EFFECT OF DIFFERENT DIETS ON GROWTH AND SURVIVAL OF ANGEL FISH *PTEROPHYLLUM SCALARE* (Lichtenstein) FRY

BY

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THESIS

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DECLARATION

I hereby declare that this thesis, entitled **EFFECT OF DIFFERENT DIETS ON GROWTH AND SURVIVAL OF ANGEL FISH, *PTEROPHYLLUM SCALARE*** (Lichtenstein) **FRY** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

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Certified that this thesis, entitled **EFFECT OF DIFFERENT DIETS ON GROWTH AND SURVIVAL OF ANGEL FISH, *PTEROPHYLLUM SCALARE*** (Lichtenstein) **FRY** is a research work done independently by **Sri. A.NEELAKANTESWAR** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

1. INTRODUCTION

More than 300 species of fish are reared worldwide and this number is continuously increasing parallel to the evolution of rearing techniques and to the mass production of feed. There is a desire to intensify research and to gain complete control over the early growth stages in both marine and freshwater fish species. Commercial production of quality fry of many species was achieved by the production of adequate live food. This diet not only provides a very diversified composition but, because of its autodigestion characteristics, facilitates nutrient uptake in the larvae.

It is generally accepted that in fish larviculture a reduction in the requirements for live foods would contribute significantly to further optimization of this sector of aquaculture production, as it would allow a more standardized production protocol and consequently a more reliable and cost effective output.

Few freshwater species have been successfully reared from first feeding exclusively on artificial diets, and in most cases success at the experimental level has yet to be reproduced on a commercial scale. Reasons for the limited success with artificial diets may be physiological (simple alimentary canal of fish larvae and hence poor efficiency in digestion and assimilation) and behavioural (very selective food preferences determined by behavioural mechanisms). Nutritional requirements are also more specific in larval stages. Therefore, replacement diets require a lot of specific characteristics with respect to particle size, physical performance in water, attractability, digestibility and nutritional composition. They must also remain cost effective.

With the aim of improving our knowledge of the quantitative nutritional requirements of larval stages of various aquacultural species, several investigators have tried to develop purified particulate diets which can be used as standard reference diets in nutritional studies. These diets not only have to meet the requirements mentioned above but also should utilize selected ingredients of defined chemical composition. This makes formulation of a nutritionally adequate diet even more difficult and expensive. A reference diet is being developed at Laboratory of Aquaculture,University of Gent, Belgium for use with different species of freshwater fish (Lavens *et al.*, 1995). Recently,

multidisciplinary approaches (enzymatic activities, histopathology of the liver, DNA/ RNA ratio, O:N ratio etc) have been developed parallel to zootechnical performance (survival, growth, food conversion ratio etc.) in order to obtain some explanation for the problems encountered in larval rearing. Such an approach allows the problem to be solved at the level of ingestion, digestion or integration and storage of nutritional material.

Compared to the fish which are used for human consumption, little attention has been paid to the growth and nutrition of ornamental species (Degani, 1993). *Pterophyllum scalare*, a much adorned aquarium fish is marketed all over the world for hobbyists. The increasing market demand and its complicated breeding behaviour has forced to increase the hatchery output of this fish.

Presently hatchery production of this species is essentially based on the supply of *Artemia* nauplii as the feed, which has its own limitations. Development of a practical larval diet may go a longway in the commercial production of this much sought after ornamental fish. The purpose of the present study is to formulate a practical diet using locally available ingredients and to examine its effect on growth and survival of *P. scalare* larvae, in comparison with that of *Artemia* nauplii and *Moina micrura*.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The success of aquaculture as a bio-industry stems from the breakthrough in larviculture of the species currently farmed. Larval nutrition continues to be a critical aspect whenever a candidate species is being evaluated. Despite the simple characteristics of gut, the larvae require sufficient food to grow several-fold during this period. A nutritionally adequate introductory feed holds the clue to successful larviculture.

2.1.Early biology of fish related to feeding

2.1.1. Endogenous nutrition

The initial nourishment to the developing fish egg is drawn from the egg yolk. The nutrient contents of the eggs vary with species, but the dynamics of the yolk absorption are similar among groups. Embryonic growth in fish depends on yolk composition, its digestion by the syncytium or analagous tissue, the absorption and transport of yolk nutrients to developing tissues for somatic organization and metabolic demands for survival. It is understood that carbohydrates, lipids and proteins are consumed prior to hatching while the latter two are catabolized also after hatching. The precise pattern depends on the fish in relation to the absolute egg composition (Heming and Buddington,1988).

Growth during the endogenous nutrition period is also influenced by abiotic factors such as temperature regimes, oxygen availability, salinity, pH and photoperiod. Within the physiologically tolerable range of these parameters, decrease in yolk utilization efficiency probably indicates increased homeostatic and maintenance cost (Heming and Buddington,1988).

In addition to yolk, some authors (Amend and Fender,1976 ; Lin and Arnold,1982; Wiggins *et al.*,1985) have described the possibility of assimilation of dissolved organic matter from the water after hatch. Other suspected non-yolk nutrient sources include peri-vitelline fluid and egg membranes (Heming and Buddington,1988). As and when the yolk reserves have been completely utilized, the feeding capabilities have developed 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 phase when the yolk has just been depleted and the emerging larva turns to exogenous nutrients for further development can be categorized as the most vital stage. In the case of Salmon, the larva carries a large yolk sac good enough for the first three weeks of development; by this time a mouth capable of accepting formulated feeds has developed (Sorgeloss and Leger,1992). Eventhough many species hatch without any oral opeing, the rapid development of the mouth facilitates a quick changeover from endogenous to exogenous nutrition. The gape of the oral opening at first feeding controls the size of the food that can be accepted by the larva (Shirota,1970) and often it is less than 0.1mm (Kohno *et al.*,1988 ; Glamuzina *et al.*,1989). As the larva grows, the mouth gape increases and larger food can be taken. The simple alimentary tract of the newly hatched larva assumes greater length and diversity on growth (Dabrowski,1984).

When the larval gut lengths are relatively small, the food passage time is short and in turn the time for digestion and absorption is also short. In Roach, *Rutilus rutilus*, the gut of the larva is only 45% of the total length, while in the adult it is 102% of the total length (Wee,1992). The gut passage time in larva at 20° C is 2.5 hours, but it is 6 hours for adult at the same temperature. In the early stages the endogenous digestive enzyme activity is very low, contributing to the increasing availability of aminoacids for protein synthesis (Lauff and Hofer,1984). Therefore, free aminoacids seem to be the best available form of nutrients for larval growth. However, the essential aminoacids supplied in the free form in live food of larval fish are in a quantity insufficient to support growth (Dabrowski and Culver, 1991).

The digestive capacity of the fish at the start of exogenous feeding has been indicated by the presence of trypsin, chymotrypsin and intestinal enzymes such as aminopeptidase, non-specific esterase and ATP- ase. Lauff and Hofer(1984) tracked the evolution of proteolytic enzymes in *Coregonus* sp., Rainbow trout and the Roach, *Rutilus rutilus* and detected an increase in activity with age, irrespective of the capacities of the fish. In the stomachless cyprinid, Roach, the level of proteolytic activity is higher

than in the other two species. In trout the low activity of pancreatic enzymes is compensated by the early development of the stomach, whereas coregonids which do not develop a stomach until 50 days after hatch depend on exogenous enzymes, particularly trypsin. Hofer (1985) and Clark *et al* (1985) also have found improved proteolytic capabilities in fish with age. Lipase activity as well as gall bladder, with potential for production of bile salts to aid digestive lipases, was found to be present at first feeding in the Red drum, *Sciaenops ocellatus* (Holt and Sun,1991).

Food digestion in the larvae is extended into the second half of the intestine. In adults only 3% of all trypsin produced is lost in the faeces and the trypsin activity in the second half of the intestine is reduced to 12% of total tryptic activity. In contrast, the tryptic activity in the second half of the larval intestine amounts to 46% of the total activity (Hofer,1985). This adaptation compensates for the relatively short gut by extending the time in which enzymes can act on the food.

Dabrowski (1991a) proposed three factors - external, central and local - as responsible for the morphologic and enzymic changes occurring during exogenous feeding of larval fish. The continuous feeding behaviour of the larvae might stimulate the intestinal mucosa and hence act as a trophic control of the absorptive cells. The central factors are basically the hormones that control the development of intestinal enzyme expression and the onset of enterocyte differentiation. Other local triggers might aid the deployment of specific enzymes like the larval pancreatic enzymes (Lauff and Hofer,1984), the activity of which might change sequentially during metamorphosis.

2.1.2.1. Role of larval foods

The diet of the larvae of most fish species is normally of animal origin (mainly zooplankton), has high nutritive value and is easily digestible. These live foods contain digestive enzymes that support and accelerate the digestive processes in the predator. In White fish (*Coregonus clupeaformis*) larvae, exogenous proteases from live foods represented upto 70% of the total proteolytic activity of the digestive tract (Lauff and Hofer,1984). Therefore, apart from the nutritional advantages that live food organisms confer, they also aid the digestive processes of the larval gut. The exogenous enzymes

may also play a role in the activation of endogenous enzymes. Artificial diet does not have such properties. Therefore, it is not surprising that artificial larval feeds may not support the level of fish growth sustained by live foods, even if the problems of size, texture, buoyancy and palatability could be solved.

It has also been shown that an artificial diet stimulated increased trypsin production in Roach larvae, to a level twice as high as that produced by natural food (*Artemia*) and of the same magnitude found in adult Roach (Hofer,1982 ; Hofer and Nasir Uddin,1985). As the protein resorption efficiency in the hind gut is poor, this leads to an increased loss of body protein and may actually contribute to low growth rates and finally mortality. Therefore, only easily digestible ingredients should be used in larval feeds.

2.2. Nutrient demands in early stages

The nutritional requirements of fish embryos and eleuthro embryos have not been defined. Nevertheless, they would be expected to match the composition of the yolk that caters the needs of the pre-feeding fish. Further, elucidation of the nutrient needs of the early stages has been hampered by difficulty in altering the composition of the endogenous yolk reserve as a means to decipher the requirements (Heming and Buddington,1988). The anatomical and physiological changes during ontogeny probably demarcate the nutrient needs specially according to developmental stages. Even after the appearance of yolk syncytium, the liver and related synthatase systems do not develop (Takahashi *et al.*, 1978) and thus the pre-feeding fish has a broader set of nutritional requirements than later life stages (Heming and Buddington,1988). Therefore, parallels cannot be drawn with the nutritional requirements of juvenile and adult fish.

Among the nutrients contained in the egg, protein, the most abundant component, resides mainly in the yolk. It provides amino acids for tissue growth and energy through catabolic processes. Lipid, the most major constituent, varies widely among the species in its content, ranging from 0.1% of the egg weight in Plaice (*Pleuronectes platessa*) to 45% in mouth breeding cichlid (*Labeotrophus* sp.) (Balon ,1977). They are used either as the structural components of cell membranes or for energy production. The energy requirements increase after hatching and are met by triglycerides and waxes. Though

only available in relatively small quantities, yolk carbohydrates are present both in the free state and as glycoproteins. Glycogen is the primary carbohydrate in all fish eggs studied to date and has also been implicated as an energy source in the early embryonic stages (Nakagawa,1970 ; Terner,1979 ; Vetter *et al.*, 1983). Carbohydrate is the nutrient utilized to the maximum, between fertilization and hatching and therefore has been attributed a nutritive role in initial cleavage (Moroz and Luzhin, 1976).

As the physiological capabilities of larvae are limited, specific diets are required, be they live food organisms or formulated feeds. A variety of live foods are currently being used in larviculture and artificial diet is the missing link in the larviculture cycle and efforts to produce it require an understanding of the nutrient needs of the developing fish. Since standard growth studies cannot be performed on early stages, current knowledge on nutrition has centered on the utilization of the yolk elements and survival and growth based on certain essential nutrients, particularly essential fatty acids (EFA) derived from live foods or provided through microdiets. Several studies have established that the polyunsaturated fatty acids of the *w*-3 and *w*-6 series are crucial to survival and growth of first feeding fish larvae (Watanabe *et al.*,1983 ; Henderson and Sargent,1985). The importance of amino acids for the developing larvae has also been emphasised by Fyhn(1990). Ronnestad (1992) concluded that the free aminoacid pool in the yolk was depleted during development and was used largely as a substrate in energy metabolism, apart from being employed at different rates for body protein synthesis. Dabrowski(1991a) suggested that body growth based on protein deposition could be monitored by the availability of dietary amino acids, their transport through intestinal mucosa and their availability at synthesis sites.

2.2.1. Lipids

Lipids are indispensable in the early stages of fish life. They are the main source of energy even from the gastrula stage in marine fish embryos (Vetter *et al.*,1983). The exponential loss in lipid reserves in larval fish on food deprivation underscores its primary role (Tandler *et al.*,1989). Many eggs have the characteristic oil globule(s) constituted by triacylglycerols (Nakagawa and Tsuchiya.,1971). Interest in the role of lipids in larval nutrition stems from the fact that essential fatty acids have been attributed with

several functions in the adult fish (Cowey and Sargent,1979 ; Watanabe, 1982 ; Kanazawa, 1985a).

It has been demonstrated that the *w*-3 PUFA are required for the normal growth and survival of larval fish. Mortality and deficiency signs such as deficient swim bladder inflation (Koven,1991), disintegration of gill epithelia, hydrops (Rodriguez *et al.*,1994) and hypomelanosis (Kanazawa , 1993 ; Rainuzzo *et al.*,1994) have been reported in larval fish which were given diets low in *w*-3 PUFA (Izquierdo,1996). Low PUFA diets resulted in incomplete metamorphosis of Summer flounder (*Paralichthys dentatus*) larvae (Bisbal and Bengston, 1991). It was revealed that eicosapentaenoic acid (EPA, 20:5*w*-3), one of the most essential fatty acids in fish, is a constituent of the cellular membranes of several developing tissues (Kanazawa *et al.*, 1982). Kanazawa (1985b) suggested that rapidly growing larval fish need relatively large amounts of exogenous EPA. In addition, the inclusion of phospholipids in larval feeds improved growth and survival. Though the precise physiological role was not demonstrated, it was proposed that the phospholipids are probably involved in the formation of new cell components. The rate of biosynthesis of phospholipids in the larvae was probably insufficient to meet the developmental requirements.

2.2.1.1. Enrichment of live feeds with PUFA

Since the nutritional components, especially the fat soluble materials, in live foods can be relatively easily modified through diets, the control of *w*-3 PUFA levels in rotifers and *Artemia* nauplii has permitted the use of these live foods to study the EFA requirements of various marine larval fish. Different techniques have been adopted by workers to enrich the live food as a means of determining the effect of *w*-3 PUFA in fish. On feeding the larvae of Ayu on rotifers cultured on omega yeast rich in linoleic acid (18:2*w*-6), linolenic acid (18:3*w*-3) and other *w*-3 PUFA, Oka *et al* (1980) found that linoleic acid had no EFA value, whereas *w*-3 PUFA was effective in enhancing the growth of this species. Le Milinaire (1984) reared the larvae of turbot (*Scophthalmus maximus*) with rotifers fed on microdiets with or without *w*-3 PUFA and proved their importance as EFA. In larval Red sea bream fed *w*-6 or *w*-3 PUFA enriched rotifers, Watanabe *et al* (1983) established a relationship between air bladder inflation and *w*-3

PUFA levels.

2.2.1.2. Importance of Polyunsaturated fatty acids

Tocher and Sargent (1984) based on the chemical profile proposed a higher w-3 PUFA requirement in the early larval stages of finfishes. The importance of docosahexaenoic acid (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 glycerophospholipids, which are essential components of biological membranes. Fluidity of the membrane in gills seem to depend more on the DHA than on the arachidonic acid content. DHA is the main component of 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 gain by the larvae (Noakes and Godin,1988). In Gilthead seabream, the DHA contents in phosphoglyceride lipids of larvae were directly correlated with growth improvement (Rodriguez *et al.*,1994). Mai *et al* (1981) reported a prostaglandin PGF_{1}, formed from DHA, as one of the major prostaglandins in the gills of freshwater trout. However, it was later demonstrated that this compound is a trihydroxylated derivative of DHA (German *et al.*, 1983). Although elevation of dietary DHA levels did not affect the growth and survival of Summer flounder larvae, it slightly improved the pigmentation and the number of fish that completed metamorphosis. In contrast, excessive levels of DHA significantly reduced total weight of Summer flounder larvae (Bisbal and Bengston,1991).

Less attention has been paid to the importance of eicosapentaenoic acid (EPA). Low growth and survival rates due to EFA deficiency are effectively prevented by elevation of the dietary EPA (Watanabe *et al.*, 1989). Labelled exogenous EPA has been shown to be incorporated in cellular membranes of the swim bladder, liver and pyloric caeca (Kanazawa *et al.*, 1982), although its function in these tissues has not been investigated.

Arachidonic acid is also selectively retained by the larvae of several species during

starvation (Rainuzzo *et al.*, 1994), suggesting the importance of this fatty acid. Although it is present in lower concentrations than EPA in fish tissues, it is regarded as the major source of prostaglandins (E_2 and F_2 alpha) for being present in high concentrations in the phosphotidylinositols of marine fish (Bell *et al.*, 1984).

Current evidence suggests that not only DHA but also other polyunsaturated fatty acids are essential for marine fish larvae. It is probable that not only is the total content of DHA or *w*-3 PUFA important, but that a balanced dietary ratio of EPA / DHA may also be necessary to obtain optimum growth rates (Izquierdo, 1996).

2.2.1.3. Phospholipids

Kanazawa *et al* (1983) described the beneficial effects of supplemental phospholipids in larval Red seabream and Knife jaw. In experiments on larval Ayu (Kanazawa et al., 1985), it was shown that phospholipids improved growth and survival irrespective of age. Kanazawa (1985b) found 1-2% lecithin as an optimum level in the diets of larval *Plecoglossus altivelis* when soy lecithin was the phospholipid source.These compounds along with *w*-3 PUFA are indispensable for normal growth and survival. The requirement for the phospholipid may arise from a limited ability of the larval fish to synthesize it from available precursors.

2.2.2. Amino acids

Several workers have revealed that free aminoacids (FAA) occur in high amounts in pelagic eggs of some marine species (Suzuki and Suyana, 1983 ; Fyhn, 1990 ; Ronnestad, 1992) and the variation observed among the species was related to egg size. In general, the FAA concentration represented about 50% of the total osmolality in the newly spawned egg. The FAA constituted 20-50% of the total aminoacids in the pelagic eggs (Ronnestad *et al.*,1992) . However, in the case of marine demersal eggs this was only about 3% and about 5% in freshwater fishes (Dabrowski *et al.*,1984). The abundance of FAA in the pelagic eggs was related to its role in the process of oocyte hydration. The relative composition of the FAA pool showed little variation and the predominant amino acids detected were leucine, lysine, valine, isoleucine, alanine and serine (Ronnestad *et al.*, 1992). The identical profiles may have resulted from the hydrolysis

of a common yolk protein-phosvitin corresponding to water uptake during swelling (Thorsen and Fyhn, 1991).

When the larva turns to exogenous food, the aminoacids necessary for energy would be provided by the ingested food. It is yet not clear if the primary intestine in the larval fish is fully able to digest food proteins in presence of the early proteolytic enzymes or whether they depend on the FAA provided by the feed (Fyhn, 1990). Kanazawa (1988) conducted some preliminary experiments with flounder (*Paralichthys olivaceus*) using various protein sources which had an overall amino acid pattern similar to the whole body of larval fish. The nutritive values of four microparticulate diets formulated with white fish meal, brown fish meal, bonito powder, yeast powder, crab meal, gluten meal and krill meal were compared with live food as control. The survival range from the test group was 36-45% and total length 19.5-19.7mm, whereas the respective data for live food as control were 52% and 21.4mm. In addition, it was found that the incidence of abnormal pigmentation was drastically reduced with the test microdiets compared to live food. When the larvae of Red seabream were fed microdiets based on sardine powder, squid powder,scallop powder and krill meal containing a balanced proportion of amino acids, recorded better survival but slightly inferior growth compared to live food (Kanazawa *et al.*, 1989). These studies have demonstrated that the essential amino acid composition of the larval fish body closely match with dietary requirements.

As the larva initiates exogenous feeding, the spurt in activity demands a great deal of energy. Since the endogenous energy reserves are finite, the fish accepts its first food, probably tempted by chemoattraction from the amino acids and other metabolites emanating from the prey organism (Tanaka *et al.*, 1991).

2.2.3. Vitamins

Reports on the vitamin needs in the early stages of fish have focussed on vitamin C. Cowey *et al* (1985) , Sato *et al* (1987) and Dabrowski (1990) have established a rapid drop in the ascorbic acid content during development. Replacement of the vitamin during first feeding seems to be vital for larval survival as the fish are unable to synthesize it . Dabrowski *et al* (1988) explained that ascorbic acid was particularly essential in

cyprinid larvae as yolk reserves are limited compared to salmonids. In addition, the body pool of ascorbate exhausted rapidly due to the high growth rate and therefore structural malformations appeared easily. Carp larvae exhibited vitamin deficiency signs such as caudal fin erosion and deformation of the gill. In order to maintain the body concentration of ascorbate during early ontogeny, Dabrowski (1990) fed White fish (*Coregonus laveratus*) with a relatively high amount of vitamin C (about 1500 µg ascorbate / g dry matter fed). It was also observed that a decrease in the body concentration of ascorbate was relatively slow in larvae fed on live food. Supplementation of ascorbic acid as ascorbyl palmitate at start feeding for *Clarius gariepinus* larvae displayed a lower stress sensitivity at 20% ascorbyl palmitate supplementation than those of 0% group (Merchie *et al.*, 1997). For juvenile fish, inclusion of high levels of vitamin C (1000-1500 µg ascorbic acid / g dry weight) has been demonstrated to enhance tolerance to environmental stressors such as aldrin toxicity (Agrawal *et al.*, 1978) and intermittent hypoxic stress (Ishibashi *et al.*, 1992), and to increase immunoresistance (Hardie *et al.*, 1991). The extra effects of ascorbic acid supplementation at high levels on stress resistance might be of importance under suboptimal rearing conditions in commercial hatcheries (handling, transportation, disease outbreaks) (Merchie *et al.*, 1997).

Studies on the chemical composition of eggs led to focus on the importance of vitamin E in salmonid larvae (Watanabe, 1990). The vitamin E content decreased by almost one half during the developmental period of 40 days from hatching in both Chum salmon (*Oncorhynchus keta*) and Coho salmon (*O.kisutch*). Hence the role of vitamins in larval survival cannot be discounted.

2.3. Hormones and larval quality

Recent evidence suggests that hormones are passed on to eggs by brood fish. This store of maternal hormones may fill the regulatory needs of fish larvae for growth, development, osmoregulation, stress response and other physiological functions prior to the functional development of their own endocrine glands. Thus, the hormonal levels in eggs/ larvae may be an important determinant of egg/larval quality (Lam, 1994).

2.3.1. Thyroid hormones

Thyroid hormones, thyroxine(T_4) and triiodo thyronine (T_3), are present in eggs in measurable quantities in all species of fish studied (Lam,1994). In eggs, T_4 level is higher than T_3 in freshwater species but lower in marine species, with diadromous or euryhaline species showing either pattern (Tagawa *et al.*, 1990). The significance of this is not clear and seasonal variations in T_4 and T_3 levels are possible (Lam, 1994). In general, the thyroid hormones show a progressive decline during embryogenesis and early larval development suggestive of utilization. Endogenous thyroid hormone production(thyroxinogenesis) appears to be turned on at around or before the time of complete yolk-sac resorption (Tagawa and Hirano, 1990).

2.3.1.1. Effects of treatment

Treatment of larvae by immersion in T_4 or T_3 solutions promoted growth, development and / or survival in several freshwater fish species : Tilapia , *Oreochromis mossambicus* (Reddy and Lam, 1992) and *O.niloticus* (Nacario, 1983) ; Carp, *Cyprinus carpio* (Lam and Sharma, 1985) ; Gold fish, *Carassius auratus* (Reddy and Lam, 1992) and Gouramy,*Colisa lalia* (Reddy and Lam, 1987).The developmental stimulating effects include fin differentiation and growth,yolk sac resorption, transition to free swimming, skin and scale formation, pigmentation, silvering and exopthalmia (in Black moor). A possible metabolic effect observed is the stimulation of heart beat (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 available from the maternal store (Reddy and Lam, 1992), thereby producing the enhanced growth and developmental effects. However, excessive or prolonged treatment had an adverse effect on growth and development (Nacario, 1983). Even with early thryroxinogenesis, iodine availability in these freshwater species may be limiting, thus allowing for efficacy of T_4 / T_3 treatment. In contrast, iodine deficiency is not a problem in marine species (Lam,1994). In such cases, T_4 / T_3 treatment of post larvae may advance particular developmental events associated with the marked elevation in T_4 / T_3. They include accelerated metamorphosis in flounder, *P.olivaceus* (Yamano *et al.*, 1991) ; transformed post larvae of Milk fish, *Chanos chanos* from being long, slender and transparent to silvery, opaque and juvenile

like (Lam *et al.*, 1985) ; accelerated the appearance of black stripes on the body and the change in habitat from pelagic to benthic in post larvae of Red seabream, *Pagrus major* (Hirata *et al.*, 1989) ; and promoted the transformation of post larvae of black 'telescopic eye' gold fish to the black exopthalmic form of the adult (Reddy and Lam, 1992).

A more practical means of thyroid hormone administration to eggs / larvae is through injection to the mother fish. This has been shown successful in Striped bass , *Morone saxatilis* (Brown and Bern, 1989).

Overdose of thyroid hormones leads to thyrotoxicosis manifested in larvae by growth retardation and abnormal development, culminating in mortality (Lam and Sharma, 1985).

2.3.2. Other hormones

The possible role of cortisol, the principal corticosteroid in early fish development is being studied (Idler and Truscott, 1972 ; Lam,1994). Cortisol has been detected in newly fertilized eggs of the flounder, *P.olivaceus*. Cortisol was also found to promote larval survival in sea bass, particularly when larvae were subjected to salinity stress (Sampathkumar *et al.*, 1993). Immersion of eggs of Brown trout Salmo trutta in 15 mg/ ml recombinant trout GH caused transient growth stimulation in the larvae from 1 to 4 weeks post hatching (Le Bail *et al.*, 1991).

2.4. Diets for larval fishes

Larviculture nutrition and feeding strategies still appear to be bottlenecks for the industrial upscaling of aquaculture of finfishes and shellfishes. Until the larva starts feeding on organisms, the cellular growth and energy needs of the developing embryo are met by the nutrients within the egg. The scarcity of suitable planktonic prey organisms when the larva commences exogenous nutrition will result in the body tissue autolysis and eventual death (Bagarinao, 1986). The natural foods include different types of phytoplankton, small zooplankters and invertebrate larvae. Some of them have been selected as food for larviculture. The criteria in deciding the food source are basically its physical qualities such as purity, availability and acceptability together with nutritional

indicators such as digestibility and energy obtainable from it (Leger *et al.*, 1987). In addition, it should be easily procurable, reproducible and economical. These definitive standards limit the choice to a few ciliate protozoans, rotifers, cladocerans, copepod nauplii and small planktonic invertebrate larvae such as trocophores and veligers(Dhert and Sorgeloss, 1995). Apart from this, formulated microdiets are also being offered to several larval fish.

2.4.1. Live foods

2.4.1.1. Micro algae

Microalgae form the primary link in the food chain of aquatic animals. Nearly 20 different species of diatoms and flagellates in the size range of 2-20 µm are popular for the production of specific types of larval aquaculture species or for feeding zooplankton, used as live food. Addition of various microalgae to the water during first feeding of fish larvae frequently has resulted in improved growth and survival during the larval stage (Howell, 1979 ; Scott and Middleton, 1979 ; Jones *et al.*, 1981; Naas *et al.*, 1992).

The green water technique has been used successfully in conjunction with the culture of various larval fishes and shrimps. The technique involves maintenance of sufficient concentrations of phytoplanktonic algae in the larval culture tanks to provide green colour. Fish larvae do not filter feed but are carnivorous hunters, as a result of which microalgae do not constitute a major food source at the start of feeding. Several attempts have been made to explain possible beneficial effects of this green water technique. In cod (Meeran, 1982) and halibut (Reitan *et al.*, 1991), it has been shown that the larvae take up substantial numbers of microalgae during the initial days of yolk absorption ('green stomachs') which may support the idea that they are used as direct food source at the start of feeding. Fortuitous ingestion may be a source of micronutrients, which are not available through the administered rotifers or brine shrimp nauplii. Also they may supply exogenous enzymes which could assist in the digestion of zooplankton food ingested by the fish larvae, which at the start of feeding have only a primitive digestive system. This technique has been applied in the culture of Common carp, *Cyprinus carpio* ; Bighead carp, *Aristichthys nobilis* (Fermin and Recometa, 1988) and

Atlantic halibut, *Hippoglossus hippoglossus* (Naas *et al.*, 1992).

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

For the maintenance of algal cultures ,stock cultures kept under sterile conditions and batch cultures of increasing size are required. Furthermore, problems still exist with respect to contamination as well as consistent nutritional quality between batches(Olsen,1989). Several subsitute products as algal replacement diets are under experimental stages. Examples are 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) .

2.4.1.2. Rotifers

Rotifers, the smallest metazoans, which are planktonic and halophilic, comprise *Synchaetus* sp. that is found in open environment along coastal areas and *Brachionus plicatilis* and *Hexartha fennica* that are found in closed bodies like lagoons and salt marshes (Pourriot, 1990). Since the establishment of culture possibilities (Ito, 1960), *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. The rotifers with improved nutritional quality were produced by culturing them on a combination of marine chlorella and baker's yeast (*Saccharomyces cerevisiae*) (Kitajima *et al.*, 1979). Nutritional studies based on rotifers have revealed that the digestibility of rotifer protein was as high as 84-94%. The net protein utilisation(NPU) was also relatively high when tested in Rainbow trout and carp (Watanabe *et al.*, 1983). The variations in the ω-3 PUFA content often induced by the culture conditions strongly affect the quality and inturn the survival of larval fish which prey on them (Fukusho, 1989). Using

emulsified formulations, the dietary value could be improved not only in terms of *w*-3 PUFA, but also fat soluble vitamins (Watanabe *et al.*, 1983). The quality enhancement techniques are applied as early as 24 hours prior to offering them to the fish larvae (Watanabe *et al.*, 1983). Using microcapsules, Walford and Lam(1987) successfully enriched rotifers to contain a high concentration of EPA and DHA eight hours after feeding.

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

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

2.4.1.3. *Artemia*

Since its discovery by Seale (1933) aquacultural practices have promoted *Artemia* as the most favoured food for larval organisms. Apart from the nutritional qualities, it has various other attributes that makes it an ideal choice for aquaculturists. Prominent among them is its availability in two forms and their wide size range. In addition, they could be used as carriers to deliver essential nutrients, pigments, prophylactics and therapeutics to fish larvae (Leger *et al.*, 1987).

The early nauplii have the maximum food value, losing upto 30% with age (Sorgeloss *et al.*, 1996). They are to be judiciously utilized as food for the particular stage of the larval fish. Cold stored freshly hatched nauplii kept for periods of 24 hours and longer have been advocated for a better quality food ration (Sorgeloss and

Leger,1992). A positive correlation between *Artemia* nauplii size and larval fish mortality (Beck and Bengston, 1982) emphasises that the selection of the *Artemia* strain to be offered as food should depend on the farmed species. This is because of the large variations observed in the nauplii size among the various geographical strains (Van haecke and Sorgeloss, 1990).

On evaluating the nutritional qualities of *Artemia* in rainbow trout and carp, Watanabe *et al* (1983) found that live food was well digested and high net protein utilisation and protein efficiency ratio were obtained. The enhanced digestion may actually be aided by the autolytic action of the endogenous enzymes- amylase and trypsin of Artemia (Samain *et al.*, 1980).

2.4.1.3.1. Bioencapsulation

Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. This method, also called *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with essential nutrients (like *w*-3 PUFA and vitamin C).

2.4.1.3.1.1. Polyunsaturated 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 obtained from emulsified concentrates (50-60mg/g DW *w*-3 PUFA) after 24 hour enrichment period. Recent studies with various species of marine fish have revealed that high dietary levels of total PUFA can have a negative effect, and that DHA is more important than EPA for various physiological functions, including survival, growth and pigmentation success (Watanabe and Kiron, 1994). However, the enrichment of *Artemia* with DHA is difficult 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 essential dietary component in larviculture (Dabrowski, 1992). Several biological (e.g. skeletal development, growth and survival) as well as physiological functions (e.g. resistance to toxicants and stress, immunoactivity) are enhanced in larvae from supplemental dietary ascorbate (Merchie *et al.*, 1996). Ascorbic acid 2-sulphate (AAS), a stable derivative of AA, was discovered in dormant cysts of *Artemia* by Mead and Finamore (1969). Cysts of various batches and strains of *Artemia* differed considerably in AAS content (296-517 µg AA/g DW) (Merchie *et al.*, 1995). The amount of AA, liberated in freshly hatched nauplii reflects 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).

Tests have been conducted to incorporate extra ascorbic acid into *Artemia* nauplii in a stable and bioavailable form. Applying a standard enrichment procedure (Leger *et al.*, 1987) and experimental selfemulsifying concentrates containing 10-20% ascorbyl palmitate (AP) , levels upto 2.5 mg free AA/g DW can be incorporated into brine shrimp nauplii within 24 hours (Merchie *et al.*, 1995). These concentrations did not drop when the 24 hour enriched nauplii were stored for another 24 hour in seawater at 28 ° C or 4° C. Merchie *et al*(1997) verified that the growth effect of the AP boosted *Artemia* diet was the result of the extra AA incorporation and not of the concomitant palmitic acid (PA), which was set free after hydrolysis of AP in the *Artemia* nauplii, and which could possibly be used as a supplemental energy source.

2.4.1.4. Cladocerans

2.4.1.4.1. *Daphnia* spp.

Daphnia is an important food source in freshwater aquaculture and the ornamental fish industry. They can also be used in mariculture as replacement live feed for *Artemia*. The nutritional value of *Daphnia* is highly dependent on the chemical composition of their food source (Dhert and Sorgeloss,1995). *Daphnia* cultures tend to collapse after 10-30 generations, if they are fed solely with *Chlorella, Chlamydomonas*,mixtures of yeast and *Scenedesmus* or *Scenedesmus* and *Chlamydomonas*. This can be overcome

by adding vitamin mixtures and trace elements to the culture medium. *Daphnia* contains a broad spectrum 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.

The freshwater cladoceran, *Moina* spp., can be mass cultured (Shim, 1988) and has often been used as live food for a large variety of fish larvae(Watanabe *et al.*, 1983; Fermin and Recomenta, 1988).*Moina* is also known to be a suitable live food for rearing tropical aquarium fish larvae to adult (Volkart, 1994). It was found to be a suitable diet for seabass larvae and juveniles (Maneewongsa and Tattaman,1983), fry of mullet (Nandy, 1979) and fry and fingerlings of Rainbow trout (Norman *et al.*,1979). The ω-3 PUFA composition of *Moina* spp. varies with the culture medium, but it can be upgraded nutritionally by employing emulsified lipids (Dhert and Sorgeloss, 1995). The *Moina* cultured on poultry manure had a high content of EPA (Shim, 1988).

2.4.1.5.Copepods

Fish larvae of about 7mm are offered marine copepods such as *Tigriopus* sp., *Acartia* sp., *Eurytemora* sp., *Euterpina* sp., *Oithona* sp., and *Paracalanus* sp. The suitability of the copepod of the genus *Tisbe* as a microfaunal food organism in mariculture has been demonstrated by Uhlig (1984). Watanabe *et al*(1983) found that the proximate and mineral composition of these live foods were altered by the culture media, but there were no significant differences in the aminoacid composition. Their dietary value interms of NPU and PER in Rainbow trout and carp was high. *Tigriopus* contained relatively high amounts of EPA and DHA, irrespective of culture media and food organisms, suggesting its high nutritional value.

From the nutritional point of view, copepods are far superior to *Artemia* nauplii ; their lower proteolytic activity and better fatty acid composition make them an excellent food with high energy content (Dhert and Sorgeloss, 1995).It is thus not surprising that their use in aquaculture has often resulted 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 provide the nutritional requirements of fish larvae(Jungwirth *et al.*, 1989). Kentouri (1981) reported the successful rearing of several marine species particularly of sea bass (*Dicentrarchus labrax*) with frozen zooplankton. White fish(*Coregonus laveratus*) was reared successfully with frozen zooplankton beyond metamorphosis using a feeding apparatus which prevents leaching of proteins (Medgyesy and Wieser, 1982). The successful first feeding of freeze dried zooplankton to the fry of Danube salmon (*Hucho hucho*) suggests that it may have been a source of exogenous enzyme activity (De Verga and Bohm, 1992).

2.4.1.7. Others

The high protein content of the nematode, *Panegrellus redivivus*, has helped to establish it as a viable food source for larval fish (Kahan *et al.*, 1980). They pointed out its advantages over *Artemia*, in that it doesnot consume algae and can survive over 72 hours in sea water. Eventhough the amino acid profile matches that of *Artemia* (Kahan *et al.*, 1980), there are several differences in the fattyacid composition (Biedenbach *et al.*, 1989). The EPA content is nearly a third of that in *Artemia*, while DHA is almost the same or little higher.Rouse *et al* (1992) succeeded in enhancing the nutritional quality in terms of EPA and DHA by employing a culture medium consisting of wheat flour, yeast and fish oil. When cultured under proper medium conditions, nematodes could be an alternative live food that is inexpensive and consistently nutritious. The experimental use of free living nematode, *Panegrellus redivivus* as larval food has been successful for carp, *Cyprinus carpio* and Silver carp, *Hypopthalmicthys molitrix* (Dhert and Sorgeloss, 1995).

Aquatic oligochaetes are important constituents in the natural food of freshwater fish and the use of tubificid, *Branchiura sowerbyi* in aquaculture, including its culture has been reported (Aston, 1984). Another species *Tubifex tubifex* has been offered as food for elvers of *Anguilla nebulosa* (Reddy *et al.*, 1977) and cat fish, *Mystus vittatus* (Arunachalam and Reddy, 1981). Terrestrial oligochaetes such as *Eisenia foetida*

(Tacon *et al.*, 1983) and *Dendrodrilus subrubicundus* (Stafford and Tacon, 1984) have been tested on trout. Bouguenec and Giani (1989) demonstrated the possibilities of mass production of *Enchytraeus* sp. and its suitability as food in the culture of early stages of ornamental fish, *Brachydanio rerio*, Roach, *Rutilus rutilus* and carnivorous perch, *Perca fluviatilis*.

2.4.2. Formulated feeds

2.4.2.1. Physical requirements of formulated feeds

There are several unavoidable problems associated with natural live food organisms. The availability of live food is subject to seasonal variation and composition. Moreover, the need for the culture of live food organisms or their collection from natural sources is an unnecessary encumbrance. Artificial diets, on the other hand, can be quality controlled during formulation, can be manufactured on a large scale and can be distributed easily to ensure regular supplies. Also, artificial feeds can be sterilized by conventional methods, resulting in the risk of importing pathogens or parasites into the hatchery.

The artificial feed should have the correct particle size. Feed particle size has been found to affect growth rate in several fish species. In several species (e.g., European eels, Atlantic salmon, Pacific salmon, Chinook salmon and Arctic charr) the particle size optimal for growth relatilve to fish size was determined following feeding experiments and described relative to fish length, fish fork and mouth size (Fowler and Burrows, 1971 ; Wankowski and Thorpe, 1979; Knights, 1983 ; Tabachek, 1988). 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 larvae must be able to chemically and optically recognise the feed as food (Appelbaum,1980). 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). Each feed particle must have the complete composition of the feed as a whole. If these conditions are not met, it may lead 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.

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

2.4.2.2. Weaning

Weaning is a critical stage in larval culture because successful transition from a live food to a prepared feed is dependant on feed quality and the larvae themselves (Devresse *et al.*, 1991). The best time to initiate weaning is soon after larvae switch to exogenous feeding. It is important for aquacultural purposes because it is economically advantageous. Dry feed can be stored refrigerated or frozen. Much of the labour, cost and time expended in rearing and dispensing of live food can be eliminated because commercial dry feed can be used in automatic feeders (Bromley, 1981).

Few species have been successfully reared from first feeding exclusively on artificial diets, and in most cases success at the experimental level has yet to be reproduced on a commercial scale. In general, freshwater larvae are fairly large at hatching and can adapt to dry feeds relatively easily. This is true particularly for the salmonids (12-25mm at hatching) which possess a functional stomach at first feeding and do not require live prey at this stage. Among other freshwater species, the most promising results have been achieved with coregonid larvae, which in many experimental studies have been reared exclusively on dry diets (Champigneuille, 1988). *Coregonus laveratus* larvae can be reared from first feeding on a yeast based dry diet, with good growth and survival (Champigneuille, 1988) . Common carp ,*Cyprinus carpio* (Charlon and Bergot, 1984 ; Charlon *et al.*, 1986), Ayu, *Plecoglossus altivelis* (Kanazawa *et al.*, 1985) and barbel, *Barbus barbus* (Wolnicki and Gorny, 1995b) are also reared exclusively on artificial diets.

Early larvae can be successfully fed artificial diets as a partial replacement for, or supplement to, live foods. In some cases, cofeeding live and artificial diets can produce 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). 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 feed may be indispensable, as has been found for tench (Wolnicki and Korwin-kossakowski, 1993) and minnow (Kestemont and Stalmans, 1992). Qin *et al* (1997) proposed that snake head, *Channa striatus* could be trained to accept formulated feeds using *Artemia* nauplii supplemented with formulated feed for 30 days,then gradually eliminate live food over a 7 to 10 period or to feed exclusively with *Artemia* nauplii for 30 days,followed by 7-10 days mixed feeding with both *Artemia* and formulated feed, then switch completely to formulated feed. Santiago and Reyes (1989) showed that the combination of *Brachionus* sp. and artificial diet was the best feeding regime in enhancing the growth of the Bighead carp, *Aristichthys nobilis* fry.

The assimilation of dry diets was improved by the addition of live foods regardless of ingestion rates or larval age in all the above works. Dabrowski (1984) and Kolkovski *et al* (1993) proposed that the digestive enzymes of live foods may assist in larval digestion. Live food assists in larval digestive process via a contribution of gastric hormones that can improve the gastric activation (Kolkovski *et al.*,1995). This hypothesis could be related to the delay in larval development and maturation of some digestive processes in larvae, such as the onset of pancreas secretory functions and enterocyte differentiation, found in sea bass larvae when *Artemia* was removed from the rearing tanks 25 days after hatching (Cahu *et al.*, 1995). The presence of live food improves artificial diet performance in larvae suggesting that a non-specific factor, like visual or olfactory stimuli, could be responsible, probably associated with increased peristalsis which triggers the rest of the larval digestive process.

2.5. Oxygen consumption, ammonia excretion and O:N ratios

The knowledge of the metabolic changes occurring after feeding would give more insight into some of the nutritional constraints on physiological processes in early stages of fish reared on live and dry diets (Dabrowski *et al.*, 1984). The effect of feeding on the magnitude of post-prandial increase in oxygen consumption has been investigated in

fishes by Muir and Niimi (1972), Oozeki and Hirano(1994),Steinarsson and Moksness (1996) ; in non-feeding larvae (Dabrowski *et al.*, 1984) and in fish grown on live *Artemia* (Forstner *et al.*, 1983). The higher the proportion of dietary protein, higher the post prandial oxygen consumption (Jobling and Davies, 1980). So, post prandial increase in oxygen consumption could be used for comparing the nutritive value of different diet formulations(Jobling, 1981).

Similarly, one of the end products of nitrogen metabolism, ammonia could be used to measure the efficiency of dietary protein utilization (Garcia *et al.*, 1981). A higher rate of ammonia excretion indicates poor amino acid balance (Lovell, 1989). The variations in ammonia excretion rates are affected by temperature, fish size and feeding rates (Steinarsson and Moksness, 1996).

Reports on O:N ratio are scarce in fishes. However, reports on the post prandial energy and nitrogen metabolism in early life history of fish and on the fate of dietary ingredients are available in marine fish larvae (Buckley and Dillman,1982 ; Houde and Schekter,1983). Post prandial metabolic changes in larval and juvenile freshwater fish under various feeding regimes have been studied with carp (Kaushik and Dabrowski,1983), pike (Kaushik *et al.*, 1984) and coregonids (Dabrowski and Kaushik,1984). In warm water fish larvae, post prandial ammonia excretion increased more than 20 fold whereas the oxygen consumption rate tripled (Dabrowski and Kaushik, 1984). A decreased O:N ratio indicates an increased protein metabolism (Capuzzo and Lancester, 1979) which indicates poor utilization of protein for growth of fish. Carolin and Susheela (1991) obtained higher O:N ratios for clam meat, squid meal and fish meal indicating the superiority of these protein sources for *Chanos chanos* fry. Stolbov *et al*(1996) obtained low O:N ratios for black sea fishes under hypoxic conditions indicating the utilisation of protein as the predominant substrate in energy metabolism.

2.6. Biology of Angel fish, *Pterophyllum scalare* (Lichtenstein)

The Angel fish, *Pterophyllum scalare* is one of the favourites among the aquarium fish hobbyists. The body is compressed with black vertical bars passing through the overall length of the body. 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. Although it is observed to breed in aquarium tanks throughout the year, the main breeding season is between June and November (Mathew *et al.*, 1996). The changes during the cycle of oogenesis of *P.scalare* have been studied in detail by Degani and Yehuda(1996). The average cycle lasts for 11 days , during which the oocyte passes through vitellogenesis, maturation and spawning. The duration of spawning and the quality of eggs (i.e., the percentage of larvae hatched) are affected by the age of the fish and environmental conditions. *P.scalare*, a multispawning fish differs from seasonal spawners and one time spawners (Luquet and Watanabe,1986). Mathew *et al*(1996) reported that the fish breeds repeatedly at varying intervals ranging from 9 to 279 days. The number of eggs varied from 100-1204, per spawning for fishes of 39mm/3g to 72mm/18g, with an average of 465 eggs. In the adult female *P.scalare* oogenesis occurs all the time so the diet, particularly live food, is very important. 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 2-3 days and start free swimming in about 7 days and start accepting food (Mathew *et al.*,1996). In contrast, marine angel fish, *Centropyge interruptus* becomes free swimming five days after hatching and the larvae measured 2.4-2.6mm in total length (Hioki and Suzuki, 1987). The natural diet of the first feeding *P.scalare* larva consists of various species of invertebrates (Degani, 1993). They can be fed with live *Artemia* naupllii, *Daphnia*, chopped Tubifex and finely ground flake diets under artificial rearing conditions (Volkart, 1994). Angel fish (0.5g) fed high fish and shrimp meal and no pigment diet registered a higher growth compared to the fish fed low fish and shrimp meal and with pigment diet (Boonyaratpalin and Lovell, 1977). Degani (1993) suggested that the angel fish requires a high protein diet (40-50%). There was no difference in growth rates between various levels of protein but the addition of *Artemia* to the diets caused a significant increase in growth of the fish(Degani, 1993). Mathew and Sherief (1996) 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 the rearing of Angel fish, *P. scalare*.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The objective of the study was to evaluate the effect of different diets on the growth and survival of Angel fish, *Pterophyllum scalare* larvae. The experiment was done twice in order to verify the consistency of the results.

3.1. Experimental rearing facilities

The experiment was conducted in the wet lab of the Department of Aquaculture, College of Fisheries, Panangad. Square shaped glass tanks of 30 X 30 X 30 cm dimension were used for rearing the experimental animals. The tanks were placed in the wet lab which had provision for subdued light. Intake water was stored in a FRP tank before using it for filling the experimental tanks.

3.2. Experimental animals

One day old, free swimming hatchlings of Angel fish, *Pterophyllum scalare* (Lichtenstein) of the same brood were obtained from the college aquarium. A few numbers of hatchlings were weighed randomly in an electronic balance and ten numbers each were distributed randomly to all the experimental tanks. The initial weight of the hatchlings ranged from 1.24 mg to 1.31 mg and the standard length ranged from 4.3 to 4.6 mm.

3.3. Different test diets used in the study

Particulars of the different test diets used in the experiment are given in Table 1.

3.3.1. *Artemia* nauplii

These were obtained by hatching the artemia cyst (Bio marine brand). The cysts were hatched in saline water with vigorous aeration. After 24 hours the nauplii were collected and rinsed in freshwater before feeding them to hatchlings(Lavens and Sorgeloss, 1996). The mean size of Artemia nauplii fed to the larvae during the study period was 444 $\pm$ 21 μ.

3.3.2. *Moina micrura*

Pure culture of *Moina micrura* was maintained in outdoor circular cement tank of 0.352 ton capacity. The tank was cleaned properly and filled with freshwater. The fertilization schedule suggested by James *et al* (1991) was applied in the culture water. The composition and quantity of the fertlizers are given in Table 2. A small quantity of *Chlorella* was inoculated in the culture tank. *Moina micrura* was isolated from the plankton samples of earthen ponds and 25 nos./l were added to the water two days after the addition of *Chlorella* . Half the volume of the water was exchanged once in a week and fertilization solution was applied once in a fortnight when the density was reduced. The mean size of Moina *micrura* fed to the larvae during the study period was 370.6 ± 47.2 μ.

3.3.3. Artificial diet

3.3.3.1. Diet formulation

The feed was formulated using the locally available ingredients so as to get approximately 30% crude protein content. The ingredients used were clam meat powder, hen's egg yolk, oats, wheat flour, yeast and vitamin-mineral mixture.

Clam meat powder was obtained by powdering the clam meat after sundrying. Boiled egg yolk was used in preparing the feed. White oats used were of commercial food grade quality (marketed under the brand name Champion White Oats).

Yeast used was the baker's yeast of food grade. Vitamins and minerals were supplemented through supplevit-M (Sarabhai chemicals, Bombay). All the ingredients were finally powdered and sieved through 250 μ mesh and stored in polythene bags.

3.3.3.2. Diet preparation

The diet was prepared by weighing the respective ingredients accurately in an electronic balance. Table 3 gives the proportion of the ingredients used in the preparation of the artificial 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 @125 ml/100g of feed and mixed well in the mortar. The dough was

Table 1. Different test diets used in the experiment.

Artemia nauplii	T_1
Moina micrura	T_2
Artificial diet	T_3
Artemia nauplii + *Moina micrura*	T_4
Artemia nauplii + Artificial diet	T_5

Table 2. The fertilization schedule for *Moina micrura* culture.

Ingredients	Dose (ppm)
Groundnut oil cake	100
Rice bran	100
Super phosphate	24
Urea	12

Table 3. Composition of Artificial diet.

Ingredients	% by weight
Clam meat	30
Wheat flour	28
Egg yolk	27
Oats	12
Yeast	2
Vitamin-mineral mixture	1

transferred into a glass beaker and steam cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was rapidly cooled under an electric fan and again mixed well in a dry mortar along with supplevit-M. The well homogenized mixture was spread on a clean, dry tray as a thin layer and sundried for a period of 8 hours. The dried feed was powdered and packed in airtight container.

3.3.4. Mixed diets

Combinations of *Artemia* nauplii + *Moina micrura* and *Artemia* nauplii + artificial feed were the other two test diets.

3.4. Proximate composition of feed ingredients and test diets

Proximate composition of the feed ingredients and test diets were analysed to evaluate the nutrient status. The result is based on the mean of two samples and is expressed on a dry matter basis.

The moisture content was estimated by drying the sample at 105° C until a constant weight was reached. Microkjeldahl's method (AOAC,1980) was used to estimate the crude protein content. The nitrogen content was multiplied by a factor of 6.25 to get the 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 estimation of crude fat in the *Moina micrura* was done using hexane-isopropanol (Radin,1981). Crude fibre was estimated by the method described by Pearson (1976). The ash content was estimated by burning the sample at 550° C± 10° C for 6 hours in a muffle furnace. The carbohydrate content was determined by difference in dry weight (Hasting, 1976).

3.5. Experimental design and procedure

Square shaped glass tanks of 30 X 30 X 30 cm were used for conducting the experiment. A total of 20 tanks with four replications for each treatment were used. Treatments were allocated to each experimental unit by random allocation method.

The feed was provided *ad libitum* twice daily, once in the morning hours and the other in the evening hours. The combination diets were provided with the dry diet in the

morning hours and the live feed in the evening. The left over feed was removed and bottom of the tank cleaned properly before each subsequent feeding. The water in the tanks was exchanged daily to maintain good water quality. During the experimental period the larvae were subjected to growth assessment every week. At the end of the feeding study the larvae were starved for one day, the number in each tank was counted and weighed collectively and the average final weight was determined.

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 like average net weight gain, average gain in length, specific growth rate(SGR) and percentage survival were determined, in order to study the influence of different diets, on the growth and survival of *P. scalare* larvae.

3.7.1. Average net weight gain

It gives the increase in the weight of larvae during the experimental period when fed on various diets. It was calculated 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 the larvae was calculated by using the following formula.

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

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 more refined and improved growth index than absolute weight gain or percentage growth rate 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. Estimation of O:N Ratios

Angel fish larvae fed for 7 days, on different diets, were selected in duplicates from each of the treatments for experimental study. The respirometer used for the study is shown in figure 1. The fry were acclimated in the respirometer chamber for one hour before the readings were taken.The water used in the respirometer was filtered so as to make it free from plankton. At the beginning of each experiment water sample was drawn for estimation of dissolved oxygen and then the respirometer was closed. All the respirometers were placed in a water bath and maintained at the room temperature. After two hours final water sample was drawn for oxygen estimation. The difference was considered to be the oxygen consumed by the animals during the period of the experiment. The results are expressed in mg O_2 consumed/hr.

Ammonia excretion was determined using water samples drawn from the respirometer chamber prior to and after respirometry. The results are expressed as mg NH_3-N/hr.

Using oxygen consumption and ammonia excretion rates, O:N ratios of individual animals fed with different protein sources were estimated following the method given by Bayne *et al* (1985). The O:N ratios were estimated at weekly intervals during the experimental study.

3.9. Statistical analysis

ANOVA (Snedecor and Cochran,1968) was carried out for the collected data. Percentage values(X) were transformed into arc sine values ($\sin^{-1}$ X/100) for analysis.O:N ratios were statistically analysed using randomized block design. Pair-wise comparisons of the data were done using Newman-kuels test.

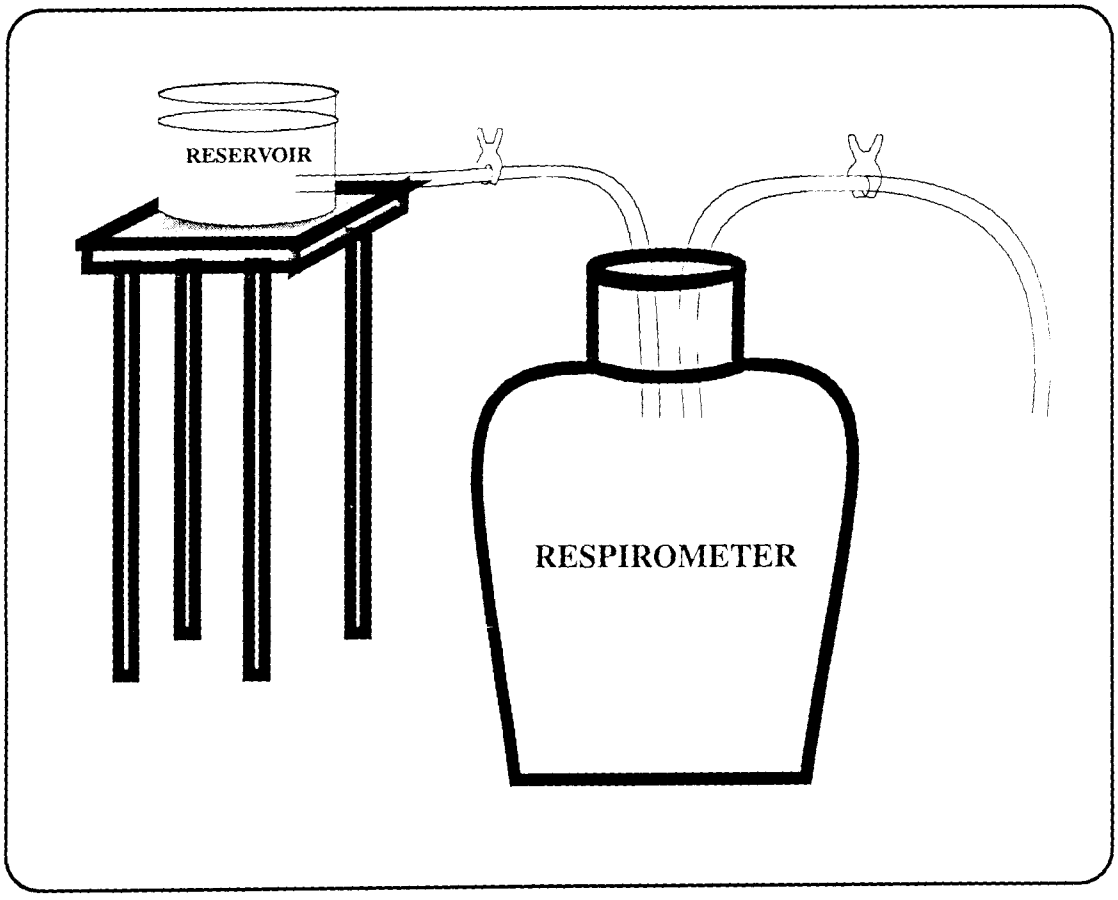

Figure 1. Respirometer used for determination of oxygen consumption and ammonia excretion rates.

RESULTS

4.RESULTS

The effect of different diets on growth and survival of Angel fish, *Pterophyllum scalare* larvae was evaluated. The details of the observations made during the study are presented below. The test diets *Artemia* nauplii, *Moina micrura*, Artificial diet, *Artemia* nauplii + *Moina micrura* and *Artemia* nauplii + Artificial diet are denoted as T_1, T_2, T_3, T_4, T_5 respectively.

4.1. Proximate composition of the feed ingredients and feeds

The proximate composition of the feed ingredients and the feeds used in the study are shown in table 4. The analysis of proximate composition of the artificial diet showed that it contained 29.71% protein, 14.09% fat, 0.4% crude fibre, 3.96% ash, 51.84% carbohydrate as nitrogen free extract and 7.0% moisture.

4.2. Efficiency of various test diets

4.2.1. Growth

The data regarding the weight gain in the larvae fed on different diets are given in table 5a and 5b for experiments I and II respectively.. The average live weight gain of larvae fed with different diets followed a similar pattern in both the experiments with the treatment T_4 giving the maximum growth and the treatment T_3 giving the minimum. The graphical representation of growth observed in the two experiments are given in figure 2. Analysis of variance (Table 6a and 6b) showed that the growth of the larvae was significantly different between the treatments.

The data on the growth in standard length of larvae fed on different diets are given in table 7a and 7b for experiments I and II respectively. Maximum average gain in length was shown by larvae in T_4, fed on *Artemia* nauplii + *Moina micrura*, followed by T_2 (*Moina micrura*), T_1 (*Artemia* nauplii), T_5 (*Artemia* nauplii + Artificial diet) and T_3 (Artificial diet) in both the experiments. However, the percentage gain in length was lower in the second experiment compared to the first. The increase in standard length of larvae fed on different test diets are graphically represented in figure 3.

Table 4. Proximate composition of feed ingredients and different test diets on dry weight basis.

Ingredients	Crude protein	Fat	Ash	Fibre	NFE
Clam meat	54.50	10.55	10.90	0.39	23.66
Wheat flour	13.43	1.18	1.03	0.57	83.79
Egg yolk	26.27	68.17	3.18	-	2.38
Yeast	42.77	3.39	7.70	0.29	45.85
Oats	13.63	7.79	1.90	0.50	76.18
Test diets					
Artemia nauplii[a]	56.6	18.9	9.7	14.8	-
Moina micrura	63.19	2.6	0.9	-	33.3
Artificial diet	29.71	14.09	3.96	0.4	51.84

a : Creswell, R.L. (1993)

Table 5a. Growth of *P.scalare* larvae fed on different diets.

Treatment	Replication	Average Initial weight (mg)	Average Final weight (mg)	Gain in Weight (mg)	Average live weight gain (mg)	Percentage weight gain	Average percentage weight gain
T_1	1	1.30	150.32	149.02	151.32 ± 2.42	11463.07	11832.92 ± 230.23
	2	1.25	152.31	151.06		12084.80	
	3	1.31	156.45	155.14		11842.75	
	4	1.29	155.34	154.04		11941.08	
T_2	1	1.27	156.25	154.98	158.13 ± 2.91	12203.15	12280.83 ± 149.85
	2	1.29	160.35	159.06		12330.23	
	3	1.30	163.75	162.45		12496.15	
	4	1.29	157.30	156.01		12093.79	
T_3	1	1.31	140.76	139.45	143.16 ± 2.41	10645.03	11036.25 ± 276.44
	2	1.32	145.32	144.00		10909.09	
	3	1.29	147.42	146.13		11327.90	
	4	1.27	144.31	143.04		11262.99	
T_4	1	1.27	160.71	159.44	162.99 ± 2.95	12554.33	12634.13 ± 137.19
	2	1.29	162.35	161.06		12485.27	
	3	1.30	165.71	164.41		12646.92	
	4	1.30	168.35	167.05		12850.00	
T_5	1	1.26	151.32	150.06	153.52 ± 2.60	11909.52	11970.48 ± 171.90
	2	1.28	155.75	154.47		12067.96	
	3	1.29	158.42	157.13		12180.62	
	4	1.30	153.71	152.41		11723.84	

Table 5b. Growth of *P. scalare* larvae fed on different diets.

Treatment	Replication	Average initial weight (mg)	Average final weight (mg)	Gain in weight (mg)	Average live weight gain (mg)	Percentage weight gain	Average percentage weight gain
T_1	1	1.27	140.35	139.08	137.35 ± 2.55	10951.18	10711.44 ± 252.06
	2	1.27	137.22	135.95		10704.72	
	3	1.29	141.72	140.43		10886.04	
	4	1.30	135.25	133.95		10303.84	
T_2	1	1.27	143.75	142.48	142.02 ± 2.46	11218.89	11052.78 ± 212.88
	2	1.29	146.27	144.98		11238.75	
	3	1.29	139.42	138.13		10707.75	
	4	1.29	143.78	142.49		11045.73	
T_3	1	1.27	136.27	135.00	135.16 ± 1.71	10629.92	10559.72 ± 77.99
	2	1.29	137.69	136.40		10573.64	
	3	1.27	133.72	132.45		10429.13	
	4	1.29	138.11	136.82		10606.20	
T_4	1	1.30	158.31	157.01	163.29 ± 4.59	10277.69	12284.29 ± 1166.96
	2	1.27	165.12	163.85		12901.57	
	3	1.29	171.21	169.92		13172.09	
	4	1.27	163.65	162.38		12785.82	
T_5	1	1.27	140.21	138.94	136.32 ± 3.64	10940.15	10652.75 ± 364.06
	2	1.29	132.72	131.43		10188.37	
	3	1.27	141.86	140.59		11070.07	
	4	1.29	135.61	134.32		10412.40	

Table 6a. Analysis of variance of the data on growth in milligrams of *P. scalare* larvae fed on different diets.

Source	d.f.	S.S.	M.S.S.	F-ratio
Diets	4	874.07	218.51	22.98**
Error	15	142.60	9.50	
Total	19	1016.68		

Comparison of treatment means
Standard error : 1.542

Treatments	T_3	T_1	T_5	T_2	T_4
Mean	143.16	152.32	153.52	158.13	162.99

Under scored means are not significantly different
** significant at 1% level

Table 6b. Analysis of variance of the data on growth in milligrams of *P.scalare* larvae fed on different diets.

Source	d.f	S.S	M.S.S	F-ratio
Diets	4	2201.45	550.36	41.38**
Error	15	199.53	13.30	
Total	19	2400.99		

Comparison of treatment means
Standard error : 1.823

Treatments	T_3	T_5	T_1	T_2	T_4
Means	135.16	136.32	137.35	142.02	163.29

Underscored means are not significantly different
** significant at 1% level

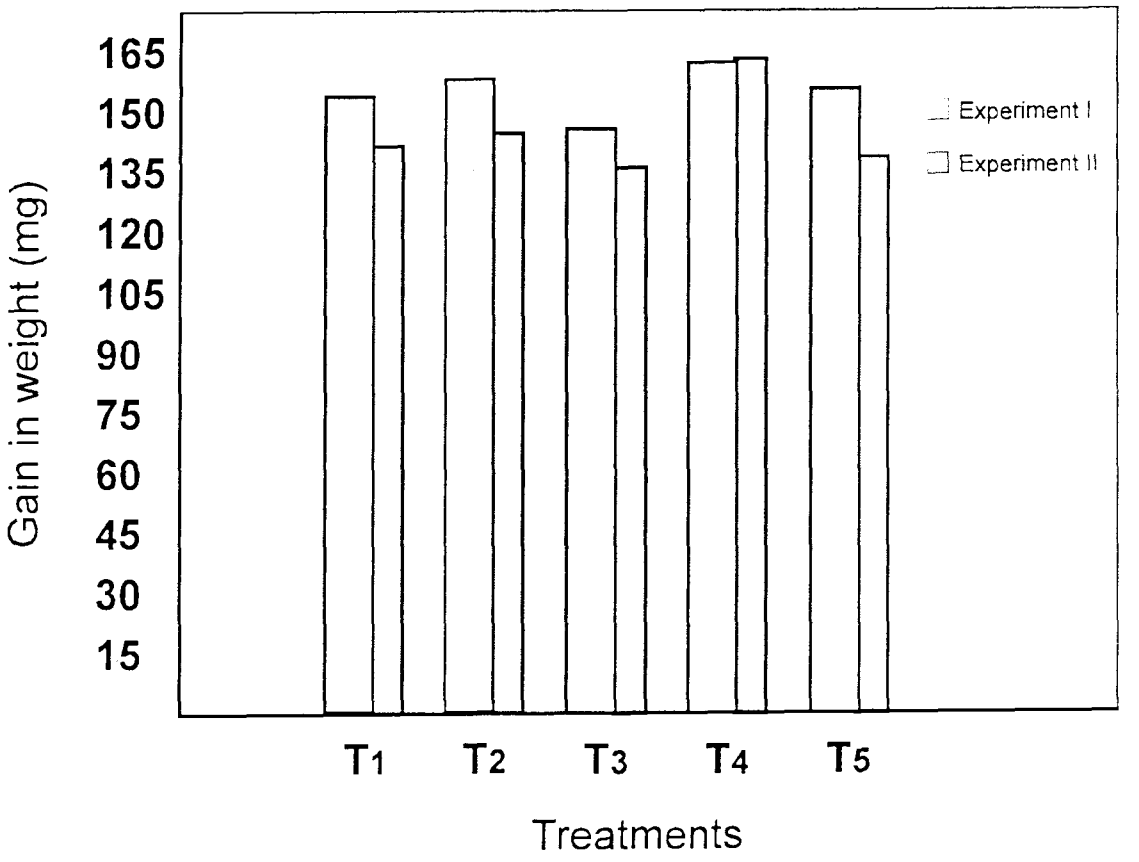

Figure 2. Growth in weight of *P.scalare* larvae fed on different diets.

Table 7a. Growth in standard length 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 (mm)	Percentage length gain	Average percentage length gain
T_1	1	4.5	18.4	13.9	14.2 ± 0.25	308.88	322.89 ± 10.94
	2	4.4	18.5	14.1		320.45	
	3	4.3	18.9	14.6		339.53	
	4	4.4	18.6	14.2		322.72	
T_2	1	4.5	18.9	14.4	14.5 ± 0.11	320.00	322.85 ± 5.60
	2	4.6	19.1	14.5		315.21	
	3	4.5	19.2	14.7		326.66	
	4	4.4	18.9	14.5		329.54	
T_3	1	4.4	17.3	12.9	12.9 ± 0.08	293.18	290.03 ± 4.89
	2	4.6	17.6	13.0		282.60	
	3	4.5	17.5	13.0		288.88	
	4	4.4	17.4	13.0		295.45	
T_4	1	4.4	19.1	14.7	14.8 ± 0.12	334.09	337.56 ± 4.32
	2	4.3	19.1	14.8		344.18	
	3	4.4	19.3	14.9		338.63	
	4	4.5	19.5	15.0		333.33	
T_5	1	4.3	18.5	14.2	14.2 ± 0.21	330.23	316.97 ± 9.96
	2	4.6	18.6	14.0		304.34	
	3	4.5	19.0	14.5		322.22	
	4	4.5	18.5	14.0		311.11	

Table 7b. Growth in standard length 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 (mm)	Percentage length gain	Average percentage length gain
T_1	1	4.3	14.5	10.2	9.87 ± 0.93	237.21	230.95 ± 21.10
	2	4.2	13.6	9.4		223.80	
	3	4.3	15.5	11.2		260.46	
	4	4.3	13.0	8.7		202.32	
T_2	1	4.3	16.0	11.7	11.32 ± 0.89	272.09	261.77 ± 19.60
	2	4.4	16.5	12.1		275.00	
	3	4.3	14.1	9.8		227.90	
	4	4.3	16.0	11.7		272.09	
T_3	1	4.3	13.5	9.2	9.27 ± 0.37	213.95	217.01 ± 9.78
	2	4.2	13.7	9.5		226.19	
	3	4.3	13.0	8.7		202.32	
	4	4.3	14.0	9.7		225.58	
T_4	1	4.3	18.0	13.7	14.57 ± 0.53	318.60	339.01 ± 12.73
	2	4.3	19.0	14.7		341.86	
	3	4.4	19.5	15.1		343.18	
	4	4.2	19.0	14.8		352.38	
T_5	1	4.3	14.4	10.1	9.65 ± 1.10	234.88	223.25 ± 26.05
	2	4.3	12.8	8.5		197.67	
	3	4.3	15.5	11.2		260.46	
	4	4.4	13.2	8.8		200.00	

Table 8a. Analysis of variance of the data on growth in standard length of *P. scalare* larvae fed on different diets.

Source	d.f	S.S.	M.S.S.	F-ratio
Diets	4	8.06	2.01	57.43**
Error	15	0.53	0.035	
Total	19	8.59		

Comparison of treatment means
Standard error: 0.0935

Treatments	T_3	T_2	T_1	T_5	T_4
Means	12.9	14.5	14.2	14.2	14.8

Underscored means are not significantly different
** significant at 1% level

Table 8b. Analysis of variance of the data on growth in standard length of *P. scalare* larvae fed on different diets.

Source	d.f.	S.S	M.S.S	F-ratio
Diets	4	75.73	18.93	21.76**
Error	15	13.0	0.87	
Total	19	88.73		

Comparison of treatment means
Standard error: 0.466

Treatments	T_3	T_5	T_1	T_2	T_4
Means	9.27	9.65	9.87	11.32	14.57

Underscored means are not significantly different
** significant at 1% level

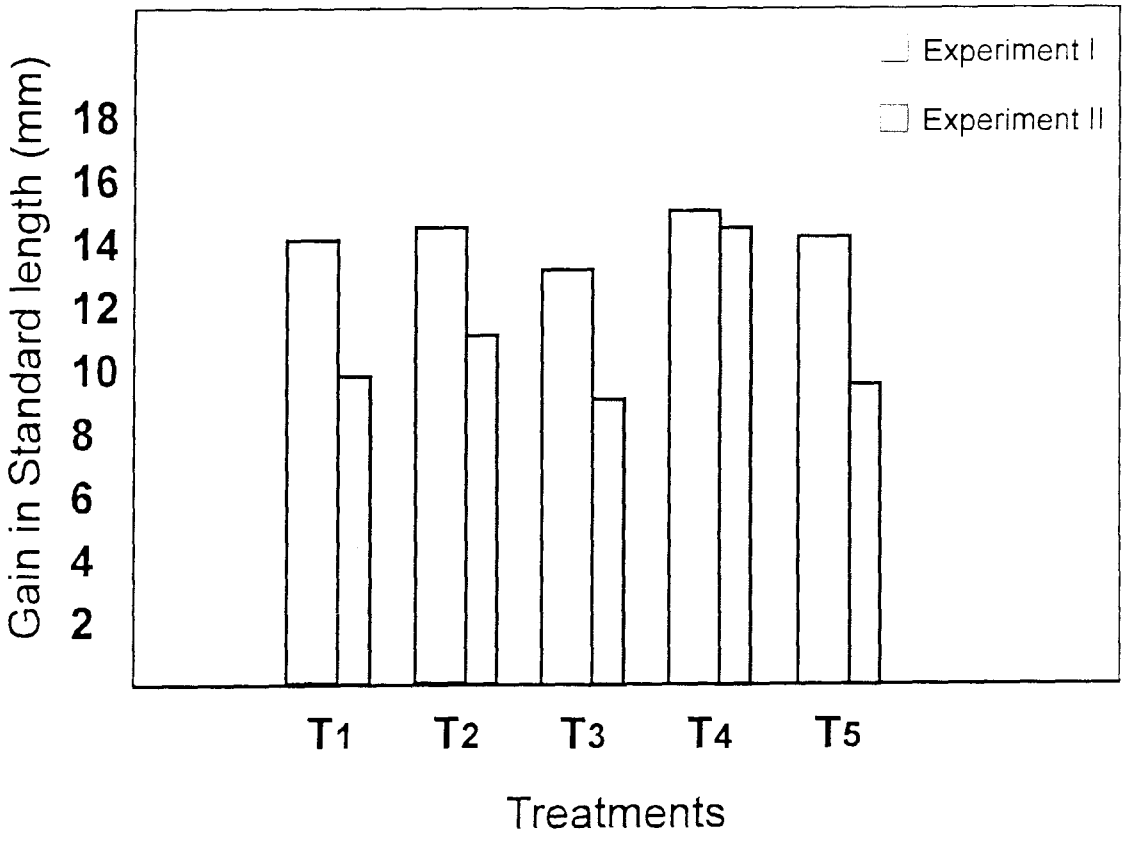

Figure 3. Growth in length of *P.scalare* larvae fed on different diets.

Analysis of variance (Table 8a and 8b) showed that the increase in standard length of larvae was significantly different among the various treatments.

4.2.2. Specific growth rate

The data on SGR are given in table 9a and 9b, respectively, for the first and second experiments. The maximum SGR was recorded for the diet containing *Artemia* nauplii + *Moina micrura* and minimum for artificial diet. The trend was similar in both the experiments. The SGR of larvae fed on different diets are graphically represented in figure 4.

Analysis of variance of the data showed that there is significant difference between the treatments (Table 10a and 10b).

4.2.3. Survival

The percentage survival values of *P. scalare* larvae in various treatments are given in table 11a and 11b, for experiments I and II respectively. In the first experiment, highest average survival was obtained in treatments T_4 and T_2 (95%), while the lowest was recorded in T_3 (75%). In the second experiment also T_4 showed the highest average survival (80%), and T_3 the lowest (65%), but T_2 showed a comparatively lower rate (72.5%). Graphical representation of percentage survival values for different diets are given in figure 5.

Analysis of variance (Table 12a and 12b) of the data showed no significant difference between the treatments.

4.3. Water quality parameters

The range of temperature, pH, dissolved oxygen, hardness and ammonia nitrogen in the experimental tanks during the period of study are given in table 13a and 13b respectively for experiments I and II.

Table 9a. 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 ± S.D
T_1	1	1.30	150.32	15.83	15.93 ± 0.06
	2	1.25	152.31	16.00	
	3	1.31	156.45	15.94	
	4	1.29	155.34	15.96	
T_2	1	1.27	156.25	16.04	16.06 ± 0.04
	2	1.29	160.35	16.07	
	3	1.30	163.75	16.12	
	4	1.29	157.30	16.01	
T_3	1	1.31	140.76	15.59	15.70 ± 0.08
	2	1.32	145.32	15.67	
	3	1.29	147.42	15.79	
	4	1.27	144.31	15.77	
T_4	1	1.27	160.71	16.13	16.15 ± 0.04
	2	1.29	162.35	16.12	
	3	1.30	165.71	16.15	
	4	1.30	168.35	16.21	
T_5	1	1.26	151.32	15.96	15.97 ± 0.05
	2	1.28	155.75	16.00	
	3	1.29	158.42	16.03	
	4	1.30	153.71	15.90	

Table 9b.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 ± S.D
T_1	1	1.27	140.35	15.68	15.61 ± 0.07
	2	1.27	137.22	15.60	
	3	1.29	141.72	15.66	
	4	1.30	135.25	15.48	
T_2	1	1.27	143.75	15.76	15.71 ± 0.06
	2	1.29	146.27	15.77	
	3	1.29	139.42	15.61	
	4	1.29	143.78	15.71	
T_3	1	1.27	136.27	15.58	15.56 ± 0.02
	2	1.29	137.69	15.56	
	3	1.27	133.72	15.52	
	4	1.29	138.11	15.57	
T_4	1	1.30	158.11	16.00	16.17 ± 0.10
	2	1.27	165.12	16.23	
	3	1.29	171.21	16.29	
	4	1.27	163.65	16.19	
T_5	1	1.27	140.21	15.68	15.59 ± 0.11
	2	1.29	132.72	15.45	
	3	1.27	141.86	15.72	
	4	1.29	135.61	15.52	

Table 10a. Analysis of variance of the data on specific growth rate of *P. scalare* larvae fed on different diets.

Source	d.f	S.S	M.S.S	F-ratio
Diets	4	0.446	0.1115	27.87**
Error	15	0.064	0.004	
Total	19	0.51		

Comparison of treatment means
Standard error: 0.0316

Treatments	T_3	T_1	T_5	T_2	T_4
Means	15.70	15.93	15.97	16.06	16.15

Underscored means are not significantly different
** significant at 1% level

Table 10b. Analysis of variance of the data on specific growth rate of *P. scalare* larvae fed on different diets.

Source	d.f	S.S	M.S.S	F-ratio
Diets	4	1.07	0.2675	30.75**
Error	15	0.13	0.0087	
Total	19	1.20		

Comparison of treatment means
Standard error: 0. 0466

Treatments	T_3	T_5	T_1	T_2	T_4
Means	15.56	15.59	15.61	15.71	16.11

Underscored means are not significantly different
** significant at 1% level

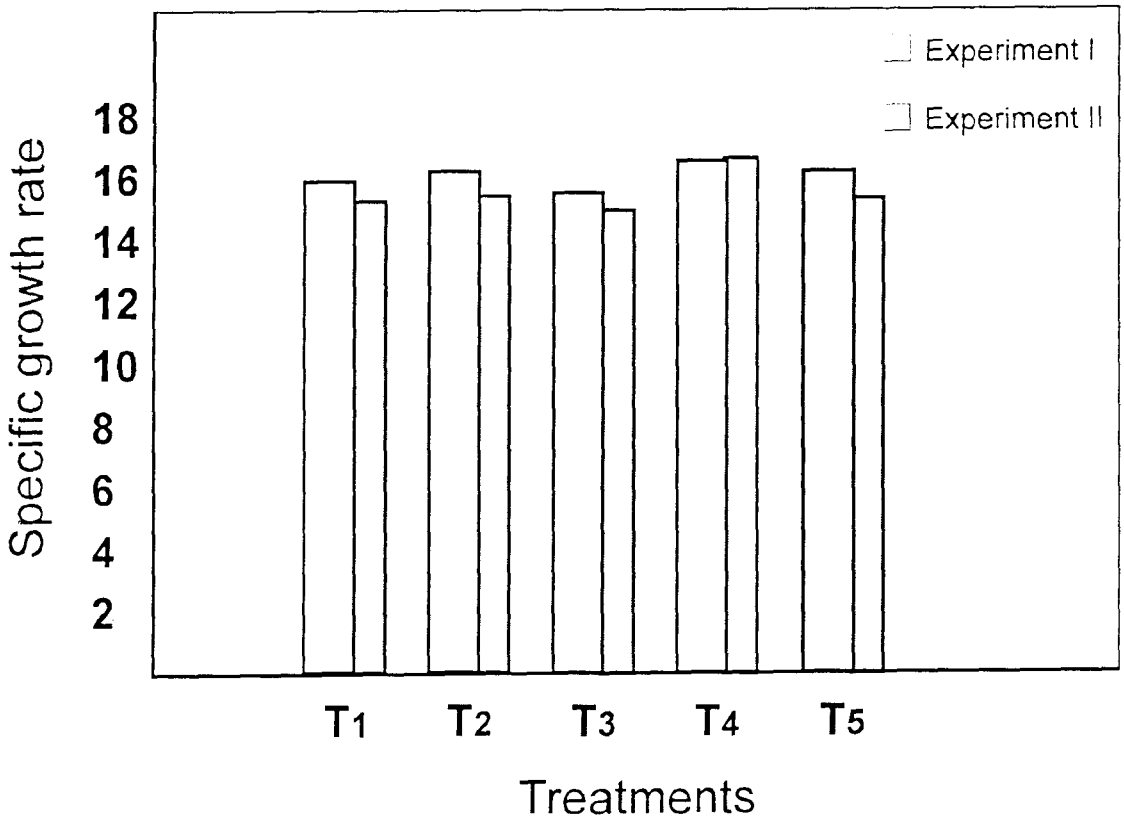

Figure 4. Specific growth rate of *P.scalare* larvae fed on different diets.

Table 11a. Percentage survival of *P.scalare* larvae fed on different diets.

Treatment	Replication	% survival	Mean ± S.D
T_1	1	100	
	2	90	90
	3	90	±
	4	80	7.1
T_2	1	100	
	2	90	95
	3	90	±
	4	100	5.0
T_3	1	70	
	2	80	75
	3	60	±
	4	90	11.2
T_4	1	100	
	2	90	95
	3	90	±
	4	100	5.0
T_5	1	80	
	2	90	87.5
	3	90	±
	4	90	4.3

Table 11b. Percentage survival of *P.scalare* larvae fed on different diets.

Treatment	Replication	% survival	Mean ± S.D.
T_1	1	70	
	2	70	72.5
	3	70	±
	4	80	4.3
T_2	1	80	
	2	70	72.5
	3	70	±
	4	70	4.3
T_3	1	70	
	2	60	65.0
	3	60	±
	4	70	5.0
T_4	1	90	
	2	80	80
	3	70	±
	4	80	7.1
T_5	1	70	
	2	70	67.5
	3	60	±
	4	70	4.3

Table 12a. 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	4	1148.86	287.21	3.194 ^{NS}
Error	15	1348.74	89.92	
Total	19	2497.61		

N.S.: Not significant

Table 12b. Analysis of variance of the data on percentage survival of *P. scalare* larvae fed on different diets. (Data subjected to Arc Sine transformation)

Source	d.f.	S.S	M.S.S	F-ratio
Diets	4	530	132.5	3.78 ^{NS}
Error	15	525	35	
Total	19	1055		

N.S.: Not significant

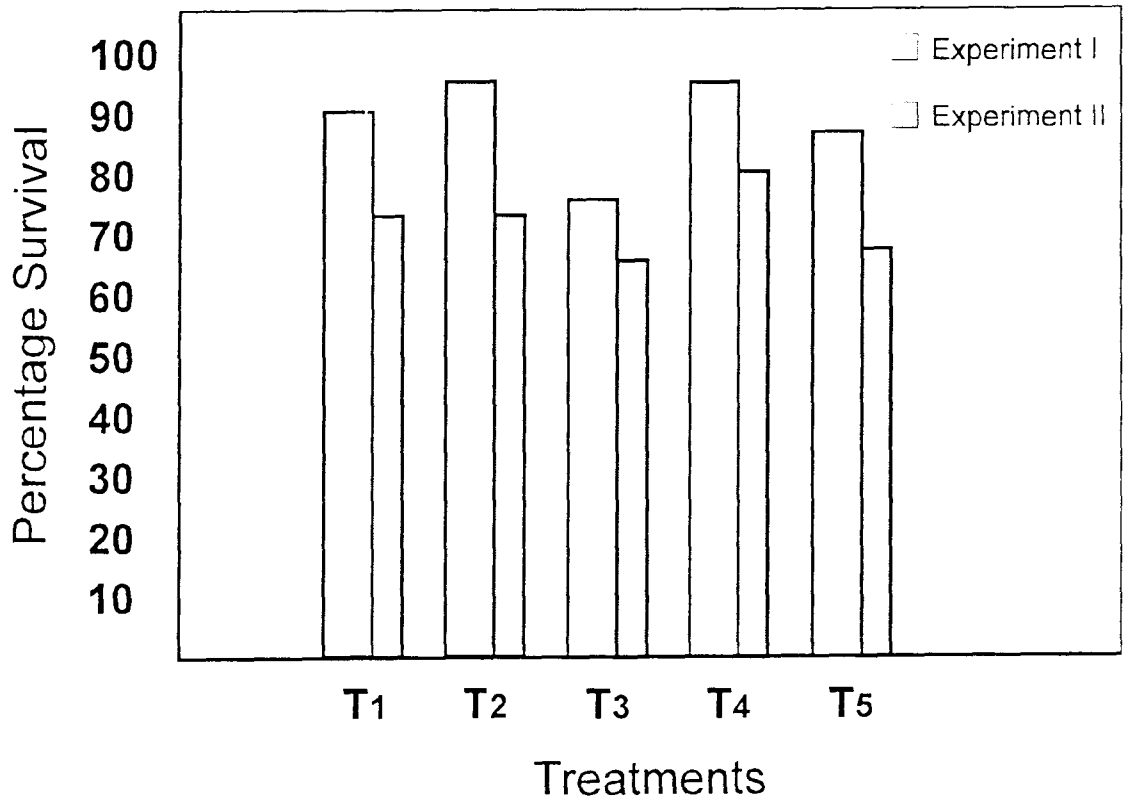

Figure 5. Percentage survival of *P.scalare* larvae fed on different diets.

4.3.1. Temperature

The maximum and minimum temperatures recorded during the study period were 30^0 C and 25^0 C, respectively. The weekly mean temperature values ranged from 26.5^0 to 27.5^0 C during the first experiment, while it ranged from 26.5^0 to 27.7^0 C during the second experiment.

4.3.2. pH

The weekly mean pH ranged between 6.0 and 7.0 during both the I and II experiments.

4.3.3. Dissolved oxygen

A minimum of 3.5 ppm and a maximum of 4.6 ppm were obtained during the first experiment, with weekly mean values ranging from 4.08 to 4.22 ppm.

During the second experiment, a minimum of 3.5 ppm and a maximum of 5.4 ppm were observed. Weekly mean values ranged from 4.10 to 4.94 ppm.

4.3.4. Hardness

Weekly mean values of hardness ranged from 117.4 to 132.5 ppm and 120.0 to 142.3 ppm during the first and second experiments respectively. A maximum of 150 ppm and a minimum of 105 ppm were observed during the experimental period.

4.3.5. Ammonia nitrogen

A minimum of 0.045 ppm and a maximum of 0.13 ppm were observed during the first experiment, with the weekly mean values fluctuating between 0.071 and 0.094 ppm.

A minimum of 0.03 ppm and a maximum of 0.09 ppm were observed during the second experiment. Weekly mean values during the period ranged from 0.05 to 0.076 ppm.

Table13a. Water quality parameters during experimental period.

Parameter	Weeks	1	2	3	4
Temperature (°C)	Mean Range ± S.D	26.71 25.5-28.5 0.9	26.50 25.5-27.5 0.7	27.35 26.0-28.5 0.8	27.50 26.0-28.5 0.7
pH	Mean Range ±S.D.	6.0 5.0-6.5 0.5	6.5 6.0-7.0 0.3	7.0 6.5-7.5 0.4	7.0 7.0 -
Dissolved oxygen (ppm)	Mean Range ±S.D	4.18 4.0-4.5 0.16	4.10 3.5-4.6 0.32	4.22 4.0-4.5 0.13	4.08 3.8-4.3 0.15
Hardness (ppm)	Mean Range ±S.D	122.5 120-128 2.71	117.4 115-120 1.76	127.7 125-130 3.20	132.5 128-138 2.40
NH_3-N (ppm)	Mean Range ±S.D	0.076 0.074-0.085 0.05	0.077 0.045-0.112 0.02	0.094 0.068-0.13 0.02	0.071 0.05-0.09 0.01

Table 13b. Water quality parameters during the experimental period.

Parameter	Weeks	1	2	3	4
Temperature (°C)	Mean Range ±S.D	26.71 25.0-28.0 1.19	27.71 25.5-30.0 1.76	26.78 25.0-28.0 1.27	26.57 25.5-28.0 0.86
pH	Mean Range ±S.D	6.5 6.0-7.0 0.3	7.0 6.5-7.5 0.3	6.0 5.5-7.0 0.8	6.0 5.5-6.5 0.4
Dissolved oxygen (ppm)	Mean Range ±S.D	4.94 4.8-5.2 0.14	4.56 4.2-5.4 0.27	4.10 3.5-4.5 0.29	4.52 4.2-5.0 0.22
Hardness (ppm)	Mean Range ±S.D	130.4 125-135 3.2	142.3 135-150 6.0	120.0 105-130 7.6	123.0 115.5-132 5.9
NH_3-N (ppm)	Mean Range ±S.D	0.05 0.03-0.07 0.01	0.06 0.04-0.07 0.01	0.076 0.07-0.09 0.007	0.075 0.07-0.08 0.006

4.4. O:N Ratio

The data on oxygen consumption, ammonia nitrogen excretion and O:N ratio of *P. scalare* larvae fed on different diets are given in table 14a and 14b for experiments I and II respectively.

The oxygen consumption values of the larvae fed on different diets showed a similar trend in both the experiments, with maximum value for treatment T_4 and minimum for T_3. The values were significantly different between treatments in both the experiments (Table 15a and 15b).

Ammonia excretion rates of the larvae in the different treatments were not similar in the two experiments. In experiment I, maximum value was recorded for T_5, followed by T_3, T_1, T_2 and T_4. Analysis of variance(Table 15a) showed a significant difference between the treatments. However, in experiment II maximum value was observed in T_5 followed by T_1, T_3, T_4 and T_2. Analysis of variance (Table 15b) showed no significant difference between the treatments.

O:N ratios showed significant difference between the treatments (Table 15a and 15b) but the trend was not similar in the two experiments. The highest ratio was in treatment T_4 and the lowest in T_3 in the first experiment. In the second experiment the highest ratio was recorded for treatment T_4 itself. However, the lowest ratio was in T_5. Figure 6 shows the graphical representation of the O:N ratios.

Table 14a. Oxygen consumption rates, ammonia excretion rates and O:N ratios of *P.scalare* larvae fed on different diets.

		Treatments (Mean ± S.D)				
Week	Parameter	T_1	T_2	T_3	T_4	T_5
I	Length(mm)	9.0 ± 0.1	9.1 ± 0.2	9.0 ± 0.1	9.7 ± 0.3	9.1 ± 0.1
	Weight(mg)	42.31±1.9	48.21±2.0	38.91±1.9	51.01±1.8	43.10±2.1
	Oxygen consumption rate(mgO_2/hr)	0.033± 0.001	0.038± 0.018	0.020± 0.0	0.058± 0.002	0.020± 0.0
	Ammonia excretion rate(mg NH_3-N/hr)	0.0039± 0.0002	0.0019± 0.0	0.0089± 0.0006	0.001± 0.0	0.0097± 0.0012
	O:N ratio	8.32±0.33	19.86±1.68	2.30±0.14	57.50±1.76	2.13±0.27
II	Length(mm)	13.4±0.1	14.2±0.2	13.2±0.1	15.1±0.1	13.1±0.2
	Weight(mg)	92.15±1.9	97.21±2.0	89.10±1.7	103.21±2.1	101.3±1.7
	Oxygen consumption rate(mgO_2/hr)	0.045±0.0	0.047±0.0	0.023±0.002	0.070±0.0	0.033±0.002
	Ammonia excretion rate(mgNH_3-N/hr)	0.0044± 0.0003	0.0017± 0.0001	0.0090± 0.0009	0.0014± 0.0001	0.0013± 0.0016
	O:N ratio	10.41±0.59	27.17±0.54	2.51±0.05	51.93±1.36	2.58±0.19
III	Length(mm)	15.9±0.1	16.5±0.1	15.6±0.2	17.5±0.2	16.8±0.2
	weight(mg)	128.90±2.1	132.50±1.8	126.57±2.7	137.60±2.2	129.40±2.3
	Oxygen consumption rate(mgO_2/hr)	0.055± 0.001	0.063± 0.002	0.035± 0.001	0.084± 0.001	0.046± 0.001
	Ammonia excretion rate(mgNH_3-N/hr)	0.0078± 0.0007	0.0021± 0.0	0.0140± 0.0001	0.0020± 0.0001	0.0175± 0.0001
	O:N ratio	7.13±0.42	29.76±0.84	2.49±0.13	41.95±1.04	2.65±0.05
IV	Length(mm)	18.6±0.3	19.0±0.1	17.4±0.5	19.3±0.1	18.7±0.2
	Weight(mg)	153.60±2.4	159.41±2.9	144.45±2.4	162.90±2.9	154.80±2.6
	Oxygen consumption rate(mgO_2/hr)	0.057± 0.0	0.073± 0.002	0.040± 0.0	0.087± 0.0	0.045± 0.0
	Ammonia excretion rate(mgNH_3/hr)	0.0099± 0.0004	0.0023± 0.0001	0.0175± 0.0005	0.0032± 0.0002	0.0169± 0.0006
	O:N ratio	5.81±0.22	32.26±1.29	2.29±0.11	27.96±1.56	2.67±0.08
Mean	Oxygen consumption rate(mgO_2/hr)	0.048± 0.005	0.055± 0.007	0.029± 0.004	0.075± 0.006	0.036± 0.005
	Ammonia excretion rate(mg NH_3/hr)	0.0065± 0.0012	0.0020± 0.0001	0.0124± 0.0018	0.0019± 0.0004	0.0143± 0.0016
	O:N ratio	7.92±0.85	27.26±2.32	2.39±0.05	44.84±5.61	2.51±0.11

Table14b.Oxygen consumption rates, ammonia excretion rates and O:N ratios of *P.scalare* larvae fed on different diets.

		Treatments(Mean± S.D)				
Week	Parameter	T_1	T_2	T_3	T_4	T_5
I	Length(mm)	9.5±0.1	9.7±0.3	9.1±0.2	11.5±0.2	10.0±0.3
	Weight(mg)	30.57±2.5	40.67±0.7	32.71±2.1	65.12±1.9	42.10±2.5
	Oxygen consumption rate(mgO_2/hr)	0.045± 0.0	0.051± 0.001	0.035± 0.004	0.070± 0.004	0.048± 0.002
	Ammonia excretion rate($mgNH_3$-N/hr)	0.0055± 0.0004	0.0027± 0.0002	0.0043± 0.0005	0.0030± 0.0007	0.0065± 0.0011
	O:N ratio	8.25±0.53	19.13±1.78	8.72±1.92	25.63±4.86	7.82±1.52
II	Length(mm)	12.1±0.2	12.2±0.5	11.2±0.5	13.3±0.4	10.9±0.2
	Weight(mg)	66.24±2.3	92.71±3.4	62.81±3.0	112.21±1.5	85.2±2.3
	Oxygen consumption rates(mgO_2/hr)	0.067± 0.001	0.079± 0.003	0.048± 0.002	0.078± 0.005	0.055± 0.004
	Ammonia excretion rate($mgNH_3$-N/hr)	0.0068± 0.0002	0.0033± 0.0005	0.0050± 0.0007	0.0030± 0.0004	0.0075± 0.0004
	O:N ratio	9.88±0.41	25.13±4.51	9.79±1.03	26.34±1.46	7.38±0.79
III	Length(mm)	13.5±0.5	13.7±0.6	11.9±0.3	15.7±0.3	11.7±0.2
	Weight(mg)	114.68±1.9	121.67±2.7	109.7±2.1	147.3±2.1	105.31±1.9
	Oxygen consumption rate(mgO_2/hr)	0.077± 0.001	0.086± 0.001	0.078± 0.002	0.090± 0.0	0.075± 0.004
	Ammonia excretion rate($mgNH_3$-N/hr)	0.0093± 0.0005	0.0044± 0.0006	0.0060± 0.0007	0.0035± 0.0004	0.0089± 0.0001
	O:N ratio	8.35±0.60	20.28±2.78	13.22±1.26	26.25±2.65	8.42±0.33
IV	Length(mm)	14.2±0.9	15.6±0.9	13.6±0.4	18.8±0.5	13.9±1.1
	Weight(mg)	137.35±2.5	142.02±2.5	135.16±1.7	163.29±4.6	136.32±3.6
	Oxygen consumption rate(mgO_2/hr)	0.088± 0.002	0.110± 0.007	0.093± 0.001	0.135± 0.011	0.095± 0.004
	Ammonia excretion rate($mgNH_3$-N/hr)	0.0115± 0.0004	0.0038± 0.0001	0.0070± 0.0	0.0060± 0.0002	0.0130± 0.0014
	O:N ratio	7.63±0.38	29.15±2.93	13.3±0.21	22.71±0.91	7.55±1.09
Mean	Oxygen consumption rate(mgO_2/hr)	0.069± 0.008	0.082± 0.011	0.064± 0.012	0.093± 0.013	0.068± 0.009
	Ammonia excretion rate($mgNH_3$-N/hr)	0.0083± 0.0012	0.0036± 0.0003	0.0056± 0.0005	0.0039± 0.0006	0.0089± 0.0012
	O:N ratio	8.53±0.41	23.42±2.0	11.26±1.02	25.23±0.74	7.79±0.20

Table15a. Analysis of variance of the data on oxygen consumption rates, ammonia excretion rates and O:N ratios of *P.scalare* larvae fed on different diets.

Parameter	Source	d.f.	S.S	M.S.S	F-ratio
Oxygen consumption	Diets	4	0.01	0.0025	250**
	Weeks	3	0.0045	0.0015	150**
	Interaction	12	0.0002	0.000016	1.6
	Between replicates	19	0.0148		
	Error	20	0.0002	0.00001	
	Total	39	0.015		
Ammonia [b] excretion	Diets	4	105320.9	26330.23	23.47**
	Weeks	3	15869.48	5289.83	4.72**
	Interaction	12	8194.9	682.9	0.6
	Between replicates	19	129385.3		
	Error	20	22429.2	1121.5	
	Total	39	151814.9		
O:N ratio	Diets	4	11086.3	2771.56	102.84**
	Weeks	3	126.24	42.08	1.56
	Interaction	12	323.36	26.95	9.10
	Between replicates	19	12289.6		
	Error	20	59.32	2.96	
	Total	39	12348.91		

**Significant at 1% level

b : The NH_3 excretion values are multiplied with a common factor 10000 for the convenience of statistical analysis.

Comparison of treatment means

Oxygen consumption

Standard error : 0.0016

Treatements	T_3	T_5	T_1	T_2	T_4
Means	0.029	0.036	0.048	0.055	0.075

Ammonia excretion

Standard error : 10.80

Treatments	T_4	T_2	T_1	T_3	T_5
Means	0.002	0.016	0.007	0.012	0.014

O:N ratio

Standard error : 0.86

Treatments	T_3	T_5	T_1	T_2	T_4
Means	2.39	2.50	7.92	27.26	44.83

Underscored means are not significantly different

Table 15b. Analysis of variance of the data on oxygen consumption rates, ammonia excretion rates and O:N ratios of *P.scalare* larvae fed on diferent diets.

Parameter	Source	d.f	S.S	M.S.S	F-ratio
Oxygen consumption	Diets	4	0.0048	0.0012	21.81**
	Weeks	3	0.0162	0.0054	98.18**
	Interaction	12	0.001	0.000083	1.5
	Between replicates	19	0.0224		
	Error	20	0.0011	0.000055	
	Total	39	0.0235		
Ammonia [b] excretion	Diets	4	19690.9	4922.73	2.01
	Weeks	3	8652.68	2884.23	1.18
	Interaction	12	2934.7	244.56	0.1
	Between replicates	19	31278.28		
	Error	20	48775.5	2438.76	
	Total	39	80053.77		
O:N ratio	Diets	4	2265.72	566.43	33.2**
	Weeks	3	26.77	8.92	0.5
	Interaction	12	158.84	0.77	
	Between replicates	19	2451.33		
	Error	20	340.5	17.0	
	Total	39	2791.83		

**Significant at 1% level

b : The ammonia excretion values are multiplied with a common factor 10000 for the convenience of statistical analysis.

Comparison of treatment means

Oxygen consumption

Standard error : 0.004

Treatments	T_3	T_5	T_1	T_2	T_4
Means	0.063	0.068	0.068	0.081	0.093

Ammonia excretion rates

Standard error : 24.69

Treatments	T_1	T_2	T_3	T_4	T_5
Means	0.008	0.003	0.005	0.004	0.008

O:N ratio

Standard error : 2.06

Treatments	T_5	T_1	T_3	T_2	T_4
Means	7.78	8.53	11.25	23.42	25.23

Underscored means are not significantly different

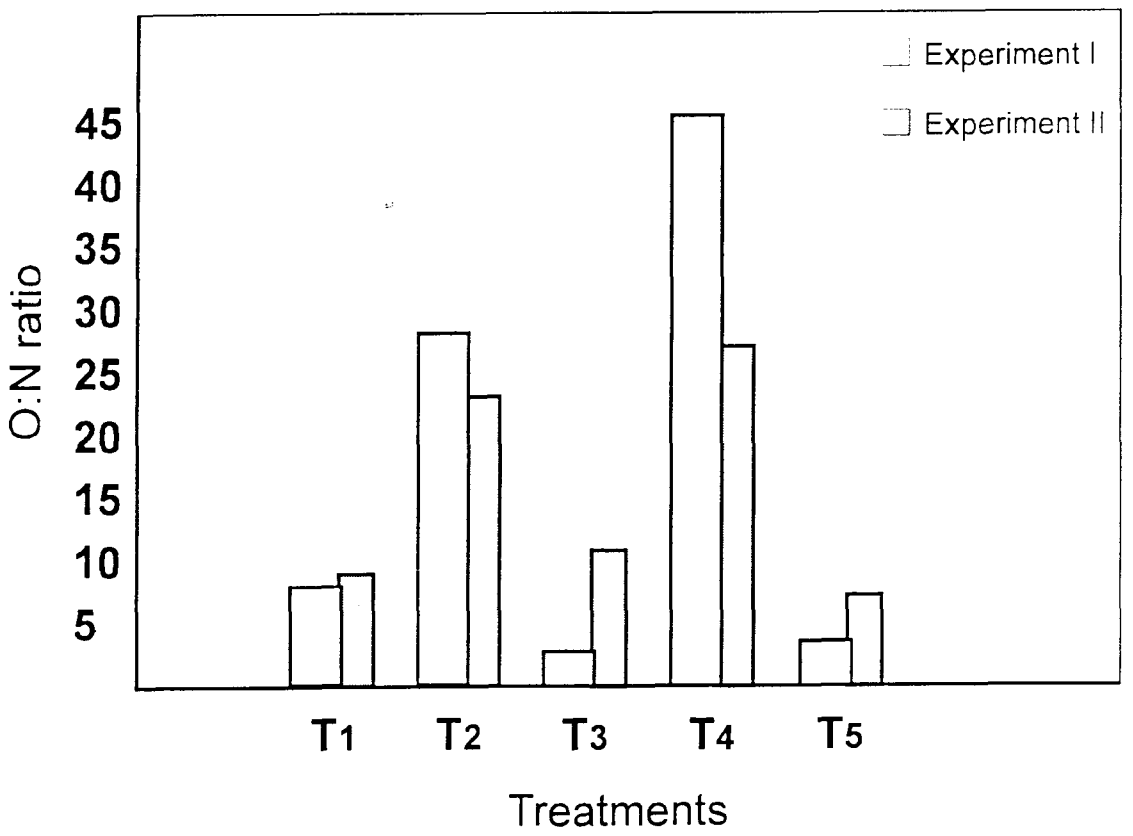

Figure 6. O:N ratios of *P.scalare* larvae fed on different diets.

DISCUSSION

5. DISCUSSION

5.1. Protein requirement in Angel fish,*P.scalare*

The requirement of 40-50% protein in the diet of Angel fish juveniles has been suggested by Degani (1993). The ingredient composition of the diet was fish meal, wheat meal, milk powder and vitamins. The diet prepared by Boonrayatpalin and Lovell (1977) for Angel fish using shrimp meal, fish meal, soya bean meal, rice polish, wheat bran, fish oil and vitamin and mineral mixture contained 40% protein. Degani and Yehuda (1996) formulated two artificial diets for Angel fish with 78% and 57% crude protein contents using two ingredient combinations. (1) Turkey heart, poultry liver, vegetarian mixture and vitamins; and (2) Fish meal, wheat meal, soya bean meal, yolk meal, milk meal, guar and vitamins, respectively. Mathew and Sherief (1996) reported that clam meal is an ideal animal protein source for Angel fish. Degani (1990) used egg yolk, yeast and shrimp meal as the sole diets for Blue gourami (*Trichogaster trichopterus*) larvae. Abi-ayad and Kestemont (1994) used commercial diet with 51% protein content for start feeding of Gold fish, *Carassius auratus* larvae. Fernando *et al* (1991) used wheat bran, oats, fish meal, ground dried shrimp and skimmed milk, as ingredients for the diet prepared for larval livebearers with the protein content of the whole feed ranging from 15 to 34%.

Proximate analysis of the test diets used in the present study revealed that the artificial feed used contained 29.7% protein. The ingredients included were clam meat, wheat flour, egg yolk, yeast, oats and vitamin and mineral mixture. All these ingredients are used widely in ornamental fish feed formulation.

Studies on the nutritional requirements of larval fmfishes are scanty due to the low survival and growth rates generally obtained when purified diets are used. Consequently, the artificial feeds for small larvae are generally formulated empirically using different raw ingredients (Lavens *et al.*, 1994). The protein requirements vary with the ingredient combinations in the diet. Apart from this, the digestibility of the ingredients is more important than the higher protein contents in the diets (Degani, 1993). The comparatively good performance of the artificial diet in the present study might be due to the better digestibility of the ingredients used.

5.2. Water quality parameters

5.2.1. Temperature

The effect of temperature in larval rearing of ornamental fishes has been reported by many workers. Degani (1991) reported that temperature is a very important parameter for the growth of larvae and juveniles of Blue gourami, *Trichogaster trichopterus*. The temperatures of 25° or 27° C were found better than 23° C for the growth of larvae and juveniles. Gold fish larvae reared at 28° C at maximum daily food ration were four times larger than those reared at 20° C, while the survival rate was lower at 28° C than at 20 °C and 24° C (Kestemont, 1995). Degani (1993) maintained a temperature of 27° C for breeding and larval rearing of Angel fish, *P. scalare*. The mean weekly range of temperature observed during the present experimental period was 25.5 to 30.0° C. The temperature fluctuations were gradual and in a limited range throughout the study period. Generally no optimum temperatures for growth and survival are reported for the ornamental fishes in the tropical waters due to the prevailing high temperatures.

5.2.2. pH

Axelrod and Vorderwinkler (1979) reported that a slightly alkaline water was found to be suitable for hatching and larval rearing of angel fish. Degani (1990) reported no significant effect of pH on dietary utilization, growth and survival of Blue gourami, *Trichogaster trichopterus* larvae. During the present study the weekly range of pH values ranged from 5.0 to 7.5 with no marked effect on growth and survival, as observed by Degani (1990).

5.2.3. Dissolved oxygen

In the rearing of Goldfish, *Carassius auratus* larvae, Kestemont (1995) indicated that by maintaining dissolved oxygen at about saturation the conditions seemed to be ideal for the rearing of small fish larvae, both concerning the rearing and feeding facilities and the mixed diet provided. These results confirm those obtained by Charlon and Bergot (1984) and Kestemont and Stalmans (1992) in their investigations with common carp (*Cyprinus carpio*) and European minnow larvae (*Phoxinus phoxinus*) respectively. The weekly range of dis-

solved oxygen content in the experimental tanks was 3.5 to 5.3 ppm during study period. Daily water exchange and cleaning of the tank bottom mitigated the low dissolved oxygen content in the water. Degani (1990) found no significant effect of dissolved oxygen on utilization of different diets.

Bergot (1986) reported that artificial feeds change the relation which exists between the animal and its environment. The deterioration of water quality and tank cleanness due to the use of formulated feeds may also affect the survival and growth in the early stages. But in the present study the larvae fed artificial diet performed well with no significant difference in survival and growth when compared with that of live foods and mixed diets. These results show that artificial feed, if judiciously used will not seriously alter the balance between animal and its environment.

5.2.4. Hardness

During the present study mean weekly range of hardness recorded was from 115 to 150 ppm. Degani (1990) reported a hardness of 189-195 ppm in the larval rearing water of *Trichogaster trichopterus*. Poor survival of fry was reported in low hardness (less than 20 ppm) waters by Mitchell and Collins, 1997.

5.2.5. Ammonia nitrogen

Degani(1993) recorded low concentration of ammonia while rearing *Trichogaster trichopterus* larvae. But the nitrite and nitrate concentrations were very high with inverse relationship causing low growth at high concentrations. He reported that some of the artificial food sink to the bottom and this leads to the development of large number of bacteria. It is possible that, when eating from the bottom, the larvae encounter a high concentration of nitrite,which leads to low survival and growth rates. In the present study also ammonia concentration was very low (0.03 to 0.09 ppm). The artificial diet didn't make much difference in growth and survival because left over feed was removed before every subsequent feeding thus keeping a very low bacterial load in the water.

5.3. Growth

In the present study *P. scalare* larvae recorded the highest growth in terms of gain in length and weight when fed with live foods, followed by mixed diet and the lowest for the artificial diet. In both the experiments the growth of larvae fed with *Artemia* nauplii + *moina micrura* were significantly different from all the other treatments. In the first experiment no significant difference was found between the treatments T_1 (*Artemia* nauplii), T_2 (*Moina micrura*) and T_5 (*Artemia* nauplii + Artificial diet). But treatment T_3 (Artificial diet) which showed the lowest growth was significantly different from all the other treatments. However, in the second experiment treatment T_3 showed comparatively better growth, with no significant difference with T_1, T_2 and T_5 treatments.

These results are in consonance with that obtained by Abi-ayad and Kestemont (1994) in Gold fish (*Carassius auratus*) ; Degani (1990 and 1993) in Blue gourami (*Trichogaster trichopterus*) and Angel fish (*Pterophyllum scalare*) ; Fermin and Recometa (1988) in Bighead carp (*Aristichthys nobilis*) fry ; Wolnicki and Gorny (1995 a,c) in ide (*Leuciscus idus*) and tench (*Tinca tinca*) ; Kestemont and Stalmans (1992) in minnow larvae ; Csengeri *et al* (1995) in cyprinid larvae and Qin (1997) in *Channa striatus* fry. In all these cases maximum growth was obtained in larvae fed exclusively on live foods, followed by mixed diet of live food and artificial diet.

In Gold fish (*Carassius auratus*) larvae improved growth rate was obtained by feeding mixed diet (live *Artemia* nauplii and dry food) instead of dry food alone (Abi-ayad and Kestemont, 1994). However, the ratio of live food/ dry feed may be different in dispensed and ingested diets, particularly if small larvae selected *Artemia* nauplii and neglected dry feed. Better growth was obtained with mixed diet of *Artemia* nauplii and artificial diet in comparison to that of artificial diet alone in the present study also. Degani (1993) reported that the addition of small quantity of live food to the artificial diet significantly raised the growth rate in juvenile *P.scalare*. Santiago and Reyes (1989) showed that the combination of *Brachionus* sp. and artificial diet was the best feeding regime in enhancing the growth of Bighead carp fry.

Several hypotheses have been proposed to explain the improvement in the assimilation of dry diets by the addition of live foods regardless of ingestion rates or larval age. Digestive enzymes of live foods may assist in larval digestion, as has been proposed by Dabrowski

(1984) ; Lauff and Hofer (1984) and Kolkovski *et al* (1993). Abi-ayad and Kestemont (1994) found higher tryptic activity in Gold fish (*C. auratus*) larvae fed with natural and mixed diets than those fed on artificial diet alone. Due to their low tryptic activity, the larvae fed on artificial diet alone would have utilized less efficiently the protein contained in the feed and consequently grew slowly.

The presence of live food in general improves the performance of artificial diet in larvae suggesting that a nonspecific factor, visual or olfactory stimuli, could be responsible for this, probably associated with increased peristalsis which triggers the rest of the larval digestive process.

In the second experiment, the performance of artificial diet (135.16 mg) was at par with the mixed diet of *Artemia* nauplii and artificial diet (136.32 mg) and live foods (137.35 mg (T1), 142.02 mg (T2)), as was observed in larval 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); sturgeon (*Acipensor baeri*) larvae (Dabrowski *et al.*, 1985); catfish (*Clarius gariepinus*) (Uys and Hecht, 1985) and razorback sucker larvae (*Xyrauchen texanus*) (Tyus and Severson, 1990). This shows that live foods can be effectively replaced by formulated diets in larval rearing of *P.scalare*, without much difference in growth.

In the present study the growth obtained by feeding artificial feed alone was 91-93% of that obtained with live foods. Similar results were obtained in *Cyprinus carpio* (Charlon and Bergot, 1984; Charlon *et al.*, 1986), *Coregonus laveratus* (Champigneuille, 1988) and *Micropterus dolomieni* (Jones *et al.*, 1993) using microparticulate diets in larval first feeding. The results obtained in the present study are significant in that complete replacement of live feeds with formulated diets is possible for the larval rearing of angel fish.

5.4. Specific growth rate

The mean SGR obtained for T_4 and T_2, T_5 and T_2 and T_1 and T_5 in the first experiment did not show significant difference among them, but all these treatments were significantly different from treatment T_3. However, the SGR was much higher in T_4, while it did not vary

significantly among the other treatments. Similarly Kestemont *et al* (1989) and Abi-ayad and Kestemont (1994) also didn't find any difference in SGR of Gold fish (*C. auratus*) larvae fed on artificial diet and mixed diet. The latter workers observed that the alimentary tract of the gold fish larvae at that age was sufficiently developed to digest and assimilate artificial feed. In the present study also, the improvement in the SGR of treatment T_3 might have been due to the development of the digestive tract.

5.5. Survival

The overall survival rate of the larvae varied in the two experiments, with comparatively lower value in the second experiment. This is probably because the hatchlings used for the second experiment were from the first breeding of a pair. It is observed through experience that survival rates are generally low in the first breeding of a pair(Mathew., Pers. Comm.). In both the experiments the highest survival rates were obtained for treatment T_4 and the lowest for T_3(artificial diet). However the rates were not significantly different between treatments. Similar observations were made by Abi-ayad and Kestemont (1994) for Gold fish (*C.auratus*)larvae; Degani (1990 and 1993) for *Trichogaster trichopterus* and Wolnicki and Gorny (1995b) in larval barbel (*Barbus barbus*).

The survival rates (65 and 75%) obtained for the artificial diet in the present study, which did not significantly differ from that of live foods and mixed diets, suggests that artificial feed also can be considered as an alternative food for the rearing of angel fish larvae. These results can be considered significant because generally survival rates are very low (0-40%) when artificial feeds are used for larval rearing (Jones *et al* ., 1993). Only known exceptions are in the case of *Cyprinus carpio* (Charlon and Bergot, 1984; Charlon *et al*., 1986 and Slaminska and Pryzybyl, 1986) with 90% survival and in *Coregonus laveratus* (Champigneuille, 1988) with 85-95 % survival using microencapsulated diets.

5.6. O:N Ratio

The increase in oxygen consumption rate is primarily due to the proportion of digestible protein in the diet. The higher the proportion of digestible protein in the diet, the greater was the relative magnitude of the effect (Jobling and Davies, 1980). Ingestion of carbohydrates

and lipids will elicit only a less oxygen consumption when compared to protein (Lovell, 1989). A high rate of protein metabolism relative to carbohydrate and lipid metabolism results in increased oxygen consumption, which is generally indicative of the nutritional status of the animal (Bayne *et al.*, 1985). The oxygen consumption rate of the larvae in the different treatments in the present study were in the order of *Artemia* nauplii + *Moina micrura* > *Moina micrura* > *Artemia* nauplii > *Artemia* nauplii + Artificial diet > Artificial diet. The low oxygen consumption rates of the larvae fed artificial diet probably may be due to less digestibility of dry diets compared to live foods as reported by Dabrowski and Kaushik (1984) for coregonid larvae fed dry diets. Lauff and Hofer (1984) pointed out the inefficiencies in digestive system of the larvae for the low efficiency of dry diets.

The rate of ammonia excretion is dependent on the amount and quality of protein fed (Lovell, 1989). The percentage of nitrogen excretion by coregonid larvae fedon *Artemia* nauplii was only 12.3 of the total nitrogen, while it ranged from 14.5 to 21.9 in the case of fish fed dry diets (Dabrowski and Kaushik, 1984). In the present study, difference in ammonia excretion rates of larvae fed different diets was not conspicuous. This shows that the protein quality of artificial diet roughly matches with that of the live foods thus maintaining a good nutritional state of the animal.

The apparent net protein utilization (NPU), i.e., body protein increment per protein consumed, was 2-3 times higher in larvae fed *Artemia* than those of dry diets. But there was no relation between the protein utilization and the ratios of nitrogen excreted/nitrogen consumed as far as dry diet utilization is concerned (Dabrowski and Kaushik, 1984). The low NPU by the larvae fed dry diets could also suggest that dietary protein digestion is low in comparison with live food.

The higher O:N ratios for the larvae fed live foods in the present study indicate a predominance of lipid and / or carbohydrate catabolism over protein degradation. However, lower ratios observed for the mixed diet of *Artemia* nauplii + artificial diet and artificial diet alone show increased protein metabolism which indicates poor utilization of protein for growth. In contrast, in the second experiment the ratio for artificial diet was higher than for *artemia* nauplii and *Artemia* nauplii + artificial diet. The improved O:N ratio for the artificial diet fed larvae indicates efficient utilisation of the protein, which is also reflected in having not much

difference in growth of larvae with that of larvae fed on live foods.

Thus, O:N ratio which indicates the nutritional status of the animal in utilising various nutrient sources helps in choosing the best available foods for rearing of fishes. In the present study, larvae fed on live foods recorded highest ratios indicating increased NPU, as is reflected in the maximum growth of the larvae, whereas, larvae fed on artificial diet and mixed diet of live food and artificial diet showed low ratios indicating less growth when compared to that of live food. Even though the O:N ratios of larvae fed on artificial diet are low, the growth and survival rates of these larvae were comparable with that of the larvae fed on mixed diets and live foods.Thus, by subsituting artificial feed for live foods in early or late early stages of Angel fish, a compromise between the loss in growth and cost involved in providing live foods can be achieved.

SUMMARY

6. SUMMARY

The present study was aimed at evaluating the effect of different diets on growth and survival of Angel fish, *Pterophyllum scalare* (Lichtenstein) larvae. The methodology, important results and conclusions 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 used in the experiment were *Artemia* nauplii, *Moina micrura*, Artificial diet, *Artemia* nauplii + *Moina micrura* and *Artemia* nauplii + Artificial feed, designated as T_1,T_2,T_3,T_4,T_5 respectively. The proximate composition of the artificial diet was 29.71% protein, 14.09% fat, 3.96% ash, 0.4% fibre, 51.84% carbohydrate as NFE and 7.0% moisture.

3. The duration of the experiment was 30 days and the experiment was done twice in order to find out the consistency of the result. The different parameters observed in order to find out the efficiency of the diets were growth (average gain in weight and length), specific growth rate, survival and O:N ratio.

4. There was not much difference in the growth and survival of the larvae fed live foods, mixed diets and artificial diet.

5. The growth attained by the larvae fed on artificial diet was 91-93% of that obtained with live foods and mixed diets.

6. Maximum specific growth rate of the larvae was obtained with the mixed diet of *Artemia* nauplii and *Moina micrura* (16.15 and 16.17). Although lowest value was with artificial diet (15.70 and 15.56), it was not significantly different from the other treatments, particularly in the second experiment.

7. The O:N ratio of the larvae fed on artificial diet, particularly in the second experiment (11.26) was not significantly different from the larvae fed *on Artemia* nauplii (8.53) and mixed diet of *Artemia* nauplii + artificial diet (7.78). This shows that the nutritional state of

the larvae fed with artificial diet was comparable with that of the live foods and mixed diet.

8. The results show that the artificial diet can effectively be used as an alternative for the live foods like *Artemia* nauplii or *Moina micrura*, which are costly and difficult to procure in time, in the larval rearing of Angel fish.

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EFFECT OF DIFFERENT DIETS ON GROWTH AND SURVIVAL OF ANGEL FISH *PTEROPHYLLUM SCALARE* (Lichtenstein) FRY

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ABSTRACT OF THE THESIS

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ABSTRACT

The effect of different diets on the growth and survival of Angel fish, *Pterophyllum scalare* (Lichtenstein) larvae was studied. One day old free swimming hatchlings were used in the study for a period of 30 days. The different diets used were *Artemia* nauplii, *Moina micrura*, artificial diet, *Artemia* nauplii + *Moina micrura* and *Artemia* nauplii + artificial diet, with four replicates for each treatment. The experiment was repeated twice in order to determine the consistency of the result.

The results showed that the growth of larvae, in terms of gain in length and weight and specific growth rate, was maximum with the mixed diet of live foods. The differences in growth between artificial diet and mixed diet of *Artemia* nauplii + artificial diet were not consistent. No significant difference was found in the survival rate of larvae fed different diets. O:N ratios showed that the nutritional state of the larvae fed artificial diet was more or less similar to that fed mixed diet of *Artemia* nauplii + artificial diet, which in turn was comparable with that fed live foods. Thus, the artificial diet has been found to be an effective replacement diet in the larval rearing of Angel fish.