COMPARATIVE STUDY ON GROWTH PERFORMANCE OF RED BELLIED PIRANHA (*Pygocentrus nattereri*) UNDER MONOCULTURE WITH SUPPLEMENTARY FEEDING OF VARIABLE PROTEIN INPUTS

A Thesis
Submitted to the
West Bengal University of Animal and Fishery Sciences
in partial fulfilment of the requirements for the award of the degree of

Master of Fishery Science
in
AQUACULTURE

By

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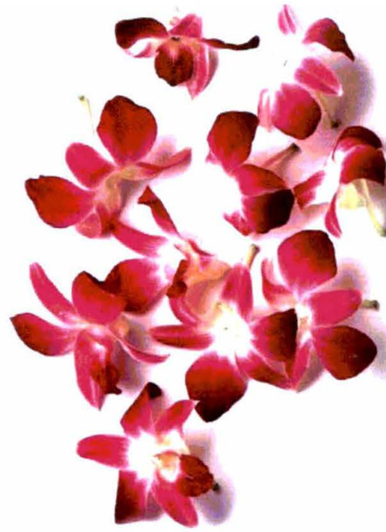

Dedicated to
Maa-Baba

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CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"Comparative study on growth performance of red bellied piranha (*Pygocentrus nattereri*) under monoculture with supplementary feeding of variable protein inputs"** *submitted by* **Mr. Subhendu Haldar** *in partial fulfillment of the requirements for the degree of* ***Master of Fishery Science (Aquaculture)*** *in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafied research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

(Dr. S.K. Das)
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APPROVAL SHEET

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE (AQUACULTURE)

We, the undersigned, have been satisfied with the performance of **Subhendu Haldar,** in the Viva-Voce Examination, conducted today, the .4th Jan... 2010....., ~~2009~~, recommended that the thesis be accepted for the award of the Degree of Master of Fishery Science in Aquaculture.

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Place: Chakgaria

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(Subhendu Haldar)

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CHAPTER 1

INTRODUCTION

In recent years, Piranhas make their presence felt not only as aquarium fish but also as formidable species of culture in some parts of West Bengal. With close morphological resemblance to Pacu (*Piaractus brachypomus*) it is sometimes very difficult to the farmers to distinguish between Piranha and Pacu. As a result, the former is gradually being introduced as a cultivable species by the farmers because of their ornamental traits without knowing the ecological consequences of this predominantly carnivorous species.

Piranha (*Pygocentrus nattereri*) is a typical tropical fresh water fish (Pauly, 1994) which is abundant in lotic (river) as well as in lentic (lagoon) water bodies. The large individuals occupy the rivers, whereas, smaller ones stay in lagoons. Piranhas are infamous for their formidable dentition and predatory habits (Freeman *et el.,* 2007). Differently called as red bellied pirhana, red pirhana, caribe in different geographical locations they belong to the order Characiformes. They comprise a monophyletic group within the Serrasalminae, a sub family of the Characidae and are comprised of 15 genera and 80 valid species (Machado-Allison ,1983, 1985; Jegu, 2003). Kner (1860) described *Pygocentrus nattereri* which was formerly known as *Serrasalmus nattereri* after Johann Natterer (1787-1843), the famous Australian naturalist (Pauly, 1994).

The red bellied pirhana is variable in its coloration with location, population and age (Fink, 1993). The red bellied piranha gets its common name from stocky silver green body with a reddish belly. In case of juvenile, the dorsal and lateral portion of the body from just behind the head to the caudal fin base are still grey, with red orange pigmented area, increasing in intensity in the belly region between isthmus and anal fin. Head is dorsally dark grey; the lower jaw is anteriorly dark grey and posteriorly is red or orange like the abdomen. The pectoral and pelvic fins are red orange; dorsal fin is dark grey; adipose fin is black proximally; the caudal fin is blackish grey. Colour pattern changes as size increases and the thickening body tissue tends to cause the black internal line of the anal fin to disappear. Also, number of body spots and the density of melanophores increase with growth. In case of adult and large specimens, the red or orange colour was pale and limited in the area to the ventral belly and the body of the fish was entirely black and bright burnished-gold "splanges" over much of the lateral body (Fink, 1993).

Female red-bellied piranhas have a greater growth potential than their male counterparts, which is a common feature in the Serrasalminae (Araujo-Lima and Goulding, 1997; Loubens and Panfili, 1997; 2001). The red bellied piranha normally attains about 15 to 25 cm long (Rahman *et al.,* 2008). Maximum size in terms of standard length and weight of the red bellied piranha was stated to be of 26 cm and 3,850g, respectively (Lauzanne and Loubens, 1985).

Sexing of red bellied piranha is very difficult due to lack of reliable, prominent externally visible difference between male and female. Riehl and Baensch (1991) found that the males have more "blunt like" heads, but are more slender than females. Darker coloring and nice purple spots are found in female during breeding but the color change is not occurred in male. Female may be larger than male as female sexual maturity at a significantly larger than male (Pauly, 1994).

Most knowledge on the reproductive biology of this species was originated from aquarium specimens (Pauly, 1994; Schulte, 1998). It is unknown whether the life-history strategies of *P. nattereri* vary among white, clear and black water (Sioli, 1984). Red bellied piranha is a batch spawner (Pauly, 1994), nest builder and only male exhibits showing parental care. The females of red bellied piranhas are bimodal (Duponchelle, 2007).

Piranhas, are mutilating predators whose diet includes whole fish and fragments of fish flesh, fins, and scales, as well as fruits and seeds (Sazima and Machado, 1990; Almeida *et al.*, 1998, Agostinho, 2001). Piranhas prefer fins during their juvenile and sub-adult stages and gradually change to flesh or whole fish as they grow (Machado-Allison and Garcia, 1986; Nico and Taphorn, 1988). There are reports of ontogenetic shifts in diet, with juveniles feeding mostly on larval and adult insects, fish scales and fins, and adults feeding mostly on flesh (Zelditch and Fink, 1995). Furthermore, *P. nattereri*, as several other species of piranha, preys upon sick and injured fishes and scavenges over cadavers of fishes and other vertebrates, thereby playing an important ecological function as 'cleaning and health squads' (Goulding, 1980; Sazima and Guimares, 1987; Pauly, 1994). The teeth of the red bellied piranha are very sharp and specialized to take mouth-sized chunks of flesh out of organisms. In this regard, there have been reports of group attacks on large mammals, including humans (Moe, 1964). Even the adult piranhas are known to attack young of the same species (Nico and Taphorn, 1988). Jegu and Dos Santos (1988) reported that due to its carnivorous characteristics the intestine was relatively short which is attributable to the possible lack of efficiency in cellulose digestion by carnivorous fish, increasing the amount of time that vegetative matter stays in the gut of *P. nattereri* (Hildebrand, 1995).

A number of piranhas belonging to genus *Pygocentrus* are voracious predators. Different piranha species select prey species in different proportion, and that the differences are associated with the body form and swimming behaviour of predator and prey (Winemiller, 1993). According to Sazima and Machado (1989) there was no significant relationship between the frequency of predator attacks and the relative abundance of the prey. Community composition was the main factor determining the attack frequency, which differed according to environment.

As the species is highly carnivorous, as a formidable predator it might pose ecological alterations if cultured uncontrolled in open environment. Therefore, the present study has been designed with the following objectives:

i) to investigate the monospecies culture potential under controlled condition,

ii) to compare the growth performance under variable regimes of protein supplementation,

iii) to investigate the growth performance under predator-prey combination using tilapia (*Oreochromis mossambicas*) fry as prey, and

iv) to study the environmental conditions with special reference to physicochemical and biological parameters under variable feeding regimes of monoculture of Piranha (*Pygocentrus nattereri*).

CHAPTER 2

REVIEW OF LITERATURES

Piranhas are Neotropical fresh water fishes belonging to the order Characiformes and infamous for their formidable dentition and predatory habits (Freeman *et el.,* 2007). According to Machado-Allison (1983, 1985) they comprise a monophyletic group within the Serrasalminae, a sub family of the Characidae and is comprised of 15 genera and 80 valid species (Jegu, 2003). The most rich genus is *Serrasalmus* (24 species, perhaps as many as 28), followed by *Pristobrycon* (5), *Pygocentrus* (3), possibly (4), and *Pygopristis* (1) (Machado-Allison, 1985; Machado-Allison and Fink, 1996; Jegu, 2003).

Serrasalmus nattereri (Family: Characidae) was originally described by Kner (1860) as *Pygocentrus nattereri* and is named after Johann Natterer (1787-1843) (Pauly, 1994), an Australian naturalist who sampled Brazillian animals for nearly 18 years (Anon, 1845). Synonyms include *P. altus, P. stigmaterythraeus, Rooseveltiella nattereri and Serrasalmo piranha* (Riehl and Baensch, 1991).

2.1. Systematic Position

According to Kner (1860) the systematic position of *P. nattereri* is as follows:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Actinopterygii (ray-finned fishes)
Order: Characiformes
Family: Characidae (Characins)
Subfamily: Serrasalminae
Genus: *Pygocentrus*
Species: *Pygocentrus nattereri*
Common Name: Red bellied piranha

According to Pauly (1994) *P. nattereri* bears a number of common names in different countries, notably 'palometa' (Argentina, Bolivia), 'pana' (Peru), 'palometa de rio' (Uruguay), 'caribe boca de locha' (Vanazuela), 'red piranha' (Guyana), 'Natterers Sagesalmler' (Germany) and 'rupchanda'in Bangladesh (Rahman *et al.,* 2008).

2.2. Distribution

The red-bellied piranha *Pygocentrus nattereri* is widely distributed across tropical South America. It has been reported in all major tributaries of the Amazon (Solimoes, Madeira, Negro, Tapajos, Xingu and Tocantins), as well as in the Sa˜ o Franscisco, Orinoco, Paraguay-Parana , Del Plata Rivers (Gery, 1964; Lauzane and Loubens, 1985; Lowe-McConnell, 1987; Lauzane *et al.*, 1990; Fink, 1993; Machado-Allison and Fink, 1995; Hubert and Renno, 2006), coastal rivers of Guianas and Brazil (Moe, 1964; Fink, 1993; Bennet *et al.*, 1997; Uetanabaro *et al.,* 1993). It frequently occurs in the white water rivers draining the Andes and in the clear waters from the Brazilian and Guyana shields, but it is rare in black waters (Fink, 1993; Jegu, 2003). It has also been reported in Florida (Courtenay *et al.*, 1974) and Texas in U.S.A. (Howells, 1992). However, this species is not established in the Gulf of Mexico drainages (Courtenay *et al.*, 1984; Courtenay *et al.*, 1991; Shafland, 1996). *Pygocentrus nattereri* was introduced in due course in countries like Thailand, China, Singapore and the Philippines (Rahman *et al.,* 2008).

2.3. Habitat

Pygocentrus nattereri is typical tropical fresh water fish (Pauly, 1994) which is abundant in lotic (river) as well as in lentic (lagoon) water bodies. The large individuals occupy the rivers, whereas, smaller ones stay in lagoons. A higher abundance of young individuals in the lagoons is possibly associated with the great availability of aquatic macrophytes as aquatic macrophytes constitute the main habitat for juvenile fish and are utilized as refuge against predation, also as a rich food source (Junk *et al.,* 1997). The use of floating beds of macrophytes as refuge has been noted for other young Characiformes as well (Junk, 1973; Lowe-Mc Connell, 1975). Several studies confirmed that juvenile piranhas appear to utilize aquatic macrophytes for feeding and protection in the Amazon (Araujo-Lima *et al.,* 1986; Sanchez-Botero and Araujo-Lima, 2001).

In South America, *Pygocentrus nattereri* is typically found in whitewater streams (Saint-Paul, 2000) and in contrast, the species is not found typically in blackwater streams (Fink, 1993; Saint-Paul *et al.*, March, 2000). This is because the nutrient-rich white waters are more productive and harbour more biomass than black and clear waters (Lowe-McConnell, 1987; Junk *et al.*, 1989; Junk, 1997; Saint-Paul *et* *al*., 2000). As white waters provide better trophic conditions than clear waters, the size at maturity, fecundity, reproductive effort, condition and growth of *P. nattereri* was greater in the more productive white water than in the less-productive clear water (Duponchelle *et al.,* 2007).

Pygocentrus nattereri is reported to tolerant of variable water condition (Fink, 1993). They live within a temperature range of about 73°F - 82°F (23°C - 28°C) (Bennett *et al.,* 1997), pH 6 - 7.5 (Utenabaro *et al.,*1993) and hardness of 10° to 20° dH (Utenabaro *et al.,*1993). Several studies reported that during breeding of *P. nattereri* the water chemistry parameter should have pH: 6.5-7.5, hardness: 4-14 dH and temperature: 70-77°F (21-25°C) (Duponchelle *et al.,* 2007).

2.4. Meristic Characters

The meristic characters of *P. nattereri* was well documented by Kner (1860) and Fink (1993) which are as follows:

Table: 1. Meristic characters of *P. nattereri*

Fins	
Dorsal	
Attributes	no striking attributes
Fins number	1
Finlets No.	Dorsal 0 – 0
Ventral	0 – 0
Spines total	0 – 0
Soft-rays	total 16 – 18
Adipose fin	Present
Caudal fin	
Attributes	more or less truncate
Anal fin(s)	Fins number 1
Spines total	0 – 0
Soft-rays total	27 – 30
Paired fins	
Pectoral	Attributes more or less normal
Spines	0
Soft-rays	
Pelvic	Atributes more or less normal
Position	abdominal beneath origin of D1
Spines	0
Soft-rays	

2.5. Colouration

The red bellied piranha is variable in its colouration and is difficult to diagnose morphologically as the physical characteristics vary with location, population and age (Fink, 1993). The red bellied piranha gets its common name from stocky silver green body with a reddish belly. A change in color pattern does seem to develop as size increases and the thickening body tissue tends to cause the black internal line of the anal fin to disappear. Both the number of body spots and the density of melanophores increases with growth (Fink, 1993). In case of juvenile, the dorsal and lateral portion of the body from just behind the head to the caudal fin base are still grey, with red orange pigmented area, increasing in intensity bin the belly region between isthmus and anal fin. Head is dorsally dark grey. The lower jaw is anteriorly dark grey and posteriorly is the same red or orange as the abdomen. The pectoral and pelvic fins are red orange; dorsal fin is dark grey; adipose fin is black proximally; the caudal fin is blackish grey.

Fink (1993) reported that the extent and colour of the belly pigmentation is variable ontogenetically, individually, geographically and according to water condition in which fish live. In case of adult and large specimens, the red or orange colour was pale and limited in the area to the ventral belly and the body of the fish was entirely black and bright burnished-gold "splanges" over much of the lateral body (Fink, 1993).

2.6. Breeding Ecology

Theoretical and empirical studies indicated positive correlation between food availability and reproductive output in *Pygocentrus nattereri* (Gadgil and Bossert, 1970; Wootton, 1998). Variations in food availability can induce changes in fecundity, growth rate, age and size at first maturity in fishes under experimental conditions (Scott, 1962; Bagenal, 1969; Wootton, 1973; Hislop *et al.*, 1978; Reznick, 1983, 1990; Stearns and Crandall, 1984; Townshend and Wootton, 1984).

However, the life-history strategies in natural environments have received little attention except for scant information on the seasonality of breeding and size at first sexual maturity in British Guiana (Lowe-McConnell, 1964), Mamore River, Bolivia, (Loubens and Aquim, 1986) or breeding behaviour in Paraguay River basin, Brazil, (Utenabaro *et al.*, 1993). Most knowledge on the reproductive biology of this species has been originated from aquarium specimens (Pauly, 1994; Schulte, 1998). In particular, it is currently unknown whether the life-history strategies of *P. nattereri* vary among white, clear and black water (Sioli, 1984).

Red bellied piranha is a batch spawner (Pauly, 1994), nest builder and only male exhibits parental care. The male dug the nest in the bottom which is bowl shaped and measured 15cm in diameter and were 4-5 cm deep. These holes were dug amongst the grass and eggs were attached in clusters to the grass root and stems (Uetanabaro *et al.*, 1993).

The females of red bellied piranhas are bimodal (Duponchelle, 2007). The first mode consisted of oocytes 1.4 mm in diameter and the second one of oocytes with diameters ranging from 1.5 to 2.3 mm. The presence of oocytes at different developmental stages in ripe females of *P. nattereri* suggested that they can spawn at least twice during the breeding period. Similar ovarian characteristics were reported and multiple spawning was suspected for another piranha, *Serrasalmus spilopleura* in the Itumbiara Reservoir, Brazil (Lamas and Godinho, 1996). Duponchelle *et al.* (2007) viewed that might be important for piranhas to spawn as early as possible during the rising waters, because it allows time for a second spawn during favourable conditions for offspring survival.

Female and male red bellied piranhas matured as yearlings and males become mature a few months before females (Duponchelle *et al.,* 2007). The sizes at first maturity observed ranging from 130 to 140 mm for females and 109 to 114 mm for males (Duponchelle *et al.,* 2007). The gonadal maturation in *P. nattereri* started in July to August. The first maturing females at early vitellogenesis stage were observed with the first rains, just before beginning of the increased rainfall levels (Duponchelle *et al.,* 2007). In riverine condition the water depth was 35 cm and water temperature was 27^0-28^0C, reported by Uetanabaro *et al.*, 1993.

The breeding season of *P. nattereri* was strongly seasonal (Duponchelle *et al.,* 2007) started in August to September and extended over 5–6 months but peaked over no more than 2 months, in November and December. The breeding period of P. nattereri was desynchronized with water level and coincided better with the rainfall and photoperiod cycles in the wild. In riverine condition, the breeding started during the dry season, it peaked at the start of the flood period and ended before water levels were maximal (Uetanabaro *et al.*, 1993). By contrast, the beginning of the breeding season coincided with the first rainfalls. It also coincided with the time of the year when day-length was increasing. The strong decline in the proportion of females in reproductive condition throughout January and February took place during periods, where rainfalls were still abundant and water level was rising. By contrast, it coincided with the time of the year when day-length started decreasing.

A set of environmental stimuli may be more reliable in cueing reproduction than a single stimulus, helping to ensure that the proper conditions are met for maximizing reproductive success (Bromage *et al.*, 2001). The red-bellied piranhas are known to lay their eggs on newly submerged vegetation (Uetanabaro et al., 1993), so spawning under rising waters, as already observed for other piranha species was expected (Vazzoler and Menezes, 1992; Ruffino and Isaac, 1995). The availability of submerged vegetation during early rising waters might be the ultimate cue that triggers spawning. the breeding intensity of *P. nattereri* decreased from January onwards responding to decreasing day length. So, the photoperiod may be a key environmental factor determining the periodicity of reproduction in natural populations of red-bellied piranha.

In *P. nattereri* the sex ratio was skewed in favour of females. Female-biased sex ratios were also reported for 10 out of 16 species studied by Loubens and Aquim (1986) in the Mamore River including *P. nattereri* and other characids (Loubens and Aquim, 1986). After seasonal flood *P. nattereri* swam side by side interacting (Uetanabaro *et al.*, 1993) with each other and also showing courtship display swimming in circles. The most common interaction observed between individuals was a fish following another, but without showing overt aggressive behaviour. It was also seen that two individuals swam in circles in opposite directions (Uetanabaro *et al.*, 1993) while maintaining their ventral surfaces in close proximity to one another, which resulted in ventral to ventral interactions among the male and female. No aggressive interaction was observed among members of the swimming group as reported by Schmitt (1984) for red bellied piranha breeding in aquaria.

Spawning generally occurred after mating and eggs were laid on tree roots trailing in the waters (Lowe-Mc Connell, 1964, 1975). The eggs were attached in clusters to the grass roots and stem (Uetanabaro *et al.*, 1993). The eggs were transparent-golden and stick to the nest (Pauly, 1994). In captive condition female can produce 500-1000 eggs per spawning (Pauly, 1994), but in wild the fecundity ranged from 4000-5000 which adhere to plant and were not attacked by the parent fishes (Mills and Vevers, 1989; Pauly, 1994).

The male dug the nest in the bottom which are bowl shaped (Uetanabaro *et al.*, 1993) and measured 15cm in diameter and were 4-5 cm deep. These holes were dug amongst the grass and eggs were attached in clusters to the grass root and stems (Uetanabaro *et al.*, 1993).

The larvae hatch after 8 days (Mills and Vevers, 1989), and start feeding after 4-5 days, i.e. once their yolk sac is consumed. The juveniles have black spots and their only red coloration is on a spot near and lower part of the operculum, and on the anal fin (Pauly, 1994). Larvae and early juveniles are found by both day and night among the roots of the water hyacinth, *Eichornia crassipes* for shelter (Sazima and Zamprogno, 1985), a rich foraging place and potential rafting dispersal. Piranha larvae up to 19mm feed mainly on aquatic arthropods, slowly searched for inside the root tangle; large juveniles tend to leave the plants and patrol more open areas.

2.7. Age and Growth

In the subfamily Serrasalminae, females generally attain a larger size than males, as exemplified by *C. macroponum* and *P. brachypomus* (Araujo-Lima and Goulding, 1997; Loubens and Panfili, 1997; 2001). This pattern of between-sex growth dimorphism also applies to *P. nattereri*. Female red-bellied piranhas have a greater growth potential than their male counterparts, which is a common feature in the Serrasalminae (Araujo-Lima and Goulding, 1997; Loubens and Panfili, 1997; 2001). Sexing of red bellied piranha is very difficult due to lack of reliable, prominent externally visible difference between male and female (Paysan, 1975; Pauly, 1994). Riehl and Baensch (1991) found that the males have more "blunt – like" heads, but are more slender than females. The female is ballooned out and male is a lot skinner. Darker coloring and nice purple spots are found in female during breeding but the color change is not occurred in male, very light in color. Females may be larger than male (Uetanabaro *et al.*, 1993) as females sexual maturity at a significantly larger than males (Pauly, 1994).

The red bellied piranha normally attains about 15 to 25 cm long (Rahman *et al.,* 2008). Maximum size in terms of standard length and weight of the red bellied piranha was stated to be of 26 cm and 3,850g, respectively (Lauzanne and Loubens, 1985). In earlier studies, Lowe-Mc Connell (1964) observed that red bellied piranha reached 4-9 cm standard length in their 4 months age, 12 cm in 7 month age and 16 cm in 12 month age in wild condition with maximum size attaining 26 cm. However, Nico and Taphorn (1986) reported that red bellied piranha reaches 5-8 inches in their second rainy season.

Several studies reported that in aquaria, *P. nattereri* reached 4.5 cm after 2 month (Schutte, 1988) and that when aged eight month the largest fish were 120mm (5 inches) long. In polyculture pond, Rahman (2008) observed that the average weight and length of piranha was 1102 g and 39.1 cm, respectively and the growth rate varied from 9.89% to 188.89 %. The growth of fishes at the early stage was rapid and growth rate was in increasing trend during February. This is due to the change of season, start of rainfall and availability of more food items in the pond. In another study Merona (1984) reported that growth rate of *P. nattereri* remains high until the fish attains 19-22 cm, when the fish are about 9 to 12 months old. Depending on where and when the young hatched in the flooded area, the encountered tropic conditions may vary and account for important growth differences among individuals during the first year (Douponchelle *et al.*, 2007).

The otolith opaque zone is usually formed during the period of fast growth under high waters, when fishes have access to the plentiful floodplain in the savanah and forest (Lowe-McConnell, 1964, 1987; Junk *et al.*, 1989; Junk, 1997). The translucent (stainable) mark corresponds to a period of slow growth during the drought and the early flooding season. This alternation of periods of fast and slow growth is widespread not only among characids (*C. macropomum* and *P. brachypomus*; Loubens and Panfili, 1997; 2001). According to Douponchelle *et al* (2007) the growth of *P. nattereri* was rapid in the first year which helps maximum egg production during the first spawning season. The size variation at a given age in *P. nattereri* was largest during the first 2 years of life.

2.8. Food and Feeding Habit

Piranhas, are mutilating predators (Agostinho, 2001) whose diet includes whole fish and fragments of fish flesh, fins, and scales, as well as fruits and seeds (Goulding, 1980; Nico and Taphorn, 1988; Winemiller, 1989; Sazima and Machado, 1990; Almeida *et al.*, 1998). Piranhas prefer fins during their juvenile and sub-adult stages and gradually change to flesh or whole fish as they grow (Machado-Allison and Garcia, 1986; Nico and Taphorn, 1988; Almeida *et al.*, 1998). In the Amazon, juvenile piranhas appear to utilize aquatic macrophytes for feeding and protection also (Araujo-Lima *et al.*, 1986; Sanchez-Botero and Araujo-Lima, 2001). There are reports of ontogenetic shifts in diet, with juveniles feeding mostly on larval and adult insects, fish scales and fins, and adults feeding mostly on flesh (Zelditch and Fink, 1995). The teeth of the red bellied piranha are very sharp and specialized to take mouth-sized chunks of flesh out of organisms. In this regard, there have been reports of group attacks on large mammals, including humans (Moe, 1964). Even the adult piranhas are known to attack young of the same species (Nico and Taphorn, 1988).

The red bellied piranha is one of among the most aggressive piranhas (Moe, 1963; Lopes *et al.*, 1991) and also an opportunistic carnivore. It has been reported that *P. nattereri* is reputedly voracious predator (Pauly, 1994), similar to the most other sharp teeth piranhas (Duponchelle *et al.*, 2007). Several studies, however, confirmed that its diet primarily comprised of fishes, but also arthropods, molluscs, other invertebrates and plant material (Bonetto *et al.*, 1967; Nico and Taphorn, 1988; Winemiller, 1989; Winemiller and Kelso-Winemiller, 1993; Pauly, 1994). Furthermore, *P. nattereri*, as several other species of piranha, preys upon sick and injured fishes and scavenges over cadavers of fishes and other vertebrates, thereby playing an important ecological function as 'cleaning and health squads' (Goulding, 1980; Sazima and Guimares, 1987; Schulte, 1988; Sazima and Machado, 1990; Pauly, 1994). In fact, plant material had often been found to be a major dietary component (Bennett *et al.*, 1997) of red bellied piranhas (Schulte, 1988; Sazima and Machado, 1990).

Several studies reported that in Amazon lagoons, the vegetarian materials was the most frequent food item in the gut content of *P. nattereri*. Luengo (1965) and, Jegu and Dos Santos (1988) reported that due to its carnivorous characteristics the intestine was relatively short which is attributable to the possible lack of efficiency in cellulose digestion by carnivorous fish, increasing the amount of time that vegetative matter stays in the gut of *P. nattereri* (Hildebrand, 1995). *P. nattereri* bitted small pieces of fishes or potential food items in the surface among the aquatic macrophytes, resulted in the high frequency of vegetative matter found in the stomach (Sazima and Machado, 1990).

Foraging methods vary in different life stages of *P. nattereri*. Smaller fish 80-110 mm were active mainly during day time. At dawn, late afternoon and early evening the larger fish (150-240 mm) search for food. *Pygocentrus nattereri* groups gather in vegetation in order to wait for prey. The group typically includes around 20-30 fishes. Small tight groups of upto 20-30 large individuals had a definite diurnal resting place usually sheltered among the vegetation. Teeth replacement on alternating sides of jaw allows continuous feeding (Sazima and Machado, 1990). Its powerful dentition can inflict serious bites and it has a highly evolved auditory capacity and a 'lurking', then 'dashing' behavior during daytime. In the daytime *P. nattereri* can be seen lurking or ambushing prey. Two other methods for obtaining food employed by *P. nattereri* are chasing and scavenging. The hunting mode of chasing was seen after the fish lie and wait in vegetation. Aquarium studies of *P. nattereri* also reported that large juveniles and small adults has two peak feeding periods during day light (Bellamy, 1968; Zbinden, 1973)

2.9. Piranha as Predator

Piranhas exhibit a variety of feeding preferences (Winemiller, 1993) including whole fish, chunks of fish flesh, fish fins, scales, and fruit and seeds (Goulding, 1980; Nico, 1991; Nico and Taphorn, 1988; Winemiller, 1989). A number of piranhas belonging to genus *Pygocentrus* are voracious predators. Different piranha species select prey species in different proportion and that the differences are associated with the body form and swimming behavior of predator and prey (Winemiller, 1993).

P. nattereri was generally found in small groups (Pitcher, 1986) of upto 20 individuals and was popularly believed to be a dangerous pack-hunting fish (Schulte, 1988). Several studies reported that the shoaling behavior in this species were mainly for prey attack. But an individual may suddenly launched on attack towards a potential prey in which it generally was followed by one or two companions (Sazima and Machado, 1989). According to Sazima and Machado (1989) during the night' single large individuals were seen patrolling a given area. Intra and interspecific agonistic behavior included chase and side-display, head to tail rounds were observed twice during the night among conspecific.

According to Sazima and Machado (1989), the main categories of hunting modes towards fish prey were observed: lurk or ambush, chase and scavenging. *P. nattereri* used scan and pick tactic on prey fishes when foraging on vegetation or on the bottom (Keenleyside, 1979). Lurking was employed by *P. nattereri* during day time and ambushed from with in the abundant aquatic vegetation and dashed after passing prey mainly from behind. Chasing after small fish was reported mainly for P. nattereri preceded by lying in wait within the vegetation. Scavenging was observed for *P. nattereri* as a natural occurrence and by provisioning with decomposing fish.

Agostinho and Marques (2001) studied that the intensity of piranha attack depends upon strategies of prey selection and capture (Markl, 1972; Sazima and Machado, 1990; Winemiller and Kelso-Winemiller, 1993), intensity of attacks on fins (Northcote *et al.*, 1986, 1987; Winemiller and Kelso-Winemiller, 1993), avoidance strategies by prey (Sazima and Machado, 1990; Winemiller, 1990) and the influence of abiotic factors (Agostinho *et al.*, 1997). According to Sazima and Machado (1989) there was no significant relationship between the frequency of predator attacks and the relative abundance of the prey. Community composition was the main factor determining the attack frequency, which differed according to environment. The frequency of attack per environment is a function of ichthyofauna composition of the rather than a function of relative abundance of prey per predator (Sazima and Machado, 1989). Prey selection by piranhas may be influenced by other factors. Nico and Morales (1994) emphasized that food quality influenced prey selection. High frequency of attacks on individuals of a given species may indicate either greater vulnerability of prey or preference of the predator (Winemiller and Kelso-Winemiller, 1993).

Several studies reported that greater frequency of attacks was observed on the fins of species which have their bodies covered by large resistant scales and bony plates (Agostinho and Marques, 2001). Cichlids were the group most frequently attacked by piranhas reported in other studies (Northcote *et al.*, 1987; Winemiller and

Kelso- Winemiller, 1993). But according to Winemiller and Winemiller (1993) the south American cichlid fishes suffered lower levels of fin predation because of their caudal (tail) mimicry of the head region. It is believed that piranhas attack the tail of a prey fish first in order to increase the vulnerability and reduce its mobility (Foxx, 1972; Markl, 1972; Zbinden, 1973).

According to Sazima and Machado (1989), the species which are usually attacked by *P. nattereri* belong to Apteronotidae, Sternopygidae, Gymnotidae, Auchenipteridae, Rhamphichthyidae, Pimelodidae, Hypophthalmidae, Ageneiosidae, Characidae, Cynodontidae, Doradidae, Loricariidae, Sciaenidae, Callichthyidae , Serrasalmidae , Erythrinidae, Urimatidae, Anostomidae, Cichlidae, Prochilodontidae, Soleidae.

Review of literatures clearly indicated lack of information regarding the biology of Piranhas under culturable conditions. The informations particularly with respect to its feeding behaviour, feed composition as well as feeding preferences are mostly based on the observations in natural waters as well as from aquarium culture conditions. Therefore, it is necessary to investigate the growth and feeding responses under culturable conditions in captivity so as to have actual understanding regarding its culture potential albeit in restricted manner.

CHAPTER 3

MATERIALS & METHODS

The present study on the comparative growth response of red bellied piranha (*Pygocentrus nattereri*) under monoculture condition with different feeding regimes like a) supplementary feeding daily @ 5% of body weight with 40% protein (MLP), b) supplementary feeding daily @ 5% of body weight with 50% protein (MHP), c) supplementary feeding daily @ 5% of body weight with early fry (0.75 ± 0.25 g) of tilapia, *Oreochromis mossambicas* as predator-prey combination (PP) and d) with no feed as control (C) has been carried out in the Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Panchasayar, Kolkata-94. The duration of the study lasted for four months from May to August, 2009.

3.1. Preparations of cistern

Twelve outdoor experimental tanks (180 l) were selected for the present investigation. After thorough washing and sun drying the tanks were provided with agricultural soil base of 15 cm. They were then filled with ground water (pH -7.5) and were manured with cowdung @ 10,000 kg/ha. as practiced in traditional pond preparation for fish farming in the locality. The requisite amount of cow dung were mixed with water in a bucket and dispensed in the form of slurry into the cisterns. They were then randomly grouped into four batches in triplicate for the four systems designed and kept undisturbed for 10 days.

3.2. Identification of Fish

As the test fish red bellied piranha (*Pygocentrus nattereri*) (Plate.1) bears some morphological resemblance to that of red bellied pacu (*Piaractus brachypomus*) (Plate.1) and very often there is misunderstanding between the two particularly during their early stages, the fish fry collected from the nearby market was confirmed of its taxonomical identification. This was done by sending the sample to the Zoological Survey of India (ZSI), Kolkata (Annexure-I).

3.3. Stocking of fish

Healthy fry of red bellied piranha, *Pygocentrus nattereri* (40 ± 0.5 mm; 2 ± 0.21g) were collected from Naihati Fish Seed Market and acclimatized in experimental tanks for 7 days. Stocking of fish was done in all the four batches of

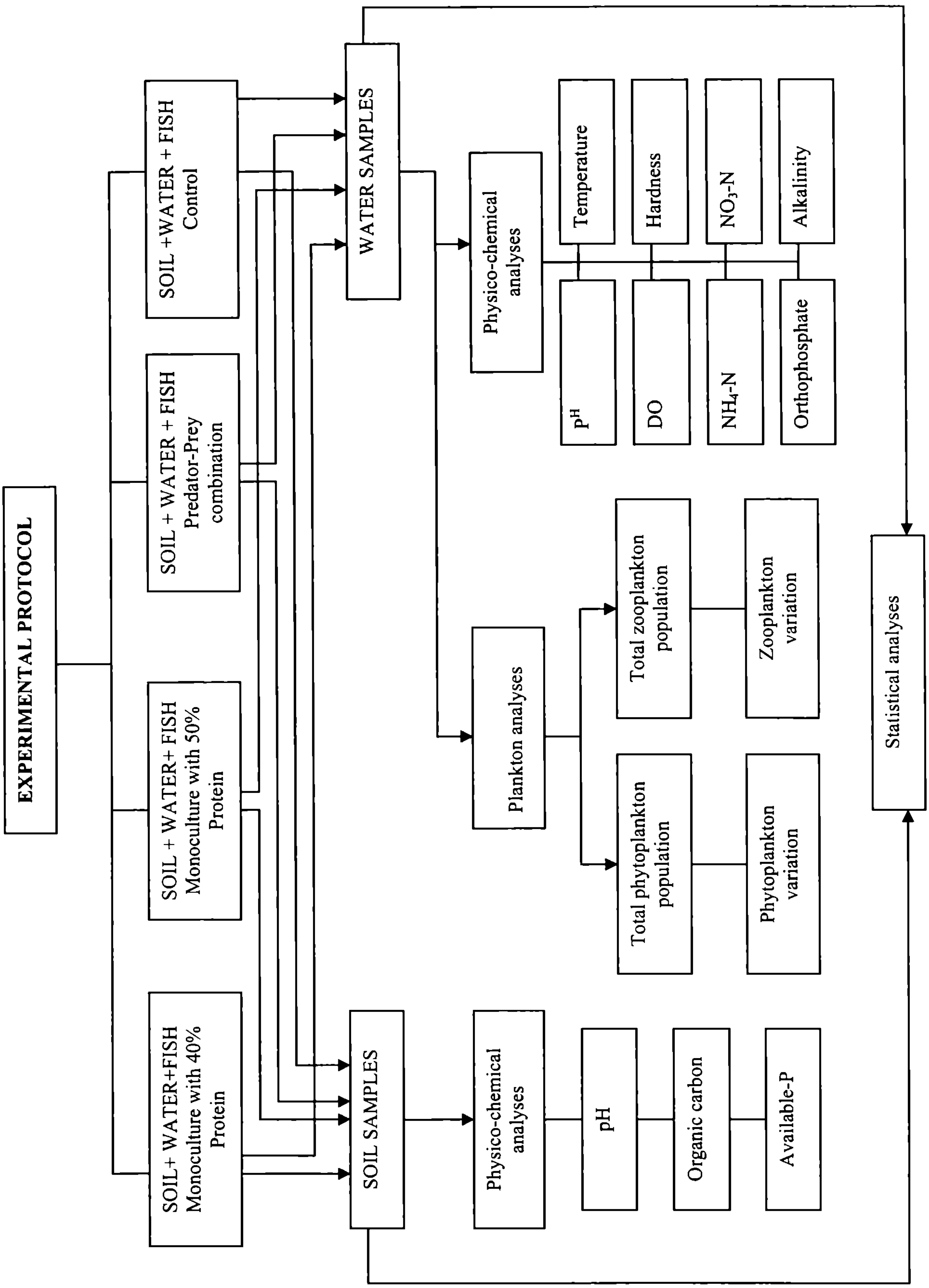


Fig. 1. Experimental protocol followed in the present investigation.

PLATE-1

Red bellied piranha (*Pygocentrus nattereri*)

Red bellied pacu (*Piaractus brachypomus*)

tanks @ 20 nos./tank, 10 days after application of cowdung when the colour of the water changes to greenish blue indicating development of planktonic organisms. They were reared for 120 days.

3.4. Feed preparation

Supplementary feeds of 40% and 50% protein content were prepared by using the Pearson square method. Total protein input was equally distributed into animal and plant sources. Freshly collected fish meal (45% protein) and mustard oil cake (30% protein) were used as animal and plant sources of protein input in the designed feed respectively (Plate.2). Rice polish was used as carbohydrate source as well as filler whereas; equal mixture of groundnut oil and cod liver oil @ 6% was used to supplement essential fatty acids. The feeds were also fortified with vitamin-mineral mixture @ 1g/kg. Different ingredients for protein and carbohydrate supplementations as required upon calculations were weighed, powered by using a mixer-grinder and mixed thoroughly. The required quantity of oil and vitamin-mineral mixtures were then sprayed upon the mash and mixed well again.

3.5. Feeding

Fish were fed twice daily (9.00 a.m. and 5.00 a.m.) in MLP and MHP where the required amount of feed were divided into $2/3^{rd}$ and $1/3^{rd}$ parts and broadcasted over the water surface in the morning(8=00 AM) and afternoon (4=00 PM) respectively. In the predator-prey combination (PP) the required number of live early tilapia fry to equate approximately 5% of body of the stocked piranha were supplied daily in a single installment during the morning (8=00 AM).

3.6. Water replenishment

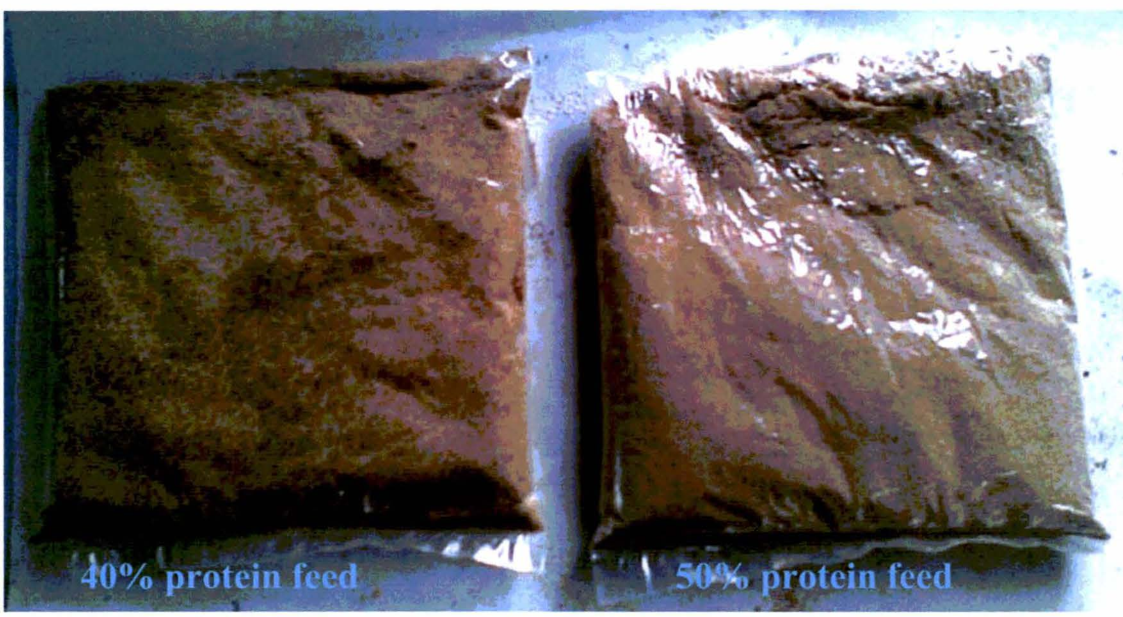
A fixed level of water was maintained in the experimental cisterns by periodic addition of ground water to compensate the losses due to evaporation and sampling. Also, 10% of water was exchanged weekly in all the experimental tanks by siphoning from the bottom layer of each tank.

3.7. Collection of water samples

Water samples were collected at 15 days interval from each of the tanks at a fixed hour of the day (10.30 AM) by completely dipping the collection bottle at 15 cm depth for physico-chemical analyses. During collection of water samples, cautions

PLATE-2

Feed ingredients used for feed preparation



Prepared supplementary feed contain 40% and 50% protein

were taken so as to prevent air bubbling, which might influence water parameters such as dissolve oxygen.

3.8. Collection of soil samples

Soil samples from each of the cisterns were collected by using a mini grab sampler. They were then mixed, air-dried, pulverized with pestle and mortar and sieved through 150 μm mesh sieve and stored in labeled polythene packets for analyses.

3.9. Collection of Plankton

A conical plankton net made of bolting silk cloth (no. 21 with 77 meshes per square centimeter) was used to collect the plankton sample. About 5 l of water from each of the cistern was collected from randomly selected locations with the help of a 500 ml beaker and pooled together for filtering through plankton in 4% formalin solution and stored in labeled vials for subsequent quantitative and qualitative analysis.

3.10. Analyses of samples

3.10.1. Water quality

3.10.1.1. Temperature

The water temperature was measured using a centigrade thermometer on spot and expressed as °C.

3.10.1 2. pH

pH of water samples was estimated by a digital pH meter (ADCO) on spot.

3.10.1.3. Dissolved oxygen

For estimation of dissolved oxygen content of water, the samples were collected with all necessary precautions. Winkler's method was followed for the same (APHA, 1995).

3.10.1.4. Total alkalinity

Total alkalinity of water samples was estimated using phenolphthalein and methyl orange as indicator and N/50 H_2SO_4 as titrant (APHA, 1995).

3.10.1.5. Total hardness

Total hardness of water samples was measured on the sampling day by titrating the samples against EDTA (Ethylene Di-amine Tetra Acetic acid) after adding ammonia buffer and Eriochrome Black T (APHA, 1995) as indicator.

3.10.1.6. Ammonia- nitrogen

After proper filtration of the sample, phenol solution, sodium nitropruside solution and oxidizing solution were added to the sample. The samples were then wrapped with paper and kept at room temperature (22-27 °C) in subdued light for at least 1 hour. A blue colour appeared which was stable for 24 hrs. The ammonia concentration of the samples was directly estimated through a double beam UV-vis-Spectrophotometer (CECIL CE-4002) at 543 nm wavelengths.

3.10.1.7. Nitrate-nitrogen (NO_3-N)

After proper filtration of the samples, 1(N) hydrochloric acid was added to each of the sample and after 10 minutes nitrate nitrogen concentration was directly estimated through a double beam UV-vis-Spectrophotometer (CECIL CE-4002) at 220 and 275 nm wavelengths (APHA 1995).

3.10.1.8. Orthophosphate

The orthophosphate level of water was determined colorimetrically (UV-vis-Spectrophotometer (CECIL CE-4002) at 690 nm following the stannous chloride method (APHA, 1995).

3.10.1.9. Productivity

The dark and light bottle technique described by Winberg (1963) was followed for the measurement of primary productivity of phytoplankton. Water samples were collected in 125 ml borosil glass bottle in triplicate from each cistern taking all necessary precautions during filling to prevent air bubble from remaining in the bottle. All the bottles were then exposed at the surface layer of water under normal light conditions for 4-5 hours of day light depending upon the photo period.

The oxygen content of all the dark and light bottles was monitored using modified Winkler's method. The calculation described by Vollenweider (1974) was used to measure the rate of primary production. The results of primary production in terms of oxygen was converted into mg carbon by multiplying with a factor 0.375 (Natarajan and Pathak, 1983).

3.10.2. Soil quality

3.10.2.1. pH

The pH was determined with a digital pH meter (ADCO) using 1:2 suspensions of soil and water (APHA, 1995).

3.10.2.2. Organic carbon

For estimation of organic carbon, air-dried powdered sediment sample (500 g) was digested with 1 N $K_2Cr_2O_7$ (20 ml) and concentrated H_2SO_4 (20 ml) and kept for 30 minutes at dark. The digested sample was then diluted with 150 ml distilled water and 10 ml ortho-phophoric acid and 1 ml diphenyl amine indicator was added. It was then titrated against 0.5 N ferrous ammonium sulphate (Mohr's salt) until brilliant green colour appeared (Walkey and Black, 1934).

3.10.2.3. Available phosphorus

Available phosphorus was determined using 1:20 soil to Olsen's extractant (0.5 $NaHCO_3$ adjusted to pH 8.5) (Olsen *et al.*, 1954) followed by Dickman and Bray's (1940) Chlorostannous reduced molybdophosphoric blue colour method in hypochloric acid system as described by Jackson (1967).

3.11. Analyses of Plankton

Formaldehyde preserved plankton samples were analysed by 'Drop Count Method' (APHA, 1995) under a binocular microscope.

3.12. Fish growth

Fish growth was recorded at 15 days intervals from each cistern. Half of the stocked fish were caught randomly with a handnet and their weight (g) increments were recorded for estimation of average weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). They were then released again as quickly as possible for further growth. The following estimates were done as:

Average weight gain = (final weight - initial weight)

Specific growth rate (SGR) = 100 x [(ln final average weight - ln initial average weight)/ days]

Feed conversion ratio (FCR) = (total dry feed fed in g /fish weight gain in g)

Protein Efficiency ratio (PER) = (g wet weight gain /g crude protein fed)

3.13. Statistical analysis

All the results were subjected to statistical analysis. One way analysis of variance (ANOVA) were applied to test the significance among the treatment followed by the Paired two sample t-test to find out significance in difference between every possible pair of treatment combination. Correlation co-efficient (r) test was applied to establish relationship between selective parameters using appropriate software.

CHAPTER 4

RESULTS

4.1. Fish growth

4.1.1. Body weight

The weight of fish continued to increase in all the systems and the maximum weight at the time of harvest were achieved in PP (42.2g) followed by MHP (31.8g), MLP (23.7g) and C (18.3g). Fish growth in different treatment manifested strongly significant difference (F = 27.96; P < 0.001) during the period of investigation. Paired two sample t-test also exhibited significant differences in every possible combination of system ($t \geq 4.69$; $P < 0.01$) (Fig. 2).

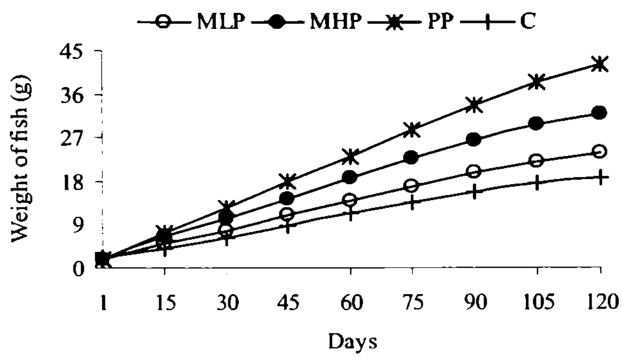


Fig. 2: Temporal changes in the body weight of fish (g) under different treatments employed.

4.1.2. Weight gain (g)

The absolute weight gain over time in different treatments indicated variable response. In MLP and MHP the gain in weight continued upto day 60 after which there was a decreasing tendency whereas in PP the value remained stationary upto day 90 followed by a fall (Fig. 3).

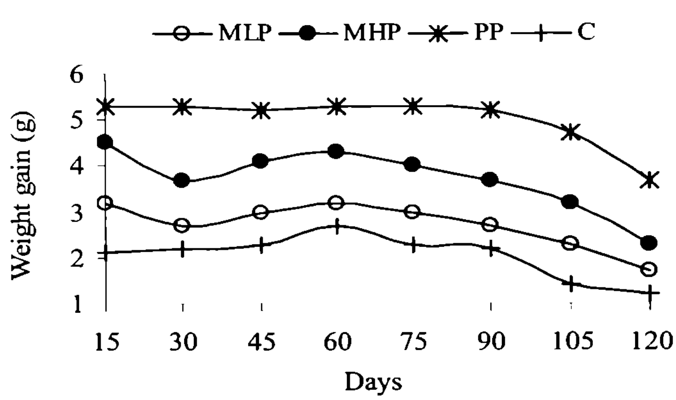


Fig. 3: Temporal changes in weight gain (g) of fish in different treatments employed.

The maximum weight gain (5.3g) was observed in PP followed by MHP (4.7g), MLP (3.2g) and C (2.7g). Overall treatment difference ($F = 478.71$; $P < 0.001$) as well as differences between any pair of treatments ($t \geq 14.74$; $P < 0.001$) remained highly significant.

4.1.3. Growth rate

The absolute growth rate was maximum (100 - 265%) during the 1st 15days of culture in any of the systems employed (Fig. 4). A sharp fall in the values of absolute growth rate in all the treatments up to day 30 followed by gradual decline in any case was manifested. Differences among the treatment reveals insignificance ($P > 0.05$).

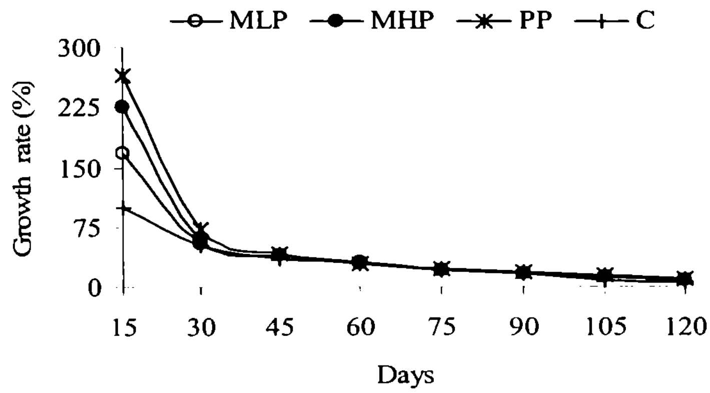


Fig. 4: Temporal changes of growth rate of fish in different treatments employed.

4.1.4. Specific growth rate (SGR)

The temporal trend of specific growth rate exhibited identical response to that of absolute growth rate (Fig. 5). However, differences among the treatments remained significant ($F = 5.14$; $P < 0.05$).

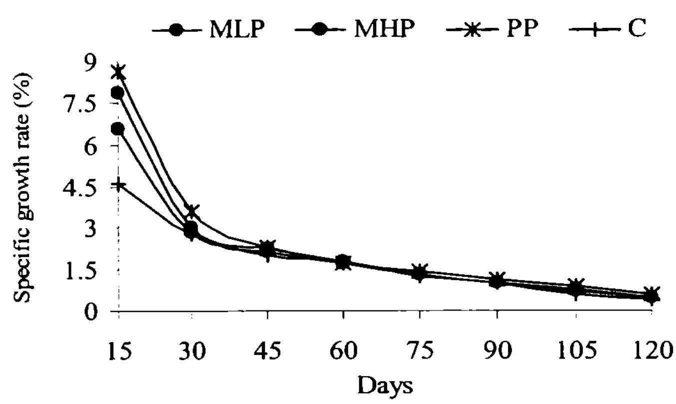


Fig. 5: Temporal changes in the values of specific growth rate of fish in different treatments employed.

4.1.5. Feed conversion ratio (FCR)

An increasing trend in the values of FCR was observed in both the systems where formulated feed was applied (MLP and MHP). The overall FCR in MLP (3.83) though remained slightly higher than MHP (3.71). There was no significant difference between them (P > 0.05) (Fig. 6).

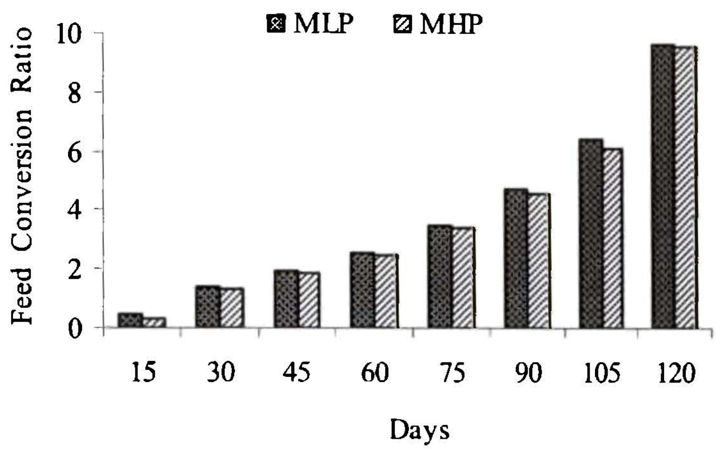


Fig. 6: Temporal changes in the values of feed conversion ratio (FCR) in different treatments employed.

4.1.6. Protein efficiency ratio (PER)

In contrast to FCR, PER tended to decline throughout the period of investigation in both the systems subjected to formulated feed (MHP and MLP) application (Fig. 7). However, the average PER value in MLP (1.44) was slightly higher than MHP (1.36). Similar to FCR difference between them was insignificant (P > 0.05).

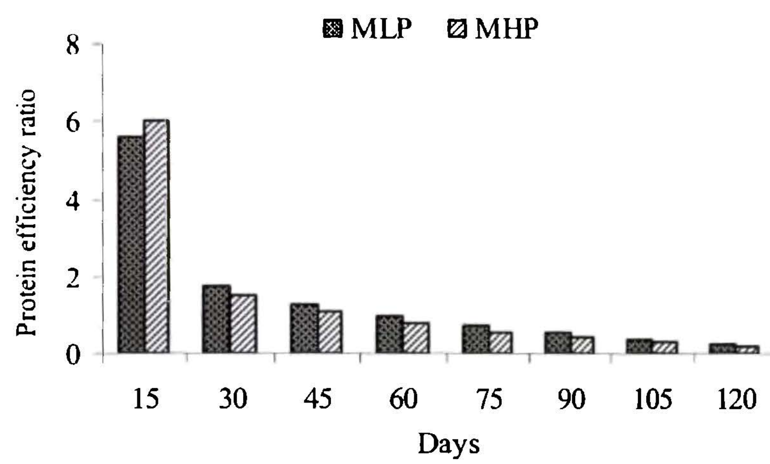


Fig. 7: Temporal changes in the values of protein efficiency ratio (PER) in different treatments employed.

4.1.7. Survival rate

The survival of fish was highest (90%) in PP followed by MHP (80%), MLP (60%), and lowest in Control (30%) (Fig. 8).

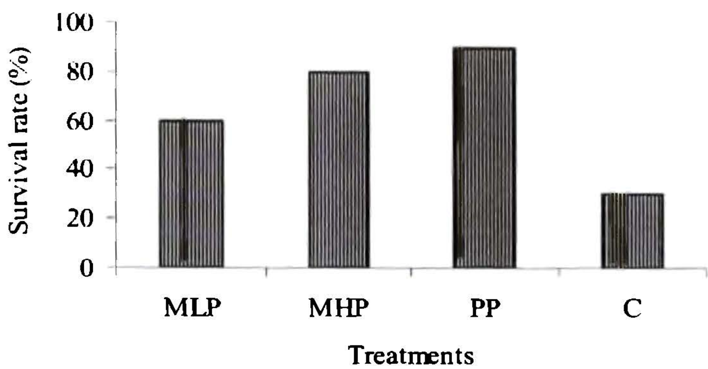


Fig. 8: Survival rate (%) of fish in different systems employed.

4.2. Water quality

4.2.1. Physico-chemical parameter

4.2.1.1. Temperature

The surface water temperature in different treatments ranged from 30.4°C to 33.7°C during the period of observation. As expected treatment differences were insignificant (P> 0.05) but temporal difference during the period exhibited strong significance (F = 34.50; P <0.01) (Fig. 9).

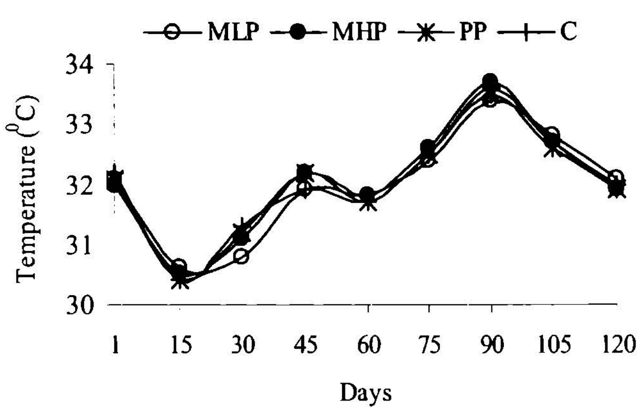


Fig. 9: Temporal changes in the temperature of water in different treatments employed.

4.2.1.2. pH

pH of water in all the treatments ranged between 7.38 to 7.89 with no significant differences among them. Though in MHP and PP water pH remained more or les stationary, a declining trend was observed in MLP and C (Fig. 10).

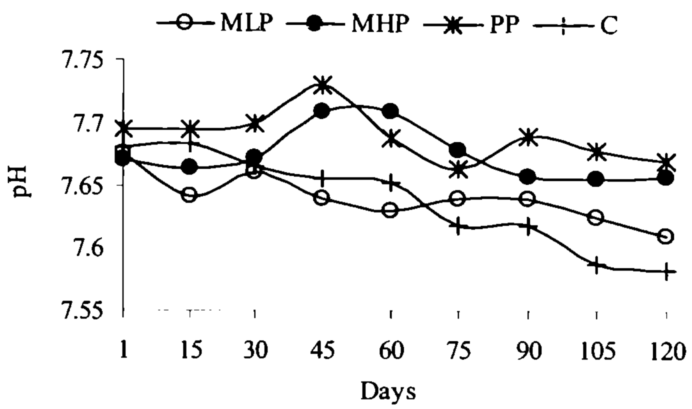


Fig. 10: Temporal changes in the pH of water in different treatments employed.

4.2.1.3. Dissolved oxygen

The concentration of dissolved oxygen gradually increased in all the treatments with maximum overall mean value in MLP (7.04 mgl^{-1}) followed by MHP (6.74 mgl^{-1}), PP (6.78 mgl^{-1}) and minimum in C (6.59 mgl^{-1}) (Fig. 11). Statistical differences (ANOVA) among the treatments remained significant ($F = 8.33$; $P < 0.05$). Also, differences between any possible pair of treatments exhibited significance ($t \geq 3.04$; $P < 0.05$).

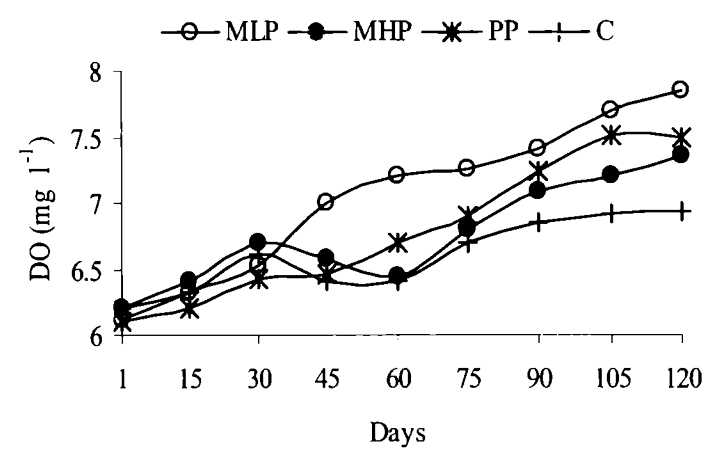


Fig. 11: Temporal changes of dissolved oxygen of water in different treatments employed.

4.2.1.4. Alkalinity

Total alkalinity of water exhibited gradual declining trend in any of the systems employed with maximum mean value of 140.34 mgl^{-1} in MHP followed by C (137.6 mgl^{-1}), PP (136.17 mgl^{-1}) and MLP (135.32 mgl^{-1}) (Fig. 12). Marginally significant difference ($F = 5.64$; $P < 0.05$) was established among the different treatments. However, treatment differences either between MLP and PP, MLP and C or between PP and C remained insignificant ($P > 0.05$).

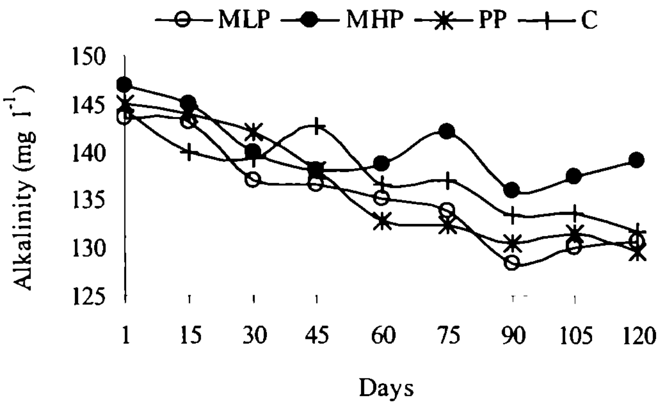


Fig. 12: Temporal changes of total alkalinity of water in different treatments employed.

4.2.1.5. Hardness

Temporal response of hardness was identical to that of alkalinity (Fig. 13). The average mean value was maximum (755.81 mgl^{-1}) and minimum (723.75mgl^{-1}) MHP and C respectively. Though, overall treatment difference ($F = 39.32$; $P < 0.001$) remained significant differences either between MLP and PP or between MLP and C were insignificant ($p > 0.05$).

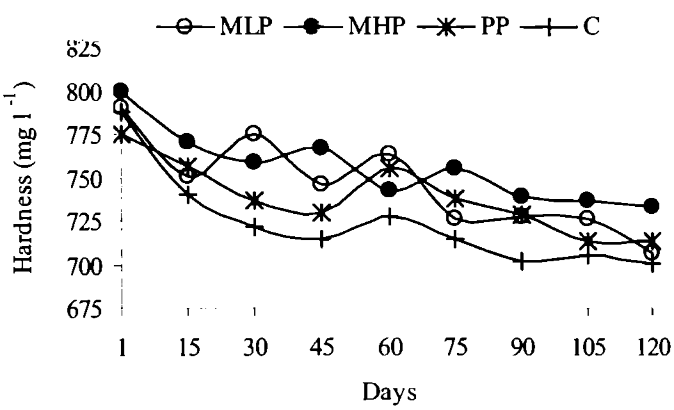


Fig. 13: Temporal changes in the Hardness of water under different treatments employed.

4.2.2. Nutrient parameter

4.2.2.1. Ammonium - N

Ammonium nitrogen in all the treatments tended to increase up to day 60-90 then remained more or less stationary. The maximum mean value (0.266mg l^{-1}) of NH_4-N was encountered in MHP followed by MLP (0.223 mg l^{-1}), PP (0.182 mg l^{-1}) and minimum in control (0.105 mg l^{-1}). The overall treatment difference was significant ($F = 104.45$; $P < 0.01$). Moreover differences between every possible combinations of treatment remain highly significant ($t \geq 4.63$; $P < 0.01$) (Fig. 14).

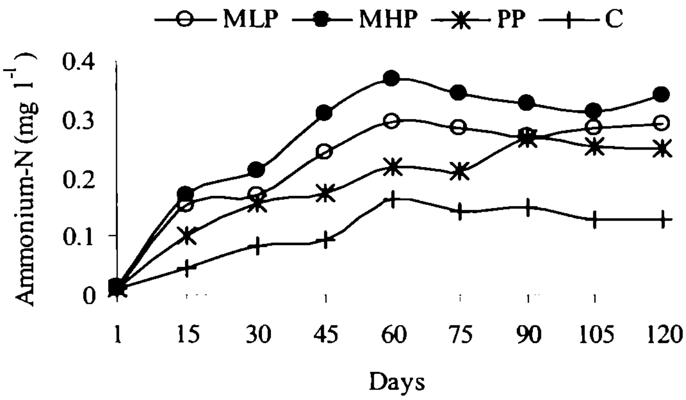


Fig. 14: Temporal changes of ammonium-N concentration of water in different treatments employed

4.2.2.2. Nitrate - N

In general, gradually increasing trend in the values of nitrate nitrogen was observed in all the system employed (Fig.15). The overall mean values in MHP (0.045 mgl^{-1}) was 15.55%, 40%, 55.5% higher than MLP (0.38 mgl^{-1}), PP (0.027 mgl^{-1}), and C (0.020 mgl^{-1}) respectively. One way analysis of variance indicated highly significant difference ($F = 124.39$; $P < 0.001$) among the treatments. Treatment differences between any of the possible combinations also remained significant ($t = 5.12$; $P < 0.001$).

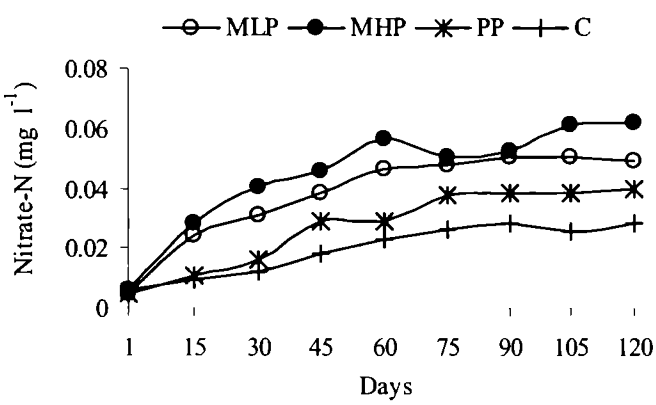


Fig. 15: Temporal changes of nitrate-N concentration of water in different treatments employed.

4.2.2.3. Ortho-phosphate

Temporal trend in the values of ortho-phosphate exhibited an increasing trend up to day 30-45 in MLP and MHP followed by a decline. The values then tended to increase again (Fig.16). In contrast, ortho-phosphate value increased up to day 15 and 60 in C and PP respectively then remained more or less stationary. The overall mean value of ortho-phosphate in MHP (0.167 mgl^{-1}) was marginally higher than MLP (0.155 mgl^{-1}) but 1.62 and 2.23 times higher than PP (0.103mgl^{-1}) and control

(0.075 mgl^{-1}) respectively. Overall treatment differences remain significant ($F = 145.92$; $P < 0.001$). Differences between any pair of treatment also remained significant ($t = 13.98$; $P < 0.001$) except between MLP and MHP ($P > 0.05$) (Fig. 16).

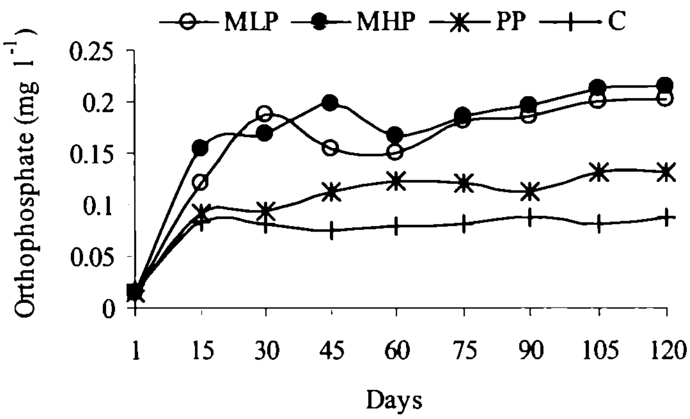


Fig. 16: Temporal changes orthophosphate concentration of water in different treatments employed.

4.2.2.4. Total inorganic nitrogen

Total inorganic nitrogen exhibited in general, an increasing trend in all the treatments during the period of investigation. The maximum mean value 0.31mgl^{-1} in MHP was higher by 16.34 %, 32.47 % and 59.8 % than MLP, PP and C respectively. Overall treatment difference was highly significant ($F = 123.95$; $P < 0.001$), also differences between any of the treatment combinations exhibited strong significance ($t \geq 8.12$; $P < 0.01$) (Fig. 17).

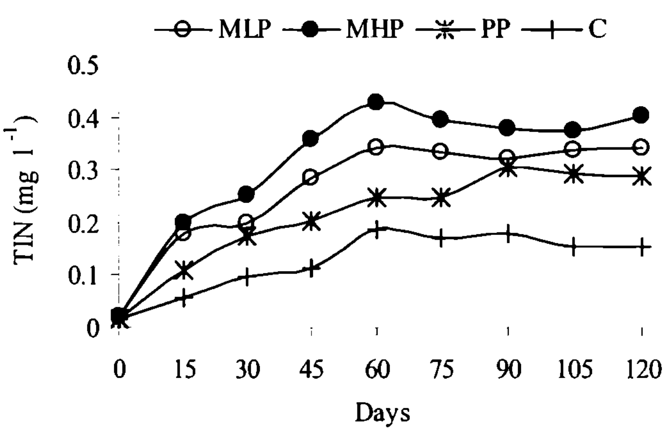


Fig. 17: Temporal changes in the total inorganic nitrogen concentration of water under different treatments employed.

4.2.2.5. N:P ratio

Ratio of total inorganic nitrogen and orthophosphate tended to increase during the 1st half of the investigation followed by a decline in MLP and MHP, whereas such increasing trend continued till day 90 in PP (Fig. 18). Unlike all the nutritional parameter, N: P ratio remained higher in PP (1:1.9) followed by MHP (1:1.8), MLP (1:1.6) and lowest in C (1:0.9). Overall treatment difference in the values of N: P ratio exhibited strong significance ($F = 40.74$; $P < 0.001$). However, differences between any pair of the treatments remained insignificant ($P > 0.05$).

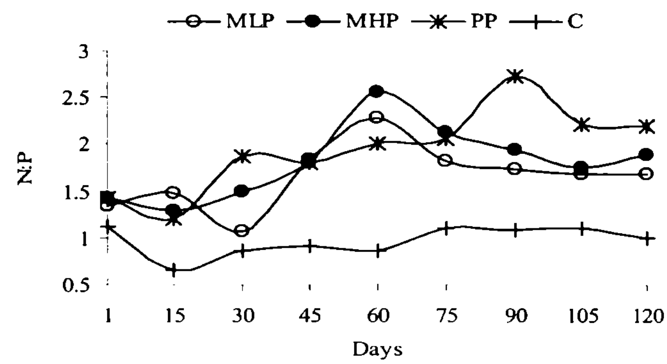


Fig. 18: Temporal changes in the N:P ratio of water under different treatments employed.

4.3. Plankton population

4.3.1. Phytoplankton

Phytoplankton population in all the treatments tended to gradually increase with maximum overall mean of 5036.5 nos. l^{-1} in MHP and minimum in C (3086 nos. l^{-1}). The population in MLP and PP did not differ much and ranged from 4489 to 4607 nos. l^{-1} (Fig. 19). Highly significant differences was observed among the treatments ($F = 85.03$; $P < 0.001$) but difference between MLP and MHP was not significant ($P > 0.05$).

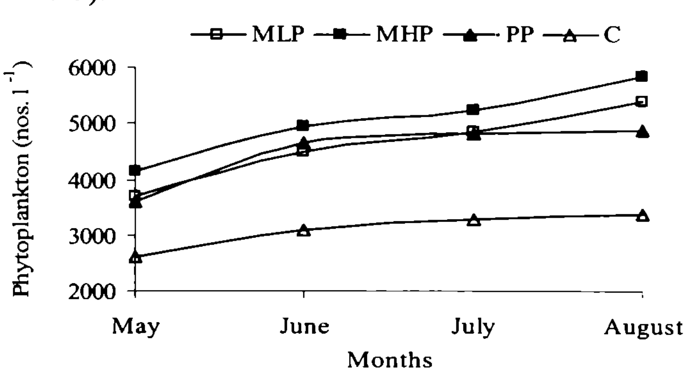


Fig. 19: Temporal variation of total phytoplankton in different treatments employed.

Phytoplankton populations in the treatments were composed by the members of Chlorophyceae (*Clostridium, Volvox, Pediastram, Chlorella,* and *Spirogyra*), Bacillariophyceae (*Cyclotella, Diatom, Synedra* and *Navicula*), Euglaenophyceae (*Phacus*) and Cyanophyceae (*Oscillatoria, Microcystis, Anabaena, Scenedesmas, Cosmerium* and *Chlamydomonas*). However, the relative response of different groups in different treatments was somewhat variable. In all the treatments Chlorophyceae dominated by 1610 to 2756 over the rests and exhibits an increasing tendency (Fig. 19). Overall differences among the different groups of phytoplankton in all the treatments remained insignificant ($P > 0.05$). Differences between Cyanophyceae and Bacillariophyceae remained insignificant ($P > 0.05$) in all the treatments except in MHP ($P < 0.05$).

Temporal changes in percentage distribution of different groups exhibited declining abundance of Chlorophyceae in MHP and PP with the population gradually gaining importance with Bacillariophyceae and Cyanophyceae (Fig. 20). However, in MLP and C phytoplankton abundance remained more or less stationary with minor variations in the other groups (Fig. 20).

4.3.2. Zooplankton

Likewise phytoplankton, zooplankton population in all the systems tended to increase but the magnitude was more pronounced in MHP (Fig.21). The overall mean value of zooplankton was highest (5189 nos.l^{-1}) in MHP followed by MLP (3814 nos.l^{-1}), PP (2093nosl^{-1}) and C (1154 nos.l^{-1}). Differences remained significant ($F = 56.60$; $P < 0.001$) among the treatments. Though, copepods dominated the populations in all the treatment, differences among different groups the zooplankton population in all the treatments remained insignificant ($P > 0.05$).

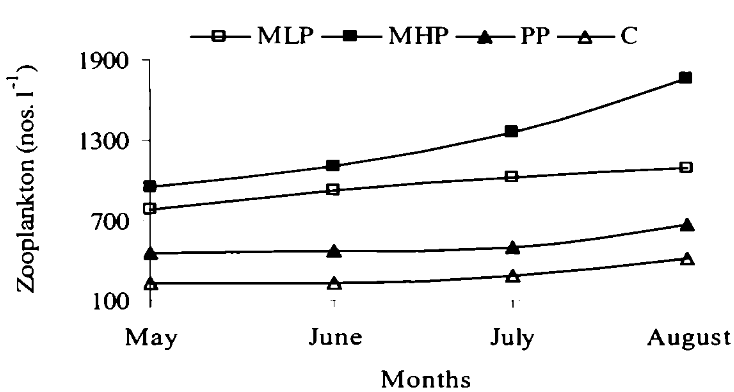


Fig. 21: Temporal variation of total zooplankton in different treatments employed.

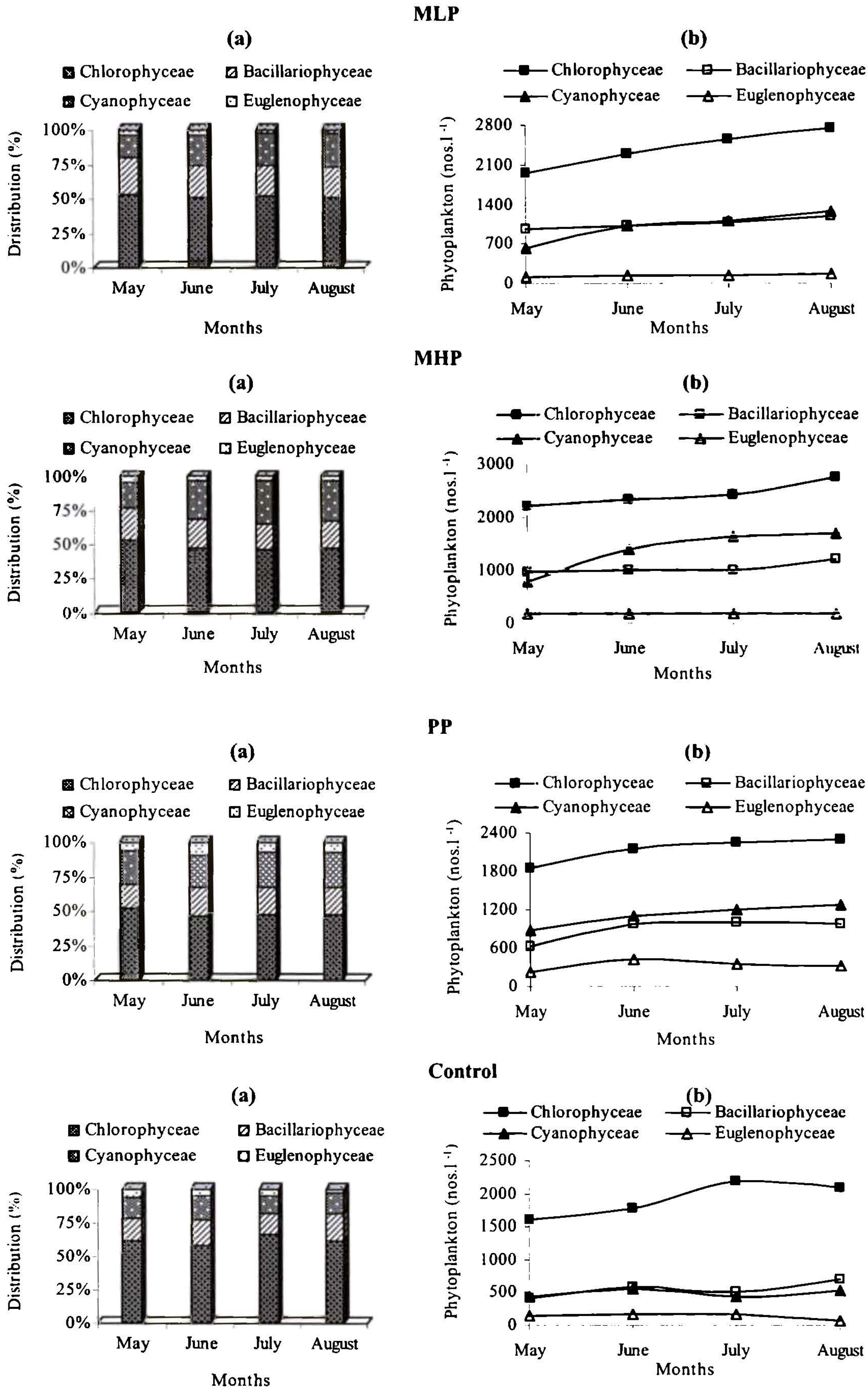


Fig. 20: Distribution (%) of different groups of phytoplankton (a) and their temporal trends (b) in different systems employed.

Copepods gradually declined in all the treatments except in control with corresponding increase of cladocerans (40% and 55.67%) both in MLP and PP and rotifers (85.23 %) in MHP respectively (Fig. 22).

4.4. Primary production

4.4.1. Gross primary production (GPP)

Gross primary production exhibited increasing trend in all the treatments with maximal mean value of 2.22 g C $m^3 day^{-1}$ in MHP which was 8.18 to 39.54% higher than the rest of the treatments (Fig. 23). Differences in the values of GPP remained highly significant ($F = 58.40$; $P < 0.001$). However, such differences either between MLP and MHP or between PP and C remained insignificant ($P > 0.05$) (Fig. 23).

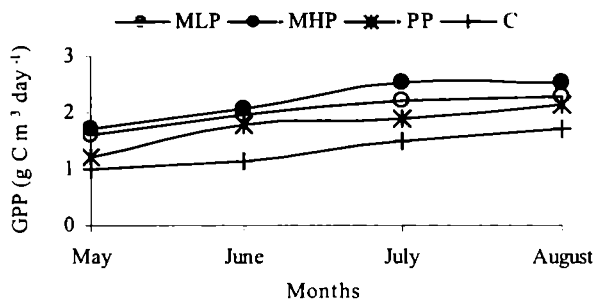


Fig. 23: Temporal variation of gross primary production in different treatments employed.

4.4.2. Net primary production (NPP)

Temporal changes in the values of NPP remained identical to those of GPP in all the treatments (Fig. 24). Likewise GPP, NPP value was maximum (1.48 g C $m^3 day^{-1}$) in MHP followed by MLP (1.35 g C $m^3 day^{-1}$), PP (1.19 g C $m^3 day^{-1}$) and C (1.04 g C $m^3 day^{-1}$). The overall difference ($F = 36.81$; $P < 0.05$) as well as paired t-test for any combination of treatments remained significant ($t \geq 5.48$; $P < 0.01$).

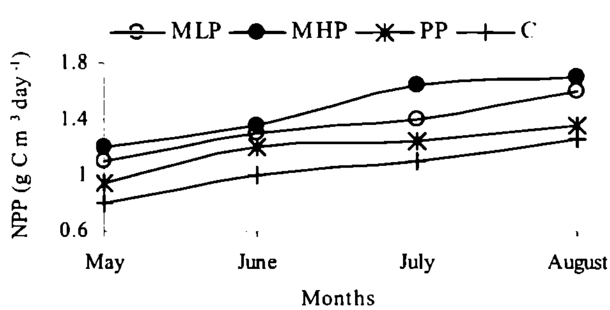


Fig. 24: Temporal variation of net primary production in different treatments employed.

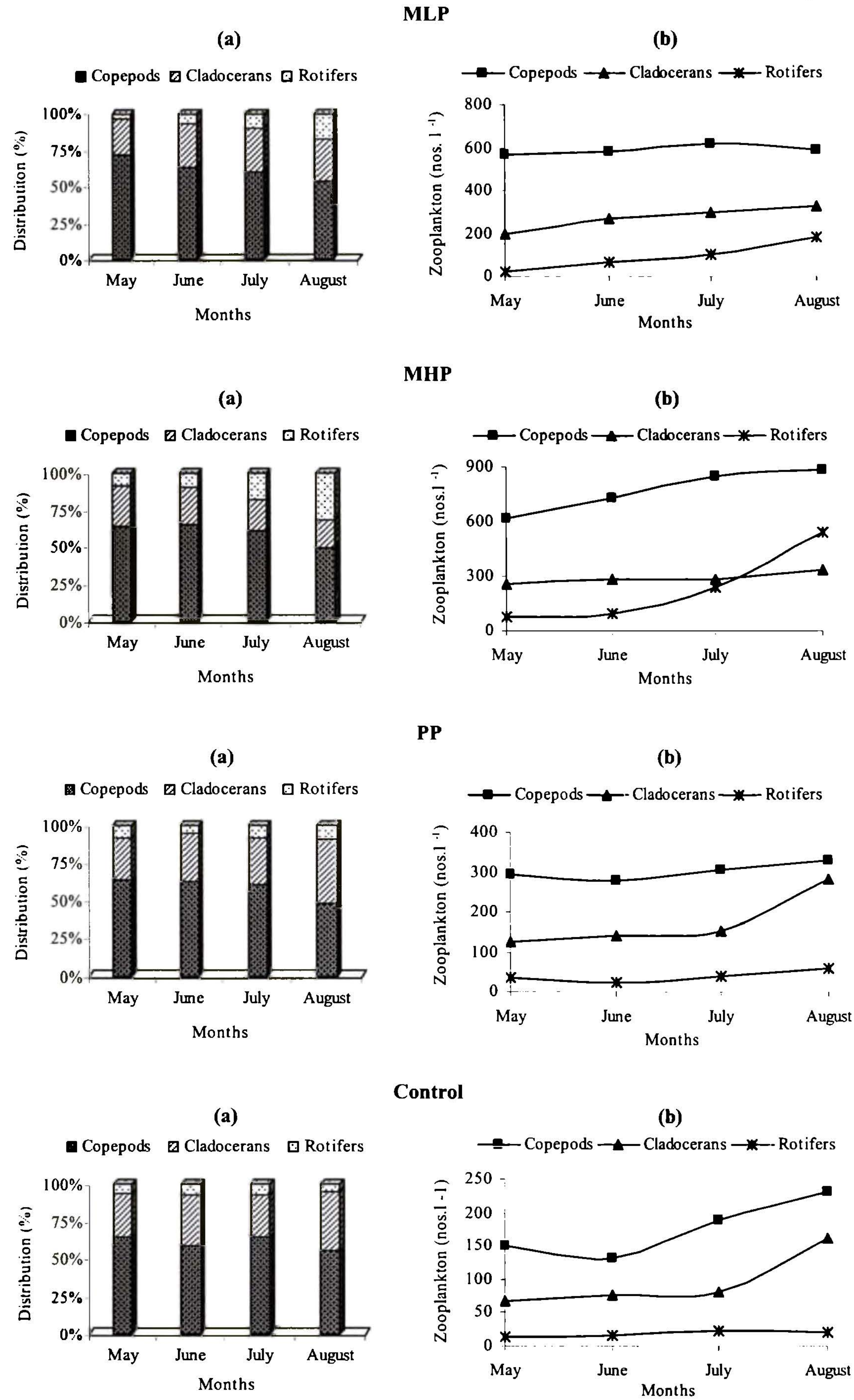


Fig. 22: Distribution (%) of different groups of zooplankton (a) and their temporal trends (b) in different systems employed.

4.4.3. Community respiration (CR)

The relative response of CR over time was identical to that of NPP. Treatment difference was significant (F = 16.63; P <0.01) with maximum value of CR in MHP (0.74 g C m^3day^{-1}) which however was not significantly different (P > 0.05) from MLP (0.72 g C m^3day^{-1}). The overall mean value of PP and C remained 0.58 g C m^3day^{-1} and 0.4 g C m^3day^{-1} respectively (Fig. 25).

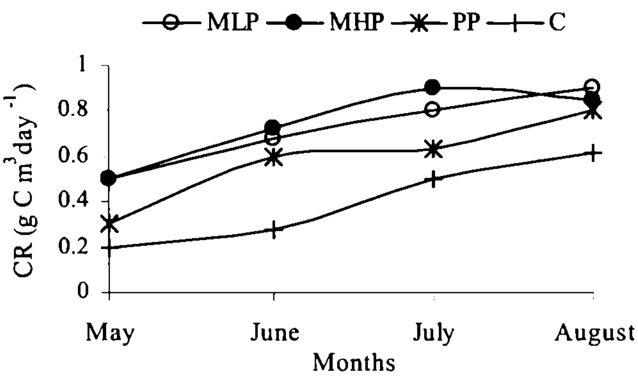


Fig. 25: Temporal variation of Community Respiration in different treatments employed.

4.5. Soil quality

4.5.1. pH

Soil pH ranged from 7.89 to 7.36 in the treatments employed during the period of investigation. A gradually declining trend was observed in all the treatments but pH values remained more or less stationary in C (Fig. 26). Overall treatment difference remained significant (F = 27.18; P < 0.001) with insignificant differences between MLP and MHP (P > 0.05).

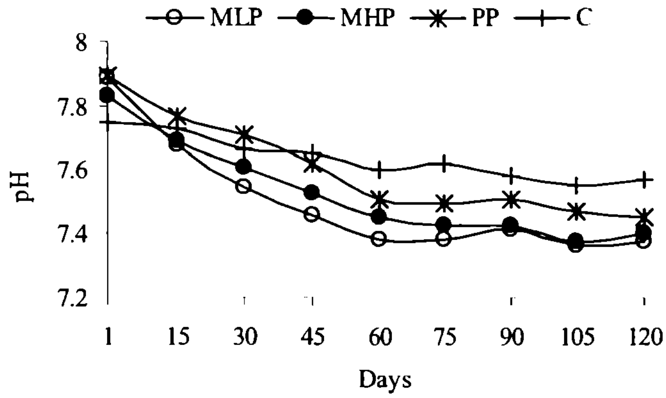


Fig. 26: Temporal changes in the pH of soil under different treatments employed.

4.5.2. Organic carbon

Organic carbon of soil tended to increase in all the treatments employed (Fig.27). The maximum (2.78 mg g^{-1}) and minimum (0.8 mg g^{-1}) mean values were observed in MHP and C respectively. Significant difference was observed among the treatments (F = 68.80; P < 0.001), however differences between MLP and MHP, PP and C remained insignificant (P > 0.05).

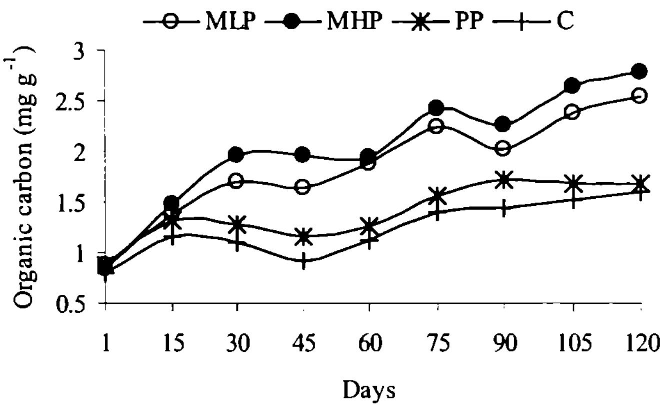


Fig. 27: Temporal changes in the organic carbon of soil under different treatments employed.

4.5.3. Available phosphorus

Available phosphorus of soil tended to increase during the initial phase after which it gradually declined (Fig. 28). The overall mean value of available phosphorus was highest in MHP (0.78 mg g^{-1}), followed by MLP (0.65 mg g^{-1}), PP (0.52 mg g^{-1}) and C (0.44 mg g^{-1}). Treatment differences remained highly significant (F = 105.03; P < 0.001).

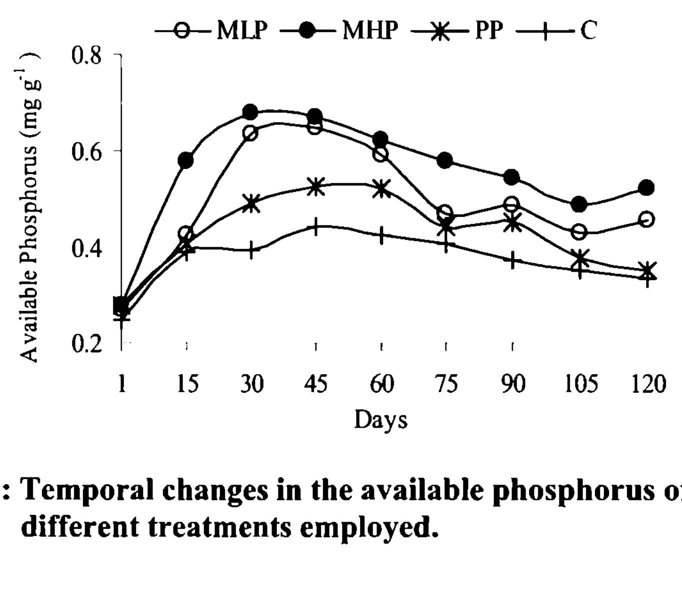


Fig. 28: Temporal changes in the available phosphorus of soil under different treatments employed.

CHAPTER 5

DISCUSSION

The results of the study clearly indicated that the weight increment of piranha (*Pygocentrus nattereri*) under monoculture condition was a function of protein supplementation either through artificial feeding or through live feeding (Fig. 2). As a result, under controlled condition where the test fish was forced to thrive only upon the autochthonous food particles, the growth was minimum. By subtracting the growth because of autochthonous food particles in the control system, the resultant growth because of supplementation of low dose protein (40%), high dose protein (50%) and protein through live feed exhibited a highly significant direct relationship (Y = 9.25x-4.23) and such relationship was explained by 99% (Fig. 29).

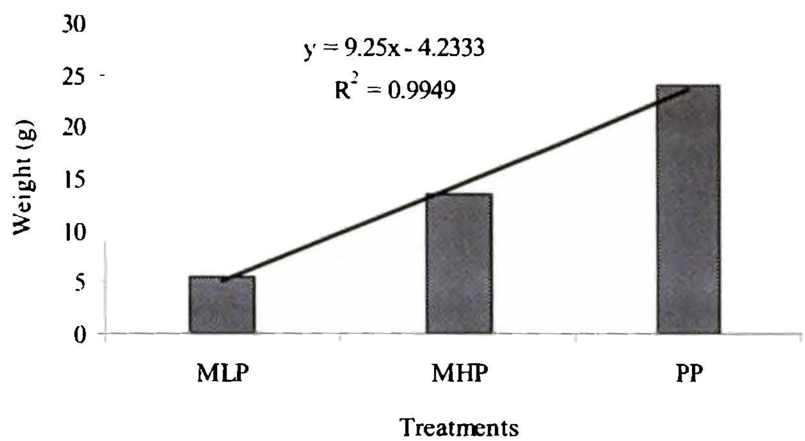


Fig. 29: Relationship between weight gain out of feed (g) with variability of protein in different treatments.

Again, supplementation of 10% more protein in the diet in MHP resulted in 60% higher growth compared to MLP. But the maximum growth which was attributable to the supplementation of live feed as prey in PP was indicative of the superiority of the protein quality and compliance with the natural instinct of the test fish as well. A number of piranhas belonging to genus *Pygocentrus* are voracious predators (Pauly, 1994), opportunistic carnivores (Lopes *et al.*, 1991) whose diet includes whole fish and fragments of fish flesh, fins and scales (Almeida *et al.* 1994). Different piranha species select prey species in different proportion and that the differences are associated with the body form and swimming behavior of predator and prey (Winemiller, 1993). In the present investigation, tilapia (*Oreochromis mossambicas*) belonging to cichlids was used as prey for the test fish which again has been supportive for attaining its best growth. Because cichlids were the group most frequently attacked by piranha reported in several studies (Northcote *et al.*, 1987; Winemiller and Kelso-Winemiller, 1993). Also piranhas are mutilating predators (Agostinho, 2001) as established in the present investigation. Mutilated tail of tilapia (Plate. 2) provided to the test fish as prey supported the above faith.

The suitable feeding regime in predator prey combination was again confirmed as the fortnightly weight gain was continuous upto day 75 in PP (Fig. 3). But in case of MLP and MHP weight gain in general, continued upto day 60 and finally a decreasing trend was observed (Fig. 3). Therefore, it was evident that live feed supplementation supported the weight gain as well as growth rate of the test fish in much better way. Also, the highest survival rate of the fish in this treatment was because of the better food quality in the form of tilapia fry (Fig. 8). Live tilapia early fry as prey was best fitted to the piranha because as carnivorous it has short gut (Jegu and Dos Santos, 1985) which was suitable for digestion of animal matter.

Fortnightly growth rate as well as specific growth rate sharply declined in all the treatments as the test fish grew older and the relationship established as $y = 204.55x^{-0.0272x}$ and $y = 5.8775e^{-0.0212x}$ respectively (Fig. 30, 31). Also such relationship was explained with much higher percentage (89-98%). It indicates that as the body weight increased over time, relative rate in weight gain hereby growth rate decrease.

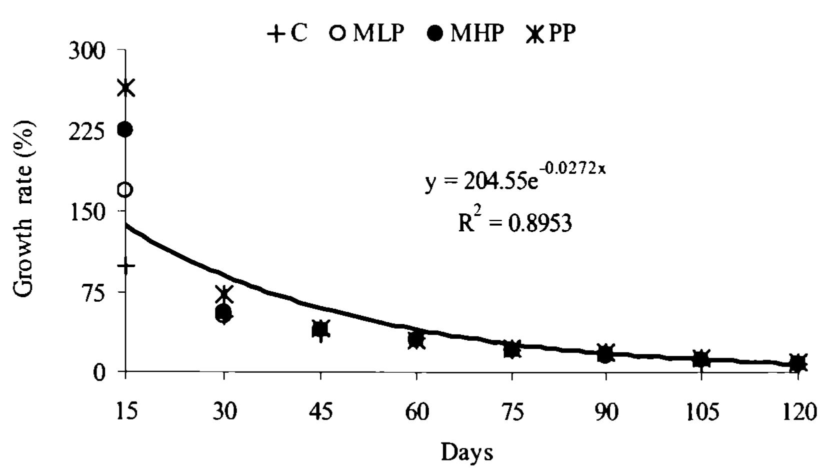
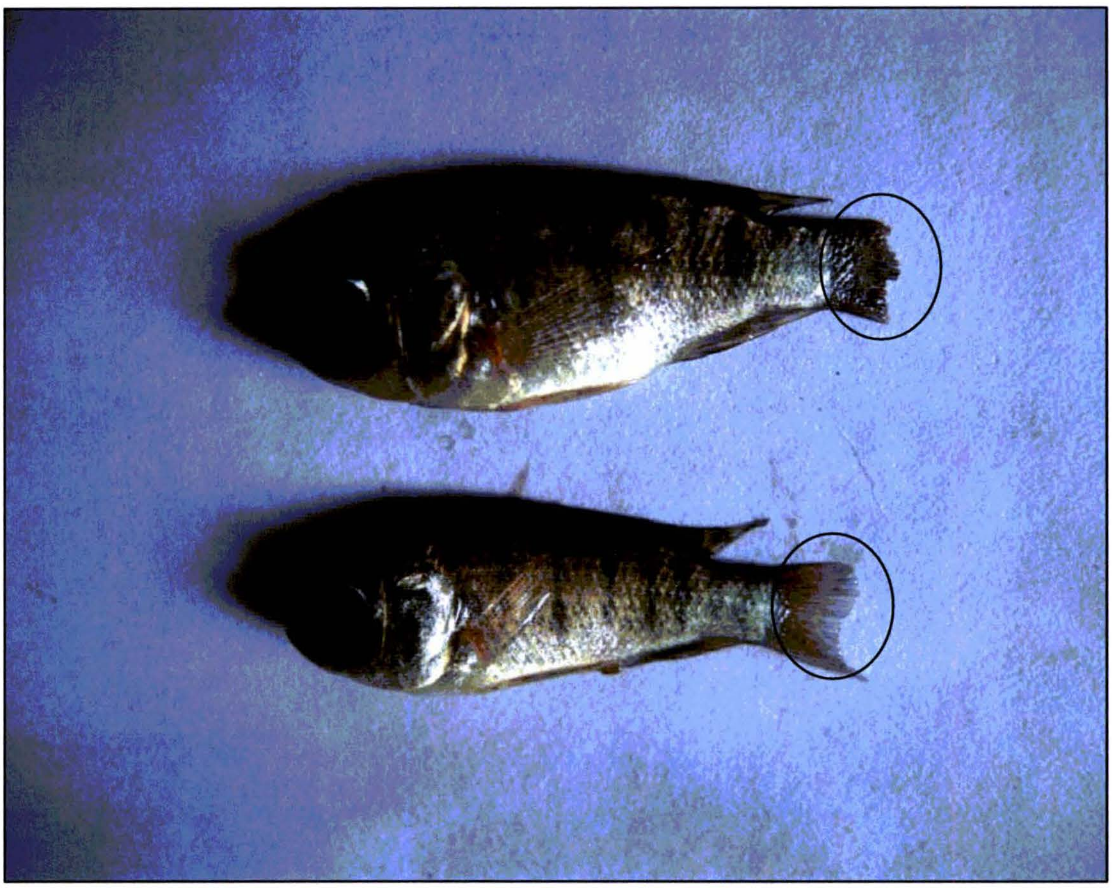


Fig. 30: Modular relationship between growth rate (%) with period of investigation (days) in different treatments.

PLATE- 3



Tail of tilapia eaten by piranha during the experiment

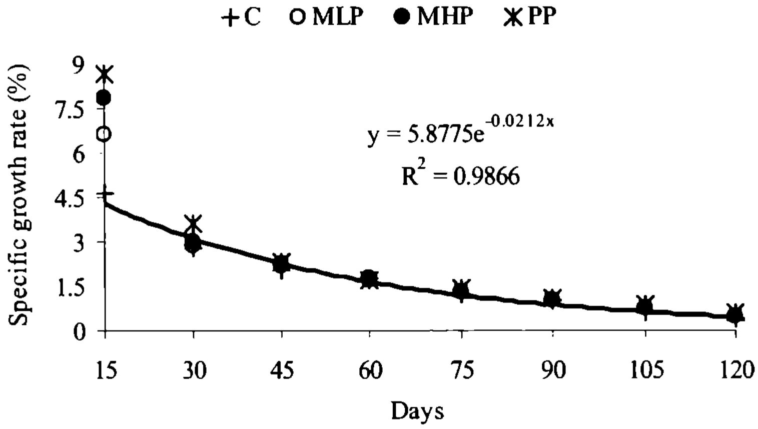


Fig. 31: Modular relationship between specific growth rate (%) with period of investigation (days) in different treatments.

A 10% variation in the protein percentage of supplementary diet in MLP and MHP did not contribute to the feed conversion ratio (FCR) (Fig. 6). In both the cases as the fish became older, the FCR becoming higher. This implies that culture of red bellied piranha with supplementary feeding beyond 60 days will not be economical when the FCR value was around 2.0 under pond based culture system (Fig. 32). Stickney (2005) considered 1.5 to 2.0 as good FCR for most aquatic organisms and for carnivorous fish like catfish the FCR is around 1.8 (De Silva and Anderson, 1995).

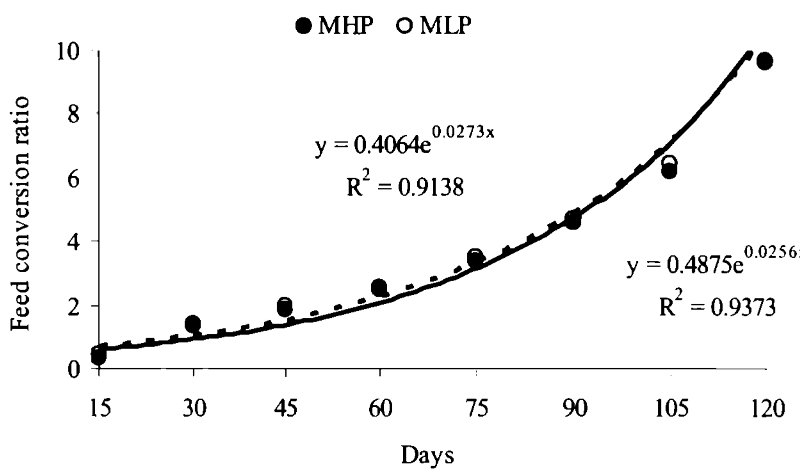


Fig. 32: Relationship between feed conversion ratio and period of investigation in different treatments.

Again, changes in protein percentage did not affected protein efficiency ratio (PER) (Fig. 7) in MLP and MHP. As PER is indicative of the protein quality (De Silva and Anderson, 1995) and because in low and high dose (MLP and MHP) the ingredients used for protein supplementation, PER did not varied significantly. PER declined exponentially both in MLP and MHP with age (Fig. 33).

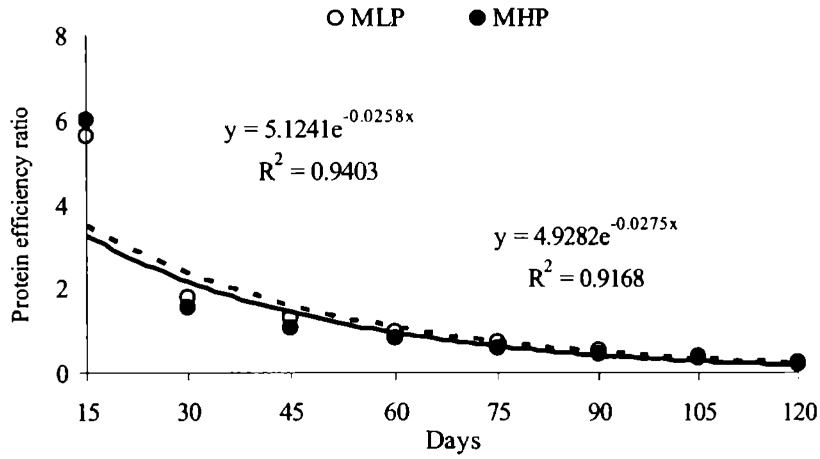


Fig. 33: Relationship between protein efficiency ratio and period of investigation in different treatments.

It is assumed that requirement as well as metabolism of protein by the test fish was inversely related with age. De Silva and Anderson (1995) opined that protein requirement of fish diminish as the fish grows older.

Supplementation of artificial feed relatively with high protein content either in MLP (40%), MHP (50%) or in PP (live protein) significantly contributed in the nutrient profile both of water and soil. As a result, all the nutrient parameters of water (Fig. 14, 15, 16, 17, 18) and soil (Fig. 27, 28) tended to increase as the culture period progressed. This is because of the nitrogenous metabolites as well as the degradation products of the unused feed in all the systems employed. The relatively higher concentration of nutrients particularly the nitrogenous materials in MHP was because of the higher supplementation of protein thereby higher supplementation of nitrogen in the feed. The metabolic nitrogenous load in the system as a result of high protein supplementation is well documented (Goddard, 1996).

Feeding piranha either with supplementary feed or with live feed not only contributed directly to fish growth but also favoured physiological as well as biological condition of the systems which is attributable to the fact that both phytoplankton and zooplankton community magnified significantly in the treatments compared to the control (Fig. 19, 21). Again, this is confirmed from the fact that both gross primary productivity and net primary productivity (Fig. 23, 24) in the systems provided with feed supplementation was significantly higher.

However, such favourable physiochemical and biological conditions towards growth were better established in the supplementary diet with artificial feed rather than feeding with live feed. This might be because of the more intensity of uneaten feed loss as well as mineralization of the uneaten feed in MLP and MHP.

The positive correlation of all the nutrient parameters of the systems with fish growth (Table. 2) in all the treatments excepting control is again supportive of the fact that the feed supplementation besides directly contributing to the fish growth supported the systems indirectly through supplementation of available nutrients towards autochthonous production mediated principally through primary production.

Among the other physicochemical parameters the relationship between fish growth and dissolved oxygen was found to be positively correlated in all the systems. Therefore, it may be concluded that in all the treatments employed DO remained within the congenial range (6.59 to 7.05 mg l^{-1}) which was supportive of fish growth. Boyd (1982) opined that dissolved oxygen of water should be (5 to 8 mg l^{-1}) for better fish growth.

Table: 2. Correlation between fish growth and selective physico-chemical parameters in different systems.

MLP

	Temp	pH	Alkalinity	Hardness	DO	NH_3-N	NO_3-N	TIN	Ortho-P	N:P	Soil pH	SOC	SAP	Fish growth
Fish growth	0.83[b]	0.74[a]	0.92[c]	-0.82[b]	0.98[c]	0.85[b]	0.93[c]	0.87[b]	0.77[a]	NS	0.85[b]	0.95[c]	NS	1

MHP

	Temp	pH	Alkalinity	Hardness	DO	NH_3-N	NO_3-N	TIN	Ortho-P	N:P	Soil pH	SOC	SAP	Fish growth
Fish growth	0.72[a]	NS	NS	-0.89[c]	0.88[b]	0.75[a]	0.91[c]	0.79[b]	0.85[b]	NS	0.94[c]	0.94[c]	0.76[a]	1

PP

	Temp	pH	Alkalinity	Hardness	DO	NH_3-N	NO_3-N	TIN	Ortho-P	N:P	Soil pH	SOC	SAP	Fish growth
Fish growth	0.71[a]	NS	0.94[c]	-0.78[a]	0.98[c]	0.93[c]	0.93[c]	0.94[c]	0.88[b]	0.79[b]	0.93[c]	0.85[b]	NS	1

C

	Temp	pH	Alkalinity	Hardness	DO	NH_3-N	NO_3-N	TIN	Ortho-P	N:P	Soil pH	SOC	SAP	Fish growth
Fish growth	0.78[a]	0.97[c]	0.88[b]	-0.88[b]	0.86[b]	NS	NS	0.79[b]	NS	0.84[b]	0.95[c]	0.84[b]	NS	1

TIN= Total inorganic nitrogen
SOC= Soil organic carbon
SAP= Soil available phosphorus

[a] Level of significant = 5%
[b] Level of significant = 1%
[c] Level of significant = 0.1%

CHAPTER 6

SUMMARY

The comparative growth performances of red bellied piranha (*Pygocentrus nattereri*) with different feeding regimes with respect to qualitative and quantitative protein inputs have been studied under controlled conditions of outdoor experimental tanks for a period of four months. The whole work embodied in this thesis has been documented in seven chapters viz. Introduction, Review of Literatures, Materials and Methods, Results, Discussion, Summary and References. The main findings of this investigation is summarized herein:

1. The maximum growth of the test fish (*Pygocentrus nattereri*) was observed in predator-prey (PP) combination system, which was attributable to the better protein quality through supplementation of live early fry of tilapia (*Oreochromis mossambicus*) as prey.
2. Compliance with its natural feeding habit as predominantly carnivore by providing suitable prey (cichlids) was supportive of it's better survival rate and growth in predator-prey (PP) combination system.
3. Supplementation of 10% more protein in the diet in MHP resulted in 60% higher growth compared to MLP.
4. Monospecies culture of Red Bellied Piranha with supplementary feeding beyond 60 days was not found to be judicious when the FCR value was around 2.
5. Supplementation of artificial feed relatively with high protein content either in MLP (40%), MHP (50%) or in PP (live protein) significantly contributed to the nutrient profile both of water and soil as all the nutrients significantly increased over time.
6. Feeding piranha either with supplementary feed or with live feed not only contributed directly to fish growth but also favoured physicochemical as well as biological conditions of the systems enhancing primary production.
7. As this course bound study was conducted with several limitations, intensive investigations are needed to compare the results under natural pond culture conditions.

CHAPTER 7

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Annexure - 1

GOVERNMENT OF INDIA

Dr.A.K.Karmakar
Office-in-Charge
Freshwater Fish Section

Zoological Survey of India
27,Jawharlal Nehru Road
Kolkata – 700 016

Ref. No. F. 235-1/98-Fish/

Date: 01.05.2009

To
Dr. S. K. Das, Reader
Department of Aquaculture
Faculty of Fishery Science
West Bengal University of Animal and Fishery Sciences
P. O. Panchasayar, Kolkata-700 094.

Sub : Identification Report

Dear Sir,

With reference to your letter dated 08.04.2009, I am furnishing herewith identification report of the live fish specimen send by you through your student.

Class : Actinopterygii
Order : Characiformes
Family: Characidae
Sub-family : Serrasalminae

Pygocentrus nattereri Kner, 1858 ....1 ex.

You are requested to kindly acknowledge the receipt of the same.

Thanking you,

Yours sincerely,

A. K. Karmakar

(AK. KARMAKAR)