and the energy needs of the developing embryo are met by the nutrients within the egg until the larva starts feeding. When the larva commences exogenous nutrition, the scarcity of suitable planktonic prey organisms will result in body tissue autolysis and eventual death (Bagarinao, 1986). Different types of phytoplankton, small zooplankton and invertebrate larvae form the natural diets. The physical qualities such as purity, availability, acceptability and size together with nutritional indicators such as digestibility and nutrient / energy obtainable from it are the criteria in deciding the food source (Leger *et al.*, 1987). It should also be easily procurable, reproducible and economical. These definitive criteria limit the choice to a few ciliate protozoa, rotifers, copepod nauplii and small planktonic invertebrate larvae such as trocophores and veligers (Dhert and Sorgeloss, 1995). At present there is an increasing trend to offer formulated micro diets to several larval fish.

2.4.1 **Live foods**

2.4.1.1 **Microalgae**

The primary link in the food chain of aquatic animals is formed by microalgae. The production of specific types of larval aquaculture species and zooplankton food is dependent upon nearly 20 different species of diatoms and flagellates in the size range of 2-20 µm, which is used as live food. Improved

growth and survival during the larval stage are obtained by the addition of various microalgae to the water during first feeding of fish larvae (Howell, 1979; Scott and Middleton, 1979; Jones *et al.*, 1981; Naas *et al.*, 1992).

The use of green water technique has been successful in the culture of various larval fishes and shrimps. Maintenance of sufficient concentrations of phytoplanktonic algae in the larval culture tanks is the major technique involved. Microalgae do not constitute a major food source at the start of feeding since fish larvae do not filter feed, but they are carnivorous hunters. Several works have been done to explain possible beneficial effects of the green water technique. In Cod (Meeran, 1982) and Halibut (Reitan *et al.*, 1991), it has been shown that the larvae take up considerable numbers of micro algae during the initial days of yolk absorption ('green stomachs'), which may support the proposition that they are used as direct food source at the start of feeding. Fortuitous ingestion can be a source of micronutrients, which are not available through the administered rotifers or brine shrimp nauplii. At the start of feeding, fish larvae have only a primitive digestive system and the supply of exogenous enzymes by them could assist in the digestion of zooplankton. This technique has been used in the culture of Common carp, *Cyprius carpio*, Bighead carp, *Aristichthys nobilis* (Fermin and Recometa, 1988) and Atlantic halibut, *Hippoglossus hippoglossus* (Naas *et al* ., 1992).

Indirectly microalgae may trigger enzymatic synthesis (Hjelmeland *et al.*, 1988) and onset of feeding (Naas *et al.*, 1992). It was found that algal polysaccharides might act as non-specified immunological stimulants in the larval fish (Lavens *et al.*, 1995) and in this way may contribute to more stable forms of production. *Spirulina spp.* has been relied upon as an excellent source of many nutrients and it has been widely used as a source of pigment in ornamental fish (Dhert and Sorgeloss, 1995).

Stock cultures kept under sterile conditions and batch cultures of increasing size are required for the maintenance of algal cultures. Problems still

exist with regard to contamination as well as consistent nutritional quality between batches (Olsen, 1989). Many substitute products as algal replacement diets are under experimental stages. Freeze-dried heterotrophically grown microalgae (Liang and Verdugo, 1991), manipulated yeast (Coutteau *et al.*, 1990) and various microparticulate and microencapsulated diets (Kanazawa *et al.*, 1982) are examples.

2.4.1.2 **Rotifers**

Brachionus plicatilis has become the most extensively used zooplankton for rearing various freshwater and marine larval fish during the first and second week of feeding since the establishment of culture possibilities (Ito, 1960). A combination of marine chlorella and baker's yeast (*Saccharomyces cerevisiae*) were used to culture and produce the rotifers with improved nutritional quality (Kitajima *et al.*, 1979). The digestibility of rotifer protein was high as 84-94% was revealed by nutritional studies based on rotifers and the net protein utilisation (NPU) was relatively high when tested in Rainbow trout and Carp (Watanabe *et al.*, 1983a). Rotifers, which are widely used as live feed, supply only half of the methionine and tryptophan requirement for most fish larvae (Harel and Place, 1999). The variations in n-3 PUFA content brought about by the culture conditions strongly affect the quality and in turn the survival of larval fish, which prey on them (Fukusho, 1989). The dietary value could be improved not only in terms of n-3 PUFA but also fat-soluble vitamins by using the emulsified formulations (Watanabe *et al.*, 1983b). The quality enhancement technique is used as early as 24 hours before offering them to the fish larvae (Watanabe *et al.*, 1983a). Walford and Lam (1987) successfully enriched rotifers to contain a high concentration of EPA and DHA eight hours after feeding by using microcapsules. Brown *et al.* (1998) in his studies indicated that the enrichment and retention of ascorbic acid in rotifers fed with micro algal diets were possible and the production of rotifers rich in ascorbic acid may be particularly valuable for the culture of fish larvae, which have a high requirement for the vitamins.

The development of an artificial diet, which completely replaces algae and at the same time eliminates the need of an extra enrichment period for enhancement of the rotifers' dietary value, has been a recent break through in production technology. The dry products need to be immersed in water prior to feeding. The food suspension can be used in automatic feeding as long as 48 hours provided it is continuously aerated and stored at low temperature (Lavens *et al.*, 1995). Under these conditions, i.e. twice a week water renewals, strain adapted temperatures, ciliate removal and culture under shaded conditions, doubling of the population may even be expected every 24 hours (Lavens *et al.*, 1994).

The exclusive use of artificial diets in rotifer culture reduces the unit production costs of rotifers by more than 60%, which is revealed by the study on the economics of rotifer production in operational hatcheries (Lavens *et al.*, 1995).

2.4.1.3 ***Artemia***

Aquacultural practices have promoted *Artemia* as the most favoured food for larval organisms since its discovery by Seale (1933). It has various attributes that make it an ideal choice for aquaculturists apart from the nutritional qualities. Their availability in two forms and wide size range are two prominent attributes. In addition, they could be used as carriers to make available essential nutrients, pigments, prophylactics and therapeutics to fish larvae (Leger *et al.*, 1987).

The maximum food value is for the early nauplii, loosing up to 30% with age (Sorgeloss *et al.*, 1996). They are to be judiciously utilized as food for different stages of the larval fish. Cold stored freshly hatched nauplii kept for periods of 24 hours and longer have been said to attain a better quality food ration (Sorgeloss and Leger, 1992). There exists a positive correlation between *Artemia*

nauplii size and larval fish mortality (Beck and Bengston, 1982) and this emphasises the need for the selection of the *Artemia* strain to be offered as food, which depend on the farmed species. This is because of the large variations seen in the nauplii size among the large number of geographical strains (Vanhaecke and Sorgeloss, 1990).

Watanabe *et al.* (1983a) found that live food was well digested and high net protein utilization and protein efficiency ratio were obtained while he was evaluating the nutritional qualities of *Artemia* nauplii in Rainbow trout and carp. Enhanced digestion was obtained by the autolytic action of the endogenous enzymes, amylase and trypsin (Samain *et al.*, 1980). The consumption of *Artemia* nauplii by marine fish larvae may increase the production of bombesin, a hormone influencing digestion. When *Artemia* nauplii was given as a sole food to the larvae, the presence of bombesin increased by 300% as compared to the levels that were found in the larvae given only a micro diet (Kolkovski *et al.*, 1997).

2.4.1.3.1 **Bioencapsulation**

Prior to feeding predator larvae, it is possible to incorporate different kinds of products into the *Artemia* nauplii, taking advantage of the primitive feeding characteristic of its nauplii. For enhancing the nutritional value of *Artemia* with essential nutrients (like n-3 PUFA and vitamin C), this method called as *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries.

2.4.1.3.1.1 **Poly unsaturated fatty acids**

British, Japanese and Belgian researchers developed enrichment products and procedures using selected microalgae and /or microencapsulated products, yeast and /or emulsified preparations, self emulsifying concentrates and /or microparticulate products (Leger *et al.*, 1986). The highest enrichment levels

are got from emulsified concentrates (50 - 60 mg/DW n-3 PUFA) after a period of 24-hour enrichment. High dietary levels of total PUFA can have a negative impact and that DHA is more important than EPA for various physiological functions including survival, growth and pigmentation success which was revealed when studies with various species of marine fishes were carried out (Watanabe and Kiron, 1994). However, the enrichment of *Artemia* with DHA is not easy because of inherent catabolism of this fatty acid upon enrichment (Triantaphyllidis *et al.*, 1995).

2.4.1.3.1.2 **Ascorbic acid**

Ascorbic acid (AA) is generally considered to be an important dietary component in larviculture (Dabrowski, 1992). Supplemental dietary ascorbate has enhanced several biological (eg. skeletal development, growth and survival) as well as physiological functions (eg. resistance to toxicants and stress, immuno activity in larvae (Merchie *et al.*, 1996). A stable derivative of AA which is Ascorbic acid 2- sulphate (AAS) was discovered in dormant cysts of *Artemia* by Mead and Finamore (1969). Cysts of different batches and strains of *Artemia* differed largely in AAS content (296-517 µg AA/g DW) (Merchie *et al.*, 1995). The amount of AA, liberated in freshly hatched nauplii gives an idea of the AAS reserve present in the cysts and provides evidence for the conversion of AAS to free AA during completion of embryonic development into nauplii (Dabrowski, 1991b). Researches have been conducted to incorporate extra ascorbic acid into *Artemia* nauplii in a stable and bio available form. Using a standard enrichment procedure (Leger *et al.*, 1987) and experimental self emulsifying concentrates containing 10-20% ascorbyl palmitate (AP), levels upto 2.5 mg free AA/g DW can be added into brine shrimp nauplii within 24 hours (Merchie *et al.*, 1995).

2.4.1.4 Cladocerans

2.4.1.4.1 *Daphnia* spp.

Daphnia is a good food source in freshwater aquaculture and ornamental fish industry. They can also be used in mariculture as substitute live food for *Artemia*. Dhert and Sorgeloss (1995) revealed that the nutritional value of *Daphnia* sp. is highly dependent on the chemical composition of their food source (Dhert and Sorgeloss, 1995). If *Daphnia* sp. is fed solely with *Chlorella*, *Chlamydomonas*, mixture of Yeast and *Scenedesmus* or *Scenedesmus* and *Chlamydomonas*, then the cultures tend to collapse after 10-30 generations. This problem can be avoided by adding vitamin mixtures and trace elements to the culture medium. *Daphnia* sp. contains a wide range of digestive enzymes such as proteinases, peptidases, amylase, lipase and even cellulase, which can serve as exoenzymes in the gut of the fish (Dhert and Sorgeloss, 1995).

2.4.1.4.2 *Moina* spp.

Mass culture can be done with the freshwater cladoceran, *Moina spp.* (Shim, 1988) and has often been used as live food for a variety of fish larvae (Watanabe *et al.*, 1983a; Fermin and Recomenta, 1988). For rearing tropical aquarium fish larvae *Moina* sp. is found to be a suitable live food (Volkart, 1994). Sea bass larvae and juveniles (Maneewongsa and Tattaman, 1983), fry of mullet (Nandy, 1979) and fry and fingerlings of Rainbow trout (Norman *et al.*, 1979), can be reared using *Moina* spp.. The n-3 PUFA composition of *Moina spp.* fluctuates with the culture medium but it can be upgraded nutritionally by employing emulsified lipids (Dhert and Sorgeloss, 1995; James and Sherief, 1999). The *Moina* sp. cultured on poultry manure had a high EPA content (Shim, 1988).

2.4.1.5 **Copepods**

Marine copepods such as *Tigriopus sp; Acartia sp., Eurytemora sp.. Euterpina sp., Oithona sp.* and *Paracalanus sp.* are offered to fish larvae of about 7mm. Uhilig (1984) has demonstrated the suitability of the genus *Tisbe* as a microfaunal food organism in mariculture. The proximate and the mineral composition of these live foods were varied by the culture media, but there were no significant differences in the aminoacid composition. In Rainbow trout and Carp their dietary value in terms of NPU and PER was high. Irrespective of culture media and food organisms, *Tigriopus* contained relatively high amounts of EPA and DHA, which suggest its high nutritional value (Watanabe *et al.*, 1983a).

Copepods are far superior to *Artemia* nauplii from the nutritional point of view; their lower proteolytic activity and better fatty acid composition makes them an excellent food with high energy content (Dhert and Sorgeloss, 1995). Their use in aquaculture has often brought in better growth, survival, development and pigmentation of the fish.

2.4.1.6 **Frozen or Freeze-dried plankton**

Live, frozen or freeze dried plankton as initial food, either alone or in combination with artificial diets seem to meet the nutritional needs of fish larvae (Jungwirth *et al.*, 1989). Frozen zooplankton proved to be a successful diet for several marine species, particularly Sea bass (*Dicentrarchus labrax*) (Kentouri, 1981). Medgyesy and Wieser (1982) found that White fish (*Coregonus laveratus)* was reared successfully with frozen zooplankton beyond metamorphosis using a feeding apparatus, which prevents leaching of proteins. The fry of Danube salmon (*Hucho hucho)* was successfully fed with freeze dried zooplankton, which suggests that it might have been a source of exogenous enzyme activity (DeVerga and Bohm, 1992).

2.4.1.7 **Others**

The high protein content of the nematode *Panegrellus redivivus* has made it a viable food source for fish larvae (Kahan *et al.*, 1980). Without consuming algae, it can survive over 72 hours in seawater and this was pointed out as an advantage it had over *Artemia.* There are several differences in the fatty acid composition (Biedenbach *et al.,* 1989). The DHA is almost the same or a little higher than that in *Artemia* while the EPA content is nearly a third. By employing a culture medium consisting of wheat flour, yeast and fish oil , Rouse *et al.* (1992) succeeded in enhancing the nutritional quality in terms of EPA and DHA . Nematodes could be used as an alternative live food that is inexpensive and consistently nutritious when cultured under proper medium conditions. In Common carp, *Cyprinus carpio* and Silver carp, *Hypothalmichthys molitrix*, the experimental use of free-living nematode, *Panegrellus redivivus* as larval food has been successful (Dhert and Sorgeloss, 1995).

The natural food of freshwater fish include aquatic oligochaetes and the use of tubificid *Branchiura sowerbyi* in aquaculture, including its culture has been reported (Aston, 1984). Another species *Tubifex tubifex* has been given as food for elvers of *Anguilla nebulosa* (Reddy *et al.*, 1977) and cat fish, *Mystus vittatus* (Arunachalam and Reddy, 1981). Trouts were fed with terrestrial oligochaetes such as *Eisenia foetida* (Tacon *et al.*, 1983) and *Dendrodrilus subrubicundus* (Stafford and Tacon, 1984). In early stages of ornamental fish, *Brachydanio rario*, Roach, *Rutilus rutilus* and carnivorous perch, *Perca fluviatilis*, the possibilities of the use of *Enchytraeus sp.* as food and its chances of mass production was described (Bouguenec and Giani, 1989)

2.4.2 **Formulated Feeds**

2.4.2.1 **Physical requirements of formulated feeds**

More than two decades of research have been done on the formulation of artificial diets to replace biofood in larviculture and have met with only limited success. The efficacy of artificial diets varied widely among species. Certain diets provided good growth, feed efficiency and stress resistance. Early application of inert diets enables easy weaning on dry food. This could drastically scale down expense in larviculture as the dependence on live food production is reduced. Generally the main problems encountered are the low ingestion and poor digestion rates.

The factors to be considered for artificial larval diets have been described by Kanazawa (1988) and Walford *et al.* (1991). The normal diet ranges between 5 and 300 micrometer but the particle size and the specific gravity depends on developmental stage and the species in production. It should be stable in water for a long time, attractive for the larvae and kept in motion until the first feeding larvae can capture it. The binding material should be easily broken down to release the nutrients, which in turn have to be easily digestible in the alimentary tract of the early stages of fish. Based on these principles, early attempts at feeding with microparticualte diets were made by Adron *et al.* (1974). In several species (e.g. European eels, Atlantic salmon, Pacific salmon, Chinook salmon and Arctic charr), the particle size optimal for growth relative to fish size was determined following feeding experiments and described relative to fish length and mouth size (Fowler and Burrows, 1971; Wankowski and Thorpe, 1979; Knights, 1983; Tabachek, 1988). The larvae must be able to chemically and optically recognise the feed as food (Appelbaum, 1980). For *Clarius gariepinus*

larvae, a feed particle diameter of 2.2% of the mean initial length of the larvae or fry was found to be optimal (Uys and Hecht, 1985). The feed particles must be water stable to restrict leaching and should have a lower moisture content to promote stability during storage (Csavas *et al.*, 1979). If these conditions are not met, it may pave way to differential acceptability of the food particles by the larvae and may ultimately result in a nutrient imbalance leading to high mortality and may also cause water quality deterioration.

The feed must not be exposed to excessive and /or prolonged heat during the manufacturing process (Meyers, 1979). The complete range of nutrients must be present in a biologically available form and at optimum level and ratios (Nose, 1979). The cost and time involved in manufacturing must be reasonable. All the above criteria have to be satisfied in developing a simple yet suitable feed manufacturing process.

2.4.2.2 **Weaning**

Weaning in larval rearing is the process by which the larvae must learn to eat the prepared diets in place of the live food (Bromley, 1981). This successful transition is dependent on the feed quality and the larvae themselves (Devresse *et al.*, 1991). The best time to start weaning is soon after larvae switch to exogenous feeding. Since weaning is economically advantageous, it is important for aquacultural purposes. Refrigeration or freezing is done to store dry feed. Commercial dry feed can be used in automatic feeders and this eliminated much of the labour, cost and time expended in rearing and dispensing of live food (Bromley, 1981).

Few species have been reared successfully from the one-day-old hatchling stage exclusively on artificial diets and in most cases success at the experimental level has yet to be reproduced on a commercial scale. In general, it is easy for the freshwater larvae to adapt to dry feed since they are fairly large at hatching. This

is true particularly for the Salmonids (12 to 25 mm at hatching), which possess a functional stomach at first feeding and which do not require live prey at this stage. Among other freshwater species, the most encouraging results have been achieved with coregonid larvae, which in many experimental studies have been reared exclusively on dry diets (Champigneuille, 1988). From first feeding, *Coregonus laveratus* can be reared on a yeast based dry diet with good growth and survival (Champigneuille, 1988). Artificial feeds are exclusively used to rear Common carp, *Cyprinus carpio* (Charlon and Bergot, 1984; Charlon *et al.*, 1986) and Ayu, *Plecoglossus altivelis* (Kanazawa *et al.*, 1985).

Artificial diets can successfully be fed to early larvae as a partial replacement for, or supplement to, live foods. In some cases co- feeding live and artificial diets can bring in growth and survival in early larvae superior to that achieved with either live foods or artificial diets alone. This has been found for *Micropterus dolomieri* (Ehrlich *et al.*, 1989); *Clarius gariepinus* (Jones *et al.*, 1993) and *Carassius auratus* (Abi-ayad and Kestemont, 1994). Dabrowski, 1984 experimented on the larvae of Common carp, *Cyprinus carpio* and Silver carp, *Hypophthalmichthys molitrix.* Dry pellets in the size range of 50 - 280 micrometer were given and comparisons were made with live food supplies. He found that the rearing of common carp on a dry diet was difficult compared to that of Silver carp. Kanazawa (1988) revealed that carp, *Gnathopogon elongatus caerulescens* fed on a commercial microparticulate diet soon after hatching had good growth and survival. Larval Dover sole, *Solea solea* were reared from first feeding to metamorphosis either on *Artemia* nauplii or inert diets alone or on inert diets following pre feeding with *Artemia* nauplii (Appelbaum, 1985). Kanazawa (1988) revealed that *Pagrus major* larvae had better survival (75%) than live foods (~ 45%) when a micro bound diet based on sardine powder was fed. Good growth and survival were observed in the rearing of Striped knife jaw, *Oplegnathus fasciatus,* when fed with micro particulate diets from day five (Kanazawa, 1988).

The advantages afforded by the live foods were not considerable compared to certain formulated diets in Flounder, *Paralichthys olivaceus* larvae (Kanazawa, 1988). Juario *et al.* (1991) succeeded in weaning *Lates calcarifer* on artificial diets following more than 10 days on bio food. Wolnicki and Gorny (1995a) indicated that, for satisfactory larval growth of the Ide (*Leuciscus idus*) at a high survival rate, a supply of live food in combination with a dry food might be indispensable, as has been found for Tench (Wolnicki and Kowin - Kossakowski, 1993) and Minnow (Kestemont and Stalmans, 1992). *Channa striatus* could be trained to accept formulated feeds using *Artemia* nauplii supplemented with formulated feed for 30 days, then gradually eliminating live food over a 7 to 10 days period (Qin *et al.*, 1997). Lazo *et al.* (2000) demonstrated that Red drum larvae might be raised on a micro particulate diet from first feeding without use of zooplankton. The artificial universal starter feed is appropriate to initial feeding and rearing of European wels, *Silurus glanis* larvae up to 16 days of age, i.e. to the mean total length of 22 mm and weight of 0.1 gm. (Prokes *et al.*, 1999).

2.5 **Biology of Angel fish *Pterophyllum scalare* (Lichtenstein)**

P. scalare, the scalare or Angel fish comes from Rio Negro and Amazon basins and attains a length of 5 ". Their unusual shape, graceful movements and attractive colouring make the Angel fish one of the perennial favourites among aquarists. The body is compressed and body colour is silvery. The iris of the eye is red and a black vertical bar passes through the eye to the base of gill plate. The fins are the unusual feature, the dorsal fin being as high as the body, deeper and rounded on top. The anal fin is even deeper than the dorsal and the first ray also forms a long filament, which extends beyond the tail. The ventral fins consist of only a few rays, which sometimes are longer than the overall size of the fish (Axelrod and Vorderwinkler, 1979).

The fish attains maturity within one-year period. The main breeding season is between June and November although it is observed to breed throughout the year (Mathew *et al.*, 1999). Degani and Yehuda (1996) have studied in detail about the changes during the cycle of oogenesis of *P. scalare*. The duration of spawning and the quality of eggs (i.e. hatching %) are affected by the age of the fish and environmental condition. The average cycle extends for 11 days, during which the oocyte passes through vitellogenesis, maturation and spawning. *P. scalare*, a multi spawning fish differs from seasonal spawners and one time spawners (Luquet and Watanabe, 1986). The fish breeds repeatedly at varying intervals extending from 9 - 279 days (Mathew *et al.*, 1999). An average of 465 eggs were laid but the number of eggs varied from 100 to 1204 per spawning for fishes of 39 mm / 3g to 72 mm/18g. The diet, particularly the live food is very important in the adult female *P.scalare* since oogenesis occurs all the time. It therefore affects egg quality and the frequency of spawning more than the number of eggs per spawning (Degani and Yehuda, 1996).

The eggs hatch out in 2 to 3 days and start free swimming in about 7 days and start accepting food (Mathew *et al.*, 1999). Degani (1993) suggested that the natural diet of the first feeding *P. scalare* larva consists of various species of invertebrates. They can be fed with live *Artemia* nauplii, *Daphnia* sp., chopped Tubifex and finely ground flake diets under artificial rearing conditions (Volkart, 1994). Boonyaratpalin and Lovell (1977) revealed that Angel fish (0.5 gm) fed low fish and shrimp meal and with pigment diet registered a lower growth compared to the fish fed high fish and shrimp meal and no pigment diet.

Degani(1993) suggested that the Angel fish juveniles require a high protein diet (40-50%). There was no difference in growth rates between various levels of protein, but the addition of *Artemia* nauplii to the diets caused a significant increase in growth of the fish (Degani, 1993). Mathew and Sherief (1999) while working on the effects of dietary protein source on growth and survival of Angel fish, found that clam meat based diet gave the highest specific

growth rate and survival as compared to fish meal and slaughter house waste. They suggested clam meal as an ideal animal protein source for Angel fish, *P. scalare* rearing. Neelkanteswar (1997) suggested that clam meat based artificial diet can be effectively used as a weaning diet to substitute the live foods like *Artemia* nauplii or *Moina micrura,* which are costly and difficult to procure in the larval rearing of Angel fish.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Angel fish (*Pterophyllum scalare* Lichtenstein), a much adorned aquarium fish is marketed all over the world for hobbyists. This species now has a number of selected forms including some with a marbled pattern and others with a veil-tail development of the fins. This study was conducted to find out the optimum protein level required in the artificial feed of Angel fish larvae. Data pertaining to the maximum growth and survival of the larvae was also recorded.

3.1 **Experimental rearing facilities**

The experiment was conducted in the wet lab of the Department of Aquaculture, College of Fisheries, Panangad in glass tanks of 30 x 30 x 30 cm dimension. Filtered freshwater was stored in fibre reinforced plastic (FRP) tank and was used for the experiment. Subdued light was provided for the glass tanks in the wet lab.

3.2 **Experimental animals**

The study was done using one-day-old free-swimming hatchlings of Angel fish, *P. scalare* of the same brood. Ten hatchlings were randomly distributed to all the experimental tanks after recording their average weight in an electronic balance. The initial weight of the hatchlings ranged from 1.26 to 1.32 mg and standard length varied from 4.3 to 4.6 mm.

3.3 **Test diets used in the study**

Particulars of the test diets used in the experiment are given in Table 1 (percentage level of protein in the treatments).

3.3.1 **Feed ingredients**

Seven test diets were prepared at different protein levels- 25%, 30%, 35%, 40%, 45%, 50% and 55% - using defatted clam meat powder, egg yolk, wheat flour, lipid and vitamin-mineral mixture. Defatted clam meat powder was obtained employing the following procedure. Clam meat was sun dried and powdered. The powdered clam meat was subjected to solvent extraction using petroleum ether (60 - 80°C) in a solvent extraction apparatus till the crude fat was removed. This fat was also used as a feed ingredient. Vitamins and minerals were supplemented through Supplevit-M (Sarabhai Chemicals, Mumbai). Good quality wheat flour and eggs were purchased. Polythene bags were used to store the powdered ingredients after sieving through 250-micron mesh.

3.3.2 **Diet formulation**

The test diets were prepared by accurately weighing the respective ingredients in an electronic balance. Table 2 gives the proportion of the ingredients used in the preparation of the formulated diet. The ingredients except the Supplevit-M were mixed well in a clean, dry mortar. The dry mixture was made into a soft dough consistency by adding distilled water at the rate of 125 ml per 100 g of feed and mixed well in the mortar. The dough was transferred in to a glass beaker and steam cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was cooled well under an electric fan and again mixed well in a dry mortar along with Supplevit-M. The well-homogenized mixture was spread on clean dry trays as thin layers and sun dried for a period of 8 hours. The dried feeds were powdered and stored in airtight containers.

Table 1. Test diets used in the experiment

Percentage level of protein	Treatment
25	T_1
30	T_2
35	T_3
40	T_4
45	T_5
50	T_6
55	T_7

Table 2. Composition of Artificial diets

Ingredients	Percentage by weight						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Defatted Clam meat powder	19.1	27.6	35.4	45.2	53.9	62.7	71.3
Wheat Flour	68.1	59.6	50.8	42.0	33.3	24.5	15.9
Egg Yolk	10.8	10.8	10.8	10.8	10.8	10.8	10.8
Vitamin mineral mixture	1	1	1	1	1	1	1
Fat	1	1	1	1	1	1	1

3.4 **Proximate composition of feed ingredients and test diets**

Proximate composition of feed ingredients and test diets was analyzed to obtain the nutrient status. The results are based on the mean of three samples and are expressed on a dry matter basis.

The sample was dried at 105°C till a constant weight was arrived at to get the moisture content. Microkjeldahl's method (AOAC, 1984) was used to evaluate the crude protein content. Solvent extraction using petroleum ether (60 - 80°C) in a solvent extraction apparatus for 6 hours was carried out to estimate the crude fat. The ash content was determined by burning the sample at 550° C ± 10°C for 6 hours in a muffle furnace. The method described by Pearson (1976) was used to estimate the crude fibre. The carbohydrate content was estimated by Difference method (Hasting, 1976).

3.5 **Experimental design and procedure**

Square shaped glass tanks were used for conducting the experiment. Each treatment was replicated three times following completely randomized design. For the seven treatments a total of 21 tanks were used. Feed was given *ad libitum* with test diets twice daily. Every day before giving the feed, the leftover feed and excreta were siphoned out. The water level in the tanks was maintained by adding fresh water. Water quality parameters like temperature, pH, dissolved oxygen, hardness and ammonia were monitored weekly. Standard length and weight were recorded at weekly intervals during the 21 days time period. By the end of the feeding study the larvae were starved for one day, the number in each tank was counted and weighed collectively to determine the average final weight .

3.6 Water quality measurement

Physico-chemical parameters of the water in the rearing tanks were measured by the following methods.

1. Temperature - With bulb thermometer having an accuracy of 0.1°C
2. pH - Universal indicator method
3. Dissolved oxygen - Winkler's method (Strickland and Parsons, 1972)
4. Total hardness - EDTA - Eriochrome black-T indicator method
5. Ammonia Nitrogen - Phenol-hypochlorite spectrophotometric method (Strickland and Parsons, 1972)

3.7 Evaluation criteria

The parameters evaluated are average net weight gain, average gain in length, average percentage growth, specific growth rate and percentage survival.

3.7.1 Average net weight gain

It gives the increase in weight of the larvae during the experimental period when fed on various diets using the formula :

Average net weight gain = Average final weight - Average initial weight

3.7.2 Average gain in length

This gives the increase in standard length of larvae during the experimental period when fed on various diets. It was calculated using the formula:

Average gain in length = Average final length - Average initial length.

3.7.3 Average percentage growth

Percentage growth of larvae in weight and length was calculated using the following formula:

$$\text{Average \% growth} = \frac{\text{(Average final measurement - average initial measurement)}}{\text{Average initial measurement}} \times 100$$

3.7.4 Specific growth rate

In the present study, growth performance was also measured in terms of specific growth rate (SGR) since it is a more refined and an improved growth index than absolute growth or percentage growth as pointed out by Hepher (1988).

$$SGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where W_1 = weight at time T_1, W_2 = weight at time T_2

3.7.5 Survival rate

It is expressed in percentage.

$$\text{Survival \%} = \frac{\text{Initial number - number of dead animals}}{\text{Initial number}} \times 100$$

3.8 **Statistical analysis**

ANOVA (Snedecor and Cochran, 1968) was performed for the data collected. Percentage values (x) were transformed into arc sine values ($\sin^{-1}\sqrt{x}$ / 100) for analysis. Pair wise comparisons of the data were done using Newman-kuels test. Least square method of estimation of regression coefficients is used to establish the relationship between SGR and protein level.

RESULTS

4. RESULTS

The effect of different artificial diets on growth and survival of Angel fish, *Pterophyllum scalare* was evaluated. The details of the observations made during the study are presented below. The test diets with protein levels 24.91%, 29.83%, 34.72%, 39.86%, 44.79%, 49.85% and 54.63% are denoted as T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively.

4.1 Proximate composition of the feed ingredients and test diets.

The proximate composition of the feed ingredients (defatted clam meat powder, wheat flour, egg yolk) and the test diets used in the study, T_1, T_2, T_3, T_4, T_5, T_6 and T_7 are shown in Table 3 and Table 4 respectively.

4.2 Efficiency of various test diets

4.2.1 Growth

The data corresponding to the weight gain in the larvae fed on different diets are given in Table 5. The average live weight gain of larvae fed on diets of different protein levels showed maximum growth in treatment T_5 with 44.79% protein level and the minimum in treatment T_1 with 24.91% protein level. The graphical representation of the live weight gain is given in Fig.1. Analysis of variance showed that the growth of the larvae were significantly different between the treatments (Table 6). The data obtained on the percentage weight gain (Table 5) revealed that the maximum value was in the treatment T_5 and the minimum was in the treatment T_1. The percentage weight gain of larvae fed on different diets is presented graphically in Fig.2. The analysis of variance (Table 7) showed that the treatments differed significantly.

The data on the growth in standard length of larvae fed on different diets are given in Table 8.

Table3: Proximate composition of feed ingredients on dry weight basis.

Ingredients	Crude Protein	Fat	Ash	Fibre	NFE
Defatted clam meat powder	70.1	-	9.40	0.37	20.13
Wheat flour	13.2	1.31	1.01	0.58	83.9
Egg yolk	26.1	68.46	3.20	-	2.24

Table 4: Proximate composition of different test diets on dry weight basis.

Test diets	Crude Protein	Fat	Ash	Fibre	NFE
T_1	24.91	9.21	2.79	0.45	62.64
T_2.	29.83	9.13	3.51	0.43	57.10
T_3	34.72	8.99	4.24	0.42	51.63
T_4	39.86	8.90	4.83	0.40	46.01
T_5	44.79	8.81	5.52	0.38	40.50
T_6	49.85	8.70	6.24	0.36	34.85
T_7	54.63	8.54	6.91	0.34	29.58

Table 5: Growth of *P. scalare* larvae in weight fed on different diets.

Treatment	Replication	Average initial weight (mg)	Average final weight (mg)	Gain in weight	Average live weight gain (mg)	Percentage weight gain	Average Percentage gain
T_1	1	1.32	93.69	92.37	92.18±0.78	6997.73	7147.30 ± 106.16
	2	1.29	94.31	93.02		7210.85	
	3	1.26	92.40	91.14		7233.33	
T_2	1	1.31	97.82	96.51	98.09±2.05	7367.18	7565.27 ± 163.86
	2	1.28	98.05	96.77		7560.16	
	3	1.30	102.29	100.99		7768.46	
T_3	1	1.30	102.62	101.32	101.47 ± 1.44	7809.23	7850.47 ± 31.55
	2	1.27	101.05	99.78		7856.69	
	3	1.31	104.61	103.3		7885.50	
T_4	1	1.32	105.22	103.9	103.10 ± 2.20	7871.21	7971.62 ± 95.47
	2	1.26	101.35	100.09		79.43.65	
	3	1.30	106.60	105.3		8100.00	
T_5	1	1.27	109.75	108.48	108.72 ± 0.69	8541.73	8471.80 ± 49.48
	2	1.28	109.30	108.02		8439.06	
	3	1.30	110.95	109.65		8434.62	
T_6	1	1.30	108.85	107.55	108.22 ± 0.49	8273.08	8367.77 ± 67.77
	2	1.29	109.68	108.39		8402.33	
	3	1.29	110.01	108.72		8427.91	
T_7	1	1.29	107.93	106.66	107.83 ± 0.74	8268.22	8359.36 ± 71.00
	2	1.28	10932	108.05		8441.41	
	3	1.30	110.07	108.79		8368.46	

Table 6: Analysis of variance of the data on gain in weight in milligrams *of P. scalare* larvae fed on different diets.

Source	d.f.	S.S.	M.S.S.	F-ratio
Diets	6	692.70	115.45	42.33**
Error	14	38.18	2.73	
Total	20	730.88		

Comparison of treatment means

Standard error: 1.350

Treatments	T_1	T_2	T_3	T_4	T_7	T_6	T_5
Means	92.18	98.09	101.47	103.10	07.83	108.22	108.72

Table 7: Analysis of variance of the data on percentage gain in weight in milligrams of *P. scalare* larvae fed on different diets.

Source	d.f	SS	MS	F-ratio
Diets	6	4248323.00	708053.9	54.79**
Error	14	180918.70	12922.77	
Total	20	4429241.70		

Comparison of treatment means

Standard error: 92.818

Treatments	T_1	T_2	T_3	T_4	T_7	T_6	T_5
Means	7147.30	7565.27	7850.47	7971.62	8359.36	8367.77	8471.80

Underscored means are not significantly different

** Significant at 5% level

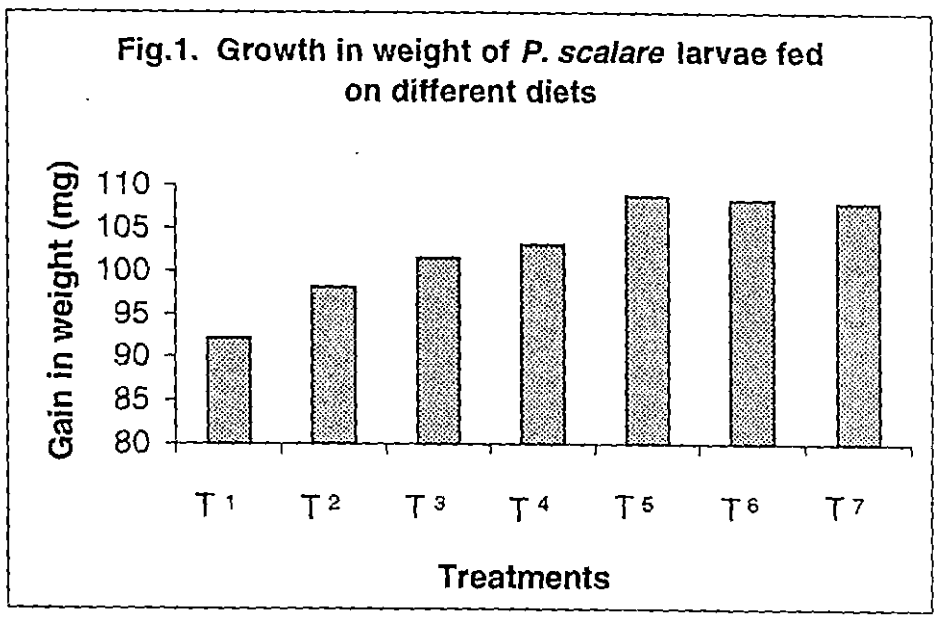
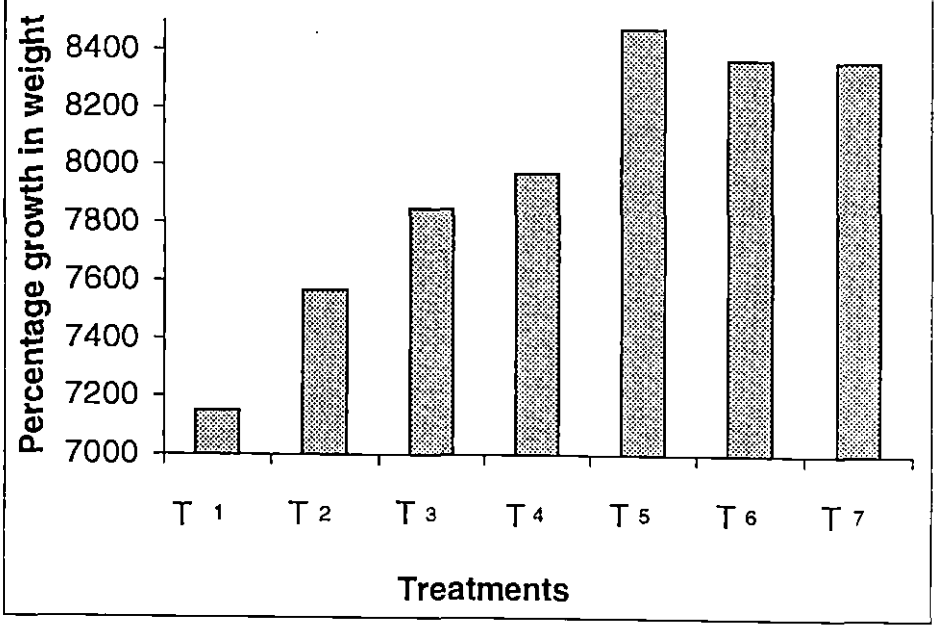


Fig.1. Growth in weight of P. scalare larvae fed on different diets
Gain in weight (mg)
110
105
100
95
90
85
80
T1
T2
T3
T4
T5
T6
T7
Treatments

Fig. 2. Percentage gain in weight of *P. scalare* larvae fed on different diets



Maximum average gain in length was observed in the treatment T_5 having 44.79% protein level and the minimum was from the treatment T_1 with 24.91% protein level. The growth in standard length of larvae fed on diets of different protein levels is represented graphically in Fig.3. Analysis of variance (Table 9) showed that the treatments differed significantly. The data pertaining to the percentage length gain (Table 8) showed that the maximum percentage length gain was observed for the diet with 44.79% protein level (T_5) and minimum for the diet with 24.91% protein level (T_1). The graphical representation of the percentage length gain is given in Fig.4. Analysis of variance (Table10) showed that there was significant difference between the treatments.

4.2.2 **Specific growth rate.**

The data on SGR are presented in Table 11. The maximum SGR was recorded for the diet with 44.79% protein level (T_5) and minimum for the diet with 24.91% protein level (T_1). The SGR of the larvae fed on different diets are graphically represented in Fig.5. Analysis of variance of the data showed that there was significant difference between the treatments (Table12). In the present study, a parabolic relationship was observed between protein level and SGR. The maximum SGR (21.163) was attained at 53.099% protein level. The relationship was established as $\mathbf{SGR = -0.00096P^2 + 0.10192P + 18.45894}$ where P represents protein level. This relationship is graphically represented in Fig.6.

4.2.3 **Survival**

The percentage survival values of Angelfish larvae in various treatments are given in Table13. The highest average survival (70%) was recorded from the treatments T_5 and T_6 while the lowest average survival was 56.67% in the treatment T_1. Graphical representation of percentage survival values for the seven diets is given in Fig.7. Analysis of variance of the data showed that there is no significant difference between the treatments (Table 14).

Table 8: Standard length gain of *P. scalare* larvae fed on different diets.

Treatment	Replication	Average initial length (mm)	Average final length (mm)	Gain in length (mm)	Average gain in length	Percentage length gain	Average percentage length gain
T_1	1	4.5	11.9	7.4		164.44	
	2	4.4	11.7	7.3	7.33±0.05	165.91	166.71±2.25
	3	4.3	11.6	7.3		169.77	
T_2	1	4.4	12.1	7.7	7.67±0.05	175.0	
	2	4.3	12.0	7.7		179.07	175.6±2.62
	3	4.4	12.0	7.6		172.73	
T_3	1	4.3	12.2	7.9		183.72	
	2	4.5	12.4	7.9	7.87±0.05	175.56	177.54±4.47
	3	4.5	12.4	7.9		173.33	
T_4	1	4.6	12.8	8.2		178.26	
	2	4.5	12.4	7.9	8.1±0.14	175.56	178.68±2.274
	3	4.5	12.7	8.2		182.22	
T_5	1	4.4	12.9	8.5		193.18	
	2	4.4	13.3	8.9	8.7±0.16	202.27	196.26±4.25
	3	4.5	13.2	8.7		193.33	
T_6	1	4.5	13.1	8.6		191.11	
	2	4.4	13.1	8.7	8.63±0.05	197.73	191.93±4.44
	3	4.6	13.2	8.6		186.96	
T_7	1	4.4	13.1	8.7		197.73	
	2	4.5	13.2	8.7	8.6±0.14	193.33	191.22±6.35
	3	4.6	13.0	8.4		182.61	

Table 9: Analysis of variance of the data on gain in standard length of *P. scalare* larvae fed on different diets.

Source	d.f.	S.S.	M.S.S.	F-ratio
Diets	6	5.16	0.86	53.08**
Error	14	0.23	0.02	
Total	20	5.39		

Comparison of treatment means

Standard error : 0.1038

Treatments	T_1	T_2	T_3	T_4	T_7	T_6	T_5
Means	7.33	7.67	7.87	8.10	8.60	8.63	8.70

Table 10 : Analysis of variance of the data on percentage gain in length of *P. scalare* larvae fed on different diets

Source	d.f.	S.S.	M.S.S.	F-ratio
Diets	6	2071.98	345.33	13.73**
Error	14	352.24	25.16	
Total	20	2424.22		

Comparison of treatment means

Standard error : 92.818

Treatments	T_1	T_2	T_3	T_4	T_7	T_6	T_5
Means	166.71	175.6	177.54	178.68	191.22	191.93	196.26

Underscored means are not significantly different

** Significant at 5% level

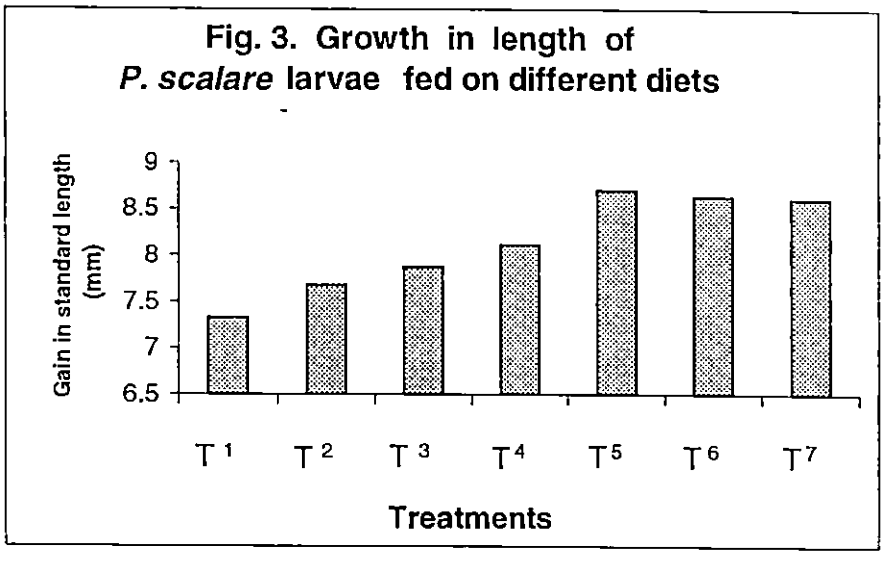


Fig. 3. Growth in length of
P. scalare larvae fed on different diets
Gain in standard length (mm)
9
8.5
8
7.5
7
6.5
T 1
T 2
T 3
T 4
T 5
T 6
T 7
Treatments

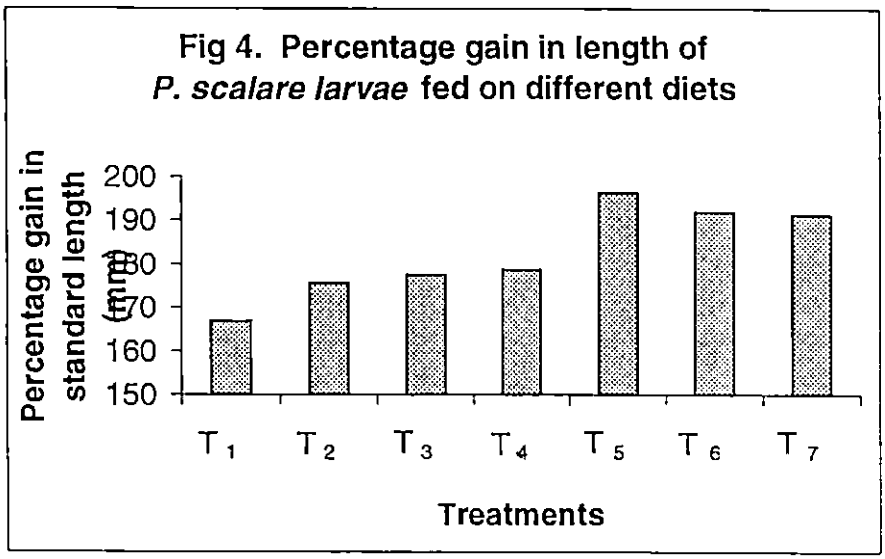


Fig 4. Percentage gain in length of
P. scalare larvae fed on different diets
Percentage gain in standard length (mm)
200
190
180
170
160
150
T1
T2
T3
T4
T5
T6
T7
Treatments

Table 11: **Specific growth rate of *P. scalare* larvae fed on different diets.**

Treatment	Replication	Average initial weight (mg)	Average final weight (mg)	Specific growth rate %	Mean ± SD
T_1	1	1.32	93.69	20.29	20.41 ± 0.009
	2	1.29	94.31	20.48	
	3	1.26	92.40	20.47	
T_2	1	1.31	97.82	20.52	20.67 ± 0.12
	2	1.28	98.05	20.67	
	3	1.30	102.29	20.81	
T_3	1	1.30	102.62	20.81	20.84 ± 0.02
	2	1.27	101.05	20.86	
	3	1.31	104.61	20.85	
T_4	1	1.32	105.22	20.86	20.92 ± 0.04
	2	1.26	101.35	20.90	
	3	1.30	110.95	21.19	
T_5	1	1.27	109.75	21.23	21.19 ± 0.04
	2	1.29	109.68	21.19	
	3	1.29	110.01	21.18	
T_6	1	1.30	108.85	21.10	21.16 ± 0.04
	2	1.29	109.68	21.19	
	3	1.29	110.01	21.18	
T_7	1	1.29	107.93	21.09	21.14 ± 0.04
	2	1.28	109.32	21.19	
	3	1.30	110.07	21.14	

Table 12: Analysis of variance of the data on specific growth rate of *P. scalare* larvae fed on different diets.

Source	d.f.	SS	M.S.S.	F-ratio
Diets	6	1.50	0.25	38.67**
Error	14	0.09	0.01	
Total	20	1.59		

Comparison of treatment means

Standard error : 0.066

Treatments	T_1	T_2	T_3	T_4	T_7	T_6	T_5
Means	20.41	20.67	20.84	20.92	21.14	21.16	21.19

Underscored means are not significantly different

** Significant at 5% level

Fig. 5. Specific growth rate of *P. scalare* larvae fed on different diets

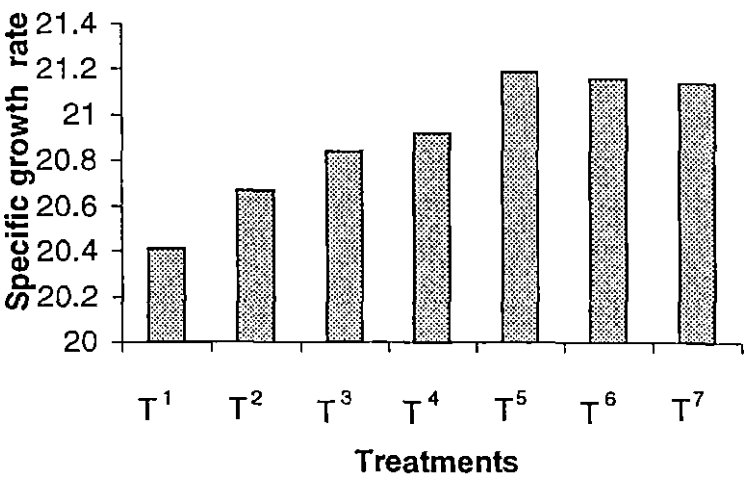


Fig. 6. Relationship between protein level and specific growth rate on rearing *P.scalare*.

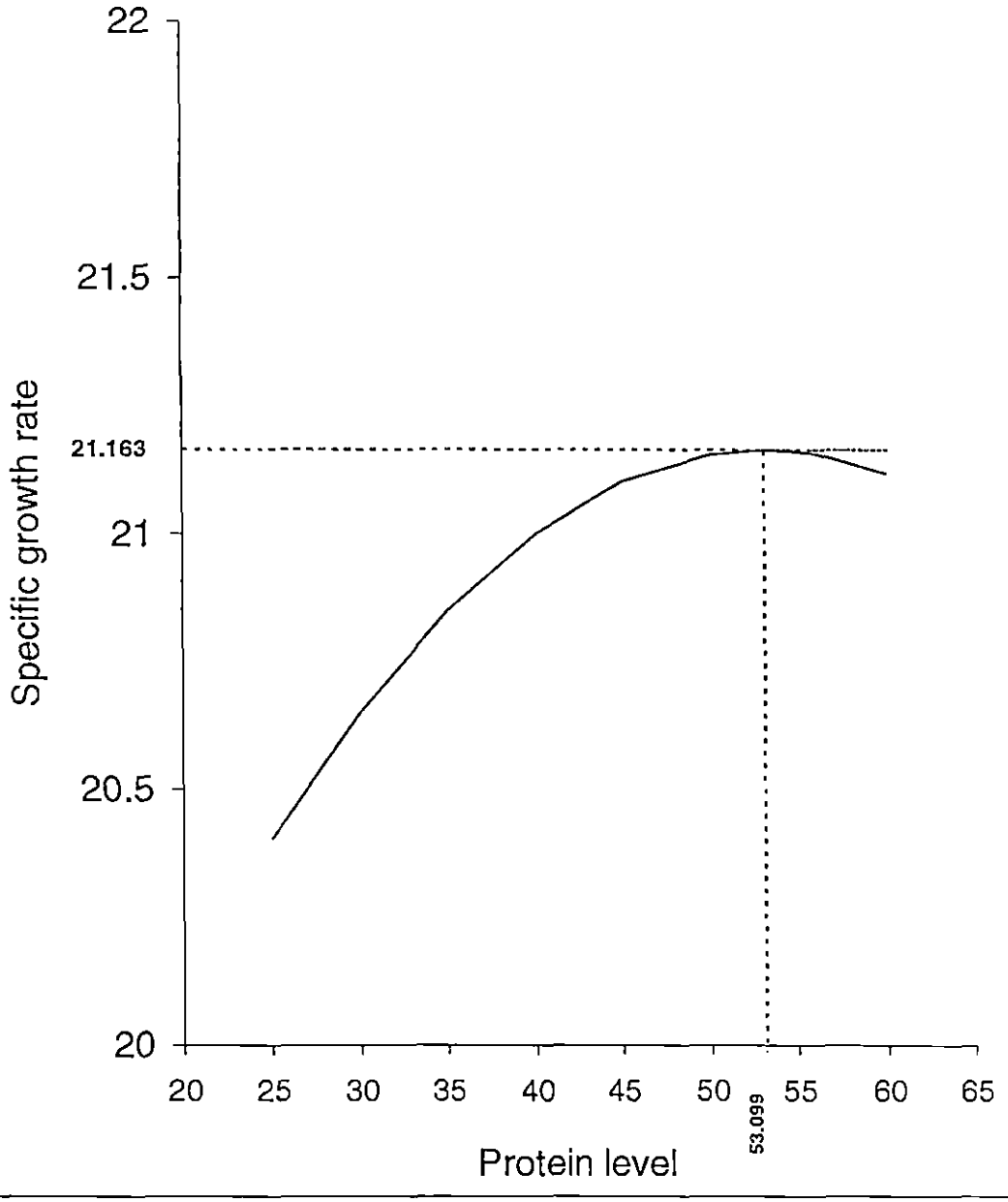


Table 13. Percentage survival of *P.scalare* larvae fed on different diets.

Treatment	Replication	Percentage survival (%)	Mean ± SD
T_1	1	50	
	2	60	56.67 ± 4.71
	3	60	
T_2	1	70	
	2	60	66.67 ± 4.71
	3	70	
T_3	1	60	
	2	70	63.3 ± 4.71
	3	60	
T_4	1	70	
	2	60	66.67 ± 4.71
	3	70	
T_5	1	70	
	2	80	70 ± 8.16
	3	60	
T_6	1	70	
	2	70	70 ± 0
	3	70	
T_7	1	60	
	2	80	66.7 ± 9.43
	3	60	

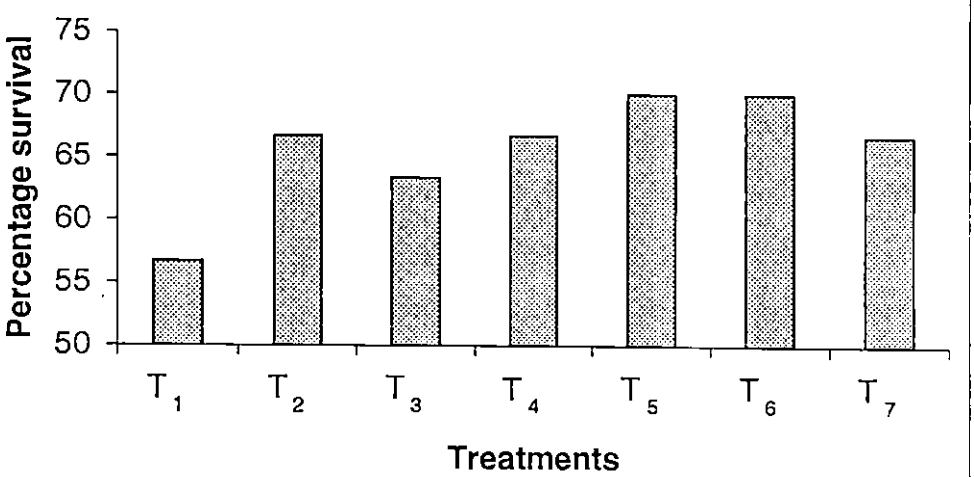
Table 14 : Analysis of variance of the data on percentage survival of *P. scalare* larvae fed on different diets.

(Data subjected to ArcSine transformation)

Source	d.f.	S.S.	M.S.S.	F-ratio
Diets	6	139.49	23.25	1.1540^{NS}
Error	14	282.03	20.14	
Total	20	421.52		

N.S.: Not significant

Fig.7. Percentage survival of *P. scalare* larvae fed on different diets



4.3 Water quality parameters

The range of temperature, pH, dissolved oxygen, hardness and ammonia nitrogen in the experimental tanks during the present study are given in Table 15.

4.3.1.Temperature

The maximum and minimum temperatures recorded during the study period were 28.1°C and 26.0°C.

4.3.2. pH

The pH values during the experimental period varied between 6.0 and 7.0.

4.3.3. Dissolved oxygen

A minimum of 4.4 ppm and a maximum of 5.4 ppm were obtained during the present study.

4.3.4. Hardness

During the experimental period the values of hardness ranged from 115 to 126 ppm.

4.3.5.Ammonia nitrogen

The ammonia nitrogen values fluctuated between 0.06 and 0.09 ppm.

Table 15. Water quality parameters during experimental period

Parameter	Weeks	1	2	3
Temperature(°C)	Mean Range ±S.D	26.1 26.0-26.2 0.1	27.35 27.2-27.5 0.15	27.95 27.8-28.1 0.15
pH	Mean Range ±S.D	6.25 6.0-6.5 0.25	6.75 6.5-7.0 0.25	7.0 7.0
Dissolved oxygen (ppm)	Mean Range ±S.D	5.2 5.0-5.4 0.2	4.8 4.6-5.0 0.2	4.6 4.4-4.7 0.15
Hardness (ppm)	Mean Range ±S.D	123.0 120-126 3	119.0 116-122 3	117.5 115-120 2.5
NH3-N (ppm)	Mean Range ±S.D	0.0625 0.06-0.065 0.0025	0.075 0.07-0.08 0.005	0.0875 0.085-0.09 0.0025

DISCUSSION

5. DISCUSSION

5.1 Protein sources and different protein levels used in rearing Angel fish, *P. scalare.*

Very few works have been carried out in the ornamental fish *P. scalare.* Degani and Yehuda (1996) formulated two artificial diets for Angel fish with 57% and 78% crude protein contents using two ingredient combinations. (1) Fish meal, wheat meal, soyabean meal, yolk meal, milk meal, guar and vitamins; (2) Turkey heart, poultry liver, vegetarian mixture and vitamins, respectively. Boonyaratpalin and Lovell (1977) prepared diet for Angel fish with 40% protein using shrimp meal, fish meal, soyabean meal, rice polish, wheat bran, fish oil and vitamin-mineral mixture. Degani (1993) conducted studies in Angel fish juveniles using 3 diets having 37%, 41% and 47% protein levels and concluded that the protein requirement was between 40% and 50%. The ingredients used in these test diets were fish meal, wheat meal, milk powder and vitamins. Mathew and Sherief (1999) prepared a diet with 30% protein using clam meal, rice bran, groundnut oil cake, tapioca flour and vitamin - mineral mixture for rearing Angel fish juveniles. Neelkanteswar (1997) while working on Angel fish larvae suggested that clam meat based diet with 29.7% protein was comparable to live feeds in growth and survival. The ingredients used in his artificial diet were clam meat, wheat flour, egg yolk, yeast oats and vitamin-mineral mixture.

Chong *et al.* (2000) used five isoenergetic semipurified diets using caesin, gelatin and danish fish meal as chief protein sources and concluded that discus (*Symphysodon spp.*) juveniles had a protein requirement of 44.9-50.1%. Lochmann and Phillips (1994) indicated that gold fish (*C. auratus*) juveniles had a protein requirement of 29% when semipurified diets containing fishmeal and caesin were used as major ingredients. Abi-ayad and Kestemont(1994) used commercial diet with 51% protein level for feeding gold fish larvae. Protein

requirement for tin foil barb juveniles was suggested to be 41.7% by Elangovan and Shim (1997). The diet ingredients used for rearing larval live bearers are wheat bran, oats, ground dried shrimp, fish meal, skimmed milk and protein content of the feed ranged from 15 to 34%, as suggested by Fernando *et al.* (1991).

Proximate composition of the test diets used in the present study revealed that the treatments T_1, T_2, T_3, T_4, T_5, T_6 and T_7 contained 24.91%, 29.83%, 34.72%, 39.86%, 44.79%, 49.85% and 54.63% protein, respectively (Table 4). The diets were prepared using defatted clam meat powder, wheat flour, egg yolk, vitamin- mineral mixture and fat (Table 2). This study reveals that Angel fish larvae require diets with high protein levels (44.79%, 49.85% and 54.63%).

5.2 **Water quality parameters**

5.2.1 **Temperature**

Degani (1993) maintained a temperature of 27°C while breeding and rearing of larvae and juveniles of Angelfish *(P. scalare)*. Neelkanteswar (1997) observed a mean weekly temperature range of 25.5 to 30°C when rearing Angel fish larvae. Although goldfish is considered as a thermophilic species, survival was lower at 28°C than at 20°C and 24°C but the larvae reared at 28°C at maximum daily food ration was four times larger than those reared at 20°C, as indicated by Kestemont (1995). Degani (1993) revealed that the growth of larvae and juveniles of *Trichogaster trichopterus* maintained at 25°C and 27°C was similar and faster than growth of those maintained at 23°C. He concluded that temperature is a very important parameter for the growth of larvae and juveniles of this species. The mean weekly range of temperature recorded during the present study was 26°C to 28.1°C. The temperature changes were gradual and only in a narrow range throughout the experimental period. The prevalence of high temperatures in the tropical waters rules out the possibility of optimum temperatures for growth and survival of ornamental fishes.

5.2.2 **pH**

Slightly alkaline water was found to be suitable for hatching and rearing of Angelfish larvae, as observed by Axelrod and Vorderwinkler (1979). Neelkanteswar (1997) while rearing Angel fish larvae observed that the pH values ranged from 6 to 7. Degani (1990) observed no significant effect of pH on growth, survival and dietary utilization of blue gourami (*T. trichopterus*) larvae. The mean weekly range of pH values range from 6 to 7 during the present study. There was no remarkable effect of pH on growth and survival, as reported by Degani (1990).

5.2.3 **Dissolved oxygen**

In the rearing of blue gourami, *T. trichopterus*, Degani (1990) indicated that there was no significant effect of dissolved oxygen on utilization of different diets. Kestermont (1995) reported that by maintaining dissolved oxygen at about saturation, the conditions seemed to be ideal for the rearing of goldfish (*C. auratus*). Neelkanteswar (1997) found that the dissolved oxygen in the water ranged from 3.5 to 5.3 ppm when Angel fish larvae were reared. The weekly range of dissolved oxygen in the experimental tanks varied from 4.6 to 5.2 ppm. during the present study. Cleaning the tank bottom along with water change could overcome the problem of low dissolved oxygen content in the water.

5.2.4 **Hardness**

Low hardness (less than 20ppm) waters result in poor survival of fry (Mitchell and Collins, 1997). The mean weekly range of hardness recorded by Neelkanteswar (1997) while rearing Angel fish larvae was from 115 to 150 ppm. During the present experiment, mean weekly range of hardness varied from 115

to 126 ppm. In the larval rearing water of *Trichogaster trichopterus*, a hardness of 189-195 ppm was reported by Degani (1990).

5.2.5 **Ammonia nitrogen**

Low ammonia concentrations were recorded by Degani (1993) while rearing blue gourami T. *trichopterus* larvae. He reported that high concentration of nitrate and nitrite led to a lower rate of growth and they are the most sensitive parameters whose control will ensure good growth rate of the larvae. There is a possibility of larvae eating unused artificial food from the bottom and they may encounter a high concentration of nitrite, which leads to low survival and growth rates. In the present experiment, the ammonia concentrations varied from 0.06 to 0.09 ppm. The low ammonia levels observed during this study could be due to the removal of waste before each feeding.

5.3 **Growth**

In the present experiment, *P.scalare* larvae recorded the highest growth in terms of gain in length (8.7mm) and gain in weight (108.72 mg) when fed with artificial diet having 44.79% protein (T_5) followed by diet with 49.85% (T_6) and 54.63% (T_7) and these treatments differed significantly from treatments with lower protein level ($P < 0.05$). Studies to find out optimum protein levels for rearing ornamental fishes are scarce. Chong *et al.* (2000) while working on discus (*Symphysodon spp.)* with mean initial weight of 4.4 - 4.6 g used isoenergetic diets with 5 protein levels (35%, 40%, 45%, 50% and 55%), the growth rate increased significantly with protein level up to 50% and then decreased. Neelkanteswar (1997) found that growth obtained by giving artificial feed alone was 91-93% of that obtained with live foods, while working on *P. scalare* larvae.

Use of artificial diet alone in larval rearing was successful in barbel (*Barbus barbus*) (Wolnicki and Gorny, 1995b); coregonid larvae (*Coregonus nasus)*(Knyazeva *et al.,* 1984); *Coregonus schnizi* (Dabrowski and Kaushik, 1985); *Coregonus laveratus* (Rosch and Appelbaum , 1985; Champignueille, 1988); sturgeon *(Acipensor baeri)* larvae (Dabrowski *et al.*, 1985); *Catla catla* (Swain *et al.,* 1999); catfish *(Clarius gariepinus)* (Uys and Hecht,1985) ; *Micropterus dolomieni* (Jones *et al.*, 1993); razor back sucker larvae (*Xyrauchen texanus*) (Tyus and Severson,1990) and *Stizostedion vitreum* larvae (Guthrie and Rust,2000).

5.4 **Specific growth rate**

The treatment T_5 with 44.79% protein level showed maximum SGR. Diets with higher protein level T_5, T_6 and T_7 (44.79%, 49.85% and 54.63%) showed significant difference in SGR compared to treatments with lower protein levels. Studies done on discus *(Symphysodon spp.)* juveniles showed a linear relationship between protein level and growth up to 45% protein, beyond which SGR did not differ significantly (Chong *et al.,* 2000). In the present study, a parabolic relationship was noticed between protein level and SGR. The maximum SGR (21.163) was attained at 53.099% protein level.

5.5 **Survival**

The highest survival rates (70%) were obtained for treatments T_5 and T_6 with 44.79% and 49.85% protein, respectively. These results can be considered excellent because generally survival rates are very low (0 to 40%) when artificial feeds are used for larval rearing (Jones *et al.,* 1993). Only known exceptions are 73% survival for *Barbus barbus* larvae fed dry diet alone (Wolnicki and Gorny, 1995b); 68 – 87% survival for *Catla catla* fed artificial dry diet (Swain *et al.*, 1999); 90% survival for *Cyprinus carpio* (Charlon and Bergot, 1984;

Charlon *et al.*, 1986 and Slaminska and Przybyl, 1986) and 85-90% survival using micro encapsulated diets in *Coregonus laveratus* (Champigneuille, 1988). Studies done by Neelkanteswar (1997) on Angelfish larvae recorded 65 – 75% survival when artificial diets were used.

SUMMARY

6. SUMMARY

The present study was conducted to evaluate the effect of different protein levels on the growth and survival of Angel fish, *Pterophyllum scalare* (Lichtenstein). The methodology, important results and conclusion of the study are as follows:

1) One day old free-swimming hatchlings of *P. scalare* were used as experimental animals.

2) The different test diets prepared using defatted clam meat powder as the chief protein source were having 24.91%, 29.83%, 34.72%, 39.86%, 44.79%, 49.85% and 54.63% protein level and they were designated as T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively.

3) The duration of the experiment was 21 days. The different parameters evaluated in order to find out the efficiency of the diets were growth (average gain in weight and length, percentage gain in weight and length), specific growth rate and survival.

4) The diets with higher protein levels (44.79%, 49.85% and 54.63%) significantly differed from the diets with lower protein levels.

5) Specific growth rate was highest for the treatment with 44.79% protein and was at par with treatments having 49.85% and 54.63% protein level and these were significantly higher than rest of the treatments. From the relationship established between protein level and SGR, the optimum level of protein attaining maximum SGR (21.163) was found to be 53.099%.

6) The results showed that artificial diet with 44.79% protein level can be efficiently and economically used as a weaning diet for rearing Angel fish larvae.

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EVALUATION OF PROTEIN REQUIREMENT OF ANGEL FISH *PTEROPHYLLUM SCALARE* (Lichtenstein) LARVAE USING DEFATTED CLAM MEAT AS THE CHIEF PROTEIN SOURCE

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ABSTRACT OF THE THESIS

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ABSTRACT

The effect of artificial diets with varying protein levels on the growth and survival of Angel fish, *Pterophyllum scalare* (Lichtenstein) larvae using defatted clam meat powder as the chief protein source was studied. One-day-old free-swimming hatchlings were used in the investigations done for a period of 21 days. The different diets used were with 24.91%, 29.83%, 34.72%, 39.86%, 44.79%, 49.85% and 54.63% protein levels with 3 replicates for each treatment.

The results showed that the growth of larvae in terms of gain in length, gain in weight and specific growth rate was maximum for the diet with 44.79% protein level. From the relationship established between protein level and SGR, the optimum level of protein attaining maximum SGR (21.163) was found to be 53.099%. No significant difference was found in the survival rate of larvae fed with different diets. Thus artificial diet with 44.79% protein level can be used efficiently for rearing Angel fish larvae.