HUSBANDRY FACTORS AFFECTING SURVIVAL AND GROWTH OF CARP (CYPRINUS CARPIO L.) FRY AND AN EVALUATION OF DIETARY INGREDIENTS AVAILABLE IN BANGLADESH FOR THE FORMULATION OF A CARP FRY DIET.

Thesis submitted to the University of Stirling

for the degree of Doctor of Philosophy

by

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Abstract

In an effort to make a positive contribution to improving nursery management, some key husbandry and water quality requirements of common carp fry were investigated. Investigations were also conducted to evaluate some dietary ingredients available in Bangladesh for the formulation of a complete diet for carp fry.

Studies on the effect of temperature and feeding rate conducted in laboratory recirculated water systems, showed that within a temperature range of 24 to 35°C, 32°C was optimal for growth and food conversion of carp fry receiving a feeding rate of 20-30% body weight/day. It was also observed that maximum growth occurred at feeding rates of 20-25%, 25-30% and 30% BW/day at rearing temperatures of 24, 28 and 32-35°C respectively.

It is suggested, based on the experimental results, that in a recirculated water system, with a water temperture of 28°C and under fully oxygenated conditions, a stocking density of 7-11g fish/litre can be maintained without any adverse effect on growth and food conversion provided the fish receive a feeding rate of 15-25% BW/day.

In an experimental evaluation of mustard, sesame, linseed, copra, leucaena and groundnut meals as dietary protein source for carp fry

it was found that linseed followed by groundnut and sesame meal gave the most promising results. When a combination of different plant protein sources was used, linseed, groundnut and sesame meals, in various combinations up to a level representing 60% replacement of fish meal protein, proved suitable in the formulation of a complete diet for carp fry.

Experimental investigations on the water quality requirements of carp fry showed that fish of size range 206-299 mg were fairly tolerant to un-ionised ammonia, the 96-h median lethal concentration (LC50) value ranged between 1.74 and 1.85 mgl^{-1} NH_3-N. Nitrite tolerance of carp fry was found to be highly variable depending on the concentration of chloride and pH of water; increased tolerance was observed with increased chloride concentration and pH of water. Nitrite tolerance , however, did not vary for carp fry weighing between 75 and 450 mg. Carp fry were found to be highly tolerant to nitrate toxicity; only concentrations of 1000 mgl^{-1} NO_3-N and above proved lethal.

The temperature tolerance of carp fry at different acclimation temperatures was also investigated. The results showed that carp fry have a high incipient lethal temperature and can withstand a large fluctuation of temperature; the upper incipient lethal temperature varied between 33.5 and 38.8 ^oC for acclimation temperatures in the range 16 to 34^oC.

The results of this study are discussed in relation to previous research publications on carp fry and to the practical problems of carp culture in Bangladesh.

CHAPTER 1

General Introduction

1.1. Role of fish culture in the developing world

In many developing countries, protein-energy malnutrition constitutes a major public health problem. Population pressure in these countries has severely depleted the traditional wild source of animal protein, e.g. fish in many parts of Asia (Davey and Wilson, 1971, cited by Edwards et al, 1983). Fish captured from the wild or harvested from traditional pond culture systems are no longer sufficient to meet the ever increasing demand for fish.

For example, over the last fifteen years, per capita consumption of fish in Bangladesh has declined by about 30%. The present production of fish in Bangladesh is estimated to be 751,000 tonnes a year, but it must be increased to 1,126,000 tonnes by the year 2005, merely to sustain per capita consumption at the present level (FAO, 1985).

Increasing fish production through intensification of aquaculture is widely regarded as one very effective means of reducing protein-energy malnutrition in many of the developing countries. But this will require improvements in applied scientific, technological and management skills and a thorough understanding of fish under intensive culture conditions.

Aquaculture research in the developed world has generated a significant amount of information on the physiological and nutritional requirements of fish under intensive culture conditions.

However, most of this research has been directed towards the salmonid species of Europe and North America and the American channel catfish (Ictalurus). Similar information for warm water, non-salmonid species is patchy, despite the fact that warm water species comprise the major group of farmed fishes in the world. Thus, there is strong justification for conducting research on the physiological and nutritional requirements of warm water species.

1.2. Aquaculture of common carp

The common carp, Cyprinus carpio Linnaeus, 1758 is, on a world-wide basis one of the most extensively cultivated of all fish species (Bardach et al, 1972; Jhingran, 1977). It is considered to be the principal cultivated warm-water fish species (Chittino, 1972).

The common carp seems to have originated in central Asia and spread to both east China and north west Europe (Balon, 1974, cited by Hepher and Pruginin, 1981). It was introduced to all the Southeast Asian countries between 1914 and 1957 and is now cultured throughout the region (Bardach et al, 1972). It is also of major importance in Japan and Israel and the countries of southern Asia (Bangladesh, India, Nepal, Pakistan and Sri Lanka).

The common carp has been farmed in central and northern Europe from the twelfth century onwards, where it has become the most important

farmed species (Meske, 1985). In eastern Europe, particularly in the Soviet Union, Poland, Czechoslovakia, Hungary and Yugoslavia - carp production is a major aquaculture industry.

1.3. Aquaculture in Bangladesh

1.3.1. Background

Fish and fisheries have always played an integral part in the economy, culture and tradition of the people of Bangladesh. Fish is the primary source of animal protein in the diet throughout the country comprising about 87% of the animal protein supplied (Mannan, 1977). The contribution of the agricultural sector as a whole to the country's gross domestic product is about 55% (Bangladesh Times, 1986), of which about 6% comes from fisheries (Yearbook of Agricultural Statistics of Bangladesh, 1985). It is generally estimated that more than 5% of the country's population is supported by fisheries and directly related activities (FAO/UNDP, 1986).

Bangladesh has excellent fishery potential because of its vast water resources. Inland water bodies of Bangladesh are highly productive and contribute more than 80% of the total fish production (Yearbook of Agricultural Statistics of Bangladesh, 1985). An estimate of the inland water resources together with their fish production potential is given in Table 1.1. In addition to the inland waters, Bangladesh

Table 1.1 Estimated inland water resources of Bangladesh with fish production potential*

Water resources	Area (hectares)	% of total inland water resources
Riverine resources		52.3
Rivers, creeks and canals	1 000 000	
Flood plains	2 800 000	
Total	3 800 000	
Small fresh water bodies		45.6
Depressions	270 000	
Ponds	158 000	
Paddy fields	2 880 000	
Total	3 308 000	
Large fresh water bodies		1.5
Oxbow lakes	21 000	
Lake Kaptai	90 000	
Total	111 000	
Brackish water	42 000	0.6
Grand Total	7 261 000	

* Adapted from FAO/UNDP (1986)

has a 480 kilometre long coast line and approximately 1 million hectares of territorial waters.

The freshwater fish fauna of Bangladesh is well developed in both variety and abundance. Inland waters of Bangladesh are inhabited by 257 species of fish (Rahman, 1974). Other aquatic animals of direct economic importance include several species of freshwater shrimp (Macrobrachium) and crabs, two species of pearl producing mussels (Lamellidens and Perreysia), one species of frog (Rana tigrina) and some turtles (Trionyx) (Karim, 1978).

The fish fauna includes the following prinicipal commercial species: Indian major caprs (Labeo rohita, Catla catla, Cirrhinus mrigala and Labeo calbasu), Indian river shad (Hilsa ilisha), several catfish (Mystus, Clarias, Pangasius, Rita, Heteropneustes, Ompok), freshwater shark (Wallago attu), climbing perch (Anabas testudineus), feather back (Notopterus), snakehead (Channa), several medium and minor carps (Tora,Puntius) and freshwater eels (Mastacembalus).

Fish farming has been a traditional practice in Bangladesh dating back several centuries when the country was ruled by Hindu Kings. But fish farming remained unimportant because wild fish were so abundant.

During the last decades however, over fishing and the increasing control of flood waters with poulders has depleted the natural stock

substantially. Production statistics suggest that total fish production in Bangladesh has declined by at least 10% since 1977 despite an almost two fold increase in the marine fish catch (Yearbook of Agricultural Statistics of Bangladesh, 1985). At the same time the demand for fish has increased because of the rapidly expanding population. The present situation is very serious. Per capita consumption of fish has declined because of poor supply and high prices, yet by the year 2000 the population is expected to be increased to 140 million, about 30-40 million more than its present level. This situation has generated renewed interest in the potential of fish culture in the country. It has been realised that intensification of aquaculture together with scientific management of the existing open water fishery resources would be the most effective means of increasing fish production to counter the worsening animal protein shortage situation.

1.3.2. Farming of major carps and its constraints

Of the many native fish species in Bangladesh suitable for aquaculture, the most commonly farmed are the Indian major carps rohu (Labeo rohita), catla (Catla catla)and mrigal (Cirrhinus mrigala). These are preferred because of their relatively fast growth, efficient herbivorous/detritivorous feeding habits and their high acceptability to the consumer public.

However, the major carps are difficult to breed in captivity and the

supply of fish seed for farming still relies heavily on recruitment of juveniles from rivers. Sexually mature fish spawn during the monsoon when the rivers are in flooded condition (April-July). Nearly 95% of the fish seed demand is met from natural spawn collection, but the spawn collected is often a mixture of desirable and undesirable species which cannot be recognised and separated at such an early stage. Culture systems based on the capture of wild stock, are, also ultimately limited by the reproductive output of the natural population. Supplies of carp spawn from the riverine spawning grounds of Bangladesh have declined significantly during the past years. This is probably due to deterioration of their spawning habitats through modification of river courses, barraging of the rivers, irrigation and embankment projects, river pollution and increase use of agricultural pesticides.

To reduce the dependence on the natural spawn, induced breeding techniques have been practiced in Bangladesh since the early 1970s. (Haque, 1974). Although there has been considerable success in the artificial propogation of major carps, production of fry through this technique has largely been limited to a few of the successful fish hatcheries established by the Government of Bangladesh (K. Khan, pers. comm.). Mass production of induced bred fry has not been achieved primarily due to the shortage of fish pituitary glands and high cost and unavailability of other mammalian and synthetic hormones.

1.3.3. Farming of common carp and its constraints

During the 1960s and early 1970s, common carp (scaled variety - 'Bangkok' strain and mirror scaled variety - Dinnyes' strain from Hungary) were introduced for culture in Bangladesh on a limited basis. Since its introduction, the common carp has gained wide acceptance throughout the country for the following reasons:

1. It is easy to spawn
2. It can spawn throughout the year
3. It has a fast growth rate
4. It can tolerate a wide range of environmental conditions
5. It is acceptable as a food fish to the consumer public
6. It can grow along with major and Chinese carps in composite farming, with the advantage that each species utilizes a different ecological niche in the pond (Chaudhuri, et al, 1975; Jhingran, 1977).

In Bangladesh, common carp are allowed to breed in ponds where they attach their eggs to grasses, aquatic weeds or any other suitable substrates. However, survival of larvae from what are 'semi-wild' spawnings is very low. To achieve better survival, special breeding techniques are now followed. Broodfish are kept in breeding hapas containing submerged or floating aquatic weeds such as Hydrilla, Najas or water hyacinth for use as a substrate for the attachment of eggs. The fertilised eggs attached to the egg collectors are then transferred to a hatching 'hapa' or tank. The incubation period is

about 48 hours at a water temperature of 27-31°C.

Hatching hapas (rectangular cloth cages) are normally maintained in the pond, without any artificial aeration or circulation of water. Mortality can occur during incubation due to a sudden rise or fluctuation in water temperature, the presence of algal blooms producing oxygen bubbles during photosynthesis which bring eggs to the surface, or poor oxygenation inside the hapa (Chaudhuri, 1983). The destruction of hapas due to adverse climatic conditions is also a commonly encountered problem by outdoor hatcheries.

Recently indoor hatcheries have been developed which use hatching jars for egg incubation and have the potential of improving egg survival considerably.

Although pond spawning techniques combined with the use of indoor hatcheries offer scope for mass production of common carp spawn, considerable difficulties still exist in rearing larvae and fry. Relatively poor growth, coupled with high losses of carp larvae and fry are frequently observed in the carp nurseries of Bangladesh (personal observation).

1.4. Rearing of carp fry

Nursery rearing of larvae and fry is one of the most important phases of carp culture (Chaudhuri, 1983). Improper management at this phase habitually leads to high mortality. EIFAC (1976) concluded

that for many of the cultivated fish species, nursery technique appear to lag behind induced breeding and hatchery technology. Mires (1976) noted that in spite of the successful application of artificial spawning techniques for common carp, tremendous losses still incurred in the hatchery tank culture from the fifth day and in the ponds during the nursing period. The author presumed that losses in nursing ponds reach as much as 70-100%,

In recent years, a considerable number of investigations have been conducted on larval rearing methods for carp. A significant proportion of these investigations have attempted to formulate an artificial diet to substitute live food for first feeding larvae (Appelbaum, 1977; Appelbaum and Dor, 1978; Dabrowska et al, 1978, 1983; Meske and Pfeffer, 1978; Dabrowska et al 1979; Ostroumova et al, 1979; Von Lukowicz, 1979; Kouvil et al, 1981; Hecht and Viljoen, 1982). Amongst several formulations tested, hydrocarbon-grown yeast, Candida lipolytica with vitamin and mineral supplements (Appelbaum, 1977; Appelbaum and Dor, 1978), and processed Candida tropicalis yeast supplemented with freeze-dried or dried animal tissues (Dabrowski et al, 1983), have shown the most promising results. Various husbandry aspects of larval rearing e.g. primary nursing, handling of larvae, temperature requirement, stocking density, rate of feeding, optimal adaptation for commercial fry diet have been studied by several other authors Sarig and Marek, 1974; Kossman, 1976; Mires, 1976; Jezierska et al, 1979; Bryant and Matty, 1980, 1981; Rothbard, 1982).

A review of the literature, indicated only a few publications dealing with husbandry and nutrition of carp fry (Mires, 1976; Tamas and Horvath, 1979; Bryant, 1980; Bryant and Matty, 1981; Rothbard, 1982). However, with a few notable exceptions (Bryant, 1980; Bryant and Matty, 1981), most of the information compiled in these publications is based on the authors' experience rather than on experimental study.

There is great scope to improve nursery production of carp by improvements to their husbandry and through better controls of environmental conditions. Husbandry factors such as rate of feeding, stocking density, frequency of feeding, size of feed particles, water flow rate etc. are important to success, while the environmental conditions of e.g. water temperature and dissolved oxygen are equally important. Huisman (1979) concluded that the low growth rate often observed in fish nurseries may be attributed to an inadequate supply of food poor husbandry techniques in general. Similarly, EIFAC (1976) concluded that low survival rates for larvae and fry constitute a major problem in nursery management, with food being a key limiting factor. EIFAC recommended that research work aimed at developing suitable fish feeds using locally available and cheap ingredients be intensified.

1.5. Aim of the research

From the above discussion, it is clear that one of the principal problems faced by the rapidly expanding aquaculture industry in many

developing countries, including Bangladesh, is the lack of appropriate fry rearing technology. The aim of the present research project was to study the effects of various husbandry factors on survival and growth of carp fry and to evaluate some foodstuffs (agricultural by-products) commercially available in Bangladesh for their suitability as ingredients for a complete carp fry diet. Carp juveniles weighing about 50-500mg, which are considered as 'fry' as defined by Alikunli (1966) were used throughout.

CHAPTER 2

The effects of husbandry factors

2.1. Introduction

The importance of carefully controlled husbandry conditions when rearing carp fry has already been discussed in section 1.4. In the nursing phase the relative rate of weight development of fish is much larger than during the ongrowing phase. Huisman (1979) indicated that for cyprinid fishes during the first days after yok sac absorption specific growth rates of up to 100% of the body weight per day are common.

It is understandable that during this period of dynamic development the animals' husbandry requirements will change rapidly. Bryant (1980) and Bryant and Matty (1981) conducted comprehensive studies on several husbandry and nutritional aspects of carp fry under laboratory conditions. Carp fry of 100-500 mg body weight showed optimal growth and food conversion at feeding rates of 10-15% body weight per day when offered graded quantities of commercial trout fry diet. The above study was conducted at a constant temperature of 24 $\pm$ 0.5^{o}C. It was observed that carp fry of similar weight showed best performance when the feed was administered in twelve equal amounts over twenty four hours(Bryant, 1980). Bryant (1980) also demonstrated that presentation of a green coloured diet to carp fry on a matching coloured background in a recirculated water system improved their growth and food conversion.

However, information on other aspects of husbandry such as stocking density, water flow requirement and optimum size of feed particles are lacking. Although optimum feeding rate of carp fry held at 24°C has been determined (Bryant and Matty, 1981), interaction between

feeding rate and environmental temperature on survivality and growth of carp fry have not been investigated. In this section, experimental feeding trials were conducted to study:

1. The optimum temperature requirement of carp fry and determine their feeding rate in relation to temperature (Experiment 2.1).

2. The effect of stocking density and water flow rate on growth and food conversion (Experiment 2.2)

3. The preferred food particle sizes for different size classes of carp fry (Experiment 2.3).

2.2 Experiment 2.1

The effect of environmental temperature and feeding rates and their interactions on the survival, growth, food conversion and body of carp fry.

2.2.1. Introduction

Different environmental factors play important roles in the growth and survivial of fish. Temperature is probably the most important single abiotic factor affecting the life of fish (Kinne, 1970; Brett, 1979). The influence of temperature on the growth of fish has been well documented (Brown, 1957; Warren and Davis, 1967; Brett et al, 1969; Niimi and Beamish, 1973; Elliott,1976; Jobling, 1983b; Kellog and Gift, 1983; Saclauso, 1985). The activities of feeding, digestion and food conversion are strongly influenced by environmental temperature, which ultimately result in variation in the growth rate. Temperature affects the rate of digestion of food (Shrable et al, 1969; Brett and Higgs, 1970; Shcherbina and Kazlauskene, 1971) by influencing the activity of the digestive enzymes (Shcherbina and Kazlauskene, 1971). Shcherbina and Kazlauskene (1971) also demonstrated that the rate of absorption of the products of digestion are influenced by the environmental temperature. Temperature also influences amino acid catabolism in the fish tissue by affecting the activity of enzymes responsible for deaminating amino acids (Cowey and Sargent), 1979). A decrease in environmental temperature, therefore, may result in the lowering of the activities of these enzymes. Above all, metabolic rate of fish and hence growth rate are directly related to the environmental temperature (Brett, 1970). Since fish are cold-blooded animals, their biological activity responds to Van't Hoff's principle: raising the temperature by 10°C approximately doubles the speed of the reaction (Meske, 1985). Therefore increasing temperature leads to an increase

in growth rate until the optimum temperature is reached. Above the optimum, growth rate declines.

Optimum temperature requirements for growth and other physiological activites vary widely among fish species (Crawshaw, 1977; Brett, 1979; Alabaster and Lloyd, 1980; Jobling, 1981b; McCauley and Casselman, 1981). Each species has an optimum temperature for growth which is probably determined by its optimum temperature for critical enzyme activity (Parker and Davis, 1981).

A considerable amount of published information is available on the temperature requirements of common carp (Table 2.1). However, wide disparity exists between the various reported values and most of the studies referred to above have not been conducted under clearly defined environmental conditions. A review of literature indicated only two publications on the effect of temperature on the growth of common carp under controlled experimental conditions (Jauncey, 1979; Coolish and Adelman, 1984). Moreover, most investigations have been confined to advanced fry, fingerling and adult stages of carp. No detailed information is available on the temperature requirement of common carp larvae, post larvae and early fry. Specific temperature requirements for maximum and/or optimum growth may vary among various age, sex or size groups within each species (McCauley and Casselman, 1981).

Most of the investigations conducted on the husbandry and nutrition

Table 2.1 The effect of water temperature on common carp

Size/age (weight/length)	Temp. (°C)	Effects	Author
Post larvae and juveniles	24-28	Most intensive feeding	Berg (1949, cited by Sarig, 1966)
Fry	16-25	Optimum for growth	Schmeing - Engbarding (1953, cited by Jezierska, et al., 1979)
5-7.5 cm	32	Final preferendum	Pitt et al. (1956)
-	23-29	Optimum for growth	Shpet and Kharitonova (1963, cited by Jauncey, 1982)
-	29	Marked reduction of feeding recorded	Shpet (1967, cited by Alabaster and Lloyd, 1980)
NR	23-30	Optimum temperature for growth in floating cages	Gribanov et al. (1968)
NR	22	Decrease in growth rate	Gribanov et al. (1968)
-	35.2	No adverse effects, normal behaviour observed	Beliaev (1969, cited by Alabaster & Lloyd, 1980)
-	33-34	Decrease in growth rate	Korneev (1964, cited by Gribanov, et al., 1968)
38 day old	27	Preferred water temp.	Askerov (1975)
Adults	29	Preferred temperature in an electronic thermo-regulatory shuttlebox	Reynolds and Casterlin (1977)
-	28 or above	Optimum temperature for growth and food conversion	Aston and Brown (1978)
5.8-8.25g (fingerling)	25-30	Optimum temperature for growth and food conversion	Jauncey (1979)
8.7 g	27	Maximum scope for growth	Coolish & Adelman (1984)

of carp larvae and fry have so far been carried out at temperatures around 23-25°C (Huisman, 1976b; Kossman, 1976; Mires, 1976; Appelbaum and Dor, 1978; Dabrowski et al, 1978; Bryant and Matty, 1980, 1981; Kouvil et al, 1981). Ambient water temperature frequently exceeds 30°C in tropical and some sub-tropical climates (e.g. 27 and 36°C are the recorded annual minima and maxima in fish ponds in Bangkok, Thailand, Edwards et al, 1984). Thus knowledge of the effect of high temperatures on the mortality, growth and food conversion efficiency of common carp fry is important to the culture of this species in warm climates.

Food ration is the most influential biotic factor affecting the survival and growth of fish (Brett, 1979). The importance of optimum feeding rate has been emphasised and investigated by several researchers for different fish species (Elliott, 1975; Huisman, 1976a; Brett 1979; Wurtsbaugh and Davis, 1977; Bryant and Matty, 1981; Allen and Wootton 1982; Macintosh and DeSilva, 1984). A number of investigations have been conducted to optimise the rate of feeding to common carp (Huisman, 1976a; Jauncey, 1979; Bryant and Matty, 1981; Goolish and Adelman, 1984). However, feeding rates are inversely proportional to the animals' age or size (Huisman, 1976b; Jauncey, 1982) and therefore vary widely between different age groups. Information on the optimum feeding rate of common carp larvae, post larvae and early fry are scanty. The role of optimum feeding in larval rearing has been discussed by Bryant and Matty (1981). The authors noted that amongst carp

juveniles underfeeding leads to impairment of growth potential and predisposes them towards disease, as well as increasing the incidence of physical defects such as abnormal operculae, scoliosis and lordosis. On the other hand, excessive feeding has been reported to increase mortality.

In the larval and fry nursing phases of fish culture, arbitrary feeding rates frequently have been adopted and practised (Huisman, 1979). The optimum feeding rate for carp post larvae and fry has been determined recently by Bryant and Matty (1981). However, the above investigation was conducted only at one temperature (24 $\pm$ 0.5°C). Ration size is reported to be influenced strongly by the environmental temperature (Brett et al, 1969; Andrews and Stickney, 1972; Shelbourn et al, 1973; Mironova, 1975; Wurtsbaugh and Davis, 1977b; Allen and Wootton, 1982). Brett et al, (1969) in their studies on young sockeye salmon, Oncorhynchus nerka, observed that optimum growth occurred at approximately 15°C for the two highest ration (4.5 and 6% BW/day), shifting progressively to a lower temperature at each lower ration. The interaction of environmental temperature and feeding rate on the growth of common carp fingerling have been investigated by Jauncey (1979) and Goolish and Adelman (1984). However, no such data exist for common carp post larvae and early fry.

The objectives of the present investigation were to examine the effects of temperature on mortality, growth, food conversion and protein utilization of common carp fry and to determine their optimum feeding rate in relation to temperature.

2.2.2. Materials and methods

To study the effect of environmental temperature and feeding rate on common carp fry, two 25-day growth trials were conducted in an experimental recirculated water system. In the first trial, feeding rates of 10, 15, 20 and 25% body weight per day (BW/day) were compared at temperatures of 24, 28 and 32°C. The results of the first trial showed that the highest temperature (32°C) in conjunction with the two higher feeding rates (20 and 25% BW/day) produced the highest growth rates. The best growth at all temperatures resulted from the 25% BW/day feeding rate. It was decided in the second trial to investigate the effect of a further increase in temperature and feeding rate above the levels used in first trial. In trial 2 feeding rates of 15, 20, 25, 30 and 35% BW/day were tested at 35°C and rates of 25, 30 and 35% BW/day were each tested at 32 and 28°C.

The results of trial 1 indicated that there would be no scope for increasing growth at 24°C by increasing the feeding rate above 25% BW/day, so the above temperature was not included in trial 2. The combinations 25% BW - 28°C and 25% BW - 32°C were included in both trials to check the reproducibility of results.

For the first trial, each treatment was tested with three replications. For the second trial, two replications were used for each treatment because of a shortage of fish. Thirty-six fish fry were used for each replicate treatment for both trials. A summary of the methodology used in the present investigation is presented in Table 2.3.

2.2.2.1. Experimental system

The experiments were conducted in a recirculated water system comprising three rows of independent tank units (Fig. 1.1 a-b). Each unit consisted of twelve 8 l polypropylene experimental tanks supplied by common inflow and outflow pipes (Fig. 1.1a). A 115 l header tank supplied freshwater by gravity to each experimental tank through an inflow pipe. Water was tangentially jetted into the experimenal tanks both to increase aeration and to induce a circular flow. The circular experimental tanks had sloping bases and because of this and the circular flow, were effectively self-cleaning. The outflow water from the experimental tank drained, through a stand pipe (Fig. 1.1d), into an outflow drain pipe which carried the water to a 115 l waste settlement tank (Fig. 1.1a). Over each stand pipe a sleeve pipe was fitted with a number of holes at the bottom so that faeces and uneaten food were sucked from the tank bottom through the holes and into the outflow pipe (Fig. 1.1d).

Solid materials from the water collected in the waste settlement tank. From the settlement tank the water passed into a 115 l filtration tank containing a plastic filter medium (Mass Transfer Ltd, Hobsons Lane, Cumbria) as a substrate for microbial growth. Water from the filtration tank then passed to a 115 l sump (Fig. 1.1c) containing a submersible 'Otter' pump (Beresford Pumps, Canley, Coventry). The pump lifted the water to a header tanks for distribution into the experimental tanks. Overflow water from the

Fig. 2.1 Diagrammatic representation of the recirculated water system, used in growth trial (Experiments 2,1, 2.2 and 4.1).

A. Side view

B. Plan

C. Bottom part showing the connection between waste settlement tank, filtration tank and sump

a: Inflow pipe

b: Clamp for inflow regulation tank

c: Experimental tanks

d: Outflow drain pipe

e: Waste settlement tank

f: Filtration tank

g: Sump

h: Header tank

i: Pipe connecting pump and header tank

P: Pump

J: Overflow pipe

K: A circular tank containing filterwool and gravel

→ Direction of water flow

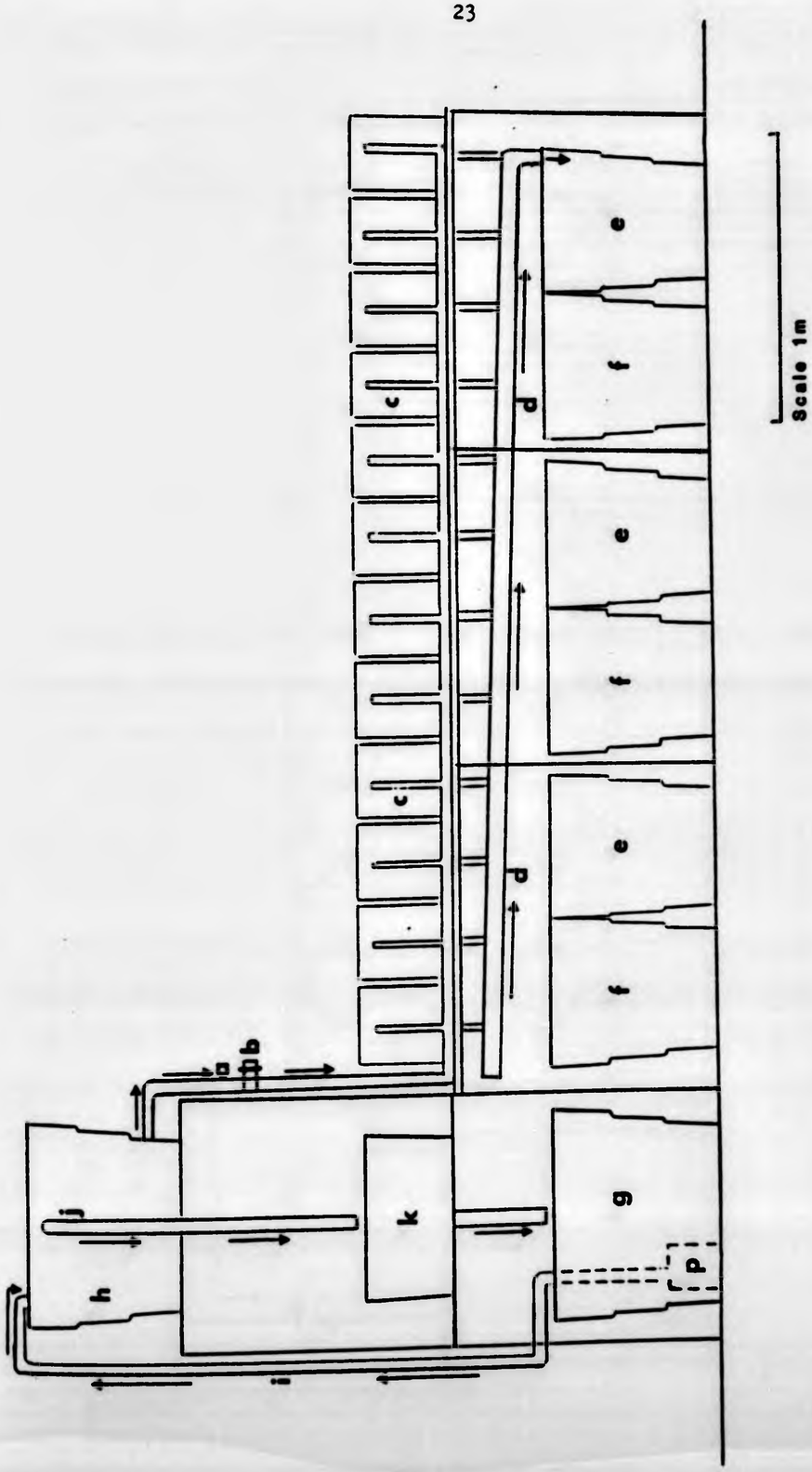

Fig. 2.1.A

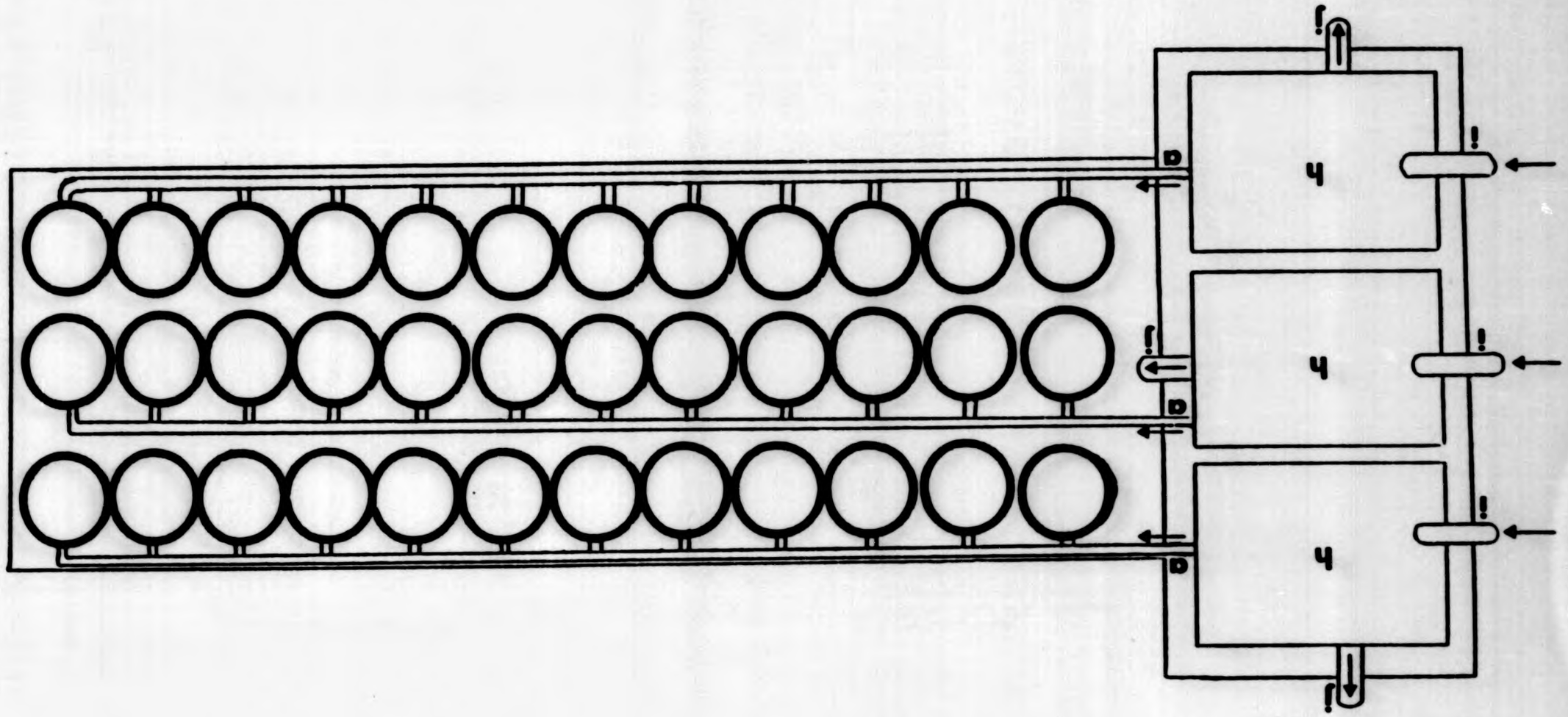

Fig. 2.1. B

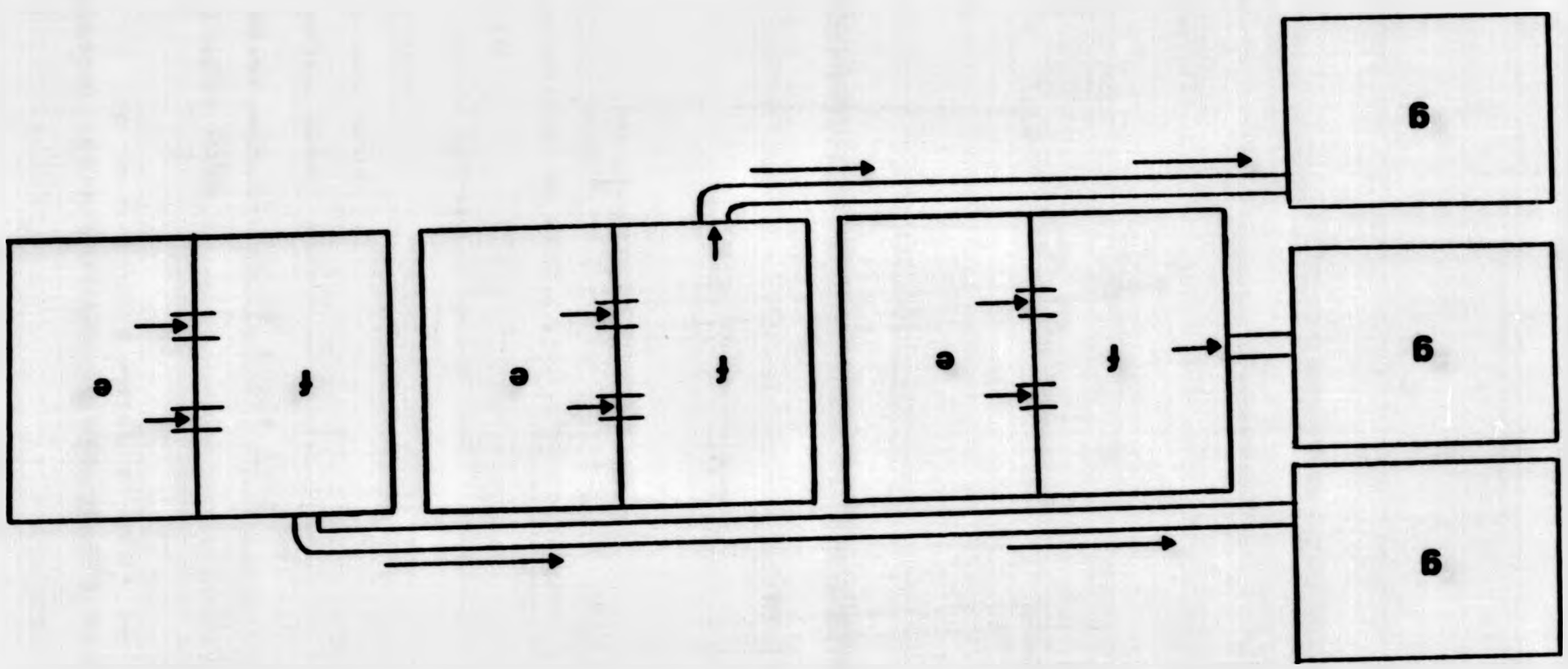

Fig. 2.1. c

Scale 1m

Fig. 2.1.D Diagramatic representation of one of the experimental tanks in the recirculated water system, used in growth trial

a: Inflow pipe

b: Valve for adjusting water flow

c: Water level

d: Stand pipe

e: Sleeve

f: Water outlfow to drain

g: Lid

——→ Direction of water flow

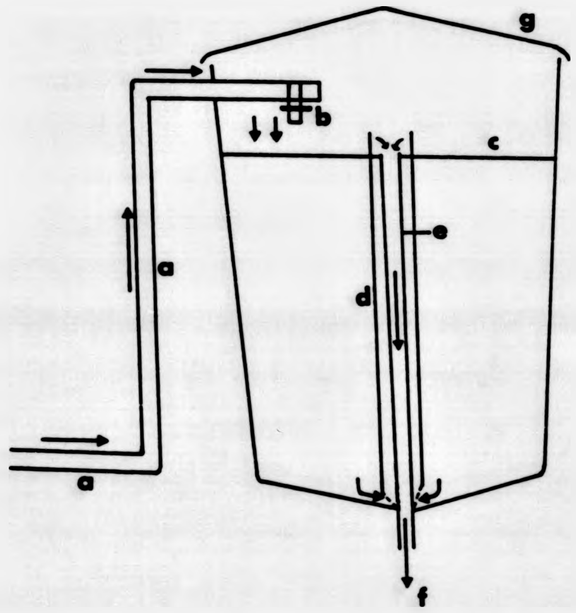

Fig. 2.1.D

header tank returned through an overflow pipe to the sump via a 25 l circular tank containing filter wool and gravel designed to trap suspended solid wastes (Fig. 1.1a).

An adequate level of dissolved oxygen was maintained by compressed air supplied through several 15 cm stone aerators submerged in each header tank. All the experimental tanks and header tanks were supported on platforms made of iron angle. The experimental system was constructed in the Tropical Aquarium Building of the Institute of Aquaculture which is maintained at an ambient temperature of 25-28°C.

Experimental temperatures of 28, 32 and 35°C were maintained using 'Nimrod' microtonic thermostatic heaters (R. Aitchisens Ltd., Edinburgh) submerged in the header tank. The experimental temperature of 24°C was produced by running a constant flow of cool water (≃16°C)through plastic tubing kept submerged in the sump. A periodic check was made of the flow of running water, which was adjusted to maintain the desired temperature. This was achieved to an accuracy of $\pm$ 1°C. Water was added periodically to the sump to compensate for evaporation.

Each experimental tank was supplied initially with about 200 ml of water per minute. The flow rate was subsequently increased in proportion to the increase in fish weight with time. The same rate of water flow was maintained in each treatment. A photoperiod of 12 hours light : 12 hours dark was operated throughout the experimental period.

2.2.2.2. Experimental animals

Fry of the mirror variety of common carp (Dinkelsbuhler strain from West Germany) were obtained from the hatchery of Newhay Fisheries, Blackburn Road, Bolton, England. Fry obtained were induced bred in a warm water (20-24°C) recirculation system. Larvae were fed with *Artemia* nauplii from 24 hours after hatching. The *Artemia* were replaced gradually by trout fry food, Omega No. 00 (Edward Baker, Sudbury, Suffolk) and the larvae were completely weaned to the artificial diet when they were about four to five weeks old. After they were completely weaned to the artificial diet (at a weight of about 20-50 mg), they were brought to Stirling by rail packed in sealed polythene bags containing water and oxygen.

2.2.2.3. Quarantine procedure

Upon arrival carp fry were kept in an isolated constant temperature room in the restricted quarantine area of the Insitute of Aquaculture. Fish fry were kept in quarantine for 14-21 days and examined for the presence of parasites, bacteria and viruses. During quarantine they were held in 80 l glass aquaria, serviced by an 'Eheim' combined water pump and filter by which water was continuously circulated. The water temperature was maintained at 24-28°C. Fish fry were fed with ground (250-500 um) commercial trout pellet (Edward Baker's Omega No. 3; protein content 49%) or a fish meal base prepared diet (protein content 50%). No disease treatment was necessary as no pathogens were detected in any of the batches of fry.

After the quarantine period the fry were either transferred to an experimental system or were maintained on 150 l stocking tanks in a recirculated water system (water temperature 28°C) in the Tropical Aquarium Building. Fish were fed with ground (500 µm and above) commercial trout pellet after stocking.

2.2.2.4. Diet formulation

Semi-purified diets were formulated to contain 50% crude protein, 10% crude lipid, 10% ash, 6% crude fibre and 25% nitrogen free extractives. These levels were based on the nutrient requirement of carp fry as summarised in Table 2.2. Herring fish meal (76.2% crude protein, 8.5% crude lipid, 13.1% ash-trial 1 and 75.0% crude protein, 10.9% crude lipid, 13.0% ash-trial 2) were used as a source of protein, to which were added other purified ingredients to form a nutritionally complete ration (as shown in Table 2.4). The diets were formulated on a dry weight basis, allowance being made for the water content of the raw ingredients during weighing. Cod liver oil and corn oil were added to the diet to achieve the desired final lipid level in the diet taking into consideration the lipid supplied incidentally by the herring meal. The amount of ash contained in the herring meal was balanced to the desired level with a mineral mix (Table 2.5). The quantity of crude fibre in fish meal was balanced to the desired level by the addition of α- cellulose. Vitamin mix (Table 2.6) and binder (sodium carboxymethyl cellulose, high viscosity) were added to the diet at the rate of 2% for each.

Table 2.2 A summary of nutrient requirement of carp fry

A. Protein

Stage/weight	% requirement	Source	Water temp. °C	Author
Young (5.8g)	38	Casein	23	Ogino and Saito (1970)
Fry (0.1-0.5g)	45	Fish meal	24	Bryant (1980)
Fry and fingerlings	43 - 47	Recommended level		National Research Council (NRC) (1983)

B. Lipid

Stage/weight	% inclusion in the diet	Source	Water Temp. (°C)	Effect	Author
Fry (0.1-0.5g)	5-15	Soyabean oil	24	Inclusion above 5% did not increase growth, food conversion or protein utilisation	Bryant (1980)
	10-15	Recommended level for commercial carp diet			NRC (1983)

C. Carbohydrate

Stage/weight	% inclusion in the diet	Source	Water Temp. (°C)	Effect	Author
Spawn, fry and fingerlings	26	Dextrin	24-32	Recommended level	Sen et al. (1978)
	25	Recommended maximum dietary digestible carbohydrate level for most fish species			Cowey and Sargent (1979)
	30	Dextrin		Maximum dietary level that did not reduce growth rate	Furuichi and Yone (1980)

Table 2.3 A summary of the methodology used to study the effects of environmental temperature and feeding rate on survival, growth and food conversion of carp fry (Experiment 2.1)

Experimental duration	:	25 days
Initial weight of fry	:	73 mg (SE ± 1.9)-trial 1 60-67 mg-trial 2
Treatments	:	Variations in water temperature and feeding rate
Temperature	:	24, 28 and 32°C - trial 1
	:	28, 32 and 35°C - trial 2
Feeding rate	:	10, 15, 20 and 25% BW/day at each temp - trial 1
	:	25, 30 and 35% BW/day at 28 and 32°C and 15, 20, 25, 30 and 35% BW/day at 35°C - trial 2
Replication	:	3/treatment - trial 1; 2/ treatment - trial 2
Stocking density	:	6/litre
Water flow rate to each tank	:	200 ml/min with corresponding increase at each subsequent sampling
Volume of water in each tank	:	6 l
Physico-chemical characteristics monitored	:	Temperature, pH, dissolved oxygen, total ammonia, nitrite and suspended solids

Table 2.4 Composition and proximate analysis of experimental diets (Experiment 2.1)

Ingredients	Trial 1	Trial 2
Composition of experimental diets (% of dry diet)		
Herring meal	65.63	66.71
Cod liver oil	2.00	1.50
Corn oil	4.00	3.00
Mineral mix	3.00	3.00
Vitamin mix	2.00	2.00
Binder*	2.00	2.00
α- cellulose	3.03	3.00
Dextrin	11.00	11.00
Corn starch	7.34	6.79
Proximate analysis of diets (% dry matter)		
Dry matter	95.13	93.46
Crude protein	50.33	51.96
Crude lipid	11.95	11.69
Ash	11.11	11.84
Crude fibre	1.94	3.79**
NFE***	19.11	18.79
Gross energy (Kcal/g)	4.64	4.65
Metabolizable energy (ME) (Kcal/g)	3.95	3.95
Protein to energy ration (mg protein/Kcal of ME)	127.46	131.54

* Sodium carboxymethyl cellulose (high viscosity)

** Calculated as crude fibre derived from test protein source and α-cellulose

*** Calculated as NFE derived from fish meal, corn starch and dextrin

Table 2.5 Composition of mineral supplement used in experimental diets (Experiments 2.1, 2.2 and 4.1)

Mineral	g/100g mix
$MgSO_4$, $7H_2O$	12.75
KCl	5.00
NaCl	6.00
$CaHPO_4$, $2H_2O$	72.78
$FeSO_4$, $7H_2O$	2.50
$ZnSO_4$, $7H_2O$	0.55
$CuSO_4$, SH_2O	0.0785
$MnSO_4$, $4H_2O$	0.25375
$CoSO_4$, $4H_2O$	0.04775
$Ca(IO_3)_2$, $6H_2O$	0.0295
$CrCl_3$, $6H_2O$	0.01275

Table 2.6 Composition of vitamin supplement used in experimental diets (Experiments 21, 2.2 and 4.1)

Vitamin	g/100g mix
Thiamine (B_1)	0.25
Riboflavin (B_2)	0.25
Panthothenic acid	0.50
Pyridoxine (B_6)	0.20
Inositol	10.0
Biotin	0.03
Folic acid	0.075
Para amino benzoic acid	0.25
Choline	20.0
Niacin (Nicotinic acid, B_3)	1.0
Cyanocobalamin (B_{12})	0.0005
Retinol palmitate (A)	10,000 IU
α-tocopherol acetate (E)	2.01
Ascorbic acid (C)	5.0
Menadione (K)	0.2
Cholecalciferol (D_3)	50,000 IU

* The mixture was made up to 100g with α-cellulose

Corn starch and dextrin were used as a source of dietary carbohydrate and to make up the ingredients to 100%.

2.2.2.5. Diet Preparation

All dietary ingredients were seived to a particle size of 500 /um or less before weighing and pelletising to ensure that a homogenous mixture was obtained. The dry ingredients were then weighed out according to the formulation (Table 2.4), placed in the bowl of a food mixer (Hobart A400 food mixer) and blended for 10 minutes. To this mixture were added weighed quantities of cod liver oil and corn oil. Blending continued for a further 10 minutes. The requisite amount of warm water was added slowly to the diet with continuous stirring until a stiff dough was obtained. The moist diet was extruded through the mincer attachment of the food mixer to obtain 3mm diameter pellets. The resultant pellets were then air dried at about 35°C using an electric fan convector heater. These pellets were ground and seived into the required particle sizes. Samples of diet were then analysed for proximate composition (Section 2.2.2.6). Diets were stored in a deep freezer (-23°C) until required.

2.2.2.6. Proximate composition analysis

Proximate composition analysis was conducted on the dietary ingredients, on the diets and on the fish at the start and end of the trials. For carcass analysis of the experimental fish, samples

from each replicate tank for each treatment were analysed separately for moisture, crude protein and crude lipid. Due to the lack of sufficient samples, pooled samples from replicate tanks were used for analysis of ash content. Therefore statistical analysis (Section 2.2.2.10) could not be performed on the ash content of the samples.

Moisture content was determined by air-drying the samples in an oven at 105°C for 12 hours. Crude protein content was determined by the micro-Kjeldahl method for determining nitrogen (A.O.A.C., 1970) and applying the empirical factor of 6.25 to the results to convert total nitrogen to total crude protein. Crude lipid content was determined by the freon (Trichlorofluoro methane 99.9%) extraction method (Korn and Macedo, 1973). Ash content was determined by igniting samples in a muffle furnace for 12 hours at a temperature of 450°C (A.O.A.C., 1970). Crude fibre was determined by the digestion method with 12.5% H_2SO_4 and 12.5% NaOH (A.O.A.C., 1970). Nitrogen free extractions (NFE) were estimated by subtracting the total of moisture, crude protein, crude lipid, ash and crude fibre from 100%.

The gross (or total) energy contents of the diet were estimated by multiplying the analysed protein, lipid and carbohydrate contents by 5.5., 9.1 and 4.1 Kcal/g respectively and adding the results together (from Brody, 1945, cited by Jauncey, 1982). The metabolizable energy contents (ME) of the diet was also calculated from the analysed values for protein, lipid and carbohydrate. The ME content of protein for carp fry was estimated as 4.5 Kcal/g as

reported for trout (Smith, 1971); that of lipid as 8.51 Kcal/g (Austreng, 1978), and that of carbohydrate as 3.49 Kcal/g as reported for carp by Chiou and Ogino (1975).

2.2.2.7. Acclimation and weighing procedures

Trial 1

One week before the start of the experiment, fish fry were transferred to the experimental system from the stocking tank for acclimation to the experimental system. During acclimation they were fed with ground (500 /um) pellet and maintained at a water temperature of 28 $\pm$ 1°C. One day before the start of the experiment, the fish were distributed randomly between the culture tanks to minimise variation in their mean weights between tanks. The required number of fry, plus a 10% excess, was allocated to each culture tank. Due to the delicate nature of the fry at the beginning of each experiment, it was not feasible to measure their individual weights because of the danger of damaging them physically. Instead 10% of the population in each tank was netted and anaesthetised with benzocaine (ethyl para-aminobenzoate) (1:20,000). They were then drained of water and gently blotted dry on a soft paper towel, and weighed individually to the nearest 1.0mg on an Oertling HB63 microbalance and thensacrificed.

All the sample measurements were pooled to obtain an average value for initial weight for the experimental population. The sampled fry

were then killed in benzocaine solution and stored at -23°C for subsequent proximate composition analysis. The total number of fish in each tank were also weighed collectively and it was found that variations in average weight between tanks were minimal (± 5mg). Variations in average weight between different tanks were minimised, if necessary, by redistribution of fish to ensure a uniform starting weight.

After the initial measurements were completed, the desired experimental temperatures were adjusted by raising or reducing the water temperature at the rate of approximately 2°C per day. These water temperatures were controlled to within ± 1°C throughout the experimental period.

Fish were weighed during the experiment every five days. The total fish of each tank were captured using a fine mesh handnet. Excess water was then removed from the fish by blotting the net on a soft paper towel; this drew most of the water from the fish sample. The fish were then transformed to a tared water-filled container and weighed collectively to the nearest 0.01g. The individual fish weights were recorded at the end of the experimental period

Trial 2

The acclimation and weighing procedures employed were as described for trial 1. However, due to the shortage of fry during this trial, 10% excess fry could not be allocated to each experimental tank and

consequently initial individual measurement of fish was not possible. Instead the total weight of fish in each tank was measured and expressed as mean weight of each tank. Fish were distributed randomly between the experimental tanks to minimise variation in their average weight. In some cases, variation in average tank weight was minimised by redistribution of fish. In this way, uniform mean weight (60-68mg) in all replicate treatments was achieved. However, due to unavailability of sufficient numbers of fry, a group of fish of lower initial weight (46mg) had to be used for one treatment (15% BW/day at 35°C temperature).

2.2.2.8. Administration of food and feeding rates

The fish were fed five times a day, every day, between 08.00 and 20.00 hours at three hourly interval. Each of the five feeds daily was administered over a period of thirty minutes to ensure consumption of the whole ration. Towards the end of the experiment in trial 1 it was noticed that fish maintained at 24°C and fed at 25% BW/day were unwilling to consume the whole amount of ration. Similarly, in trial 2, this was noticed for fish maintained at all three temperatures and at higher feeding rates (30 and 35% BW/day) towards the end of the experiment. When this occurred they were given only the amount they could consume within 30 minutes and a record of the amount of food administered was kept for subsequent calculation of food conversion and protein utilization. The quantity of food administered per day was adjusted

after each periodic weighing of the fish. The feeding rate was calculated on the basis of fresh weight of diet. Daily mortalities were recorded and feeding was adjusted accordingly. Fish were fed by hand feeding.

2.2.2.9. Measurement and analysis of water

The physical and chemical characteristics of the water in the experimental culture system were measured following standard procedures.

Temperature : Temperature in ^{o}C was measured using a nitrogen filled mercury themometer to an accuracy of $\pm$ 0.1^{o}C.

Dissolved oxygen : Dissolved oxygen concentration was measured using a YSI (Yellow Spring Instrument Co) model 57 oxygen meter, calibrated in air saturated with water, to an accuracy of $\pm$ 0.1mgl^{-1}.

pH : The pH of test water was measured with an Extech 651 digital pH/mv/temp. meter, callibrated with known phosphate buffers at an appropriate temperature, to an accuracy of $\pm$ 0.01 unit.

Total ammonia : Total ammonia-nitrogen was measured using the

nesslerization method (Stirling, 1983) or in some cases by means of an autoanalyser following the method of Jones et al (1980).

Nitrite : Nitrite-nitrogen concentration was measured by a sulfanilamide based colorimetric reaction (Mackereth et al, 1978).

Total alkalinity: Total alkalinity was measured according to Stirling (1983) and expressed as mgl^{-1} of $CaCO_3$.

Total hardness : Total hardness was measured by EDTA titrimetric method (APHA et al, 1980).

All colorimetric measurements were carried out in a Linear Read Ultraviolet Spectrophotometer (CE272 model : Cecil Instruments) or in a Uvicon 810 electronic spectrophotometer.

Suspended solids : Suspended solids were collected on filter paper (Whatman's GF/C) from 0.5-1.0 l water samples run under vacuum; and the filter papers were dried at 105°C for 24 hours (Stirling, 1983). The water temperature of the three experimenal systems were recorded once every day.

The dissolved oxygen and pH of the experimental water were measured once in every five days and ammonia, nitrite and suspended solids were measured once in every seven days.

A complete analysis of the tap water in the Tropical Aquarium

Table 2.7 Chemical characteristics of the tap water used in the tropical aquarium*

Total oxidised nitrogen	0.5
Conductivity	65.0 $uScm^{-1}$
Chloride	5.0
Total alkalinity (as $CaCO_3$)	10.0
Orthophosphate (as P)	0.01
Calcium	6.4
Magnesium	4.9
Potassium	0.5
Sodium	3.2
Copper	0.004
Iron	0.01
Lead	<0.005
Manganese	0.004
Zinc	0.025

* All values are in mg l^{-1} unless otherwise specified

Table 2.8 Physico-chemical characteristics of the experimental water in trial 1 and 2, Experiment 2.1.

Trial 1	Treatment Group A	B	C
Temperature (^{o}C)	32 ± 1	28 ± 1	24 ± 1
pH	6.39-7.66	6.53-7.83	6.39-7.70
Dissolved oxygen (mg l^{-1})	6.30-7.20	6.70-7.40	7.00-7.80
Total ammonia-nitrogen (mg l^{-1})	0.005-0.160	0.03-0.18	<0.005-0.115
Nitrite-nitrogen (mg l^{-1})	0.018-0.156	0.026-0.172	0.044-0.131
Suspended solids (mg l^{-1})	0.4-1.0	0.6-1.2	2.4-3.0

Trial 2	Treatment Group D	E	F
Temperature (^{o}C)	35 ± 1	32 ± 1	28 ± 1
pH	6.58-7.73	6.29-7.84	6.45-7.84
Dissolved oxygen (mg l^{-1})	6.10-7.10	6.50-7.60	7.10-7.80
Total ammonia-nitrogen (mg l^{-1})	0.01-0.46	0.01-0.35	0.03-0.26
Nitrite-nitrogen (mg l^{-1})	0.006-0.36	0.004-0.23	0.01-0.19
Suspended solids (mg l^{-1})	1.0-2.5	0.8-2.0	1.0-2.0

Building used to fill the experimental system was carried out with the help of the Forth River Purification Board, Causeway Old School, Alloa Road, Stirling and is presented in Table 2.7. The measured physico-chemical characteristics of the experimental water are presented in Table 2.8.

2.2.2.10. Analysis of experimental data

a. Specific growth rate (SGR)

The SGR is the rate of change in weight of fish; it was calculated as the percentage increase in body weight per day as follows:

$$SGR(\%/day) = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1} 100 \text{ (after Brown, 1957)}$$

where,

W_1 = the initial live body weight (g) at time T_1 (day)

W_2 = the final live body weight (g) at time T_2 (day)

Since the growth rate of fish varies with body size, the specific growth rate for the group of fish fry with the lower initial weight of 46mg (Trial 2 : 15% BW/day-35°C) was adjusted to refer to an initial weight of 63mg (overall mean initial weight trial 2) (See Table 2.11). The adjusted specific growth rate was calculated by the following regression equation:-

$Log_e G_w = a - 0.4 Log_w$ (after Jobling, 1983a).

where,

GW = the specific growth rate

W = the fish weight

a = intercept

To overcome the problems of variations in the growth rresponses between trial 1 and 2, a factor was used to adjust the SGR of trial 2 to refer to the SGr of trial 1. One ratio has been computed by dividing the SGR for treatment A_4 (25% BW/day - 32°C; trial 1) by treatment E_1 (25% BW/day - 32°C; trial 2) and another by dividing the SGR for treatment B_4 (24% BW/day - 28°C; trial 1) by F1 (25% BW/day - 28°C; trial 2) (see Table 2.9 and 2.11 for treatment specification). SGRs of all treatments of trial 2 were multiplied by the above factor to adjust them to the SGRs of trial 1.

b. Food conversion ratio (FCR)

The food conversion ratio is defined as the amount of dry food fed per unit live weight gain of fish. It was calculated as follows:

$$FCR = \frac{\text{Food fed (dry weight)}}{\text{Live weight gain}}$$

In calculating FCRs, the dry food weight was derived by using a correction for the analysed moisture content of the diet.

c. Protein efficiency ratio (PER)

The PER which is the gain in weight of fish per gram of crude protein consumed, gives an indication of the efficiency with which the fish were able to utilize dietary protein. This was calculated as:

$$PER = \frac{\text{Live weight gain}}{\text{Crude protein fed}}$$ (After Osborne et al, 1919)

d. Apparent net protein utilisation (ANPU)

Net protein utilization is the apparent efficiency of deposition of dietary protein as body tissue. In the present experiment, NPU was

determined by the carcass analysis method of Miller and Bender (1955). Since no correction was made for endogenous nitrogen losses during the experiments, results are expressed as apparent NPU. This was calculated as:

$$\text{ANPU (\%)} = \frac{Nb - Na}{Ni} \times 100$$

where,

Na = the body nitrogen at the start of the test,

Nb = the body nitrogen at the end of the test

Ni = the amount of nitrogen ingested

Due to practical difficulties in food administration, it was neither possible to ensure that all administered food was eaten nor possible to collect uneaten food from the experimental tanks. Therefore, calculated food conversion ratios, protein efficiency ratios and apparent net protein utilizations were derived from the amount of food offered without correction being made for wastage. However, mortalities during the experimental period were taken into consideration while calculating the food conversion and protein efficiency ratios.

e. Statistical analysis

Comparison of treatment means was carried out using a one or two-way analysis of variance, followed by testing for pairwise differences using Newman-Keul's multiple range test (SNK test) (Zar, 1974). Standard errors ($\pm$ SE) of treatment means were calculated from the

residual mean squares in the analysis of variance. For comparison of mortalities between treatments, values for percentage mortality were subjected to arcsin transformation (Zar, 1974) and the resultant data were subjected to analysis of variance as above.

2.2.3. Results

The fish in both trials acclimated well to the experimental temperatures (within 2 days of exposure) and fed actively throughout the study period. Mortalities were very low and not significantly different between treatments ($P > 0.05$).

2.2.3.1. Growth

The growth responses of carp fry in trial 1 are presented in Table 2.9 and shown graphically in Figs 2.2-2.8. At each rearing temperature growth responses increased with feeding rate (Figs 2.2-2.4 and Table 2.9). Temperature had no significant effect on the growth response at feeding rates of 10 and 15% BW/day with the exception of 10% BW/day and 32°C (Figs 2.5-2.6 and Table 2.9) In this treatment the growth response was lower than those at 28 and 24°C. However, at higher feeding rates (20 and 25% BW/day), increase in the rearing temperature improved the growth performance towards the end of the experimental period (Figs 2.7-2.8). With a 20% feeding rate, the SGR of 8.18 at 32°C was significantly higher

Table 2.9 Mortality, growth and food utilization of common carp fry reared for 25 days in trial 1, Experiment 2.1

Mean initial weight 73.0 mg (SE ± 1.9)

Treatment No.	Temp. (°C)	Feeding rate (%BW/day)	Mean final weight (mg)	Mortality (%)	SGR	FCR	PER	ANPU (%)
A1	32	10	249^{g}*	0	4.91^{h}	1.65^{ab}	1.20^{b}	14.65^{e}
A2		15	389^{f}	0.9	6.69^{f}	1.76^{bc}	1.13^{c}	14.77^{c}
A3		20	565^{c}	0.9	8.18^{c}	1.82^{cd}	1.09^{cd}	14.62^{c}
A4		25	707a	0	9.08^{a}	2.04^{e}	0.97^{e}	12.92^{d}
B1	28	10	286^{g}	0	5.46^{g}	1.56^{a}	1.27^{a}	16.75^{a}
B2		15	383^{f}	0	6.63^{f}	1.90^{d}	1.05^{d}	13.98^{c}
B3		20	499^{d}	0.9	7.69^{d}	2.06^{c}	0.96^{e}	12.81^{d}
B4		25	634^{b}	0.9	8.64^{b}	2.26^{f}	0.88^{f}	11.43^{e}
C1	24	10	279^{g}	0	5.36^{g}	1.65^{ab}	1.21^{b}	15.39^{b}
C2		15	392^{f}	1.8	6.72^{f}	1.92^{d}	1.04^{d}	13.22^{d}
C3		20	439^{e}	0.9	7.17^{e}	2.53^{g}	0.79^{g}	10.02^{f}
C4		25	522^{d}	1.8	7.86^{d}	2.80^{h}	0.71^{h}	9.27^{g}
		± SE**	11.53	1.39	0.105	0.039	0.017	0.256

* Figures in the same column with same superscripts are not significantly different ($P > 0.05$)

** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

Table 2.10 Proximate carcass composition analysis (% fresh weight) of fish samples at the start and end of trial 1, Experiment 2.1

			Moisture	Crude Protein	Crude Lipid	Ash	Total
Initial Composition			83.67	10.93	1.83	2.79	99.22
Final Composition							
Treatment No.	Temp. °C	Feeding rate (%Bw/day)					
A1	32	10	79.06^{a}	12.24^{a}	4.52^{f}	2.37	98.19
A2		15	78.57^{ab}	13.16^{b}	5.37^{e}	2.06	99.16
A3		20	78.05^{bc}	13.40^{b}	6.01^{bc}	2.03	99.49
A4		25	77.94^{bc}	13.26^{b}	6.23^{b}	2.10	99.53
B1	28	10	78.48^{ab}	13.29^{b}	5.68^{cd}	2.00	99.45
B2		15	78.00^{bc}	13.34^{b}	6.26^{b}	2.00	99.60
B3		20	77.36^{c}	13.38^{b}	6.70^{a}	1.95	99.39
B4		25	77.80^{bc}	13.20^{b}	6.60^{a}	1.94	99.54
C1	24	10	78.87^{a}	12.75^{b}	5.41^{de}	1.93	98.96
C2		15	78.03^{bc}	13.04^{b}	6.03^{bc}	1.96	99.06
C3		20	78.11^{bc}	12.91^{b}	6.78^{a}	1.70	99.50
C4		25	77.37^{c}	13.28^{b}	6.93^{a}	1.78	99.36
		± SE **	0.189	0.168	0.101		

* Figures in the same column with same superscripts are not significantly different ($P > 0.05$)

** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

Table 2.11 Mortality, growth and food utilization of common carp fry reared for 25 days in trial 2 ,Expt. 2.1

Treatment No.	Temp. (°C)	Feeding rate (%BW/day)	Mean initial weight (mg)	Mean final weight (mg)	Weight gain (mg)	Mortality (%)	SGR	FCR	PER	ANPU (%)
D1	35	15	46^{b}	238	192^{d}	6.9	*6.35(5.83)^{d}	1.86^{a}	1.04^{a}	13.19^{abc}
D2		20	60^{a}	412	352^{c}	1.4	7.70^{c}	1.91^{a}	1.01^{ab}	13.16^{abc}
D3		25	65^{a}	568	502^{abc}	0	8.64^{abc}	2.07^{ab}	0.94^{abc}	12.49^{abc}
D4		30	62^{a}	716	654^{a}	0	9.79^{a}	2.04^{ab}	0.95^{abc}	13.95^{a}
D5		35	62^{a}	613	551^{ab}	1.4	9.10^{ab}	2.61^{c}	0.75^{de}	10.06^{e}
E1	32	25	64^{a}	599	535^{ab}	0	8.95^{ab}	1.94^{a}	0.99^{ab}	13.73^{ab}
E2		30	64^{a}	710	646^{a}	1.4	9.63^{a}	2.19^{ab}	0.88^{bc}	12.55^{abc}
E3		35	62^{a}	695	633^{a}	1.4	9.67^{a}	2.45^{bc}	0.82^{cd}	10.50^{de}
F1	28	25	61^{a}	467	406^{bc}	1.4	8.13^{bc}	2.16^{ab}	0.89^{bc}	11.86^{bcd}
F2		30	63^{a}	564	501^{abc}	1.4	8.79^{abc}	2.31^{abc}	0.84^{cd}	11.34^{cde}
F3		35	67^{a}	548	481^{abc}	0	8.42^{bc}	2.91^{d}	0.67^{e}	8.73^{f}
		± SE***	1.29		35.88	1.8	0.247	0.094	0.031	0.406

* Value in the parenthesis is the adjusted specific growth rate to refer to an initial weight of 63 mg (see Section 2.2.10 for detail)

** Figures in the same column with same superscripts are not significantly different ($P>0.05$)

*** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

Table 2.12 Proximate carcass composition analysis (% fresh weight) of fish samples at the end of trial 2, Experiment 2.1

Treatment No.	Temp. °C	Feeding rate (%BW/day)	Moisture	Crude protein	Crude lipid	Ash	Total
D1	35	15	80.54^{a}*	12.74^{a}	2.66^{d}	3.01	98.95
D2		20	80.06^{a}	13.04^{a}	3.71^{c}	2.90	99.71
D3		25	78.41^{b}	13.39^{a}	4.36^{bc}	2.77	98.93
D4		30	78.20^{b}	13.69^{a}	4.79^{ab}	2.76	99.44
D5		35	78.57^{b}	13.56^{a}	4.34^{bc}	2.62	99.09
E1	32	25	78.02^{b}	13.85^{a}	5.17^{a}	2.58	99.62
E2		30	77.22^{b}	14.30^{a}	5.41^{a}	2.46	99.39
E3		35	78.49^{b}	13.36^{a}	5.36^{a}	2.26	99.47
F1	28	25	78.01^{b}	13.30^{a}	5.56^{a}	2.54	99.41
F2		30	77.81^{b}	13.57^{a}	5.38^{a}	2.53	99.29
F3		35	77.94^{b}	13.17^{a}	5.58^{a}	2.64	99.33
		± SE**	0.322	0.255	0.185		

* Figures in the same column with same superscripts are not significantly different ($P > 0.05$)

** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

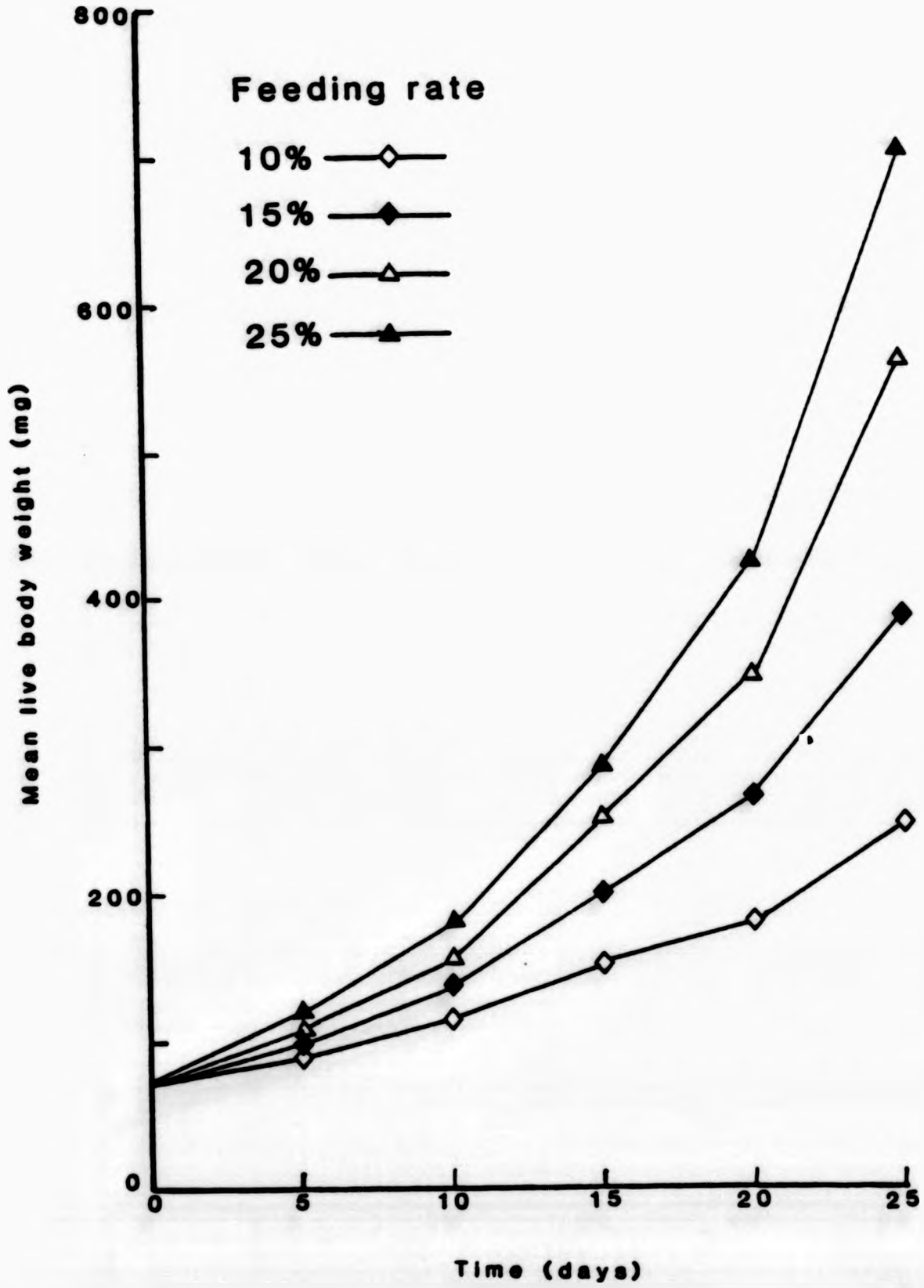

Fig. 2.2 Growth responses of carp fry at 32°C at different feeding rates in trial 1.

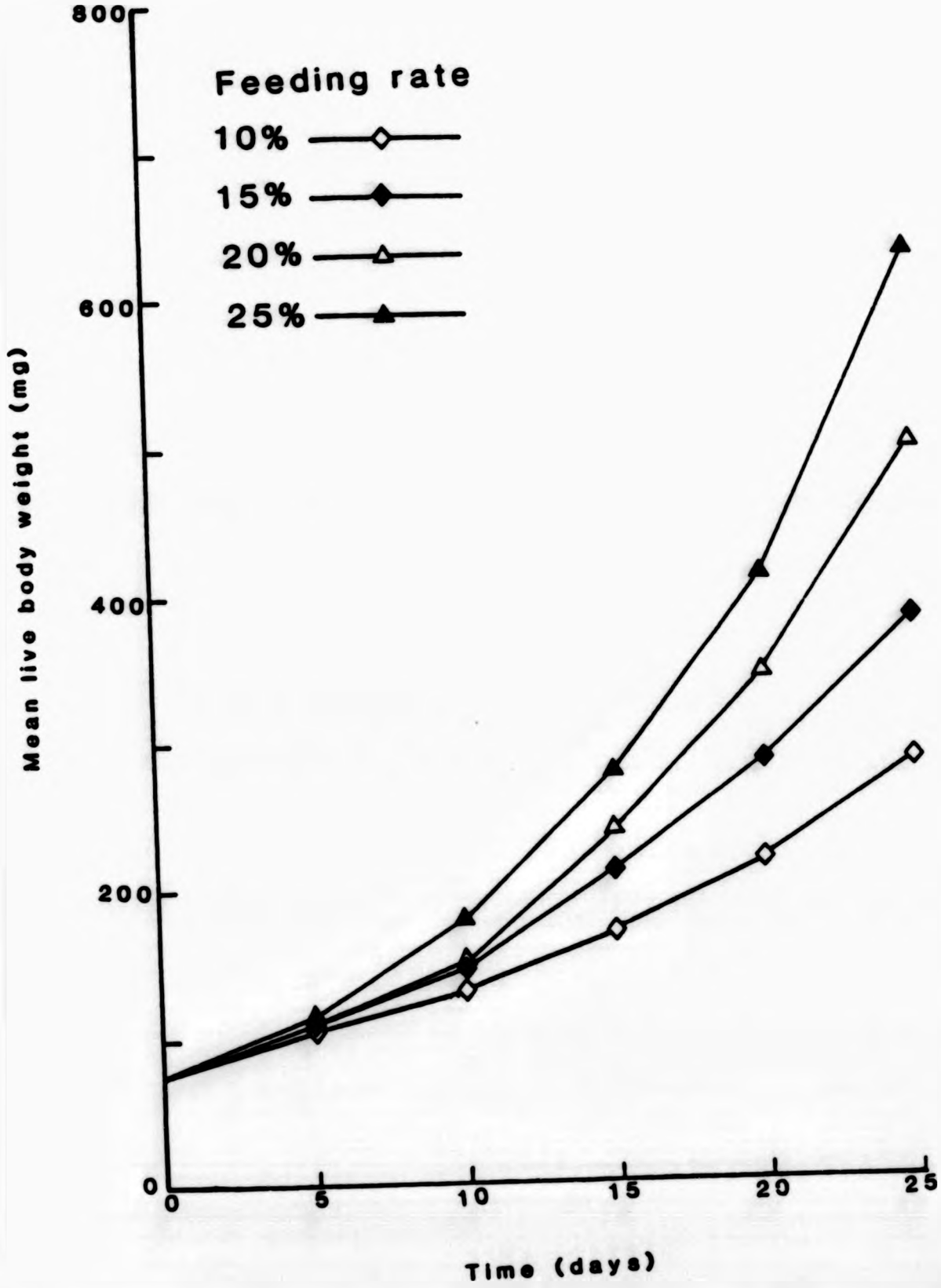

Fig. 2.3 Growth responses of carp fry at 28°C at different feeding rates in trial 1.

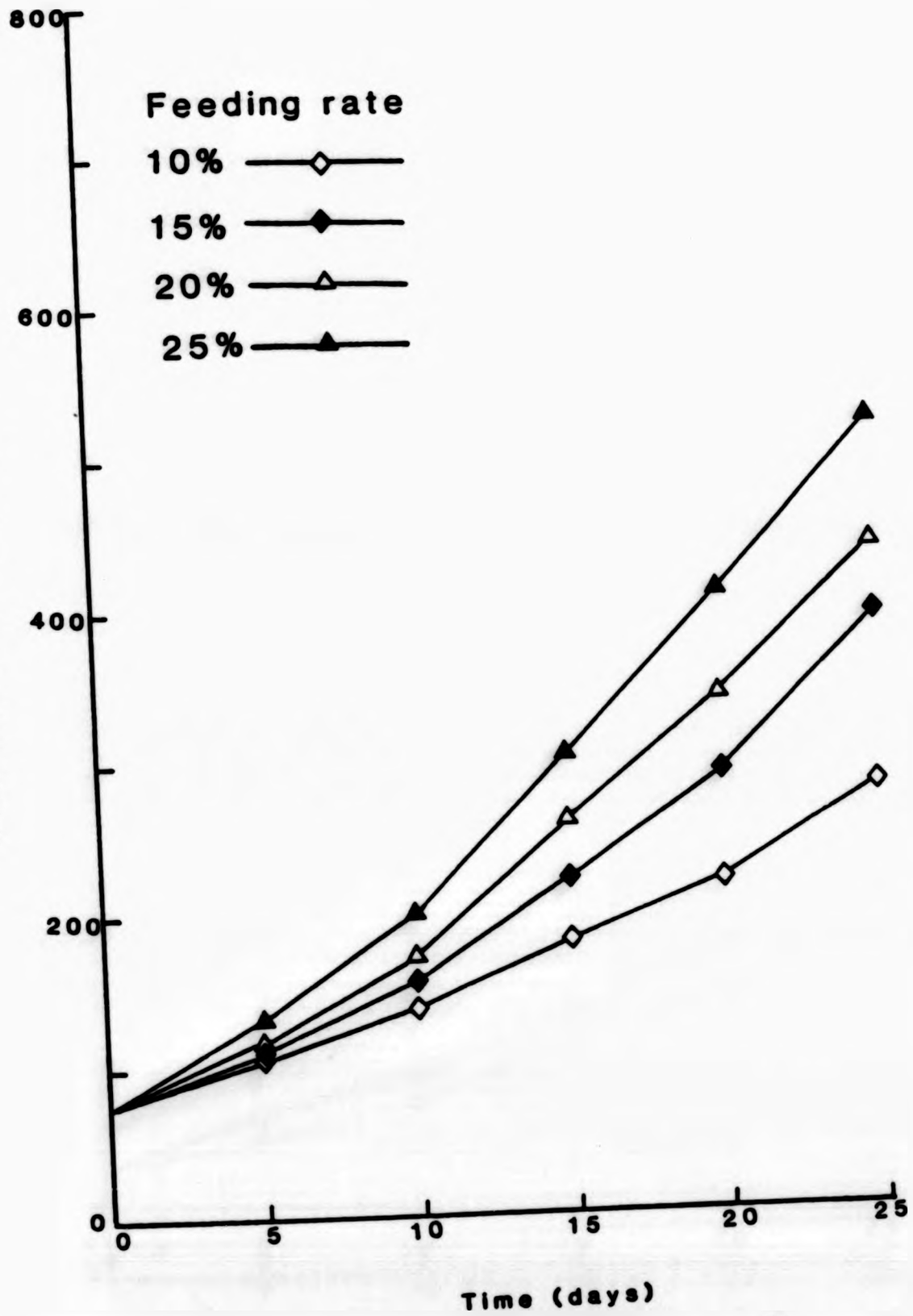

Fig. 2.4 Growth responses of carp fry at 24°C at different feeding rates in trial 1.

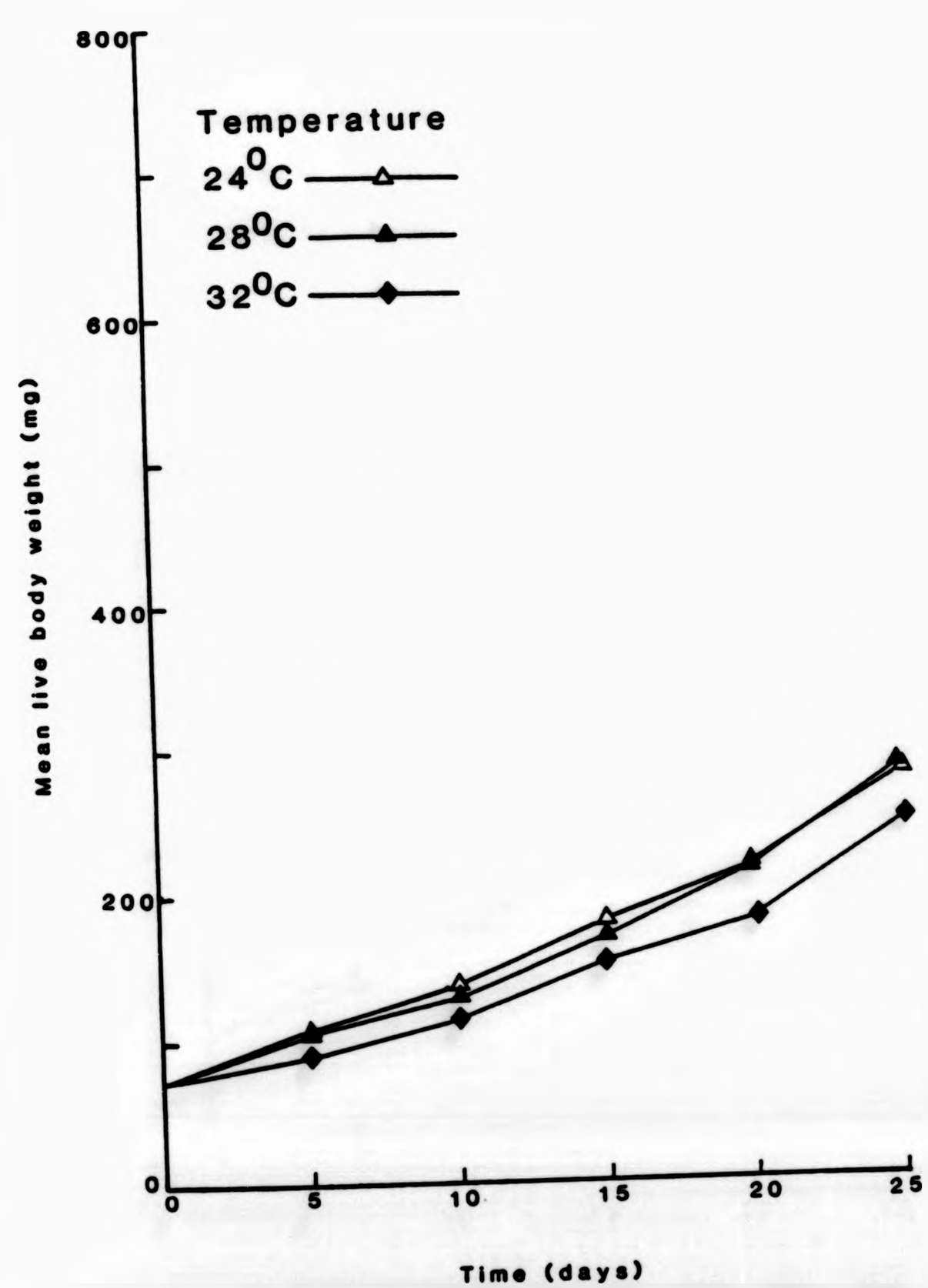

Fig. 2.5 Growth responses of carp fry fed 10% of body weight per day at different rearing temperatures in trial 1.

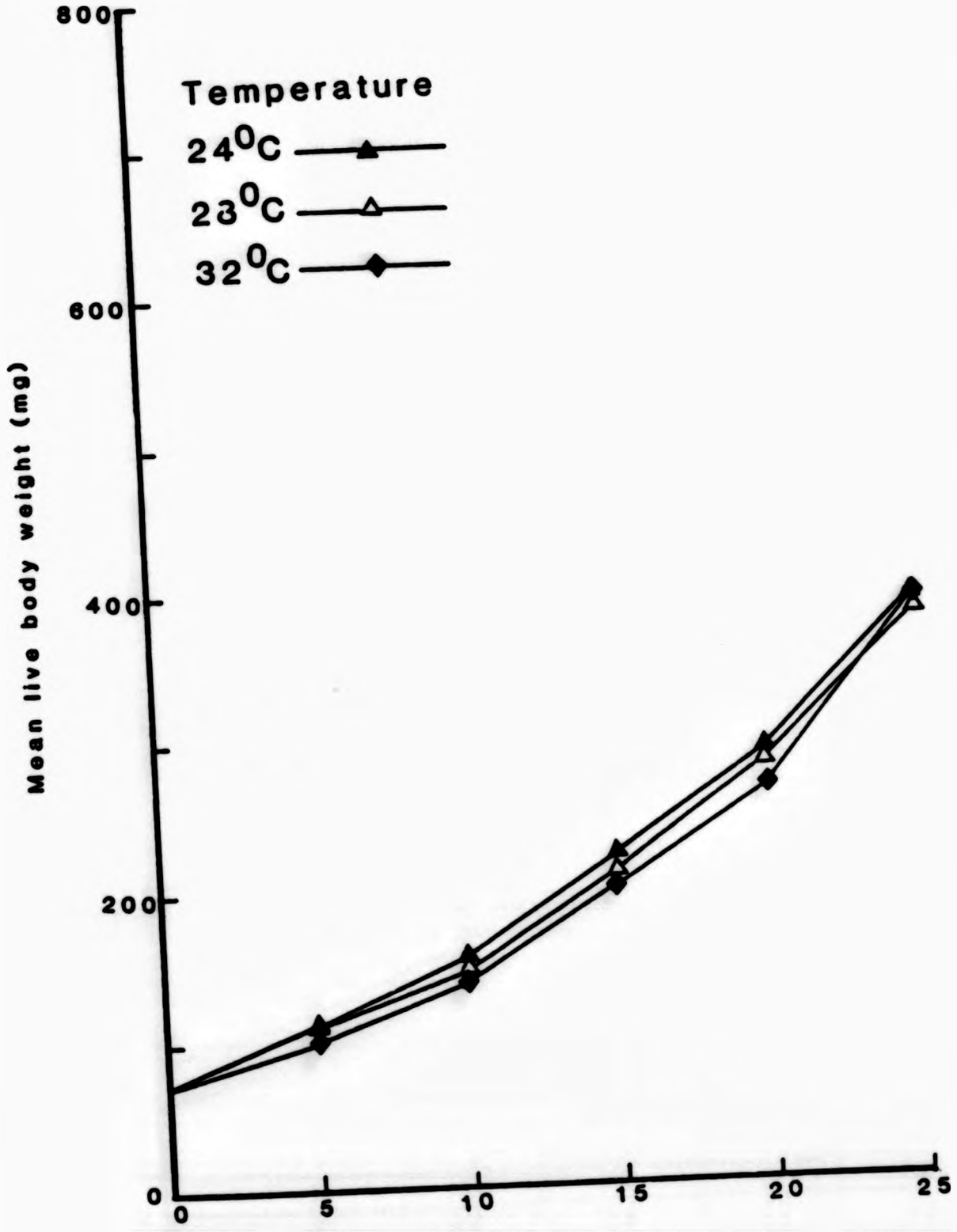

Fig. 2.6 Growth responses of carp fry fed 15% of body weight per day at different rearing temperatures in trial 1.

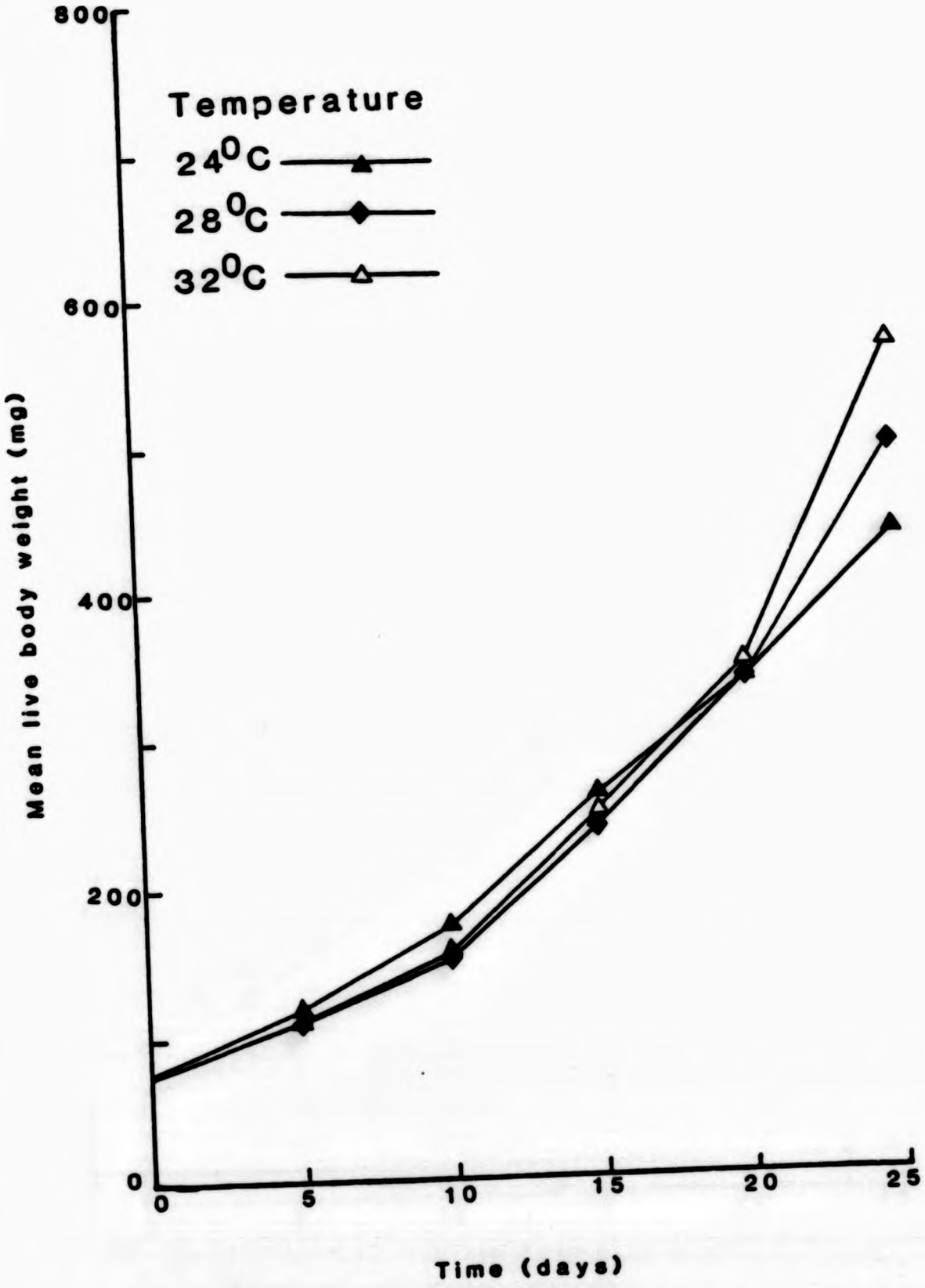

Fig. 2.7 Growth responses of carp fry fed 20% of body weight per day at different rearing temperatures in trial 1.

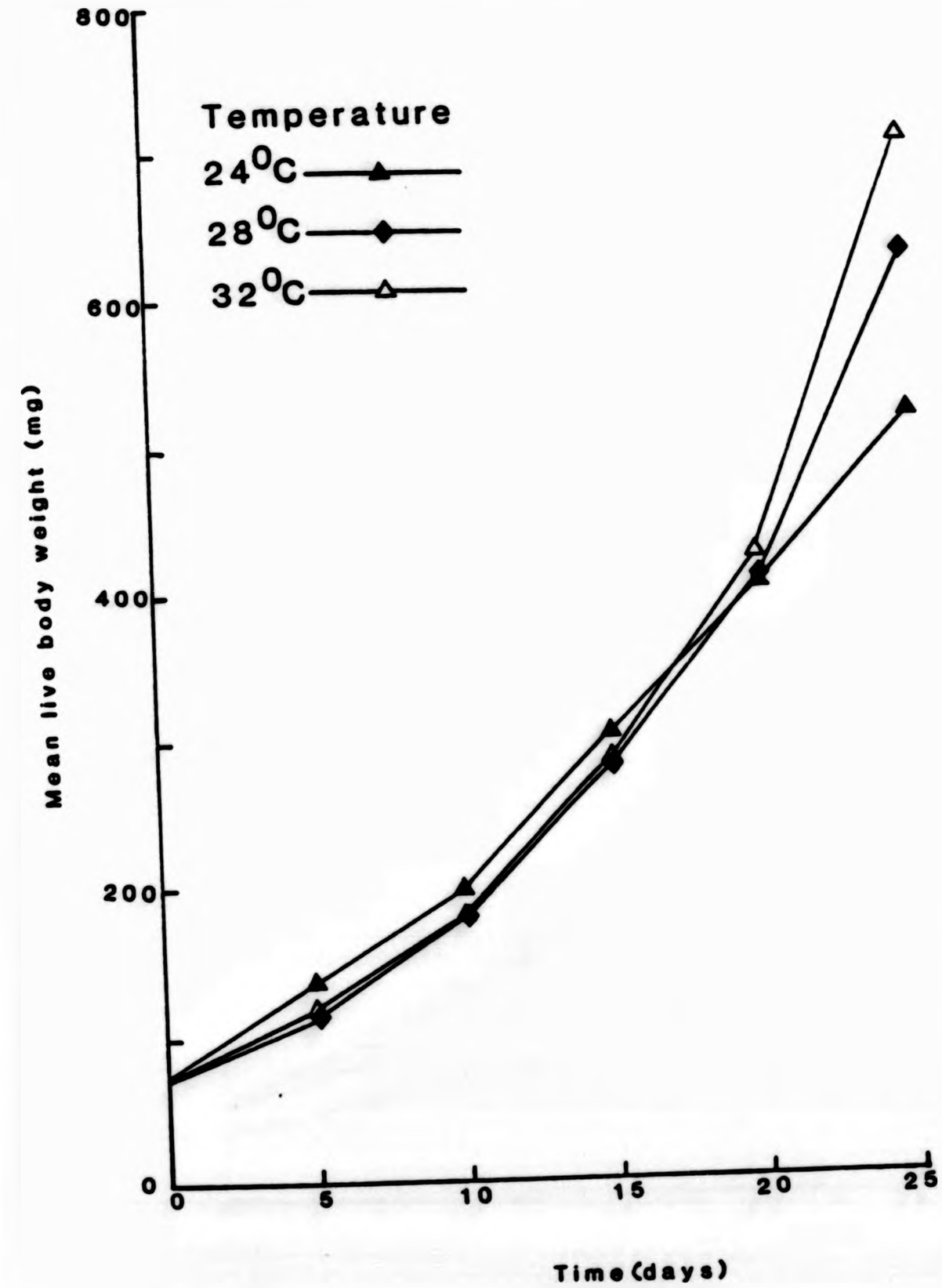

Fig. 2.8 Growth responses of carp fry fed 25% of body weight per day at different rearing temperatures in trial 1.

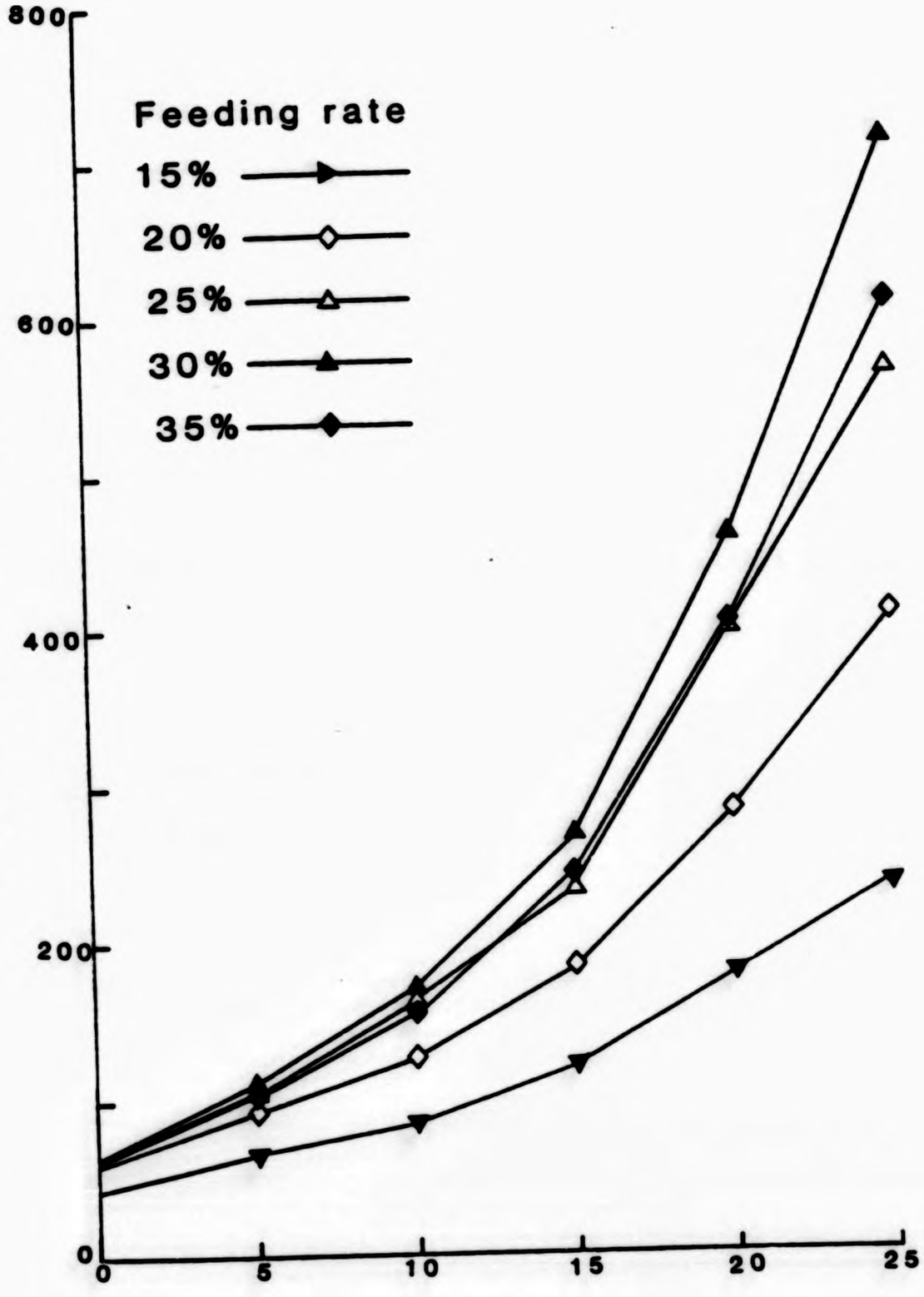

Fig. 2.9 Growth responses of carp fry at 35°C at different feeding rates in trial 2.

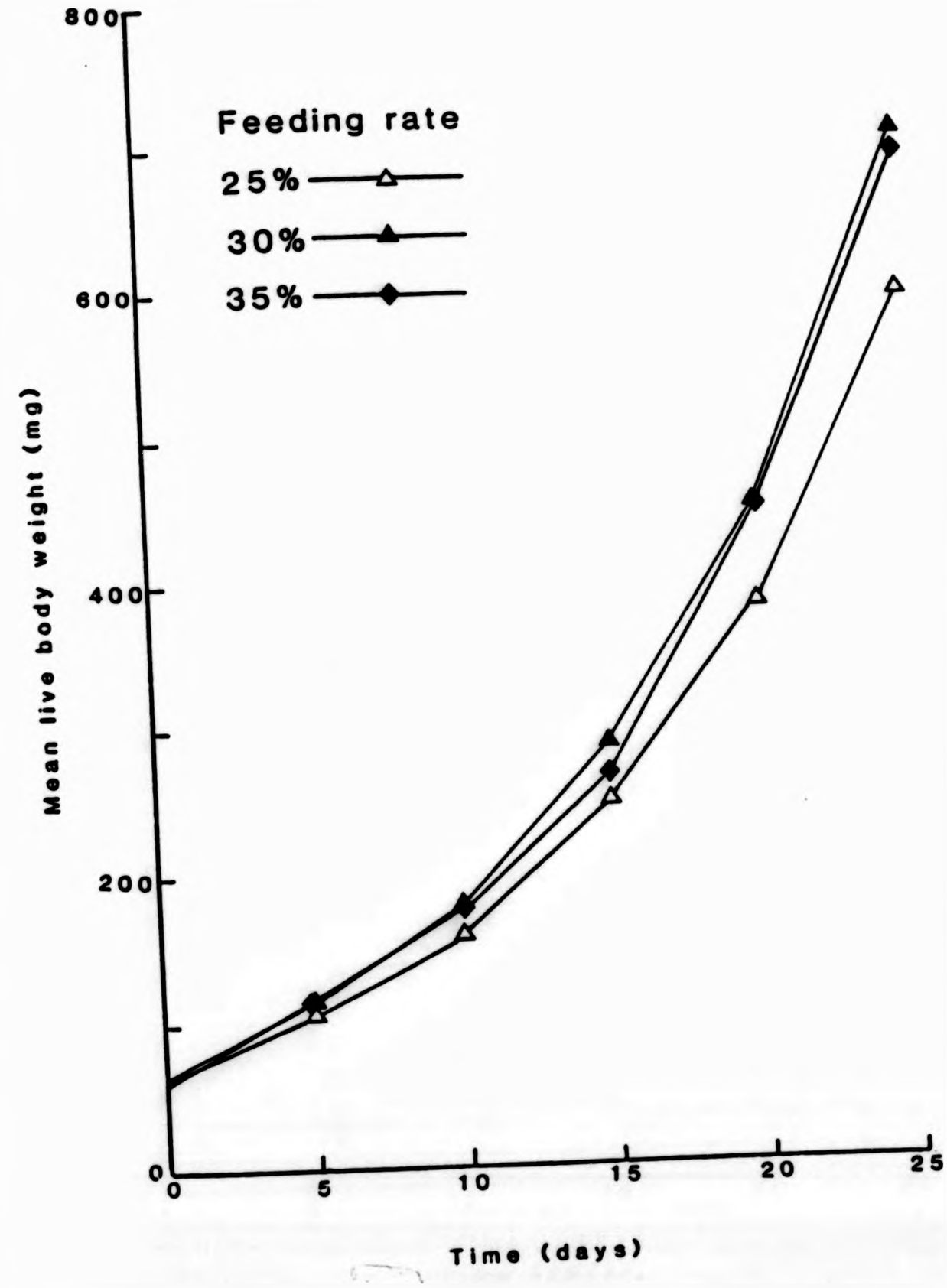

Fig. 2.10 Growth responses of carp fry at 32°C at different feeding rates in trial 2.

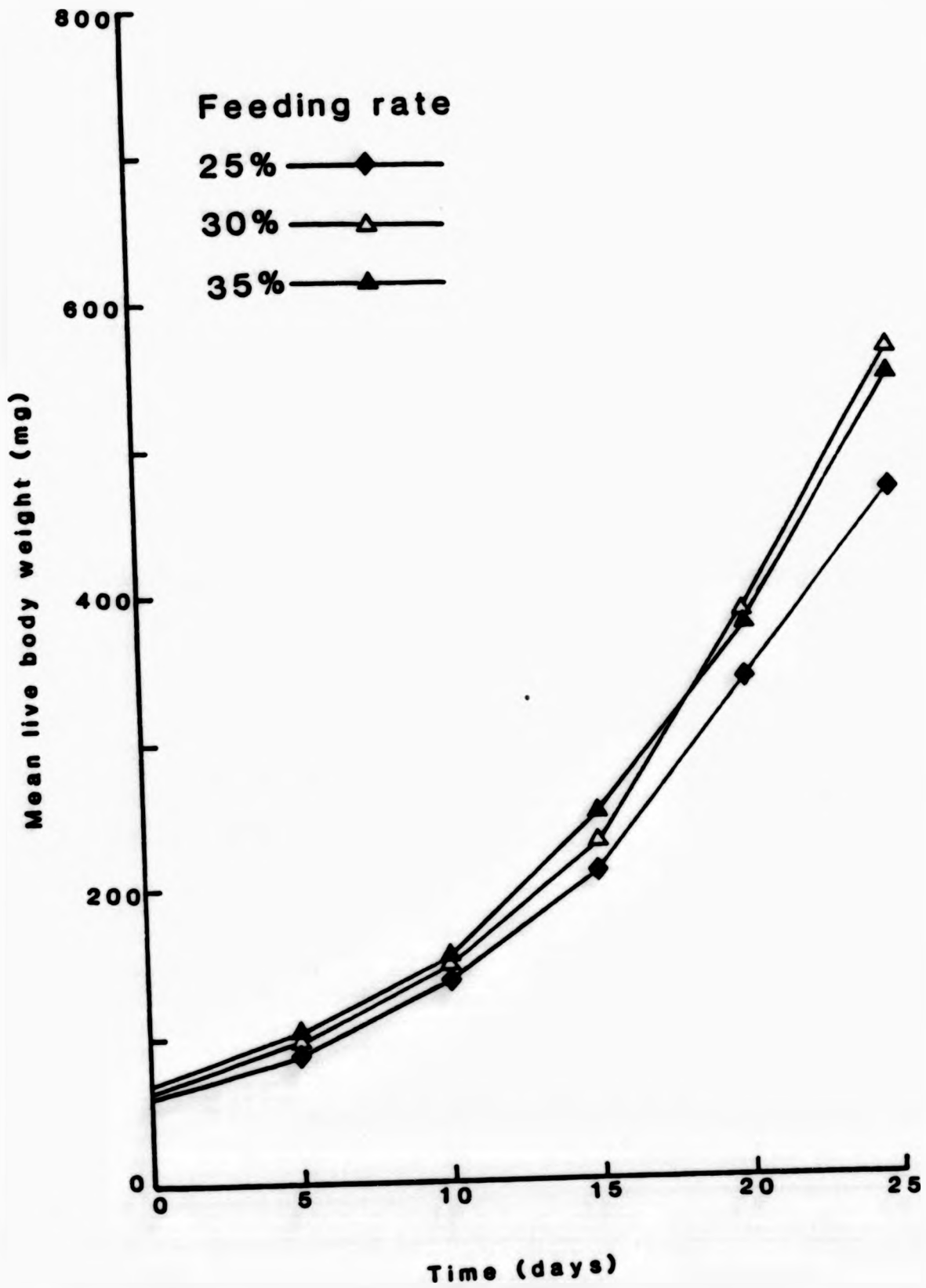

Fig. 2.11 Growth responses of carp fry at 28°C at different feeding rates in trial 2.

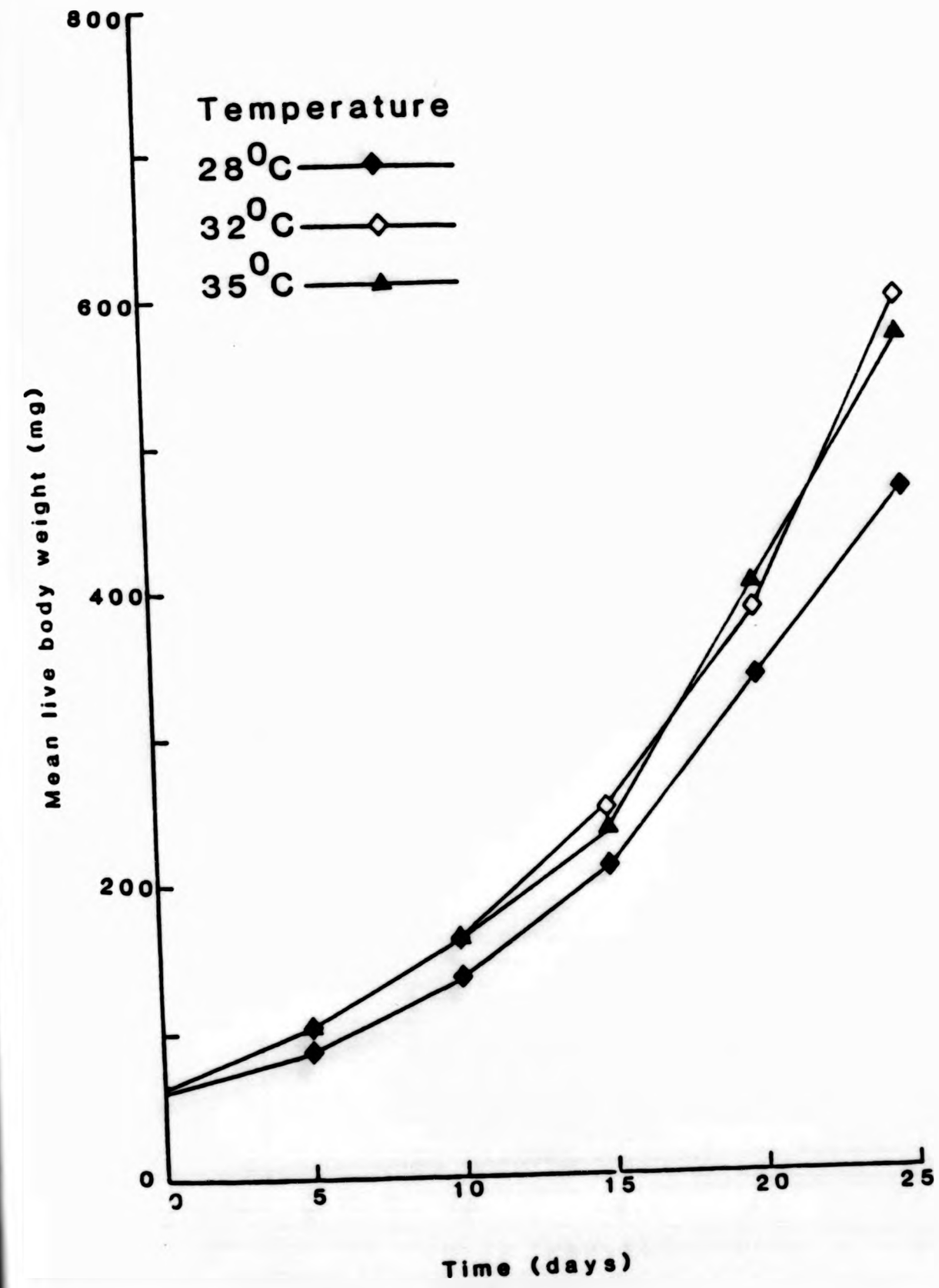

Fig. 2.12 Growth responses of carp fry fed 25% of body weight per day at different rearing temperatures in trial 2.

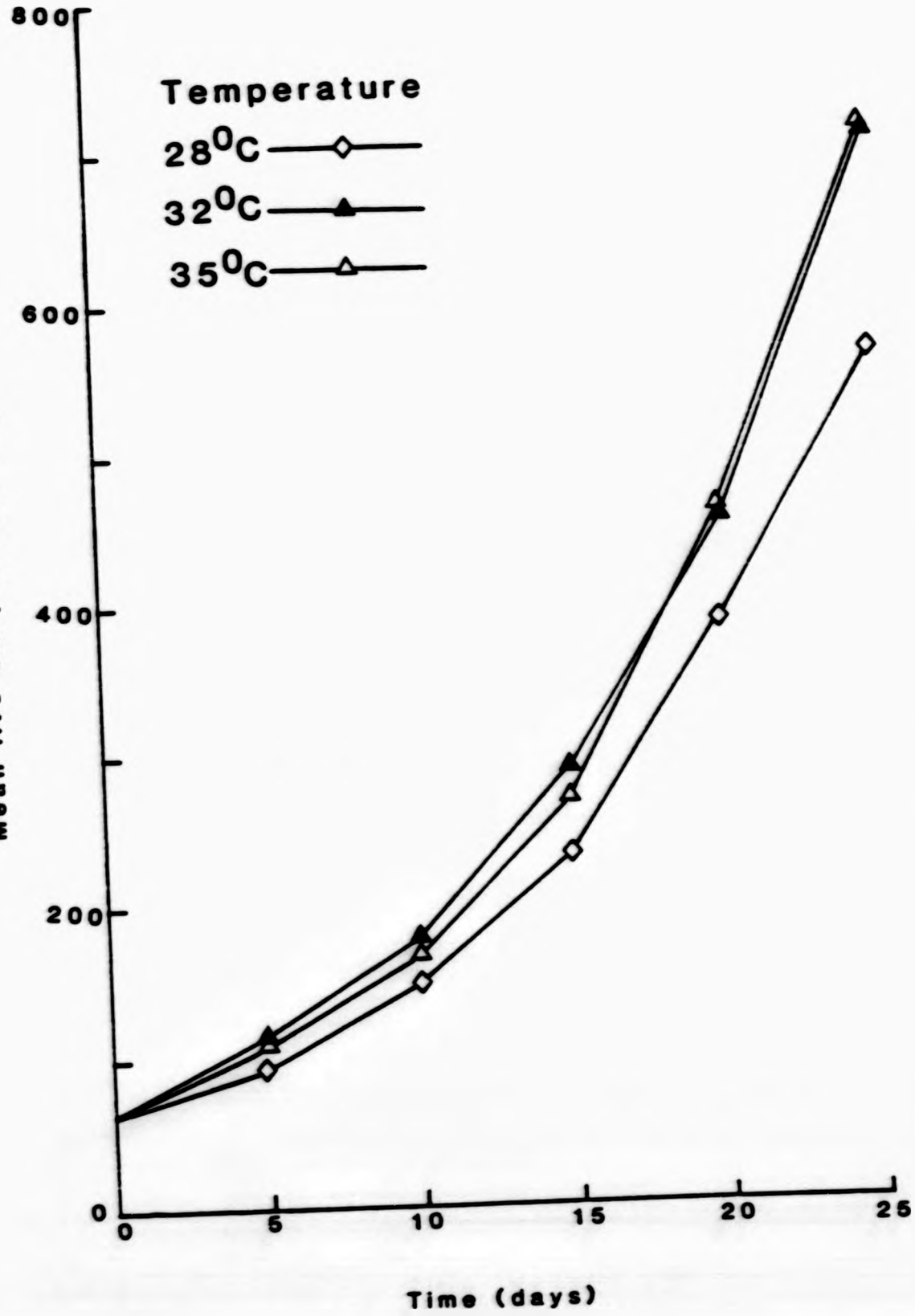

Fig. 2.13 Growth responses of carp fry fed 30% body weight per day at different rearing temperatures in trial 2.

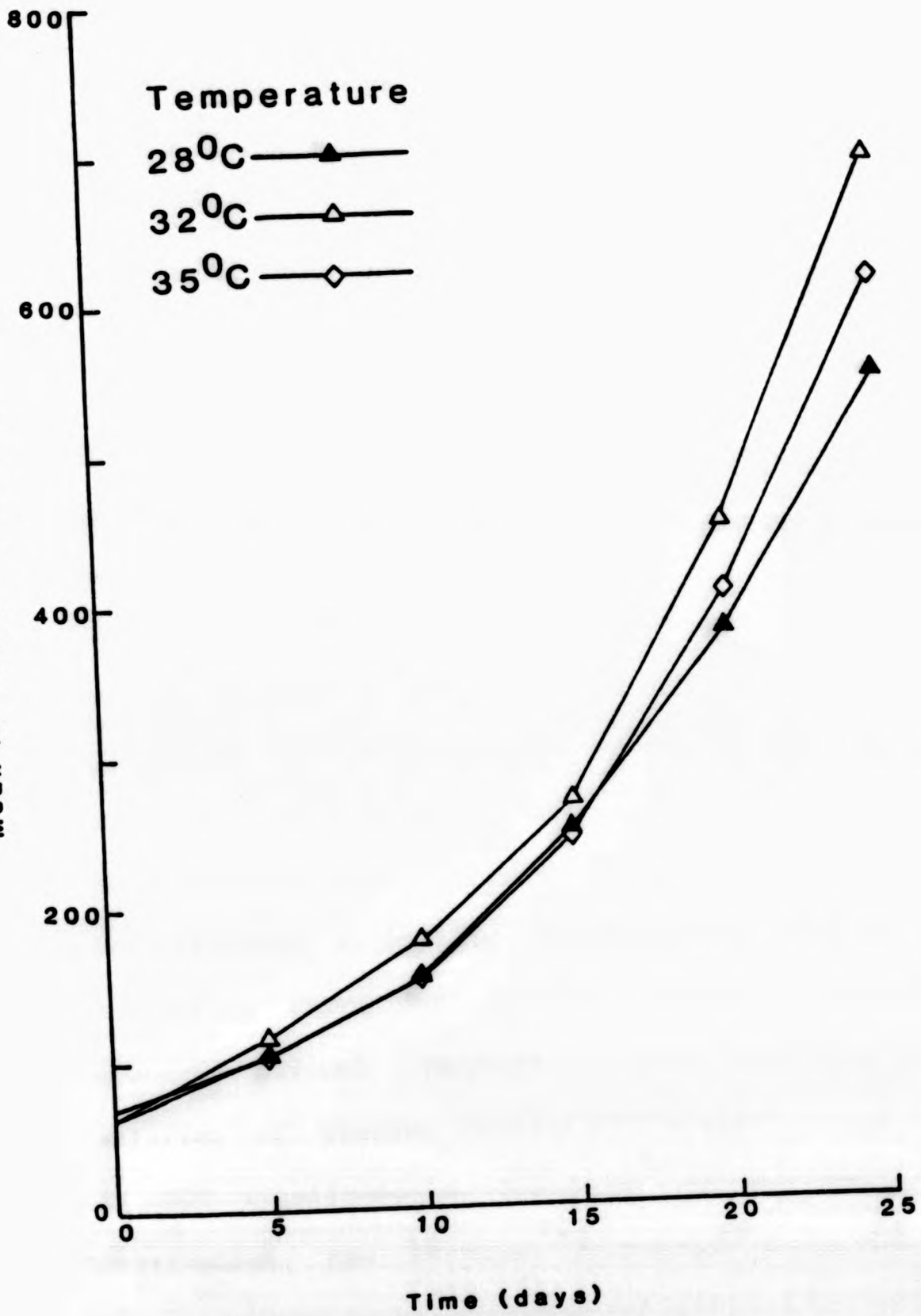

Fig. 2.14 Growth responses of carp fry fed 35% of body weight per day at different rearing temperatures in trial 2.

($P<0.05$) than the SGRs of 7.69 and 7.17 recorded at 28 and 24°C respectively (Table 2.9). Similarly at 25% feeding rate, the SGR (9.08) at 32°C was significantly higher than those at 28 and 24°C respectively (8.64 and 7.86).

The growth responses of carp fry in trial 2 are presented in Table 2.11 and illustrated graphically in Figures 2.9-2.14. The mean initial weight of carp fry was statistically the same ($P > 0.05$) for all groups except that of treatment D_1 (15% BW - 35°C) (Table 2.10). To overcome the problem of size variation, the SGR for carp fry in above treatment was adjusted to an initial weight of 63 mg (overall mean) (see section 2.2.2.10 for detail) and the adjusted SGRs used for statistical comparison. At all rearing temperatures, increase in feeding rate up to 30% BW increased both weight gain and the SGR, although in some cases the increases were not statistically significant ($P > 0.05$) (Figs 2.9-2.11 and Table 2.11). At 35°C, SGR increased from 5.83 at 15% BW to 9.79 at 30% BW. Similarly, at 32°C SGR increased from 8.95 to 9.63 and those at 28°C from 8.13 to 8.79 with increase in feeding rate from 25 to 30% BW. However, increase in feeding above 30% resulted either in a decrease or relatively unchanged growth response. (Figs 2.9-2.11 and Table 2.11). Variation of rearing temperatures did not influence the weight gain and SGR significantly ($P > 0.05$) at any of the feeding rates. Nevertheless, the increase in rearing temperature from 28 to 32 or 35°C increased the weight gain and SGR at each feeding level tested, although not always significantly.

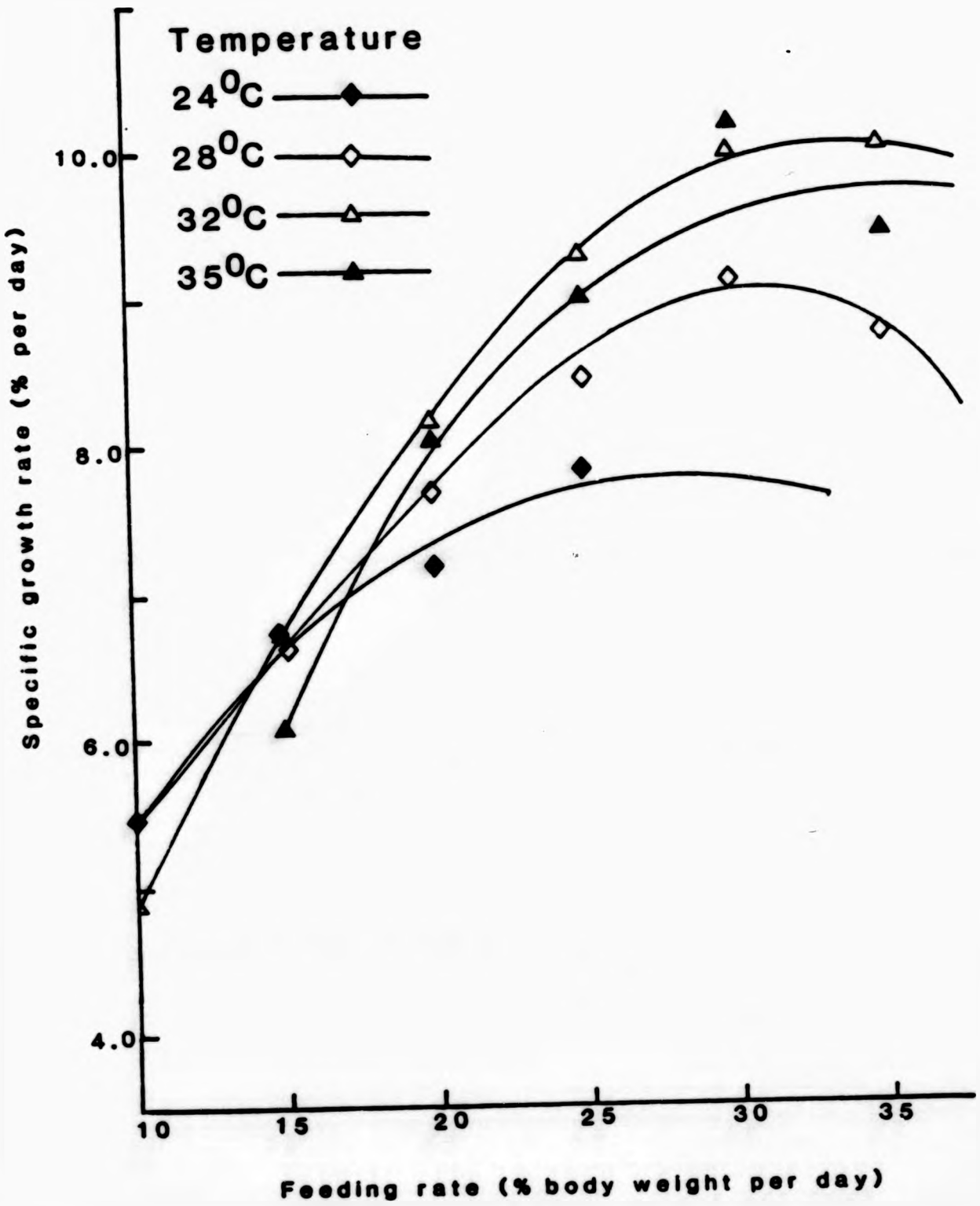

Fig. 2.15 Relation between feeding rate and growth rate of carp fry at four rearing temperatures (combined data of trial 1 and 2) (curves fitted by eye).

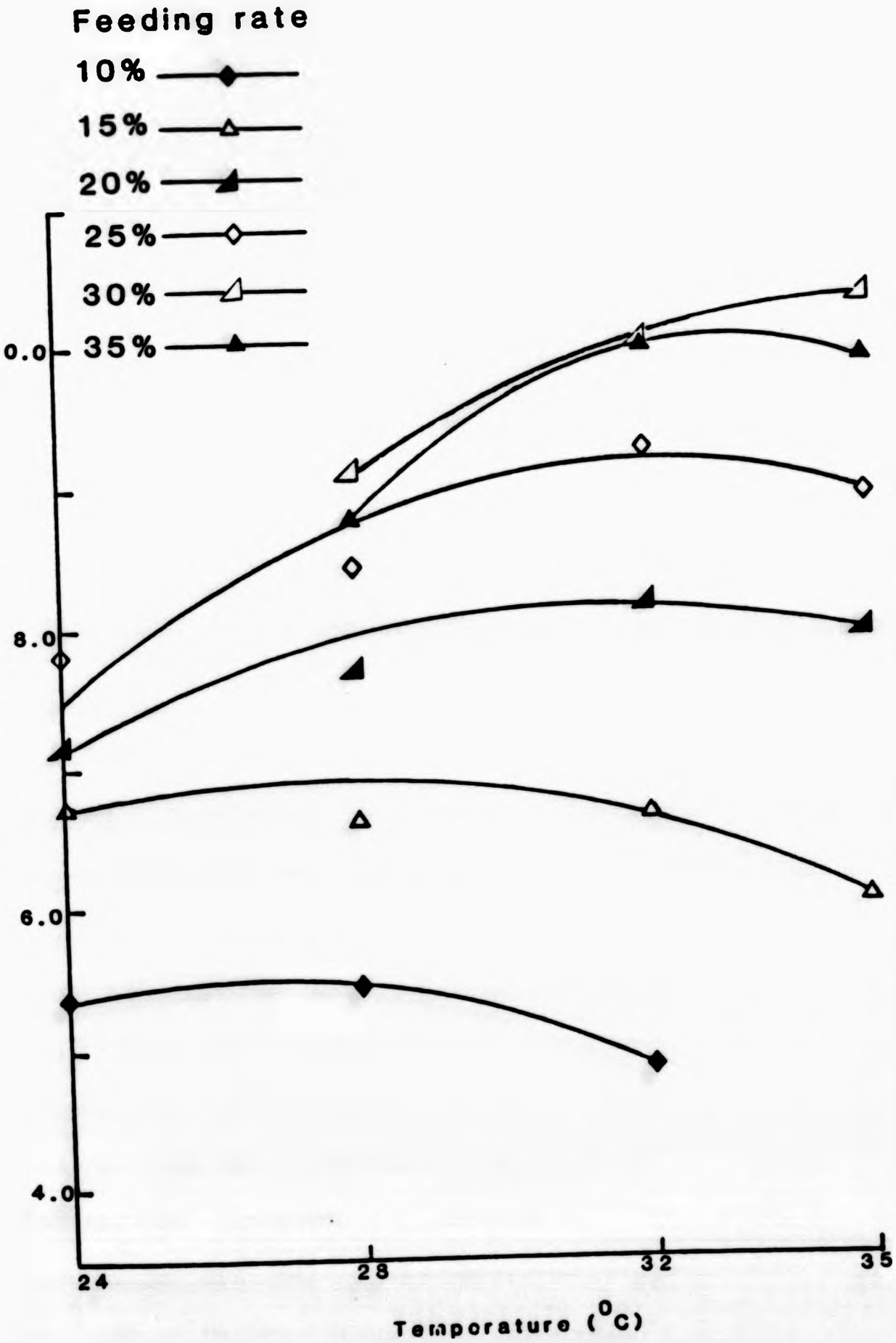

Fig. 2.16 Relationship between rearing temperature and growth rate of carp fry at six feeding rates (combined data of trial 1 and 2) (curves fitted by eye).

Combined growth data of trial 1 and 2 (see section 2.2.2.10a for detail) have been plotted to illustrate the effect of feeding rate (Fig 2.15) and temperature (Fig 2.16) on the growth rate of carp fry.

2.2.3.2. Food conversion

The calculated food conversions (FCRs) for each treatment group in trial 1 are presented in Table 2.9. The FCR values ranged from 1.56 (10% BW-28°C) to 2.80 (25% BW-24°C). At each temperature, the FCR increased with feeding rate. Raising the rearing temperature, however, improved significantly ($P < 0.05$) the FCRs at all feeding levels except the 10% level which was unaffected ($P > 0.05$) by temperature.

The FCRs from trial 2 are presented in Table 2.11. The FCR values ranged between 1.86 (15% BW-35°C) and 2.91 (35% BW-28°C). At each temperature, increasing the feeding rate resulted in an increase in FCRs, although in some cases not significantly ($P > 0.05$). At all temperatures, 35% feeding resulted in very poor food conversion. For example, at 35°C an FCR of 2.04 was recorded at 30% BW, compared to 2.61 at 35% BW. Similarly, at 28°C the FCR was 2.31 at 30% BW, whereas, it increased to 2.91 at 35% BW. Variation in rearing temperature did not result in significant differences ($P < 0.05$) in the FCRs up to 30% feeding rate. However, in general, the FCRs were poorer at 28°C than at 32°C and 35°C.

The FCRs from both trials have been plotted in Fig. 2.17. It can be seen that at all feeding rates ranging from 15 to 35%, the lowest FCRs were observed at 32°C, followed by those at 35, 28 and 24°C, with the exception of the 30% BW-35°C treatment. From Fig. 2.17 it can also be seen that, with the exception of 24°C, increasing the feeding rate from 15 to 25 or 30% at any rearing temperature, did not appreciably affect the efficiency of food conversion.

2.2.3.3. Protein utilization

The levels of protein utilization were evaluated in terms of (a) protein efficiency ration (PER) and (b) apparent net protein utilization (ANPU%).

PER and ANPU values for the different treatment groups in trial 1 are presented in Table 2.9. Since a single diet was used for all treatments, the PERs obtained at various temperatures and feeding rates were similar to the equivalent FCRs. The PER values ranged between 0.71 and 1.27, the highest value occurring at 10% BW-28°C and the lowest value at 25% BW-24°C. At each rearing temperature, the PER decreased with feeding rate and at each feeding rate, it increased with temperature (10% BW-32°C is the exception).

ANPU values tended to reflect the PER values observed for different treatment groups. The highest ANPU value (16.75%) was observed at 10% BW-28°C followed by a value of 15.39% at 10% BW-24°C with the lowest value (9.27%) occuring at 25% BW-24°C. With exception of

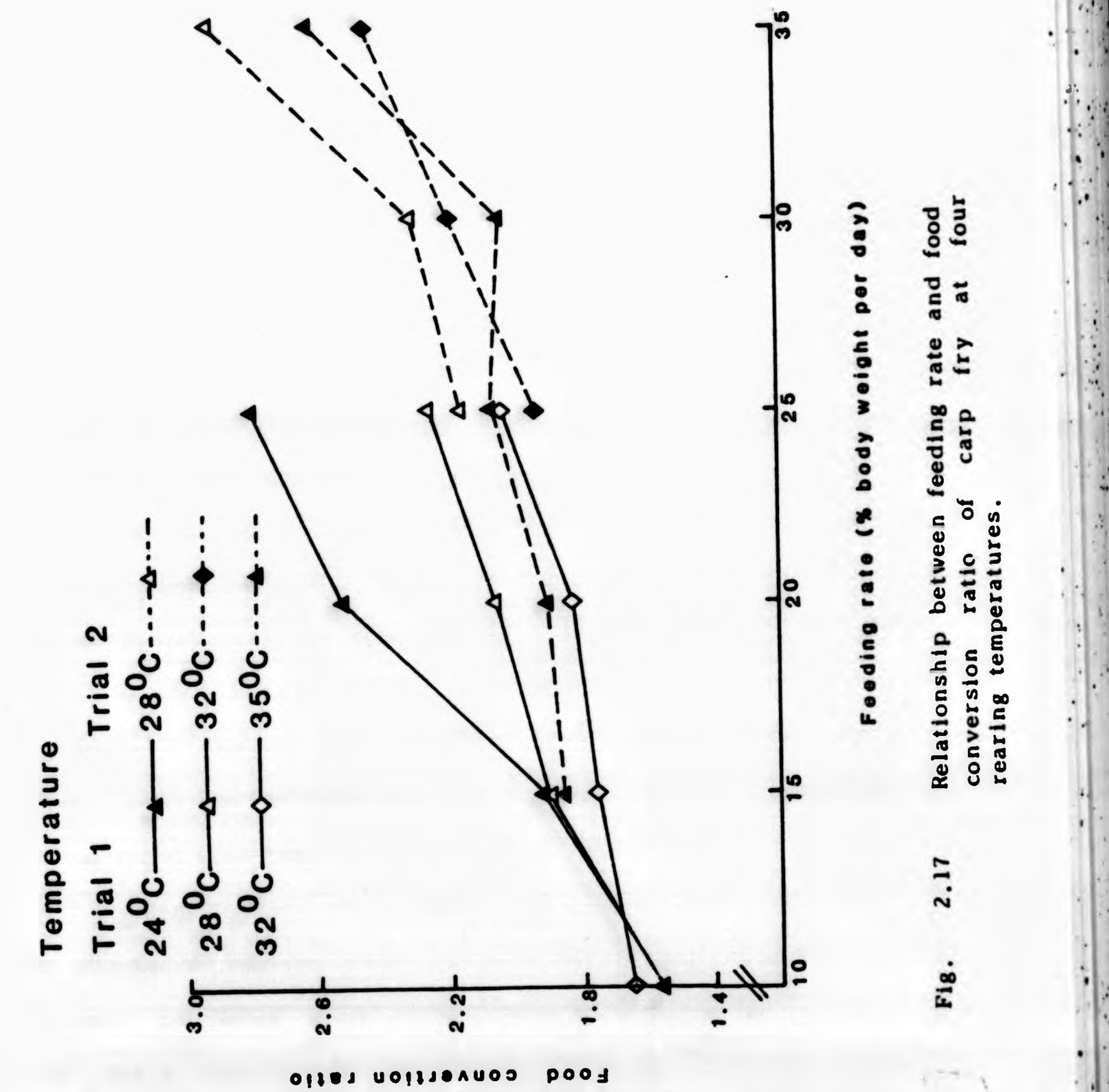

Fig. 2.17 Relationship between feeding rate and food conversion ratio of carp fry at four rearing temperatures.

32°C at each rearing temperature, ANPU values decreased with feeding rate and with the exception of 10% BW, ANPU values increased with temperature.

PER and ANPU values for different treatments in trial 2 are presented in Table 2.11. The PER values ranged between 0.67 (35% BW-28°C) and 1.04 (15% BW-35°C). The ANPU values ranged from 8.73% (35% BW-28°C) to 13.95% (30% BW-35°C).

2.2.3.4. Carcass compostion

The proximate carcass composition of fish samples taken at the start and end of trial 1 is presented in Table 2.10. Neither temperature nor feeding rate appeared to have a major effect on the carcass composition of fish. Although not statistically different ($P > 0.05$) in every case, carcass moisture content decreased and carcass lipid content increased with feeding rate at each rearing temperature. Carcass protein content did not vary significantly ($P > 0.05$) between treatments (exception 10% BW-32°C). With some exceptions, the carcass ash content decreased with feeding rate at each temperature and increased with temperature at each feeding rate.

The proximate carcass composition of fish samples from trial 2 are presented in Table 2.12. Both temperature and feeding appeared to have little influence on the carcass composition. Carcass moisture content did not vary significantly ($P > 0.05$) between different

treatments except at 15 and 20% BW-35°C. There were no significant differences ($P > 0.05$) in carcass protein content between treatments. With the exception of 35°C, the carcass lipid content was not affected by temperature or feeding rate. At 35°C, carcass lipid content increased with increase in feeding rate from 15 to 25% BW and remained relatively unchanged with further increase. Carcass ash content decreased with feeding rate and increased with temperature at each temperatures except 28°C.

2.2.4. Discussion

The resuts from these trials suggest that a rearing temperature of about 32°C is optimal for growth and food conversion of common carp fry. This is a higher value than the optimum temperature reported by other authors (Table 2.1), however, previous studies have dealt with more advanced fry, fingerlings and adult fish.

Some researchers (Beitinger and Fitzpatrick, 1979; Jobling, 1981b; McCauley and Casselman, 1981) have concluded that optimum temperatures for various physiological functions, particularly growth, coincide generally with the final thermal preferenda of the fish. For example, Brett (1952) determined the preferred temperature for sockeye salmon, Oncorhynchus nerka and subsequent investigation revealed that this temperature coincided with the temperature otpima for swimming speed, metabolic scope for activity and growth (Brett, 1971).

There are a number of accounts which report preferred temperatures for the common carp (see review by Coutant, 1977) and the values range from 27.4 to 32.0°C. However, the preferred ranges for young carp are 31.9 to 32.0°C, compared to 27.4 to 31.9°C for adults. It may be inferred from this that adult carps have a broad zone of thermal preference, whereas younger fish prefer the upper part of this range. The above statement may be substantiated by findings reported by McCormick et al, (1971) for ciscos (Coregonus artedii), Shelbourn et al, (1973), for sockeye salmon, Elliott (1975), for brown trout, Huisman et al, (1979) for carp and Mironova (1975) and Nyambi (1982) for Java tilapia (Oreochromis mossambicus). These authors reported that temperature plays a more important role in the growth of younger fish than of adults. Brett (1979) reported that all species in their younger stages show a typically rapid increase in growth rate with temperature, but their response passes through a peak (optimum temperature) and frequently falls precipitously at adversely high temperatures.

At higher feeding rates, the growth rate and food conversion of the carp fry were found to be comparable at 32 and 35°C. This contrasts with the findings of Korneev (1964) cited by Gribanov et al, (1968) and Jauncey (1979). Jauncey (1979) reported severely depressed growth of fingerling carp at 35°C compared to fish maintained at 30°C. However, as in the present study, Beliaev (1969) cited by Alabaster and Lloyd (1980) observed normal behaviour, and no apparent adverse effects of temperature, for carp (size not reported)

maintained at 35.2°C. Studies by Mironova (1975) with Java tilapia also showed that for 3-4 month old fish their maximum growth rate occurred at 31°C and the temperature most favourable for growth decreased with increase in fish size. Therefore, the comparatively higher growth rate recorded for carp fry at 32-35°C confirmed the growth potential of young fish, even at higher temperatures.

The relationship between growth and feeding rate (Fig 2.15) shows that at any rearing temperature, the SGR increased with feeding rate. From the growth curve at 24°C, it can be postulated that the scope for maximum growth did not occur above 25% feeding rate, but lay between 20 and 25% BW. At 28°C the maximum growth rate appeared to occur at feeding rates of between 25 and 30% BW. Both at 35 and 32°C, maximum growth occurred at about 30% BW. Feeding above 30% BW at both 28 and 35°C led to a decline in growth rate. Huisman (1976a) and Meske (1985) for carp, and Roberts (1976) cited by Jauncey (1979) for rainbow trout, observed a fall in growth rate with high levels of feeding.

The above observations conform to the mathematical approach to fish growth of Paloheimo and Dickie (1965, 1966a,b) in which they expressed metabolism at a given body weight as a function of food ration. These authors showed that the metabolic rate of fish increases 4-5 times as food ration increases from the maintenance level. Thus it may be concluded that excessive feeding would induce a high metabolic rate, resulting in a significanct reduction in the

amount of energy available for growth. The results of the present investigation indicate that feeding carp fry more than 30% BW per day, even at higher rearing temperatures (28-35°C), is excessive.

The results of this study show that optimum temperature for the growth of carp fry shifted upwards with increase in feeding rate. At feeding rates of 10 and 15% BW, 28°C appeared to be the most suitable temperature for maximum growth, whereas at feeding rates between 20 and 35% BW, 32°C was better. This result conforms with reports by Brett et al (1969) for sockeye salmon and by Elliott(1975) for brown trout. These authors concluded that the optimum temperature for growth decreases progressively with decreasing ration. This is because the decrease in maintenance metabolism at lower temperatures should permit better use of a restricted ration for growth. However, in the present investigation, the progressive shift of optimum temperature with ration size was not as marked as observed for salmon and trout. This was probably due to the fact that all the levels of feeding used (10 - 35% BW) were significantly higher than the maintenance ration required by carp fry at the temperatures studied. Nevertheless, at 10% feeding rate, the SGR at 32°C (4.91) was significantly lower than those at 28 and 24°C (5.46 and 5.36) (Table 2.9). Similarly, at 15% feeding rate the SGR at 35°C was considerably lower than those at other three temperatures (Fig. 2.15). Conversely, at feeding rates above 15% (20-30%) increase in rearing temperature up to 32°C did result in a higher growth rate. Similarly Allen and Wootton (1982), while studying the effect of

ration and temperature on the growth of the three spined stickleback, Gasterosteus aculeatus L., noted that at low rations, growth rate decreased with temperatures, whereas at high rations growth rate increased with temperature.

In both trials, increasing the feeding rate resulted in an increase in food conversion ratio at all rearing temperatures (Fig. 2.17). However, with exception of 24°C, increase in feeding rates from 15 to 30% BW at each rearing temperature did not greatly affect the efficiency of food utilization. At 24°C the efficiency of food utilization was reduced to a large extent at feeding levels above 15%BW. This is in agreement with finding of Bryant and Matty (1981), who reported that optimal growth and food conversion for carp fry of weight 100 mg occurred at feeding rates of between 10 and 15% BW at 24°C.

At all feeding levels, FCR improved considerably with increasing temperature, except at 35°C. Increased efficiency of food utilization with increase in rearing temperature even at lower feeding rates has been observed for channel catfish (Andrews and Stickney, 1972; Murray et al, 1977) and carp (Huisman et al, 1979; Jauncey, 1979). This may be due to the fact that the response of temperature dependent activities (e.g. rate of digestion, rate of food absorption, amino acid catabolism) results in more efficient utilization of food. And over the temperature range tested in the present study, this does not appear to have been offset by an

increase in maintenance energy expenditure.

Temperature and feeding rates influenced the body composition of carp fry in both trials of this study (Table 2.10 and 2.12). However, the influences were not as profound as reported by some other researchers (Brett et al, 1969; Andrews and Stickney, 1972; Niimi and Beamish, 1974; Murray et al, 1977; Huisman, 1976a; Huisman et al, 1979). At each rearing temperature, with some exceptions, increase in the feeding rate resulted in a decrease in carcass moisture content and an increase in carcass lipid content. However, in some cases the variations in the body composition were not statistically significant ($P>0.05$).

Increasing the feeding rate at any one temperature has been reported to decrease the carcass moisture content and increase carcass lipid content in sockeye salmon (Brett et al, 1969), large mouth bass (Micropterus salmoides (Niimi and Beamish, 1974), channel catfish (Murray et al, 1977) and carp (Huisman, 1976a; Huisman et al, 1979; Jauncey, 1979) implying that dietary energy, excess to requirements, is stored in carcass lipid. However carcass lipid content did not change significantly ($P>0.05$) at feeding rates above 20% at rearing temperatures of 24, 28 and 32°C (Trial 1 and 2, Table 2.10 and 2.12) and at feeding rates above 25% at 35°C (Trial 2 - Table 2.12). similarly, Huisman (1976a) observed no increase in carcass lipid content for fingerling carp with increase in feeding rate above 5% at 23°C.

Temperature regime also influenced carcass lipid in both trials. An increase in the rearing temperatures resulted in a decrease in carcass lipid content. In trial 1, at each feeding rate, lipid contents were significatnly lower ($P < 0.05$) at 32°C than at 28 and 24°C. Similarly, in trial 2, carcass lipid content at 35°C was significantly lower ($P < 0.05$) than at 32 and 28°C. At the higher temperatures excess energy may not have been available for deposition as lipid due to the temperature effect in metabolic energy demand.

A decrease in carcass lipid content with increase in temperature at all levels of feeding has been reported for sockeye salmon (Brett et al, 1969) and largemouth bass (Niimi and Beamish, 1974). However, Huisman et al. (1979) observed no definite relationship between carcass lipid and environmental temperature for carp. On the contrary, carcass lipid contents have been found to increase with temperature for channel catfish (Andrews and Stickney, 1972), rainbow trout, Salmo gairdneri (Papoutsoglou and Papoutsoglou, 1978) and carp (Jauncey, 1979) at higher levels of feeding.

With some exceptions, carcass ash content decreased with feeding rate at a given temperature, but increased with temperature at a given feeding rate. Decrease in the carcass ash content with feeding rate has been reported for carp (Huisman, 1976a; Huisman et al, 1979; Jauncey, 1979). Increase in ash content with increase in temperature has also been reported for carp (Huisman et al, 1979; Jauncey, 1979) and for largemouth bass (Niimi and Beamish, 1974). The probable

reason for variation in the ash content with temperature and feeding rate have not been reported.

In Summary, the results of this experiment showed the following:

1. 32°C is the most suitable rearing temperature for carp fry when feeding is within the range 20 to 30% BW/day

2. 28°C is a more suitable temperature when the feeding rate is lower (10–15% BW).

3. At rearing temperatures of 24 and 28°C, the potential for maximum growth is achievable with feeding rates of 20–25% BW and 25–30% BW respectively. For rearing temperatures of 32 and 35°C, a feeding rate of 30% BW is the most suitable for maximum growth.

2.3 Experiment 2.2

The effect of stocking density and water flow rate and their interactions on the survival, growth and food conversion of carp fry.

2.3.1. Introduction

Stocking density is an important parameter in fish husbandry which can greatly influence the success of nursery management. In fish farming, high density culture is reported to maximise the utilization of available water resources and to enhance yields. But stocking density depends on the carrying capacity of the waterbody and is strongly affected by the species and size of fish cultured and by physical factors such as water temperature and flow rate.

The carrying capacity of a static rearing system is limited by oxygen availability and the accumulation of various toxic metabolites (Haskell, 1955; Burrows; 1964; Westers and Pratt, 1977). There is a beneficial influence on the growth and viability of fish if a running water culture system is adopted as this provides continuous elimination of growth inhibiting and potentially toxic metabolites as well as the replenishment of dissolved oxygen (Chiba, 1965; Wohlfarth et al., 1971; Bryant et al., 1980).

Carrying capacity can be increased tremendously with high water flow, but economic use of water, as well as the cost of maintaining heated water for warm water fish culture, places major restrictions on the use of flow through rearing systems.

Recirculation of water in fish culture reduces discharge problems

and increases the efficiency of water use. A well insulated warm water recirculation system minimises heat loss and can make artificial heating economical (Speece, 1973; Pecor, 1979; Bryant et al., 1980).

The carrying capacity of a recirculation system is the maximum weight of fish that it can sustain above which its purification facilities cannot maintain the water quality at a standard compatible with the health requirement of the fish. The carrying capacity of a recirculation system is primarily a balance between the consumption and availability of oxygen in the water.

The metabolic oxygen requirement of fishes is one of the major sources of oxygen consumption in a recirculation system. The three major factors which routinely affect metabolic oxygen demand are water temperature, fish size and the type and quantity of feeding. The metabolic rate of fish has been reported to be linearly correlated to water temperature (Elliott, 1969; Smith, 1976, cited by Westers, 1982; Jobling, 1981a; Soofiani and Hawkins, 1982). Elliott (1969) for chinook salmon and Kausch (1969) for carp have shown that the routine metabolic rate decreases proportionately with fish size. Routine metabolic rate also varies between fish species (Davis, 1975; Westers, 1982). The elevation in metabolic rate associated with feeding has been reported to increase linearly with ration size (Elliott, 1969; Jobling, 1981a; Soofiani and Hawkins, 1982).

Oxidation of ammonia to nitrate by the process of nitrification consumes considerable amounts of oxygen. Excretion of ammonia by fish is directly related to their nitrogen intake (Westers and Pratt, 1977; Pecor, 1979; Kaushik, 1982). Therefore in a recirculation system where a high feeding rate is employed, significant amounts of oxygen may also be removed by the filter as a result of nitrification by microorganisms. The oxygen required by fish for routine metabolism and that required for metabolisation of ingested food plus the oxygen consumed in nitrification, constitute the total oxygen demand of the rearing system. The availability of oxygen in the system is primarily a function of the water flow rate and the oxygen saturation level in the inflowing water.

Investigations of the carrying capacity of recirculated water systems have been reported for trout (Speece, 1973), channel catfish (Stickney *et al*., 1972; Broussard and Simco, 1976) and tiger muskellunge (female *Esox masquinongy* x male *Esox lucius*) (Pecor, 1979). The combined effects of stocking density and water flow rate on growth and food conversion have also been studied for channel catfish (Andrews *et al*., 1971; Allen, 1974). A recent publication by Meske (1985) reports the results of several investigations on the effect of stocking density and water flow rate on growth and food conversion of common carp. However, these were conducted with advanced fry and fingerlings. Information on the optimum stocking density of common carp fry in a recirculated water system under controlled environmental condition are lacking.

A high stocking density in terms of numbers of fish, as well as in biomass terms, can adversely influence growth. Competition for space and food by fish kept under crowded conditions has often resulted in the suppression of growth of low ranking individuals from intimidation, dominance and agression by other fish (Brett, 1979). For example, even when food and water quality were not limiting, when the density of Atlantic salmon was increased the movement of the fish was inhibited which affected food availability, resulting in a reduced growth rate (Refstie and Kittelsen, 1976). Korneyeva (1969) reported that increasing the population density of carp in nursery ponds leads to greater variation in weight rather than a reduction in average weight.

Brett (1979) noted that some species of fish appear to prefer a particular stocking density even in the absence of any limitations of water quality or food supply. He recalled the observation of Brown (1946a) that at low density (3 fish/50 litre) fingerling brown trout did not feed well and appeared to lack social stimulation, whereas highly crowded conditions (1 fish/3 litre) resulted in reduced food conversion efficiency and some physical interference between fish.

Findings like these clearly have important husbandry and economic implications for fish culture. The present experiment was designed therefore to study the effect of stocking density on growth, food conversion and size variation of common carp fry and to establish

their maximum carrying capacity in recirculated water system for defined rates of water flow.

2.3.2. Materials and Methods

A 30-day growth trial was conducted in an experimental recirculated water system to study the effects of stocking density and water flow rate on the survival, growth rate and food conversion efficiency of common carp fry.

2.3.2.1. Experimental system

The experimental system described in section 2.2.2.1 was used for the present investigation, but modified, by connecting three sumps as well as three header tanks, to operate as one single unit so that the same temperature and water quality conditions existed in all tanks.

The source of experimental animals and their quarantine procedure were as described in section 2.2.2.2.-3.

2.3.2.2. General experimental procedure

Groups of carp fry were reared at stocking densities of 6, 10, and 15 fish/litre (36, 60 and 90 fish/tank respectively) and each stocking density group was subjected to three water flow rates. Initially flow rates of 100, 250 and 500ml/minute were set. These were increased correspondingly after each weighing in proportion to the increase in fish weight. The flow rates used during the experiment are explained in detail in section 2.3.2.3. Three replications were used for ech treatment group. A schematic representation of the experimental design is presented in Fig. 2.18.

Carp fry were acclimated to the experimental system three days before the start of the experiment. Healthy fish were selected and assigned randomly to nine tretment groups. The required number of fry, plus a 15% excess, was allocated to each experimental tank. After the allocation of the fry was completed, 15% of the fish in each tank were captured randomly and weighed individually to the nearest 1.0 mg. All the sample measurements were pooled to obtain an overall average initial weight for the experimental population. Fish were weighed during the experiment every five days. The fish were weighed collectively tank by tank. On termination of the experiment they were weighed individually. Details of the acclimation and weighing procedure are described in section 2.2.2.7.

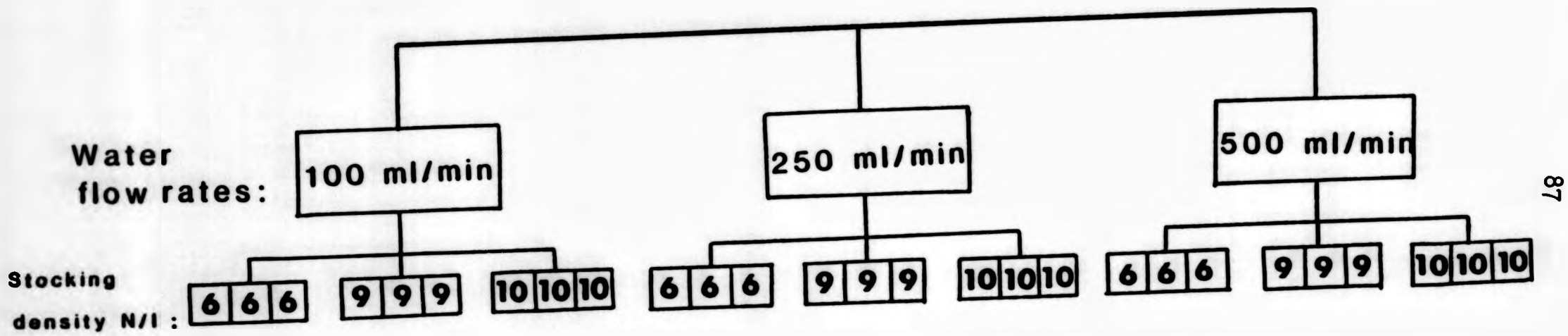

Fig. 2.18 Schematic representation of the experimental design, for Experiment 2.2. There were three density groups and each was subjected to three water flow rates. There were 3 replicates for each treatment group.

The fish were fed five times every day between 08.00 and 20.00 hours (at three hourly intervals). Each of the five daily feeds was distributed over a period of thirty minutes to ensure consumption of the whole ration. The quantity of food administered per day was adjusted after each weighing. Initially, fish were fed at a feeding rate equivalent to 25% of their live body weight per day. This was reduced during the experiment as follows:

Period of experiment (days)	Feeding rates used (% BW/day)
1 - 10	25
11 - 15	20
16 - 30	15

Feeding rate was calculated on as-fed basis and no allowance was made for the moisture content of the diet. Daily mortalities were recorded and the amount of feed adjusted accordingly. The feed given was the same as that used in trial 1 (Expt 1) (protein 50.1%, lipid 12.0%, ash 11.1% and fibre 1.9%). Details of the diet formulation and preparation are given in section 2.2.2.4-5.

A summary of the methodology used in the present investigation is presented in Table 2.13.

Table 2.13 A summary of the methodology used to study the effects of water flow rate and stocking density on survival, growth and food conversion of carp fry (Experiment 2.2).

Experimental duration	:	30 days
Initial weight of fry	:	101 mg (SE ± 3.3)
Treatments	:	Variations in stocking density and water flow rate
Stocking density	:	6, 10 and 15 fish/l of water at each flow rate
Flow rate	:	Initially 100, 250 and 500 ml/min with corresponding increase at each subsequent sampling
Replication	:	3/treatment
Feeding rates	:	15-25% BW/day
Water temperature	:	26-28°C
Volume of water in each experimental tank	:	6 l
Physico-chemical characteristics monitored	:	Dissolved oxygen, pH, total ammonia, nitrite, suspended solids and total alkalinity

2.3.2.3. Criteria for selection of water flow rates.

The flow rates used were set on the basis of the balance between the availability and the demand of oxygen in the experimental system. It was assumed that the reoxygenating capacity of the recirculated water system was 90% (of saturation). The level of dissolved oxygen in one litre of water at 28°C was therefore expected to be 7.85 x 0.9 = 7.1mg. Huisman (1974, cited by Westers, 1982) recommended $3mgl^{-1}$ as the minimum allowable level of dissolved oxygen in effluent for common carp. Therefore the estimated usable amount of oxygen available to the experimental fish was (7.1 - 3.0) = 4.1 mgl-1.

Thus per litre/min water flow (lpm), an estimated 4.1 x 60 = 246 mgO_2/hr was available.

Huisman (1974, cited by Westers, 1982) reported an oxygen consumption of 210g per kg food for carp fed a dry pelleted diet over a 16 hour feeding priod. Carp fry feeding at 25% BW/day would require 210 x 0.25 = 52.5g oxygen per day or 52.5 ÷ 16 = 3.281g or 3281 mg O_2/Kg fish/hr.

Common carp have been reported to excrete ammonia-nitrogen equivalent to 34.7% of their total nitrogen intake (Kaushik, 1982). Therefore 1Kg of fish fed a diet containing 50.1% protein at 25% BW/day would

produce 8.4g of ammonia. Based on the chemical reaction for the oxidation of ammonia to nitrate, 3.77g of oxygen are needed for every 1g of ammonia oxidised to nitrate (Bryant et al., 1980). Therefore, (3.77 x 8.4 ÷ 24) = 1.32g or 1230 mgO_2/Kg fish/hr would be consumed in the oxidation of ammonia to nitrate over a 24 hr nitrification period.

Significant amounts of oxygen may also have been removed from the culture system by the activities of heterotrophic microorganisms. Dissolved organic matter, particulate matter, waste food and faeces in a recirculated water system may be broken down by heterotrophs consuming a considerable amount of oxygen. However it is difficult to estimate even roughly this source of oxygen consumption. Wickins (1982) reported that under some circumstances, particularly after feeding, 60% of total oxygen consumed may be removed by heterotrophs. Bryant et al., (1980) noted that the typical values for the oxygen requirement for these sources in fish farm effluent ranges from 200-500 mgO_2/Kg fish/hr. However, the authors were referring to fish farms with a water temperature of around 16°C and where the rate of feeding is only about 2% BW/day. In a warm water carp hatchery, where typically the feeding rate is about 15 - 25% BW and the water temperature 28°C, the demand for oxygen by heterotrophs would be expected to be much higher. A value of 1000 mgO_2/Kg fish/hr has been used therefore for this source in calculating the total O_2 demand in the experimental system. Therefore the total O_2 demand = 3281 + 1320 + 1000 = 5601mgO_2/Kg fish/hr.

Since the estimated amount of available oxygen was 246 mgO_2/h/1pm and the total oxygen demand 5601 mgO_2/Kg fish/h a flow rate of one litre/min would have been able to sustain approximately (246/5601) = 0.0439 Kg fish/1pm. A summary of the above calculation of flow requirement have been presented in Table 2.14 for quick reference.

At a flow requirement of 0.0439 kg fish/1pm, for the 36 fish of mean weight 101 mg = 3636 mg the minimum flow rate would have been 83 ml/min. To allow a safe margin of error an initial or 'low' flow rate of 100 ml/min was selected. Two faster flow rates of 250 (medium) and 500 ml (high)/min (i.e. 2.5 and 5.0 times the minimum flow) were also selected.

As the fish grew during the experimental period the amount of food consumed increased. Flow rates to the experimental tanks were increased correspondingly to compensate for the higher oxygen requirement. The rates of water flow were adjusted after each 5-day weighing and rechecked after two to three days. Each inflow pipe in the individual tanks was fitted with valves to control the rate of water flow (Fig. 2.1d). The number of valves fitted in individual tanks were one, two and three for low, medium and high flow rates. The rate of water flow maintained throughout the experimental period are given in Table 2.15. Medium and high flow rates were maintained as fixed multiples (by 2.5 and 5.0 times) of the low flow rate except during the last 10

Table 2.14 *Calculation of flow requirements in a recirculated water system for rearing of carp fry in Experiment 2.2

1 Estimated usable amount of oxygen available to fish per litre/min water flow (lpm) at 28°C and 90% saturation	246 mgO_2/h
2 Total oxygen demand in the experimental system	
a) Oxygen consumption to metabolize ingested food (feeding rate 25% BW/day)	3281 mgO_2/Kg fish/h
b) Oxygen consumption by biological filter	1320 mgO_2/Kg fish/h
c) Oxygen consuption by heterotrophic micro-organism	1000 mgO_2/Kg fish/h
Total	5601 mgO_2/Kg fish/h

Flow requirement or loading at a flow rate of 1 lpm

$$= \frac{\text{Estimated amount of oxygen available}}{\text{Total oxygen demand}}$$

$$= \frac{246\ mgO_2/h}{5601\ mgO_2/Kg\ fish/h}$$

$$= 0.0439\ Kg\ fish/lpm$$

* See section 2.3.2.3. for details of calculations

Table 2.15 *Rates of water flow used during Experiment 2.2

Period of experiment (days)	Rates of water flow used (ml/min)		
	Low	Medium	High
1 - 5	100 ± 20	250 ± 50	500 ± 100
6 - 10	160 ± 20	400 ± 50	800 ± 100
11 - 15	200 ± 25	500 ± 50	1000 ± 100
16 - 20	240 ± 30	600 ± 75	1200 ± 150
21 - 25	300 ± 30	600 ± 75	1200 ± 150
26 - 30	350 ± 40	700 ± 80	1400 ± 150

* See section 2.3.2.3. for details of criteria for selection of flow rates

days when multiples of 2.0 and 4.0 were used instead, so that an excessively high flow rate was avoided in the experimental tanks.

2.3.2.4. Measurement and analysis of water quality

Measurements of the physico-chemical characteristics of the experimental water were carried out as described in section 2.2.2.9. pH, dissolved oxygen, total ammonia and nitrite levels were measured in each tank once every seven days. The level of suspended solids was measured once every ten days in all tanks. The above parameters were also monitored in inflow water in the header tanks. Temperature and total alkalinity were measured once in every five and ten days respectively in representative tanks and the inflow water.

2.3.2.5. Analysis of experimental data

Specific growth rate (SGR) and food conversion ratio (FCR) were calculated by the methods described in section 2.2.2.10. Comparison of treatment means was carried out by two-way analysis of variance, followed by testing for pairwise differences using Duncan's new multiple range test (Steel and Torrie, 1960). Duncan's test was used for these as the Newman-Kent's multiple range test (SNK test), used for analysis of results of other growth studies in this thesis, did not show any significant differences among treatment means even

though analysis of variance rejected the multiple hypothesis of equal means (see Table 2.19). For comparison of mortalities between treatments, values for percentage mortality were subjected to arcsin transformation (Zar, 1974) and the resultant data were subjected to analysis of variance as above.

To determine the extent of asymmetry in the size distribution of the populations under stocking densities and flow rates, the coefficient of variation and skewness values (Zar, 1974; Sokal and Rohlf, 1981) were calculated using SPSSX (Statistical Package for Social Scientist Extended). Coefficient of variation and skewness were calculated from the pooled data of three replicate tank population of each treatment. Student's t-tests (Zar, 1974) was performed to test the significance of skewness within a population and the significance of differences between different skewness values.

2.3.3. Results

2.3.3.1. Physico-chemical characteristics of the experimental water

Levels of temperature, pH, nitrite and total alkalinity were found to be almost identical in different experimental tanks and in the inflow water at every measurement. Therefore overall mean values of these parameters are presented in Table 2.16. There were some variations

Table 2.16 Physico-chemical characteristics of the recirculated water in the experimental culture system (Experiment 2.2)

	Mean	Range
Temperature (^{o}C)	27.3	26.0 - 28.4
pH	6.29	5.80 - 7.03
Dissolved oxygen * ($mg\ l^{-1}$)	7.40	7.10 - 8.00
Total ammonia -N* ($mg\ l^{-1}$)	0.19	0.05 - 0.49
Nitrite - N ($mg\ l^{-1}$)	0.10	0.01 - 0.37
Suspended solids* ($mg\ l^{-1}$)	2.9	1.4 - 4.2
Total alkalinity ($mg\ l^{-1}$)	18.75	10.5 - 23.75

* Analysed values for inflow water only. See table 2.17 for levels of dissolved oxygen, total ammonia and suspended solids in experimental tanks.

Table 2.17 Levels of total ammonia, dissoved oxygen and suspendid solids in experimental tanks at different water flow rates and different fish stocking densities (Water collected from each experimental tank were used for analysis)

Treatment No.	Water flow*	Stocking Density (N/l)	Total ammonia-N ($mg\ l^{-1}$) Mean (range)	Dissolved oxygen ($mg\ l^{-1}$) Mean (range)	Suspended solids ($mg\ l^{-1}$) Mean (range)
A_1	Low	6	0.48 (0.23 - 0.66)	6.55 (5.80 - 7.10)	12.5 (11.0 - 13.4)
A_2		10	0.61 (0.37 - 0.75)	6.30 (5.40- 7.10)	12.7 (9.2 - 16.6)
A_3		15	0.64 (0.25 - 0.91)	6.00 (5.20- 7.00)	20.4 (18.4 - 21.8)
B_1	Medium	6	0.42 (0.17 - 0.62)	7.10 (6.70 - 7.30)	6.8 (5.4 - 8.2)
B_2		10	0.53 (0.13 - 0.73)	7.00 (6.60 - 7.40)	9.10 (4.8 - 14.4)
B_3		15	0.56 (0.27 - 0.78)	6.80 (6.30 - 7.20)	10.3 (6.8 -14.0)
C_1	High	6	0.40 (0.07 - 0.59)	7.20 (6.80 - 7.70)	3.3 (2.6 - 4.2)
C_2		10	0.41 (0.10 - 0.57)	7.30 (6.80 - 7.60)	4.1 (2.4 - 5.2)
C_3		15	0.46 (0.13 - 0.65)	7.10 (6.70 - 7.50)	4.8 (3.2 - 7.2)

* See table 2.15 for details of water flow rates used

in the levels of dissolved oxygen (DO), total ammonia (TA) and suspended solids between experimental tanks and the inflow water. Mean values of the above parameters in the inflow water are presented in Table 2.16 and the value for different treatment groups are shown in Table 2.17. Both water flow rate and stocking density had little influence on ammonia concentration. Mean total ammonia concentrations ranged from 0.40 mgl^{-1} TA-N at 6 fish/litre with high flow rate to 0.64mgl^{-1} TA-N at 15 fish/litre with low flow rate. Water flow rate had more influence on both DO and suspended solids levels than had stocking density. The DO level increased while the level of suspended solids decreased with flow rates. Mean DO values ranged from 6.00 mgl^{-1} at 15 fish/litre with low flow rate to 7.30 mgl^{-1} at 10 fish/litre with high flow rate. Mean suspended solids values ranged between 3.3mgl^{-1} (6 fish/litre with high flow rate and 20.4 mgl^{-1} (15 fish/litre with low flow rate). Except at low flow rates, stocking density appeared to have little influence on either the levels of DO or suspended solids.

2.3.3.2. Mortality

Mortality of carp fry at different stocking densities and water flow rates are presented in Table 2.20. The mortality values ranged from 2.8% at 6 fish/litre with high flow rate to 12.0% at 6fish/litre with low flow rate. Two-way analysis of variance revealed that mortality was influenced significantly ($P<0.05$) neither by stocking

density nor by interaction between stocking density and water flow rates. However, water flow rates influenced mortality rates significantly ($P < 0.05$) (Table 2.19). Higher mortality occurred in the low and medium flow rates but no conclusive trend was evident.

2.3.3.3. Growth

Growth responses of carp fry during the experimental period are presented in Table 2.18. From these data it can be seen that neither stocking density nor water flow rate had much influence on growth responses.

The mean final weights and specific growth rates (SGRs) of the experimental fish are presented in Table 2.20. Both stocking density and the interaction between stocking density and water flow rate were insignificant ($P > 0.05$) in influencing the mean final weights and SGRs (Table 2.19). Water flow rate, however, did have a significant influence (Table 2.19). The mean final weights ranged from 815mg (SGR 6.96) for 6 fish/litre with high flow rate to 1000mg (SGR 7.64) for 10 fish/litre with medium flow rate. In general the growth rate of carp fry appeared to be higher at medium flow rates and lower at higher flow rates.

2.3.3.4. Food Conversion

The responses of carp fry in terms of efficiency of food utilisation

Table 2.18 Growth responses of common carp fry reared at different water flow rates and stocking densities

Treatment No.	Water flow*	Stocking density (N/1)	Mean body weight (mg) Time in days 5	10	15	20	25	30
A_1	Low	6	188	307	468	583	692	891
A_2		10	195	298	446	565	675	875
A_3		15	190	294	442	546	652	872
B_1	Medium	6	201	303	461	578	672	912
B_2		10	206	307	455	589	723	1000
B_3		15	196	310	447	565	683	973
C_1	High	6	198	296	414	519	610	815
C_2		10	184	276	390	494	591	832
C_3		15	193	294	417	536	647	909

Mean initial weight 101 mg (SE $\pm$ 3.3)

* See table 2.15 for details of water flow rates used

Table 2.19 Summary of 2-way analysis of variance of data on mortality, final weight, specific growth rate and food conversion ratio of common carp fry as a function of stocking density and water flow rate.

Source	D.F.	S.S.	M.S.	F	P
		Mortality			
Between densities	2	9.1	4.6	0.41	>0.05
Between water flow rates	2	137.6	68.8	6.14	<0.05
Interaction	4	73.5	18.4	1.64	>0.05
Error	18	200.9	11.2	-	
Total	26	421.1	-	-	
		Final Weight			
Between densities	2	9442	4721	0.83	>0.05
Between water flow rates	2	58749	29375	5.18	<0.05
Interaction	4	18334	4583	0.81	>0.05
Error	18	102153	5675	-	
Total	26	188678	-	-	
		Specific growth rate			
Between densities	2	0.1414	0.0707	0.92	>0.05
Between water flow rates	2	0.7617	0.3808	4.97	<0.05
Interaction	4	0.2647	0.0662	0.86	>0.05
Error	18	1.3781	0.0766	-	
Total	30	2.5458	-	-	
		Food conversion ratio			
Between densities	2	0.0722	0.0361	2.80	>0.05
Between water flow rates	2	0.1122	0.0561	4.35	<0.05
Interaction	4	0.0361	0.0090	0.70	>0.05
Error	18	0.2323	0.0129	-	
Total	30	0.4528	-	-	

Table 2.20 Mortality, growth rate and food utilization of common carp fry reared for 30 days at different water flow rates and stocking densities

Treatment No.	Water flow*	Stocking density (N/l)	Mortality (%)	Mean final weight (mg)	SGR	FCR
A_1	Low	6	12.0^{a**}	891^{abc}	7.25^{abc}	2.41^{abc}
A_2		10	6.7^{abc}	875^{abc}	7.19^{abc}	2.44^{ab}
A_3		15	5.6^{bc}	872^{abc}	7.18^{abc}	2.37^{abc}
B_1	Medium	6	8.3^{ab}	912^{abc}	7.31^{abc}	2.33^{abc}
B_2		10	7.8^{abc}	1000^{a}	7.64^{a}	2.22^{c}
B_3		15	8.2^{ab}	973^{ab}	7.54^{ab}	2.21^{c}
C_1	High	6	2.8^{c}	815^{c}	6.96^{c}	2.45^{a}
C_2		10	5.0^{bc}	832^{bc}	7.02^{bc}	2.33^{abc}
C_3		15	4.1^{bc}	909^{abc}	7.32^{abc}	2.23^{bc}

Mean initial weight 101 mg (SE $\pm$ 3.3)

* See table 2.15 for details of water flow rates used

** Figures in the same column with same superscripts are not significantly different ($P>0.05$; Duncan's test)

under different treatments followed closely the trend observed in the growth responses. The calculated food conversion ratios (FCRs) for each treatment group are presented in Table 2.20. The FCR values ranged from 2.21 (15 fish/litre with medium flow) to 2.45 (6 fish/litre with higher flow). Statistical analysis showed that stocking density had no significant influence ($P > 0.05$) on food conversion, whereas water flow rate effects were significant ($P < 0.05$) Although lower FCRs (2.22 and 2.21) were observed at 10 and 15 fish/litre with medium flow rate, no consistent relationship is evident between the FCRs and water flow rate.

2.3.3.5. Size variation

Coefficient of variation (CV%) and skewness values for common carp populations reared at different stocking densities and flow rates are presented in Table 2.21. The CV values ranged between 62.4% (10 fish/litre with high flow rate) and 74.6% (6 fish/litre with medium flow rate). Neither stocking density nor flow rate showed any consistent relationship to CV%. Carp fry populations reared at different treatments were highly skewed ($P < 0.001$, t-test) and the values ranged from 1.116 to 1.972. No significant differences ($P > 0.05$, t-test) were observed between skewness values for carp fry population subjected to different treatments.

Table 2.21 Mean weight (mg), coefficient of variation (C.V.) and skewness (Sk) of the weight distribution of common carp fry reared for 30 days at different water flow rates and stocking densities

			No. of animals	Mean ± S.E. (mg)	C.V.%	Sk* ± S.E.
Initial sample			241	101 ± 3.3	50.3	0.920 ± 0.157
Final sample						
Treatment No.	Flow rate**	Stocking density (N/1)				
A_1	Low	6	95	891 ± 60	65.9	1.116 ± 0.247
A_2		10	168	875 ± 46	68.4	1.586 ± 0.187
A_3		15	255	872 ± 36	65.0	1.247 ± 0.153
B_1	Medium	6	99	912 ± 69	74.6	1.972 ± 0.243
B_2		10	166	1000 ± 57	72.9	1.818 ± 0.188
B_3		15	248	973 ± 41	66.4	1.503 ± 0.155
C_1	High	6	105	815 ± 57	71.0	1.640 ± 0.236
C_2		10	171	832 ± 40	62.4	1.433 ± 0.186
C_3		15	259	909 ± 37	64.6	1.601 ± 0.152

* Skewness values in different treatment are not significantly different from each other ($P > 0.05$, t-test)

** See table 2.15 for details of water flow rates used.

2.3.4. Discussion

The ranges of water flow rate used in this trial did not influence the carrying capacity of the experimental tanks, as increase in the water flow rate did not improve the growth and food conversion of carp fry at any of the stocking densities tested. However, there was a greater probability of food being washed away from the experimental tank (if not consumed immediately by the fish) in the high flow rate conditions than in the low and medium ones. Therefore, the shorter food consumption time available to fish at high flow rate (at 6 and 10 fish/litre density) might have resulted in lower food consumption leading eventually to poorer growth. However, the view is not entirely supported by the results as there was comparatively good growth at 15 fish/litre and a high flow rate.

Even at low flow rate, stocking density did not affect growth and food conversion significantly. Apart from a few studies (Brown, 1946a; Andrew et al., 1971; Refstie and Kittelsen, 1976), most published information indicates that the growth of fish is not affected by stocking density provided food and water quality are not limiting (Magnuson 1962; Korneyeva, 1969; Wohlfarth et al., 1971; Hysmith et al., 1983; Meske, 1985). The conditions of temperature, pH, nitrite and alkalinity were almost identical in the experimental tanks and therefore are unlikely to have contributed

to growth variation between different treatments. There were some variations in the levels of DO, total ammonia and suspended solids (Table 2.17), but even the minimum concentration of DO and the maximum concentration of total ammonia and suspended solids recorded were within the levels suitable for optimum fish growth (Alabaster and Lloyd, 1980; Wickins, 1980; Wedemeyer, 1981).

Fish mortality did not show any conclusive trend during this investigation. The small number of mortalities (2.8 - 12%) observed are considered acceptable in intensive culture and perhaps included 'natural' death. Andrews *et al.* (1971) for channel catfish and Macintosh and DeSilva (1984) for tilapia, *Oreochromis mossambicus* and *O. niloticus* female x *O. aureus* male hybrids, observed no consistent relationship between mortality and stocking density.

The results of the present study suggest that the theoretically calculated (Section 2.3.4.3.) maximum sustainable capacity indicated by the water flow rate was in fact lower than the capacity actually observed. The calculated minimum flow rate for thirty-six fish was in practice able to sustain ninety fish of similar average weight. The actual sustaining capacity, otherwise termed loading (kg fish/lpm) (based on observation) was calculated as follows:

Feeding rate (% BW/day)	calculated flow requirement or loading (Kg fish/lpm)	Recirculated/observed flow requirement or loading (Kg fish/lpm)
25	0.0439	0.091 - 0.106
20	0.0525	0.130
15	0.0653	0.157 - 0.160

There are several factors which might have attributed to the observed higher loading factors. While calculating the loading, the reoxygenation capacity of the recirculation system was considered to be 90% saturation (i.e. 7.1mgl^{-1} DO at 28^{o}C), whereas the measured level of DO was higher (mean 7.40, range 7.10 - 8.00 mgl^{-} (Table 2.16). However, even if a DO value of 7.40 mgl^{-1} had been used in the calculations, the maximum loading at 25% feeding would not have exceeded 0.047 Kg fish/lpm .

Other factors which might have attributed to the higher loading are as follows: -

(a) The amount of oxygen consumed by the fish in metabolising ingested food was probably lower than the value obtained from the literature.

(b) Oxygen consumption was estimated on the basis that all food was consumed, but it is probable that considerable amounts of food remained uneaten.

(c) The experimental system was kept very clean and therefore consumption of oxygen by heterotrophic microorganisms might have been lower than the figure used in the calculations (see Section 2.3.2.3).

Westers and Pratt (1977) recommended four water changes per hour in hatcheries for intensive salmonid culture. If the same practice were followed in a carp hatchery, four water changes per hour would entail a flow rate of about 1 litre per minute in a 15 litre tank. If the 'observed loading' of the present study is applied, approximately 100, 130 and 160g of carp fry could be maintained at 25, 20 and 15% feeding respectively, (i.e. 7, 9 and 11g fish/litre).

The present findings are similar to those reported by Bryant and Matty (1980). These authors, while studying the feeding of carp larvae, observed that a maximum stocking density of about 7g/l did not affect the growth of carp larvae. The authors, however, did not report the rate of water exchange used.

NO SUCH WORD

Growth depensation among aquatic animals appears to be unaffected by variation in water flow rates. Nakamura and Kasahara (1961) investigated the effect of stocking density on the size variation of carp population and observed that stocking density had no clear influence on the degree of skewness provided an adequate amount of food was available. Similarly, Magnuson (1962) explored density-related growth depensation in population of Medaka, Oryzias latipes and concluded that neither a general depression in growth rate nor growth depensation occurred in this species as long as accumulation of waste products was prevented and food was supplied in excess. Wohlforth (1977) reviewed the results of asymmetrical frequency distribution of length and weight in several

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species of fish and concluded that greater assymmetry of the frequency distribution of fish was due to competition for limited food rather than an effect of stocking density. However in contrast, Korneyeva(1969) concluded that increasing the stocking density affected size variation by creating discrepancies in food availability and by increasing physical contact among fish.

All the experimental fry populations exhibited highly significant degrees of skewness ($P < 0.001$, t-test) in their size frequency distribution. In a communally reared carp population, the occurence of a skewed frequency distribution of body length is a common phenomenon (Nakamura and Kasahara, 1955). Since there was no apparent effect of stocking density on the size variation of carp fry in the present study, the development of skewness probably derived from an initially positively skewed population (initial Sk value $\pm$ 0.92; Table 2.21).

The population initially had a high C.V. value of 50.3%. This increased to 62.4 - 74.6% at the end of the experiment. An increase in the C.V. with time is indicative of growth suppression of certain individuals within a population. Suppression of the growth of small individuals is due principally to disproportional food acquisition by subdominant fish caused by aggression by a few 'dominant' individuals even in the presence of excess food (Magnuson, 1962; Koebele, 1985). This phenomenon has been reported for brown trout (Brown, 1946a,b),carp (Wohlfarth and Moav, 1972) and Arctic char, Salvalinus alpinus (Jobling & Wandsvik, 1983.) The results of the present study

indicate that social interactions between individuals led to increased size variation, which was independent of stocking density at least within the range tested of 6 - 15 fish / l .

2.4 Experiment 2.3

Food particle size preference of different size classes of carp fry.

2.4.1 Introduction

The success of any aquaculture industry depends largely on the efficient utilization of available resources. Foodstuffs are one of the major items of cost in aquaculture (Webber and Huguenin, 1979; Aquaculture Development Coordination Programme, 1983); therefore gains in the efficiency of feeding practices can have significant economic importance. The preparation of a complete diet in accordance with the nutritional requirements of the cultured species is clearly essential to the success of intensive aquaculture. But so is the physical nature and form of the diet and the efficiency of its acquisition by the organism (Thorpe and Wankowski, 1979; Webber and Huguenin, 1979).

Paloheimo and Dickie (1966b), in their review on fish feeding and growth, concluded that different growth efficiencies were due to differences in foraging efficiencies in different types of food rather than to major differences in the physiological conversion of foods. These authors suggested that selective food size was the major factor determining efficient utilization of rations.

In nature and in laboratory conditions, the evidence for prey-size selection in different fish species has been overwhelmingly demonstrated (Nilsson, 1957; Moore, 1974, 1976; Wankowski, 1977; Mathias and Li, 1982). This evidence has been obtained either by examining the stomach contents of fish captured from the wild or from selectivity preference experiments involving predator-prey

manipulation.

In recent years, with the advent of intensive aquaculture, pelleted artificial diets have been used increasingly in fish farming. This has resulted in the formulation of empirical rules relating feed pellet size to fish size (Hasting and Dickie, 1972). However, these empirical rules have been formulated largely on the basis of experience rather than experimentation (Wankowski and Thorpe, 1979).

Both physical and physiological factors can be postulated as having possible limitations on the size range of feed particles that can be handled satisfactorily by fish. Therefore the physical size, shape and texture of the feed should conform to the animal's physical ability to seize, engulf or otherwise ingest its food (Webber and Huguenin, 1979). Particles which are too small may not be detected or captured easily by the fish, while those that are too large may be too difficult to ingest quickly or whole.

The importance of optimum feed particle sizes in the efficiency of feeding has been emphasized by several authors (Thorpe and Wankowski, 1979; Wankowski, 1979; Wankowski and Thorpe, 1979; Dabrowski et al, 1983; Knights, 1983; Dabrowski and Bardega , 1984). Wankowski and Thorpe (1979) even established the role of food particle size in the growth of juvenile Atlantic salmon, Salmo salar. In a twenty day feeding experiment, these authors selected a range of food particle sizes based on the salmon's maximum mouth breadth and found out that there was a clear relationship between growth and feed particle size.

Moreover, in a flow through or recirculated water fish culture system, if food is not rapidly eaten, particles are usually washed away. Loss of material from large food particles, perhaps after soaking and softening, inevitably leads to wastage, pollution and nutrient leaching.

Mouth size is one of the most important factors imposing limitations on the feeding of juvenile fish in both natural and pelleted diets (Northcote, 1954; Hartman, 1958; Keast and Webb, 1966; Shirota, 1970; Hyatt, 1979; Wankowski, 1979; Knights, 1983; Dabrowski and Bardega, 1984). Northcote (1954) observed a close relationship between mouth size and diet composition in two species of Cottus. Similarly, Shirota (1970) observed a close relationship between the mouth size of larval fish and the size of their natural food in nineteen different fish species. Keast and Webb (1966) suggested that the mouth of fish should be studied because its structure dictates the size and type of prey that can be handled. Knights (1983), while studying the food particle size preferences and feeding behaviour of the European eel, Anguilla anguilla observed that the upper size limit particles for efficient feeding was determined by mouth width, the physical nature of the particles and by hunger motivation. The lower limit was determined by the eel's location abilities.

Researchers have often adopted an arbitrary food particle size for the rearing of carp larvae (Chakrabarty et al., 1971; Sarig and Marek, 1974; Kossman, 1976; Appelbaum and Dor, 1978; Hecht and

Viljoen, 1982). However, the results of a recent investigation reported on the criteria for selection of appropriate feed particle size for common carp larvae and juveniles (Dabrowski et al, 1983). The authors established the relationship between standard length and mouth size when opened at 90° and 120° angle between upper and lower jaws and predicted appropriate food particle size on the basis of these measurements. The authors, however, did not make any actual observations on selection of feed particle size by fish. Although preferred feed particle sizes are closely related to the mouth size of the animal, there are several instances where it has been reported that preferred feed particle sizes are in fact much smaller than those the animals are physically capable of ingesting (Burko, 1975 cited by Hyatt, 1979; Knights, 1983; Mills et al, 1984).

The present investigation was designed to determine the preferred food particle sizes of a commercial pelleted diet for common carp fry ranging between 15 and 500 mg weight. The minimum weight of carp fry within the size range used was based on the findings of Bryant and Matty (1981). These authors demonstrated that carp post larvae may be reared on a commercial trout fry diet from an initial body weight of approximately 15 mg.

The method used to determine the preferred feed particle size in this study was to observe feeding behaviour and consumption time of feed particles and an attempt was made to establish the possible relationship between the preferred food particle size and fish

size in relation to weight, total length and mouth size of different size classes of carp fry.

2.4.2. Materials and Methods

2.4.2.1. Experimental animals and acclimation

The source of the experimental animals and their quarantine procedures are described in section 2.2.2.2-3. After being quarantined the fry were maintained in 150l holding tanks in a recirculated water system in the Tropical Aquarium Building. During this period they were maintained at 28°C and fed with ground (125-1000 µm) commercial trout pellet (Edward Baker's Omega No. 3; protein 49%, lipid 10%, ash 10% and fibre 2.5%).

2.4.2.2. Experimental procedure

Four size classes of carp fry were used <50, 50-100, 100-200, and 200-500 mg. Means and ranges of the measured body weight, total length and standard length of each size class of carp fry are given in Table 2.22.

Four days prior to the test, fish of the respective size classes were selected from the holding tanks and maintained in 8-l culture tanks of the recirculatory experimental system described in section 2.2.2.1. Only healthy fish were chosen. The fish were fed three to four times daily to satiation with a range of food particle sizes.

Table 2.22 Body weight, total length and standard length of different size classes of carp fry and the ranges of particle size of food used in the feed particle selection study*

Size Class	Weight (mg) Mean (range)	Total length (mm) Mean (range)	Standard length (mm) Mean (range)	Particle diameter range (µm)
A	313 (210 - 466)	27.1 (24 - 31)	20.9 (18 - 24)	<125
				125-300
B	151 (105 - 209)	22.4 (20 - 25)	16.8 (14.5 - 18.5)	300-500
				500-790
C	72 (46 - 97)	18.9 (17 - 22)	14.2 (11.0 - 17.0)	790-1000
				1000-1490
D	38 (15 - 53)	16.0 (13 - 18)	12.3 (10.5 - 14.0)	1490-2000

* Particle diameter ranges given in the same row do not represent the corresponding values for each size group. See Table 2.23 for the ranges of particle size used for each group of fry.

Three to four days were allowed to ensure familiarity with relevant particle sizes and to allow acclimation to the test conditions. Commercial trout pellet (see section 2.4.2.1 for details) was used during this period as well as test diet. A water temperature of 28°C was maintained throughout the experimental period.

A water level of about 5 litres was maintained in the test tank by adjusting the stand pipe (Fig. 2.1d). This was done to facilitate close observation of feeding and behaviour. Prior to actual observation of feed particle selection, faeces and any other solid materials were removed from the tank by siphoning. Rate of water flow was reduced considerably during the study period so that smaller feed particles did not get washed away in the water flow. A group of 24 fish was used per test tank. Each feed particle size was tested in three replicates for each weight class of carp fry. To ensure the comparable levels of hunger, motivation and appetite the fish were starved for 12 hours prior to feeding and were fed only about 2-3% of body weight over the course of each observation. After the feeding observation was completed, fish were starved for 24 hours and 4-5 fish from each test tank were netted, anaesthetized with benzocaine, dried on a soft paper towel and their total length, standard length, body weight and upper jaw length measured.

Different particle sizes (Table 2.22) were obtained by grinding and seiving using close tolerance test seives. The range of particle size chosen for each group of fish was based approximately on the

maximum mouth size of fish fry in the group.

The feed particles under test were administered slowly to the tank. The time in seconds between entry of the feed particles into water and final consumption were recorded. It was noted in the case of large particles whether the fish fed from the water column or from the bottom of the tank. In some cases, particles began to disintegrate before final ingestion and the incidence of this occurence was recorded. Disintegration was caused by soaking and/or spit ejection by fish and it was not possible to observe whether all fragments were eventually ingested.

2.4.2.3. Measurements of morphometric characters

The following morphometric characters were measured:-

a) Body weight - to the nearest 0.1mg

b) Total length - to the nearest 0.5mm

c) Standard length - to the nearest 0.5mm

d) Mouth dimensions - the length of the upper jaw was measured under a stereo microscope at 10 x magnification to the nearest 0.05mm. The mouth opening or size (gape height) at an angle of 90° between the upper and lower jaw were calculated using the following formula:

Gape height $= \sqrt{2}$ AB (after Shirota, 1970) where, AB = length of the upper jaw.

Linear regression equations were calculated to describe the relationship between predicted mouth size and carp total length and body weight.

2.4.3. Results

The carp is a slow eater and usually picks up its food from the bottom by sucking. It also occasionally feeds by snapping. Two types of suction feeding are commonly encountered in carp: a) particulate intake and b) gulping (Sibbing, 1986). Particulate intake is accomplished by fast and voluminous suction, caused by expansion of the oropharyngeal and opercular cavities. Gulping, the slow and less aimed uptake of a mouthful of water with suspended food particles, is accomplished mainly by small expansion of the oropharyngeal cavity.

Consumption times for various sizes of food particles by different size classes of carp fry are given in Table 2.23. Fish fry of all size classes were found feeding mostly from the bottom of the tanks; sometimes on sinking food particles from the lower water column. Fish were noted to come occasionally towards the surface to feed on floating particles. Feeding from the surface water occurred mostly after food particles on the bottom had been consumed. An increase in the intensity of surface feeding was noted for comparatively smaller food particles sizes, as these took a

Table 2.23 *Mean consumption time (in seconds) of various sizes of feed particles by different size classes of carp fry observed in the feed particle selection study.

Size classes of carp fry		Food particle diameter (μm)						
Mean weight (mg)	Mean total length (mm)	<125	125-300	300-500	500-790	790-1000	1000-1490	1490-2000
313	27.1	-	940	700	380	307	460	838
151	22.4	-	790	339	293	320	520	-
72	18.9	-	733	343	690	915	1093	-
38**	16.0	840	546	672	1490	1730***	-	-

* Mean of three replicate observations

** Food given at 3% of body weight; for all other groups food given at 2% BW

*** Food still remaining at end of the observation period

significantly longer time to reach the bottom of the tank. In general, feeding of comparatively large feed particles from the bottom of the tanks was accomplished by particulate intake, whereas feeding on smaller suspended and floating particles was largely by gulping. An account of food consumption and feeding behaviour of different size classes of carp fry is given below:-

Size class A (mean weight 313mg, range 210-466mg); mean length 27.1mm, range 24-31mm). Food particles of 790-1000 µm diameter were consumed in the shortest time. However, the consumption time for food particles of 500-790 µm diameter was not significantly longer (t-test, $P > 0.1$). Feeding activity started as soon as both of the above food particles were administered and feeding continued throughout. A progressive delay in consumption time was seen with increase in particle diameter. Feed particles larger than 790-1000 µm took significantly longer (t-test, $P < 0.05$) time to consume. Feeding on these particles did not start immediately after administration. But after some time fish started feeding on particles which had absorbed water and become soft. Some spit ejection was observed and feed particles were eventually swallowed after softening or disintegration of particles which inevitably led to wastage of feed as well as delay in consumption time. Feed particles smaller than 500-790 µm size also showed progressive delay in consumption time principally due to delay in location. Since fish were primarily feeding from the bottom, or on sinking particles near the bottom, the slower rate of sinking of smaller feed particles led

to the delay in consumption time. However, these particles were consumed apparently with no difficulty. Nevertheless, due to the delay in locating them some of the feed particles were wasted as a result of disintegration and some of them remained uneaten. Therefore delay in location could result in loss of food by outflow in a recirculation system, or to the bottom sediments in a pond. Moreover the searching involved for smaller particles may lead to increased energy expenditure.

Size class B (mean weight 151mg, range 105-209mg; mean length 22.4mm, range 20-25mm). The shortest consumption time was recorded for food particles of 500-790 μm diameter, but the consumption times for food particles of 300-500 μm and 790-1000 μm were not significantly longer (t-test, $P > 0.1$). Fish started feeding on food particles of 300-790 μm range immediately after administration and were seen feeding continuously throughout the observation period. Although fish feeding on feed particles of 790-1000 μm did not have significantly longer feeding time (t-test, $P > 0.1$) compared to the other two particle sizes, they were seen to be more selective when feeding on this range of particle sizes. Initially they were feeding on smaller particles within this range and later started feeding on larger particles, possibly after particles were softened due to the absorption of water. Food particles of 125-300 and 1000-1490 μm took significantly longer time to consume (t-test, $P < 0.05$) than the above three food particle sizes. Behaviour displayed towards these sub-optimal and supra-optimal particle sizes were similar to those

seen in fish fry of group A.

Size class C (mean weight 72mg, range 46-97mg; mean length 18.9mm, range 17-22mm). The shortest consumption time was recorded for 300-500 µm food particle and progressive delay in consumption time was seen for larger or smaller food particles. Significantly longer consumption time for food particle of 125-300 µm was primarily due to the dispersion of particles all over the tank, which led to considerable delay in location. The probable reason for significantly longer (t-test, $P<0.05$) consumption time recorded for food particles of 500-790 µm was not very clear. No difficulty in ingestion of the food particles was noticed; only the speed of feeding was comparatively slow. Behaviour displayed towards the other supra-optimal food particles were similar to those seen in groups of larger sized fish fry.

Size class D (mean weight 38mg, range 15-53mg; mean length 16.0mm, range 13-18mm). The shortest consumption time recorded was for 125-300 um feed particles. Although the above food particles were dispersed all over the tank, this group of fry were particularly more efficient in locating the particles and ingesting them. Food particles of 125 µm diameter were also efficiently consumed. However, significantly longer (t-test, $P<0.01$) consumption time for this feed particle was due to the presence of a very large number of particles, even though the amount was the same. Moreover, towards the end of the feeding period, delays in location of food particles also contributed to longer consumption time. However, at the end of

the observation period, it was not possible to know if the total amount of food was consumed. A progressive delay in consumption time was recorded with increasing feed particle sizes. Although fish did feed on food particles of 300-500 µm diameter, the speed of feeding was slow and occasional spitting out of particles was recorded. For both reasons mentioned above, consumption time was delayed significantly (t-test, $P < 0.05$). The feeding behaviour displayed for other supra-optimal food particle sizes was similar to that noted for the other fish size groups and which consequently delayed the consumption time. For feed particles of 790-1000 µm diameter, only about half to two thirds of the total amount of food was consumed by the end of the observation period.

During the course of the experimental period, morphometric characters of carp fry of the different size classes studied were measured (see section 2.4.2.3. for details). The relationship between upper jaw length and total length, and that between upper jaw length and body weight are presented graphically in Figs. 2.19 and 2.20 respectively. The maximum mouth opening of carp larvae and fry during feeding was considered to be a 90^{o} angle between upper and lower jaw (Shirota, 1970). The relationships of predicted mouth size (gape height) at 90^{o} angle with total length and body weight were found to be highly significant (Fig. 2.21 and 2.22). These linear relationships can be described by the following equations:

1. $M = 0.0665TL + 0.0453$ (d.f. = 94; $P < 0.001$)

$r = 0.957$ (d.f. = 94; $P < 0.001$)

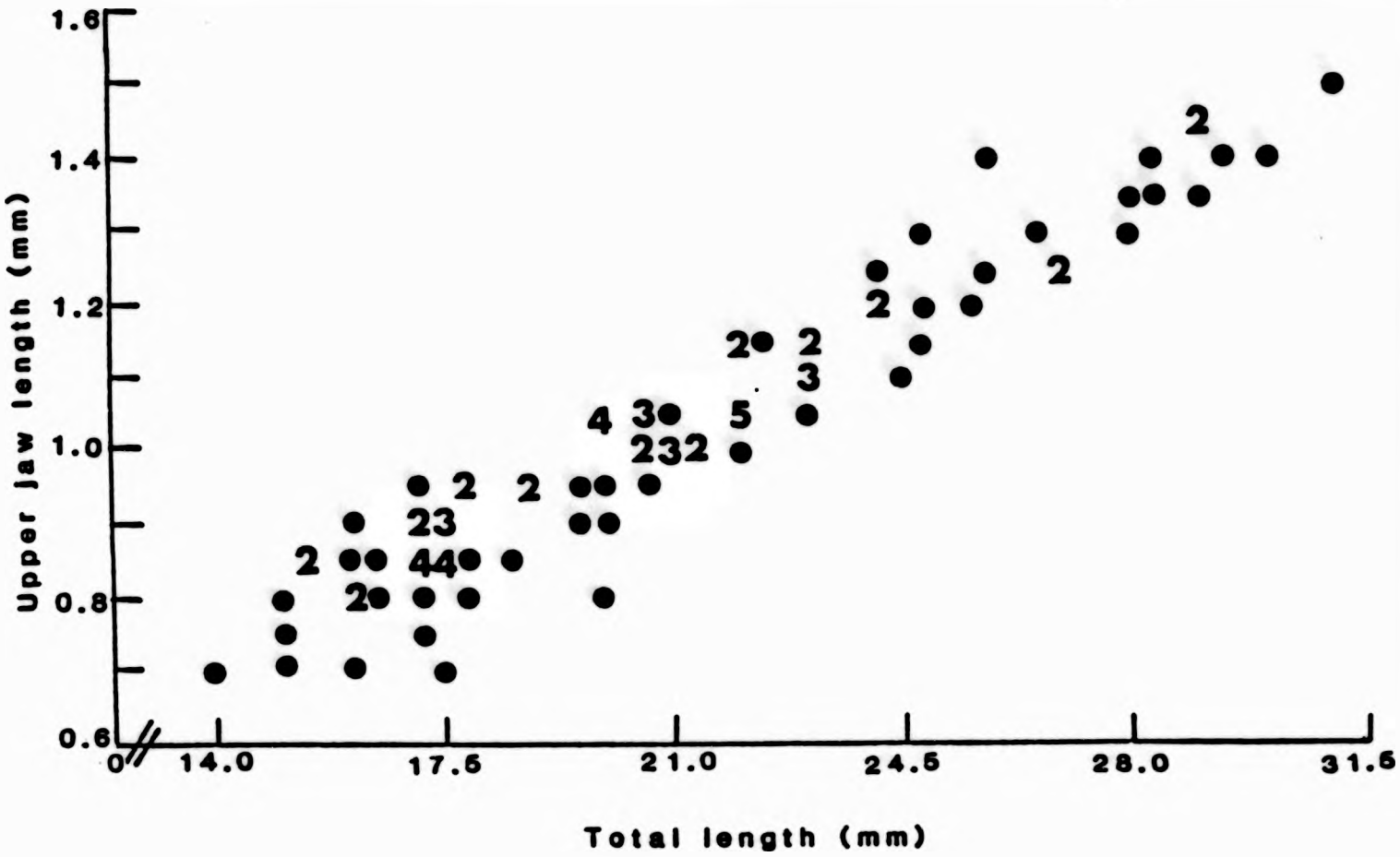

Fig. 2.19 Relationship between upper jaw length and total length of carp fry. Numbered points represent multiple observations.

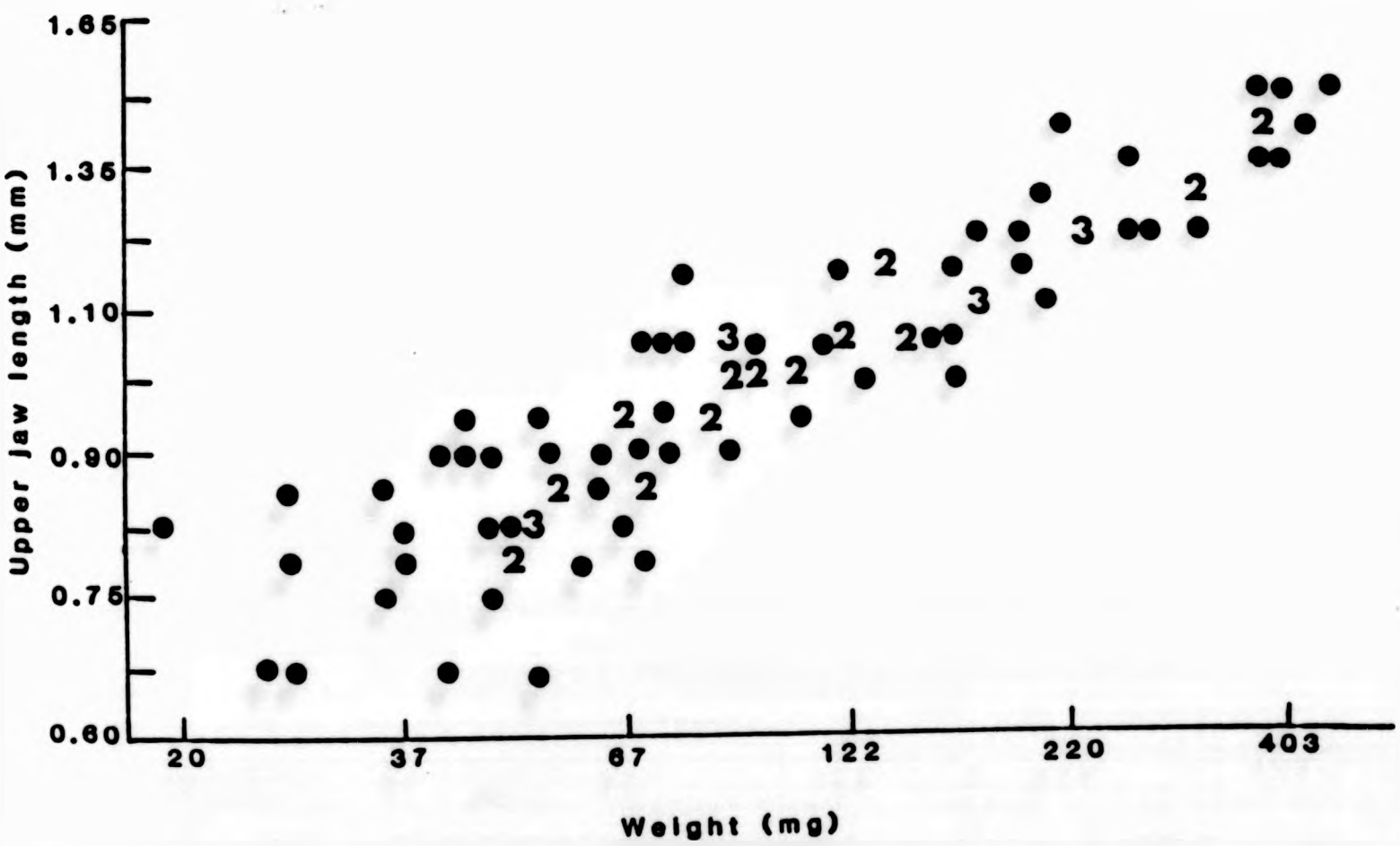

Fig. 2.20 Relationship between upper jaw length and weight of carp fry. Numbered points represent multiple observations.

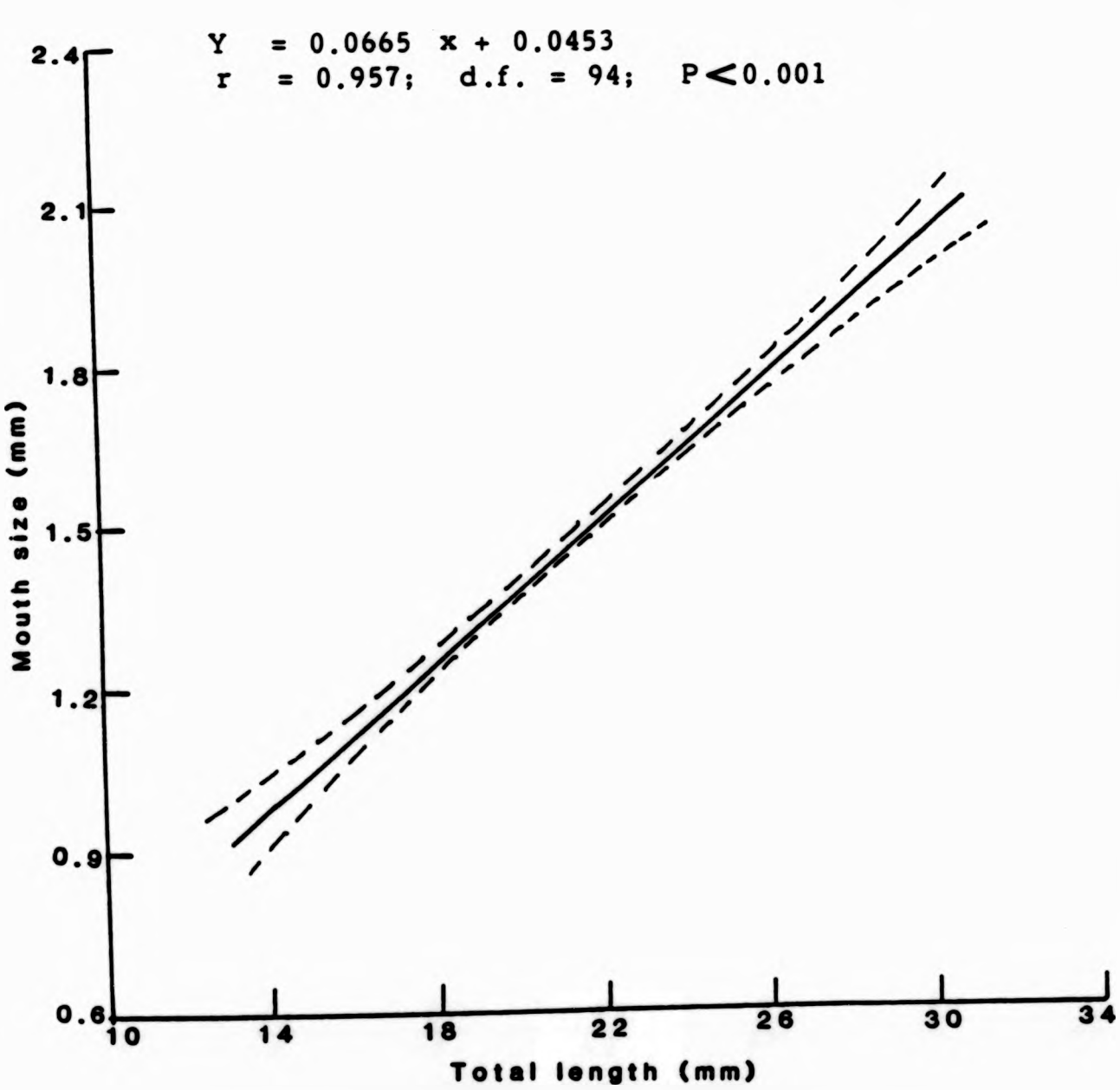

Fig. 2.21 Relationship between predicted mouth size and total length of carp fry. Broken lines indicate 95% confidence limits.

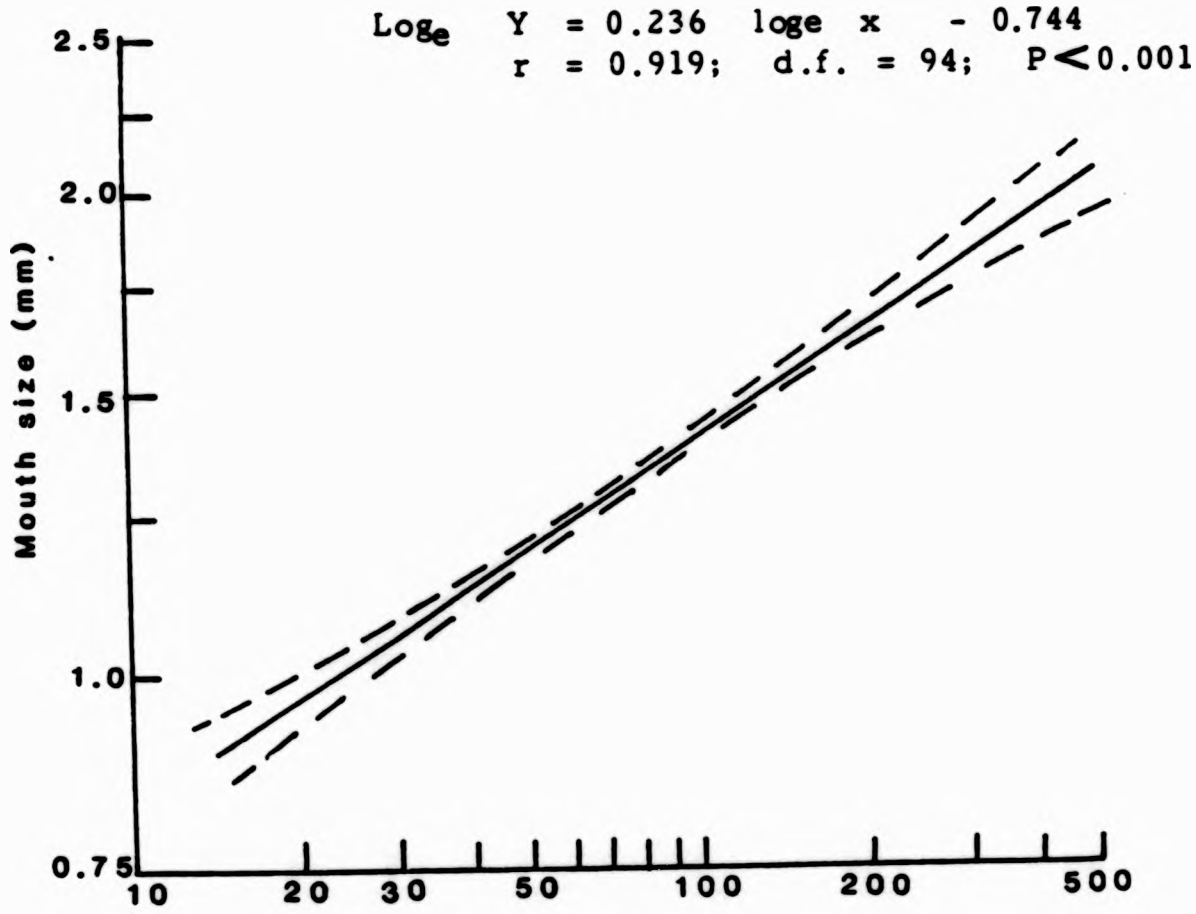

Fig. 2.22 Relationship between predicted mouth size and body weight of carp fry. Broken lines indicate 95% confidence limits.

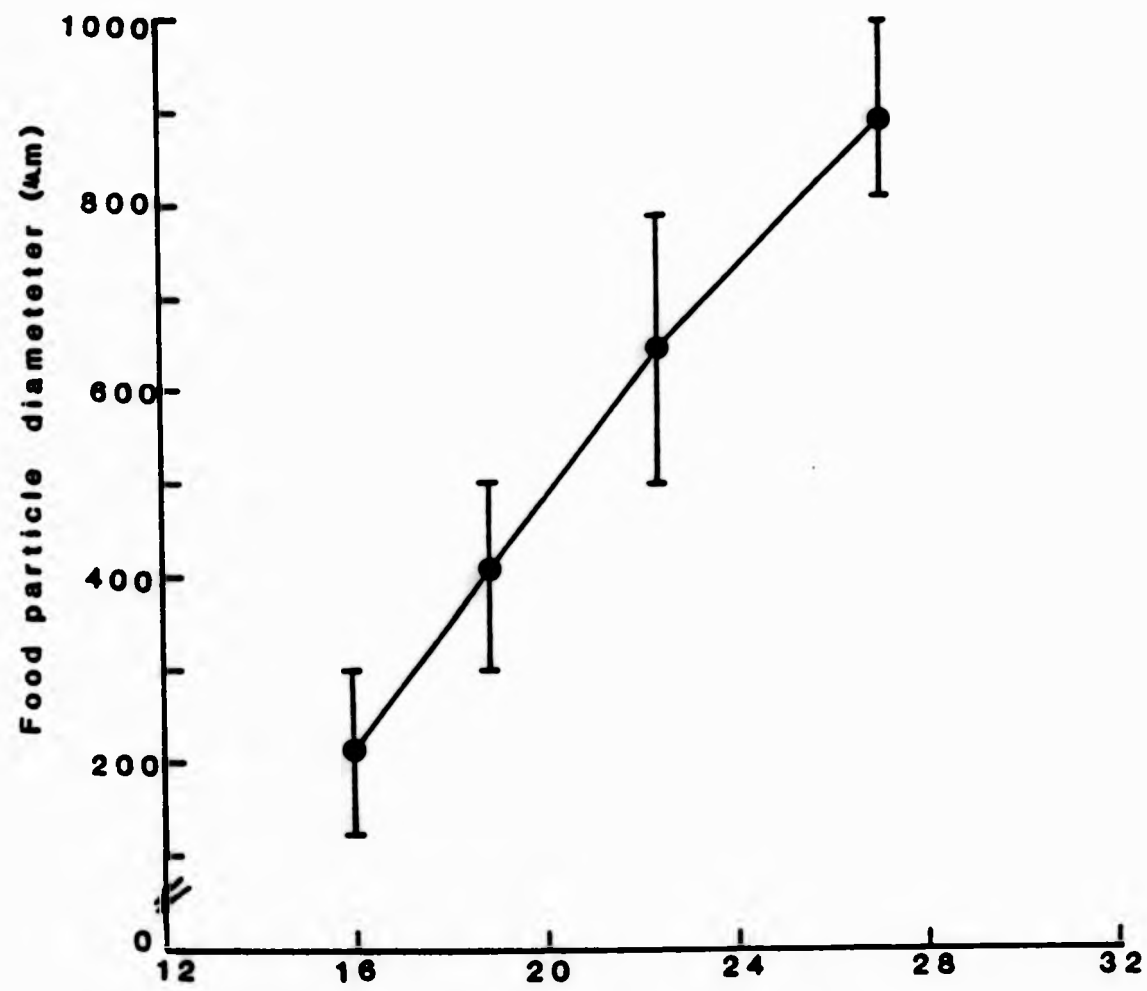

Fig. 2.23 Relationship between food particle sizes with shortest consumption time and mean total length of carp fry. Bars indicate ranges of feed particle size.

2. $\text{Log}_e M = 0.236 \log_e W - 0.744$ (d.f. = 94; $P<0.001$)

$r = 0.919$ (d.f. = 94; $P<0.001$)

Where M = mouth size in mm

W = body weight in mg

TL = total length in mm

In Fig. 2.23 the food particle sizes giving the shortest consumption times have been plotted against average fish length. From this figure, it can be seen clearly that the preferred food paticle size increases linearly with the size of the fish.

2.4.4. Discussion

This study demonstrated that for carp larvae and fry, preferred food particle size increases with fish size. On the basis of consumption time and feeding behaviour, food particle sizes ranging between 125-300 μm, 300- 500 μm, 300-790 μm and 500-1000 um diameter may be considered most suitable for carp fry of the length ranges 13-18, 17-22, 20-25 and 24-31mm respectively.

The present results indicate that if mouth size is the only limiting factor in the selection of food particle size, carp fry are in fact capable of accepting much larger food particles than they actually select. For carp fry ranging between 13-31mm length (15-466mg

Table 2.24 Predicted mouth size and ratio of mean optimum particle size to mouth size for each size group of carp fry

Size group	Body weight (mg)	Total length (mm)	Predited mouth size (mm)	Ratio of mouth size to particle size
A	313	27.1	1.85	1 : 0.41
B	151	22.4	1.53	1 : 0.36
C	72	18.9	1.30	1 : 0.31
D	38	16.0	1.11	1 : 0.19

weight), the mean preferred food particle sizes are about 0.2-0.4 that of mouth size (Table 2.24).

In many laboratory studies a preference for food particle sizes smaller than the fish's mouth size has been reported (Thorpe and Wankowski, 1979; Dabrowski et al, 1984, cited by Dabrowski and Bardega, 1984; Mills et al, 1984). Thorpe and Wankowski (1979) found that the most preferred food particle sizes for juvenile Atlantic salmon, Salmo salar were about 0.3-0.4 of mouth size. Similarly larvae of the coregonid, Coregonus pollan have been reported to select zooplankton, 0.4-0.6 of fish mouth size under laboratory conditions (Dabrowski et al, 1984, cited by Dabrowski and Bardega, 1984). Mills et al (1984), from their studies with young yellow perch, Perca falvescens, observed that in both laboratory and field tests, fish did not select the largest daphnids available although they were physically capable of doing so.

It also has been reported that variation exists between the maximum prey size that fish are capable of ingesting and the sizes that they will usually ingest under natural conditions. Hyatt (1979) recalled the laboratory observation of Burko (1975) that the three-spine stickleback, Gasterosteus aculeatus, can consume invertebrates with a maximum body width very nearly equal to its jaw width. But in the field, where a wide range of prey sizes are available, sticklebacks generally ingest prey that are significantly smaller than the maximum sizes they can handle.

Several hypotheses have been put forward to explain this phenomenon. It has often been related to the problems of manipulating and swallowing hard abrasive food (Knights, 1983). The results reported here also indicate that fish fry often accept larger than their preferred particle sizes after they have been softenend with water. Knights (1983) observed that the maximum diameters of soft paste balls consumed by larger eels corresponded well with their average mouth width, i.e. an approximately 1:1 relationship was exhibited, whereas the mean optimum particle sizes for a dry compounded diet were about 0.4-0.6 of mouth width. Similarly, Hartman (1958) observed that young rainbow trout fed natural diets, showed a nearly 1:1 ratio for smooth prey (trout fry), whereas the maximum width of prey with appendages which interfered with oral manipulation and swallowing (caddis larvae and stonefly nymphs) was only about 0.4-0.6 of mouth width. Therefore the author concluded that although mouth size imposes a limit on the size of the food swallowed, the structure and reactions of food organisms may result in a considerable discrepancy between prey width and mouth width of the fish.

The result of the present investigations do not agree with the criteria proposed by Dabrowski et al (1983) for selection of appropriate food particle sizes for common carp larvae and juveniles. These authors considered that carp larvae and fry should be able to accept food particle sizes as large as their mouth. However, a recent study by Sibbing (1986) reported that common carp (standard length 10-25cm size), due to the limitation of the size of the

chewing cavity, cannot feed on particles larger than 3-4% of their standard length, even though the maximal diameter of protruded circular mouth is about 9% of their standard length. If the preferred food particle sizes for carp fry observed in this investigation are compared to their standard length, the value obtained is 1.7-3.7% of standard length, which is consistent with the findings of Sibbing (loc. cit.).

The present observations indicated that the lower size limit of feed particles selected by carp fry was primarily dictated by the efficiency of their location. Similarly, Knights (1983) reported that for the European eel, the lower size limit of feed particles to efficient feeding has been dictated by location abilities.

On the basis of the findings discussed above, the following general recommendations are made on the optimum food particle sizes for different size classes of carp fry:

Size class of carp fry		Recommended food particle size
weight (mg)	length (mm)	(μm diameter)
115-100	13-21	125-500
100-250	21-26	300-790
250-500	26-31	500-1000

Under hatchery and nursery conditions size differences usually develop rapidly among carp fry. To overcome this problem frequent size sorting of the stock is essential. However, if frequent size

sorting is not feasible, mixtures of different particle sizes may be administered in a culture tank to satisfy the requirement of fish of different size classes.

CHAPTER 3

Short term tolerance of carp fry to water quality parameters

3.1. Introduction

The physical and chemical characteristics of water are among the most important parameters affecting the life of fish. If the value of one or more parameter exceeds the physiologically acceptable limits for the fish, it can result at sub-lethal levels in reduced growth, decreased food utilization and predispose the fish to infectious or other diseases, or even cause mortality (Fijan, 1979; Plumb, 1984). In several instances, it has been reported that poor water quality and rearing conditions can lead to 60-70% mortality of carp fingerlings (Goltz and Wierowski, 1978, cited by Fijan, 1979).

In conventional nursery and production ponds water quality has been reported to vary widely. Chakrabarty et al (1973) reported levels of DO ranging between 1.6 and 12.4 mg l^{-1} and pH between 6.8 and 9.5 in various nursery ponds in Orissa, India. The pH in ponds and open waters used for fish rearing often reaches values above 8.5 or 9.0 due to eutrophication, high stocking densities, and intensive feeding with protein rich diets (Fijan, 1979). Although the range of pH stated above is not limiting to carp, it can affect the toxicity of ammonia and nitrite considerably. A change in pH of 0.4 unit can change the mole fraction of un-ionized ammonia by more than 100%. The toxicity of un-ionized ammonia to carp fry and the effect of pH on nitrite toxicity is discussed in sections 3.2 and 3.3.

In controlled environment cultures, water temperature should not be a limiting factor. However, temperature can be limiting in field

conditions, where wide diurnal or seasonal fluctuations in water temperature can occur. Diurnal variations in water temperature ranging between 27 and 37°C were recorded during summer months in some shallow nursery ponds at Mymensingh, Bangladesh (unpublished data). Such changes are very stressful to fish and can be lethal. In a partially or totally recirculated water system, or in intensively stocked static rearing systems, the toxicity of excreted nitrogenous compounds is singly the most limiting parameter provided adequate dissolved oxygen levels are maintained (Colt and Armstrong, 1981).

Various control measures such as use of biological filters and oxygenation of water in recirculated water systems, and frequent change of water, oxygenation and use of low stocking and feeding levels in the static rearing system, are used. These measures allow individual control of some of the physico-chemical changes and it then becomes important to establish the precise nature of the individual effects on the organisms. Estimates of the tolerance of fish to these water quality parameters are essential to determine the maximum and minimum levels that will permit accurate estimate of the control measures required for economic operations.

The establishment of tolerance limits of fish to pollutants is most often experimentally determined by relating the concentrations of a pollutant to some measurable, presumably deleterious, response (Brownell, 1980). Primarily for reasons of simplicity, this has

usually been achieved by means of a short term acute lethal test conducted for a period of 96-168 hours to determine the median lethal concentration (LC50). The median lethal concentration is, primarily a measure of lethal concentration, defined as a concentration lethal for median or typical fish for an indefinite exposure time.

When one is concerned with the growth of fish in a tank, or the long-term health of a natural population, sub lethal or chronic toxicity tests are more meaningful (Brownell, 1980). In the absence of any chronic toxicity data, but when a prediction of an acceptable concentration is needed, arbitrary reductions below the LC50 may be made (Mount, 1977).

Calculations of acceptable levels of pollutants from acute lethal tests involves the use of an application factor. A value for the application factor is assigned on the basis of scientific judgement after taking into account all available evidence concerning the relation between safe levels and lethal levels for the chemical in question (Sprague, 1971). 'Safe levels' have often been assumed to be 5-10% of the 48 to 96-h LC50 and have been used successfully by several researchers and research bodies (Aquatic Life Advisory Committee, 1955; Tarzwell, 1966; United States National Technical Advisory Committee, 1968; Lloyd and Orr, 1969; U.S. Environmental Protection Agency, 1973, cited by Buckley, 1978).

Tolerance levels to different water quality parameters vary widely

among fish species. The requirement for minimum level of dissolved oxygen in the effluent for normal life of carp juvenile have been investigated by Chiba, 1965 and Itazawa, 1971. Detailed information on the tolerance level of temperature, ammonia, nitrite and nitrate are lacking for carp fry.

The objective of the present study was to determine the lethal concentration of several nitrogenous compounds and the upper lethal temperature for common carp fry under laboratory condition. Several static or recirculation bioassays were conducted to determine the median lethal concentration of ammonia (Experiment 3.1), nitrite (Experiment 3.2), nitrate (Experiment 3.3) and upper incipient lethal temperature (Experiment 3. 4) for common carp fry. Tests for the sub lethal or chronic toxicity of nitrogenous compounds could not be carried out because of the unavailability of suitable facilities for sub lethal testing.

3.2 Experiment 3.1

Estimation of median lethal concentration of un-ionized ammonia to carp fry

3.2.1. Introduction

Ammonia occurs in natural water in un-ionized (NH_3) and ionized (NH_4+) forms. It is a product of biological metabolism. It can also enter natural water from several other sources, e.g. sewage effluents, industrial wastes, and agricultural inputs. Moreover, ammonia is the principal nitrogenous excretory product of freshwater teleosts (Brockway, 1950; Burrows, 1964; Forster and Goldstein, 1969). The other nitrogenous products they excrete are urea, amino acids, amine oxide derivatives, creatine and uric acid. Ammonia and urea are the only nitrogenous compounds excreted by freshwater teleosts in significant quantities (Wood, 1958; Burrows, 1964). However urea is non-toxic to fish at the concentration normally present in hatcheries (Colt and Tchobanoglous, 1976).

Fish produce ammonia from the metabolic activity of nerve and muscle tissues, by deamination of amino acids by the liver, and by the activity of enzymes in the microflora of the gut on substrates derived from the diets and the blood (Fromm and Gillette, 1968).

Excretion of ammonia, urea, and amines by fish occurs mainly through the gills, while creatine, creatinine and uric acid are excreted through the kidneys (Brockway, 1950).

Ammonia is highly toxic to fishes and its toxicity has been fairly extensively investigated (Ball, 1967; Flis, 1968a; Colt and

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Ammonia is highly toxic to fishes and its toxicity has been fairly extensively investigated (Ball, 1967; Flis, 1968a; Colt and

Table 3.1 A summary of the toxicity of un-ionized ammonia (NH_3) to several species of fish

Fish species	Size (Weight/length)	NH_3 Conc. (mgl^{-1} NH_3-N)	Effect	Author
Rainbow trout *Salmo gairdneri*	Fertilised eggs and alevins (0-52 days)	>2.94	24-h TLm*	Rice & Stokes (1975)
	Fry after yolk sac absorption	0.06	24-h TLm*	Rice & Stokes(1975)
	Sac fry	0.25	21-day incipient LC50	Burkhalter & Kaya (1977)
	40g (23-53)	0.41	48-h incipient LC50	Ball (1967)
Cut-throat trout *S. clarkii*	1-3g	0.43-0.66	96-h LC50	Thurston *et al* (1978)
	1-3g	0.28-0.46	29-day LC50	Thurston *et al*(1978)
Coho salmon *Oncorhynchus kisutch*	6g	0.45	96-h LC50	Buckley (1978)
Chinook salmon *O. tshawtyscha*	5.1cm	0.30	24-h LC50 in freshwater	Harader & Allen(1983)
Atlantic salmon *Salmo salar*	Smolt(14.6 cm 2 + years)	0.12	24-h LC50 in freshwater	Alabaster *et al* (1979)

contd...

Table 3.1 (contd.) A summary of the toxicity of un-ionized ammonia (NH_3) to several species of fish

Fish species	Size (Weight/length)	NH_3 Conc. (mgl^{-1} NH_3-N)	Effect	Author
Channel catfish *Ictalurus punctalus*	3-4 g**	2.38	96-h LC50 at 26°C	Colt & Tchobanoglous (1976)
	3-4g**	3.13	96-h LC50 at 30°C	Colt & Tchobanoglous (1976)
	20-47g	2.36	24-h LC50 at 25°C	Robinette (1976)
	1g	1.60	96-h LC50 at 28°C	Colt & Tchobanoglous (1978)
	7-13cm standard length	1.39-1.82	24-h LC50 at 21-25°C	Tomasso *et al* (1980a)
Bream *Abramis brama*	16g	0.41	168-h incipient LC50	Ball (1967)
Perch *Perca fluviatilis*	14g	0.29	Incipient LC50	Ball (1967)
Roach *Rutilus rutilus*	9g	0.35	120-h incipient LC50	Ball (1967)
Rudd *Scardinius erythrophthalmus*	20g	0.36	144-h incipient LC50	Ball (1967)
Guppy *Poecilia reticulatus*	6.3-11.0 cm	1.24	96-h LC50	Rubin & Elmaraghy (1977)
Golden shiner *Notemigonus crysoleucas*	N.R.	0.99	96-h LC50	Baird *et al* (1979)

contd....

Table 3.1 (contd.) A summary of the toxicity of un-ionized ammonia (NH_3) to several species of fish

Fish species	Size (Weight/length)	NH_3 Conc. (mgl^{-1} NH_3-N)	Effect	Author
Tilapia, Oreochromis aureus	7-9 cm	2.35	72-h LC50	Redner & Stickney (1979)
European eel Anguilla anguilla	Elvers-0.2g Yellow eel-2.8g	1.0	10-day LC50	Sadler (1981)
Japanese eel Anguilla japonica	-	4.64	24-h LC50	Yamagata & Niwa (1982)
Red drum Sciaenops ocellatus	Larvae	0.39	96-h LC50	Holt & Arnold (1983)
Fathead minnows Pimephales promelas	0.22-1.7g	1.52-2.83	96-h LC50	Thurston et al (1983)

* Median tolerance limit (=LC50)

** Reported by Colt & Techobanoglous (1978)

N.R. Not reported

and Tchobanoglous, 1976; Thurston et al., 1978; Redner and Stickney, 1979; Sadler, 1981; Harader and Allen, 1983). In fish culture systems, especially those utilising recirculated water, the accumulation of ammonia may lead to suppression of fish growth, sub-lethal histopathological changes and even death (Burrows, 1964; Flis, 1968a, b; Robinette, 1976; Wickins, 1980; Colt and Armstrong, 1981). Wuhrmann et al. (1947) demonstrated for the first time that it was NH_3 which was toxic to fish, and that NH_4^+ had little or no toxicity. Subsequently, the toxicity of NH_3 has been confirmed experimentally by Wuhrmann and Woker (1948), Downing and Merkens (1955) and Lloyd (1961). The portion of total ammonia existing as NH_3 is to some extent dependent on temperature and the ionic strength of the medium, but primarily on the pH of the solution (Trussel, 1972; Emerson et al., 1975). The toxicity of NH_3 has been attributed to the fact that the un-ionized form can readily diffuse across gill membranes due to its lipid solubility and lack of charge, whereas the ionized form occurs as larger hydrated form with charged entities which cannot readily pass through the hydrophobic micropores in the gill membrane (Fromm and Gillete, 1968; Hampson, 1976).

Under normal circumstances ammonia levels in the blood of fish are much greater than ambient concentrations. Fromm and Gillete (1968) reported that ammonia levels in the blood of trout are 9-40 times greater than those in ambient waters. The normal ammonia concentrations in the plasma vary between different families or genera of fish. Hampson (1976) reported that in the murray eel, Muraema helema, the plasma contains 30 mgl^{-1} of ammonia-nitrogen ;

32 times the concentration in the water. In contrast, the lamprey, Lampetra fluviatilis has a plasma level of only $5mgl^{-1}$ and trout, Salmo spp. and tench, Tinca tinca, well below $3mgl^{-1}$ ammonia-nitrogen. Fish get rid of their excess ammonia from the blood through diffusion across the gill membrane (Fromm and Gillete, 1968), or by excreting it as NH_4^+ through a carrier-mediated process in exchange for Na^+ (Campbell, 1983). As external ammonia concentrations increase, the rate of outward diffusion from an animal decreases and toxicity ensues when the tolerable body load is exceeded (Armstrong et al., 1978). The release of ammonia into the blood from ambient water as a result of inward diffusion also increased the blood ammonia level.

Several authors have demonstrated that the prolonged exposure of fish to sublethal concentrations of ammonia results in severe histopathological changes in gill and kidney tissues and in the liver (Burrows, 1964; Flis, 1968a,b; Smart, 1976; Burkhalter and Kaya, 1977; Yamagata and Niwa, 1982; Thurston et al., 1984). However, in recent years, the validity of gill damage caused by sublethal concentrations of ammonia has been questioned as several authors have observed no gill lesions after prolonged exposure to sublethal concentrations of ammonia in the case of Dover sole Solea solea, turbot Scophthalmus maximus, channel catfish and rainbow trout (Alderson, 1979; Mitchel and Cech, 1983; Daust and Ferguson, 1984). Exposure to sublethal concentrations of ammonia has also been reported to cause reduced food uptake, reduced assimilation & growth

inhibition in fish (Robinette, 1976; Burkhater and Kaya, 1977; Colt and Tchobanoglous, 1978; Alderson, 1979; Sadler, 1981; Yamagata, and Niwa, 1982; Holt and Arnold, 1983). However, no significant histopathological changes in gill from exposure to acutely lethal concentrations of ammonia were observed by Sousa et al. (1974) and Smart (1976). Smart (1976) concluded that relatively minor gill damage in rainbow trout resulting from exposure to acutely lethal concentrations of ammonia was unlikely to have been a primary cause of death. Therefore it has been suggested that lethal concentrations of ammonia may cause mortality in fish by impairing cerebral energy metabolism (Smart, 1976; Arillo et al., 1981). Similarly Nemcsok et al. (1984) demonstrated that ammonia (as a toxic agent) produced metabolic disturbances which caused stress in fishes and led to enhanced blood sugar and catecholamine level in the blood sera. He also proposed that higher ammonia concentration induced anoxia in tissues.

As previously discussed NH_3 has been shown to be the principal toxic form of ammonia. However, it has been shown that NH_4^+ may also have a considerable toxicity under low pH conditions (Tabata, 1962; Armstrong et al., 1978; Thurston et al., 1981a; Yamagata and Niwa, 1982). Although Tabata (1962) demonstrated that NH_4^+ can be toxic, it has only one fiftieth of the toxicity of NH_3 to the water flea (Daphnia pulex) and even less for some species of fish. Similarly, NH_4^+ has been shown to be at least 70 times less toxic than NH_3 to prawn larvae (calculated from the data of Armstrong et al., 1978) and 250 times less toxic to rainbow

trout (calculated from the data of Thurston et al., 1981a). Moreover, Thurston et al., (1981a) reported that the acutely toxic effects of NH_3 on rainbow trout appear to be relatively constant over the pH range of 7.8 to 9.0. Similarly Yamagata and Niwa (1982) suggested that NH_3 may be responsible solely for the toxicity of ammonia in the pH range of 7.0 to 9.0. However, they suggested that at a pH lower than 7.0, NH_4^+ exhibited some toxicity to eel. Alderson (1979) also did not observe any marked effect on the growth of sole and turbot after sublethal exposure to a similar NH_3 concentration over the pH range of 6.9 to 8.1. Therefore it can be summarized that over the pH range of 7.0 to 9.0, the median lethal concentration of NH_3 remains relatively unchanged indicating that the un-ionized form may be the only toxic form of ammonia over this range. At a pH below 7.0, the percentage of the total ammonia present as NH_3 is so insignificant that the amount of total ammonia required to produce a toxic effect are relatively very high; and even if NH_4^+ has some toxic effect, such a high amount of total ammonia is unlikely to occur in a natural water body or in an intensive aquaculture system. Therefore, it can be concluded that the ionized portion of ammonia will normally pose no serious threat to cultured fishes.

The toxicity of ammonia may be affected by several other environmental factors. Temperature and pH affect ammonia toxicity by influencing the fraction of un-ionized ammonia in a solution containing total ammonia. An increase in pH and temperature leads to an increase in NH_3 and therefore, ammonia becomes more toxic at higher pH and temperature. As has been indicated previously,

pH has a greater influence than temperature in determining the un-ionized portion of ammonia. Alkalinity and free carbon dioxide only affect the toxicity of ammonia by their part in influencing the pH value of the water (Alabaster and Herbert, 1954; Lloyd, 1961).

A reduction in the level of dissolved oxygen in the water results in an increase in toxicity of ammonia and this has been demonstrated by Downing and Merkens (1955), Alabaster et al. (1979) and Thurston et al. (1981b). The most likely explanation is that fish respond to the reduced oxygen by increasing their ventilation volume, thereby bringing more pollutant into contact with the gill surface. However, Fromm and Gillete(1968) showed that the ability of oxygen to combine with haemoglobin is not affected by the ammonia concentration.

Variations in water hardness reportedly had no effect on the toxicity of ammonia to minnows (Wuhrmann and Woker, 1953 , Herbert, 1961; both cited by Alabaster and Lloyd, 1980).

It has been demonstrated by several authors that the susceptibility to ammonia varies with the age of fish. Rice and Stokes (1975) found that eggs and alevins of rainbow trout, Salmo gairdneri were not vulnerable to 2.94mgl^{-1} NH_3-N when exposed for a 24 hour period, but the alevins became increasingly sensitive to ammonia just before the absorption of their yolk sac. The 24-h median tolerance limit of rainbow trout fry is as low as 0.06 mgl^{-1} NH_3-N. Similarly, Alabaster and Lloyd(1980) citing a personal communication from D. Calamari reported that developing rainbow trout

alevins became increasingly sensitive to short-term exposures to ammonia, with the 96-h LC50 falling from 0.30 mgl^{-1} NH_3 at yolk sac absorption stage, to 0.13 mgl^{-1} NH_3 32 days later before rising to 0.36 mgl^{-1} NH_3 at the fingerling stage. In contrast, Holt and Arnold (1983) reported that NH_3 concentrations as low as 0.26 mgl^{-1} NH_3 significantly reduced the survival of newly hatched red drum, Sciaenops ocellatus, larvae (in the first two weeks), whereas concentrations twice that high were tolerated by three-week old post larvae. Thurston and Russo (1983) observed that susceptibility of rainbow trout decreased as the fish developed from yolk-sac fry to juveniles, and increased thereafter for rainbow trout, whereas toxicity of ammonia was not found to be related to size over the range 0.1-2.9g in fathead minnows (Thurston et al., 1983).

Clearly, more emphasis should be given to the study of ammonia toxicity to different stages of the life cycle of a particular fish species.

The toxicity of NH_3 also varies between species. Reported acute toxicity values in tests of 24-h and 96-h duration on salmonids range from 0.06 to 0.66 mgl^{-1} NH_3 and values for comparable tests on non-salmonids range between 0.29 and 4.64 mgl^{-1} NH_3-N (Ball, 1967; Rice and Stokes, 1975; Thurston et al., 1978; Yamagata and Niwa, 1982; Table 3.1). Although there is some published information on the toxicity of ammonia to carp fingerlings and adults (Vamos, 1963; Danecker, 1964, cited by

Table 3.2 A comparison of the toxicity of NH_3 to common Carp*

Size (weight/length)	pH	Temp (°C)	NH_3 Conc. (mgl^{-1} NH_3-N)	Effect	Author
6-8g	8.2	22-25	0.43-0.55	Toxic; sink to the bottom in 60-75 min	Vamos (1963)
-	-	16	1.23	Lethal in less than 2 days	Danecker (1964, cited by Alabaster and Lloyd, 1980)
125-260g	8.3-8.7	7-15	0.71-0.98**	10-day LC16-LC18	Flis (1968a)
125-260g	7.8-8.3	7-15	0.09**	35-day LC8	Flis (1968b)
4-5cm (1-2g approx)	7.2-7.8	27.6	1.21**	24-h TLm***	Rao et al (1975)
4-5cm	7.2-7.8	27.6	1.06**	48-h TLm***	Rao et al (1975)
4-5cm	7.2-7.8	27.6	0.96**	96-h TLm***	Rao et al (1975)

* All tests were static except that of Danecker (1964), information of which is not available

** Calculated from data given for total ammonia or ammonium chloride concentration, pH and temperature

*** Median tolerance limit (= LC50)

Alabaster and Lloyd, 1980; Flis, 1968a, b; Rao et al., 1975; Table 3.2), data on the toxicity of ammonia to common carp fry (<1.0g) are not available.

The present investigation was conducted to estimate the lethal threshold concentrations of NH_3 so as to assess the tolerance of carp fry to NH_3.

3.2.2. Materials and Methods

3.2.2.1. Experimental animals and acclimation

The source of experimental animals and their quarantine procedure are described in section 2.2.2.2-3. After being quarantined the fry were maintained in 150-L tanks in a recirculation system in the tropical aquarium building at the Institute of Aquaculture. Prior to test, they were maintained at 28°C and fed with ground (500-100μm) commercial trout pellet (Edward Baker's Omega No. 3; protein content 49%), two to three times a day. Two trials were conducted to estimate the median lethal concentration of NH_3 to carp fry. Carp fry weighing 299mg (± S E 14.2) and 206mg (± S E 8.4) were used for the first and second trials respectively.

3.2.2.2. Experimental system

Both trials were conducted in a recirculation system. The system consisted of eight independent tank units arranged in rows on a wooden platform. Each unit was comprised of six 2-l perspex tanks fitted with common inflow and outflow pipes (Fig. 3.1.) and was connected to a 10-l reservoir tank. Water from the reservoir tank was pumped by an Eheim-brand electric pump through an inflow pipe fitted with valves to supply controllable rates of water to each perspex tank. On the side of the upper region of each tank, a window was cut out and a fine mesh netting was fixed to this cut portion; this served as the water outflow. All eight reservoir tanks were maintained in a 150-l glass water bath. Thermostatically controlled immersion heaters in the water bath maintained the required temperature. A 15-cm stone aerator connected to a compressed air supply was used to maintain an adequate level of dissolved oxygen in each reservoir tank. A photoperiod of 12h light: 12h dark was maintained by an electronic timer throughout the experimental period.

3.2.2.3. Test water

To maintain uniform water quality in all the tests, synthetic dilution water was prepared with a total hardness of 50 mgl^{-1} (as $CaCo_3$) and a pH of about 7.5-8.0 as recommended by Sprague (1973) and Alabaster and Lloyd (1980). The dilution water was prepared following the procedure outlined by Ministry of Housing and Local Government (1969).

Fig. 3.1 Layout of one unit of the recirculation system used for the ammonia toxicity bioassay.

a: Inflow pipe

b: Experimental tanks (two-litre perspex tank-receiving an inflow of water at a rate of 100 ml/minute)

c: Outflow

d: Water bath (150 litres)

e: Outflow trough

f: Clamp for inflow regulaton

g: Reservoir tank (10 litres)

h: Aquarium heaters (5 x 200w)

p: Eheim brand electric pump

⟶ Direction of waterflow

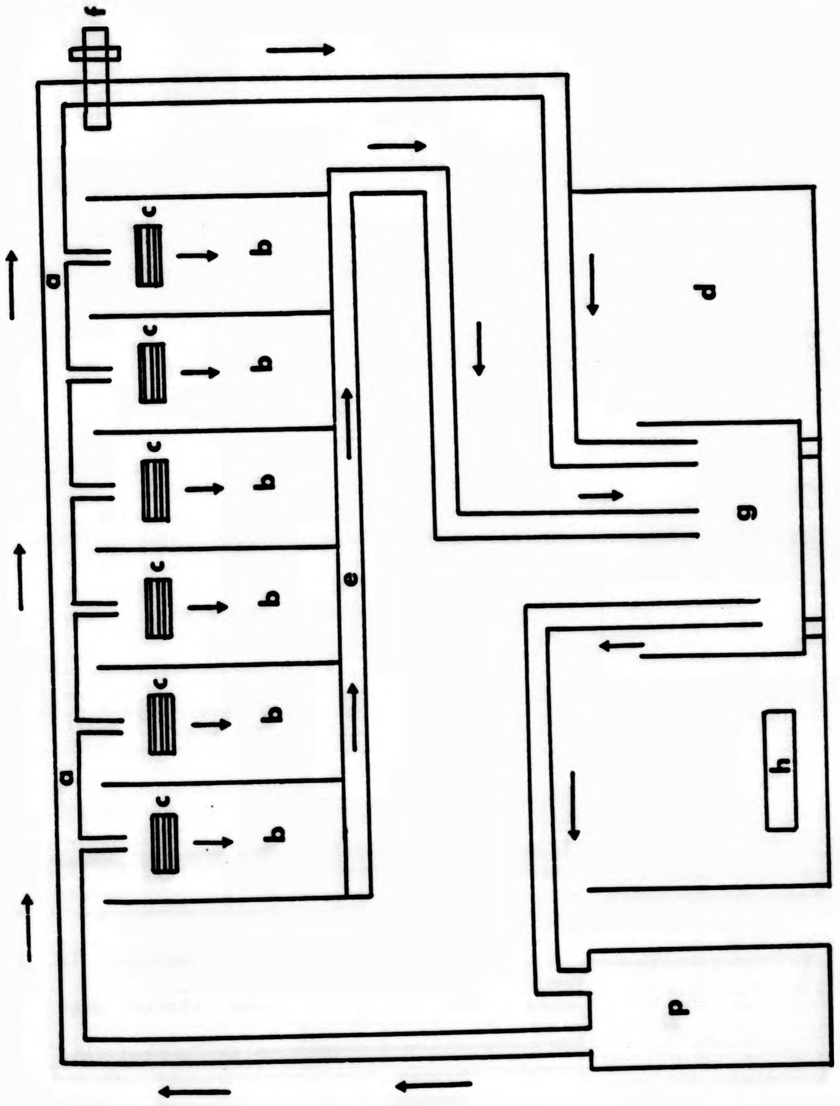


Fig. 3.1

Initially three stock solutions were prepard with de-ionized distilled water with a conductivity not exceeding 10 micromhos. Stock solution 1 contained 400g $CaCO_2,6H_2$, 36g Nacl and 11g $NaNO_3$ dissolved in de-ionized distilled water and made up to 1 litre. Stock solution 2 contained 189g Mg $SO_4,7H_2O$ and 99g Na_2SO_4 dissolved in de-ionized distilled water and made up to 1 litre. Stock solution 3 contained 34g $NaHCO_3$ dissolved in de-ionized distilled water and made up to 1 litre.

Twenty millilitres each of stock solutions 1 and 2, and 200ml of stock solution 3 were added to 100 litres of de-ionized water. a full analysis of this dilution water was carried out with the help of the Forth River Purification Board, Stirling (Table 3.3). All the chemicals used in the preparation of the dilution water were of analytical reagent (A.R.) grade.

3.2.2.4. Experimental procedure

Two trials were conducted to estimate the median lethal concentration of NH_3 to carp fry. The procedures are described below:-

a) Series of concentrations tested.

Both trials were conducted with seven concentrations of NH_3 and one control as recommended by Sprague (1973) and Alabaster and Lloyd (1980). The values of total ammonia and NH_3 concentration used

Table 3.3 Chemical characteristics of the dilution water used in ammonia toxicity bioassay *

	Mean	Range
Total oxidised nitrogen	0.35	0.3 - 0.4
Conductivity	219.5	194 - 245 $uScm^{-1}$
Suspended solids	1.5	1.0 - 2.0
Chloride	31.5	31.0 - 32.0
Total alkalinity (as $CaCO_3$)	42.5	40.0 - 45.0
Total hardness (as $CaCO_3$)	58.5	57.0 - 60.0
Orthophosphate (as P)	<0.01	<0.01 - 0.01
Calcium	7.05	6.9 - 7.2
Magnesium	3.65	3.2 - 4.1
Potassium	0.55	0.5 - 0.6
Sodium	28.3	-
Copper	0.01	0.01 - 0.01
Iron	0.02	0.02 - 0.02
Lead	<0.005	-
Manganese	<0.005	
Zinc	0.009	<0.005 - 0.017

* All values are in mgl^{-1} unless otherwise noted.

in trial 1 and 2 are given in Table 3.4 and 3.5 respectively. The concentrations were spaced at approximately logrithmic intervals (e.g. 0.1, 0.2, 0.4, 0.8, 1.6mgl^{-1}) and in such a way that a complete mortality occurred in the highest concentration used and no mortality occurred in the lowest concentration (Sprague, 1973; American Public Health Association et al., 1980). On the basis of the observed mortality in the first trial, the second trial was conducted with a series of more closely spaced concentrations of NH_3. The second trial was also intended to verify the reproducibility of the toxicity bioassay for NH_3. Ammonium chloride was used as a source of ammonia and a constant pH was maintained with 0.01M phosphate buffer (a combination of sodium dihydrogen phosphate and disodium hydrogen phosphate). All chemicals were of analytical reagent grade.

b) Replication. In both of the trials conducted, each concentration was tested in duplicate.

c) No. of experimental animals. Jenson (1972) observed that increasing the number of experimental fish significantly reduces the standard error of the LC50 until the sample size reaches about 30 fish. However, he recommended that for practical reasons a sample of 20 fish from each test level can be considered optimal. American Public Health Association et al.(1980) recommended the use of at least 10, preferably 20 or more test organisms, at each toxicant concentration.

On the basis of the above observations and recommendations, 32 fish were used per replicate tank in both trials.

d) Fish to volume of water ratio (organism loading). An organism loading of less than $1.0gl^{-1}$ of water was maintained in all the tests as recommended by American Society for Testing and Materials (1970) and American Public Health Association et al. (1980).

e) Acclimation to test conditions. Fish were maintained in stock tanks at a temperature similar to that used in the bioassay. Fish were transferred gradually from the stock tank to the dilution water by slowly changing the water. They were acclimated to the test dilution water for 24-48 hours (American Public Health Association et al., 1980) before adding the chemicals. Fish were not fed during the acclimation and test periods.

f). Randomization. Fish were randomly distributed among the test tanks as recommended by Sprague (1973).

g) Duration of the tests. Each test was conducted for a period of 168 hours to determine lethal threshold concentration (incipient LC50) as recommended by Sprague (1969, 1973), Alabaster and Lloyd (1980) and American Public Health Association et al. (1980). Sprague (1969) suggested that the lethal threshold concentration is the most useful single criterion of toxicity in fish studies.

h) Observations on mortality and criterion for death. Records of mortality were made at logarithmic time intervals (Sprague, 1973; Alabaster and Lloyd, 1980) i.e. after 1.5, 3, 6, 12, and 24 hours

from the start of the test and once daily thereafter. However in addition to these fixed time observations, several inspections were made in between these periods and fish were removed from test tanks as soon as they were found dead to prevent water quality deterioration. The cessation of opercular movements was used as the criterion for death. Fish which died during the tests and a portion of survivors at the termination of the tests were weighed individually.

i) Incidence of feeding and delayed mortality. After a test was terminated, samples of surviving fish from one tank of each concentration were placed in fresh dilution water for a further three to four days to check for delayed mortality according to recommendations of Wickins (1982). Fish from the other tank (i.e. duplicate) at each concentration were given food dyed with red colouring (Carmosine, E 122) in the test tank and their gut contents examined under a microscope after one hour to observe the incidence of feeding.

3.2.2.5 Measurement and analysis of water quality.

The physical and chemical characteristics of the test water were measured as described in section 2.2.2.9. The temperature, DO and pH of the test solution were measured everyday from each toxicant concentration. Ammonia-nitrogen and nitrite-nitrogen were measured once in every two or three days from each concentration including control. The un-ionized ammonia level was calculated using the following formula: -

NH_3 -N = $\frac{\text{Ammonia-N}}{1+10^{(pKa-pH)}}$ (after Emerson et al., 1975)

where,

Ammonia-N = the measured concentration of total ammonia

pKa = the negative logarithm of the ionization constant. Calculated pKa values for ammonia as a function of temperature were obtained from Emerson et al.,(1975)

pH = the measured pH of the solution.

the measured physico chemical characteristics of the test solutions are presented in Table 3.4 and 3.5

3.2.2.6. Analysis of experimental data

Median lethal concentration (LC50) values for different exposure times and their 95% confidence limits were calculated using a computer programme developed for the trimmed Spearman-Karber method (Hamilton et al., 1977). This method is easy to use and has been recently used in preference to conventional probit methods (Thurston et al., 1978; Russo et al., 1981; Thurston et al., 1981a; Thurston and Russo, 1983). Tests for significant differences were carried out between LC50 values using the method of American Public Health Association et al. (1980). When differences between replicate LC50 values were found to be insignificant ($P>0.05$) LC 50's and their 95% confidence limits were recalculated after combining the two replicates. Log LC 50's for each test were plotted against log exposure time to determine the lethal threshold concentration.

Table 3.4 Physico-chemical characteristics of the test solutions during the period of ammonia bioassay (trial 1) *

Solution No.	Total ammonia-N (mgl^{-1}) Mean (range)	NH_3-N** (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.03 (0.02-0.05)	0.00(1) (0.00(7)-0.00(2))	7.00 (7.00-7.10)	7.73 (7.67-7.77)
1	5.31 (4.67-5.66)	0.19 (0.17 - 0.21)	7.00 (6.90-7.20)	7.73 (7.69-7.76)
2	6.88 (6.48-7.28)	0.23 (0.22 - 0.25)	6.95 (6.90-7.00)	7.70 (7.66-7.74)
3	12.20 (12.00-12.40)	0.43 (0.43 - 0.44)	7.05 (7.00-7.10)	7.72 (7.67-7.76)
4	20.17 (17.20-21.70)	0.69 (0.58 - 0.74)	7.10 (7.00-7.20)	7.70 (7.67-7.72)
5	43.60 (39.90-46.50)	1.39 (1.27 - 1.48)	7.10 (7.00-7.30)	7.67 (7.63-7.70)
6	87.50 (85.00-90.00)	2.54 (2.47 - 2.62)	7.00 (7.00-7.10)	7.63 (7.61-7.65)
7	180.50 (173.60-187.40)	4.80 (4.62 - 4.98)	7.05 (6.90-7.20)	7.59 (7.58-7.60)

* Temperatures were maintained at 28°C; nitrite-nitrogen concentrations were insignificant ($<0.01 mgl^{-1}$)

* Calculated from total ammonia, pH and temperature

Table 3.5 Physico-chemical characteristics of the test solutions during the period of ammonia bioassay (trial 2)*

Solution No.	Total ammonia -N (mgl^{-1}) Mean (range)	NH_3-N** (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.06 (0.05 - 0.07)	0.00[2] (0.00[2] - 0.00[3])	7.30 (7.20 - 7.40)	7.79 (7.77 - 7.80)
1	12.40 (12.00 - 12.80)	0.50 (0.49 - 0.52)	7.10 (7.00- 7.10)	7.78 (7.77 - 7.79)
2	20.70 (19.10 - 22.30)	0.84 (0.78 - 0.90)	7.00 (7.00 - 7.10)	7.78 (7.76 - 7.79)
3	25.65 (24.30 - 27.00)	1.00 (0.94 - 1.05)	7.10 (7.00 - 7.10)	7.76 (7.75 - 7.77)
4	41.95 (39.70 - 44.20)	1.56 (1.47 - 1.64)	7.00 (7.00 - 7.10)	7.74 (7.73 - 7.75)
5	58.50 (58.30 - 58.70)	2.13 (2.12 - 2.13)	7.00 (7.00 - 7.20)	7.73 (7.72 - 7.74)
6	68.20 (66.20 - 70.20)	2.42 (2.35 - 2.49)	7.40 (7.20 - 7.50)	7.72 (7.71 - 7.73)
7	72.70 (71.20 - 74.20)	2.62 (2.60 - 2.64)	7.20 (7.00 - 7.40)	7.73 (7.72 - 7.73)

* Temperatures were maintained constantly at 28 ^{o}C; nitrite-nitrogen concentrations were insignificant ($<0.01 mgl^{-1}$)

** Calculated from total ammonia, pH and temperature

3.2.3. Results

The cumulative percentage mortality of carp fry at different concentration of NH_3 after 168h exposure are presented in Table 3.6. In the first trial very few mortalities were observed at concentrations ranging from 0.19 to 0.69 mgl^{-1} NH_3-N. Mortality higher than 10% occurred only at a concentration of 1.39mgl^{-1} NH_3-N. In the second trial, however, mortalities were observed only at concentrations greater than 1.0mgl^{-1} NH_3-N Two fish died in one of the duplicate control tanks in the first trial at the 144h exposure. This was equivalent to 3.1% mortality on the total control population and was well below the acceptable maximum 10% level for control mortality as recommended by Sprague (1973). No delayed mortality was observed when samples of the surviving fish from both trials were placed in clean dilution water for a further four days. Observations on the incidence of feeding were carried out only in the second trial. Active feeding was observed in all the surviving fish.

The median lethal concentrations (with 95% confidence limits) for total and un-ionized ammonia at various exposure times in the first and second trials are presented in Table 3.7 and shown graphically in Fig. 3.2. In the first trial, the 48-, 96- and 168-h LC50's for exposure to total ammonia were 57.37, 56.74 and 53.10 mgl^{-1} total ammonia-N and those to NH_3 were 1.76, 1.74 and 1.64 mgl^{-1} respectively. In the second trial, the 48-, 96- and 168-h LC50's for exposure to total ammonia were 51.21, 50.31 and 48.76mgl^{-1}

Table 3.6 The percentage mortality of common carp fry after 168 h exposure to different concentration of NH_3

	Trial 1		Trial 2	
Solution No.	NH_3 Conc. (mgl^{-1} NH_3-N)	% mortality	NH_3 Conc. (mgl^{-1} NH_3-N)	% mortality
Control	0.00[1]	3.1	0.00[2]	0
1	0.19	0	0.50	0
2	0.23	0	0.84	0
3	0.43	3.1	1.00	0
4	0.69	6.3	1.56	15.6
5	1.39	12.5	2.13	81.3
6	2.54	100	2.42	100
7	4.80	100	2.62	100

Table 3.7 Median lethal concentrations and 95% confidence limits of total and un-ionized ammonia (NH_3) for different exposure time from trials 1 and 2.

Exposure time (h)	Trial 1				Trial 2			
	Total ammonia-N (mgl^{-1}) LC 50	95% CL	NH_3 - N (mgl^{-1}) LC50	95% CL	Total ammonia-N (mgl^{-1}) LC 50	95% CL	NH_3 -N (mgl^{-1}) LC50	95% CL
1.5	95.16[a] *	80.91-110.55	2.74[a]	2.40-3.12	-	-	-	-
3.0	87.54[a]	77.77-98.60	2.56[a]	2.33-2.81	72.70	N.C.	2.61	N.C.
6.0	81.39[a]	74.22-89.26	2.39[a]	2.20-2.59	72.70	N.C	2.61	N.C.
12.0	63.63[b]	59.33-68.24	1.93[b]	1.81-2.05	71.28[a]	69.88-72.72	2.55[a]	2.49-2.61
24.0	58.04[c]	55.18-61.05	1.78[c]	1.70-1.86	59.96[b]	57.95-62.05	2.16[b]	2.10-2.23
48.0	57.37[c]	54.30-60.62	1.76[c]	1.67-1.85	51.21[c]	49.54-52.94	1.87[c]	1.82-1.93
72.0	57.37[c]	54.30-60.62	1.76[c]	1.67-1.85	50.57[c]	48.84-52.37	1.86[c]	1.80-1.93
96.0	56.74[c]	53.48-60.21	1.74[c]	1.65-1.84	50.31[c]	48.52-52.17	1.84[c]	1.78-1.91
120.0	56.02[c]	52.57-59.69	1.72[c]	1.62-1.82	49.45[c]	47.49-51.49	1.81[c]	1.74-1.88
144.0	54.32[c]	50.63-58.35	1.67[c]	1.57-1.78	49.45[c]	47.49-51.49	1.81[c]	1.74-1.88
168.0	53.10[c]	49.16-57.35	1.64[c]	1.53-1.76	48.76[c]	46.71-50.89	1.79[c]	1.71-1.86
Incipient LC50	56.50	-	1.74	-	49.50	-	1.82	-

N.C. : Not calculable

* Figures inthe same column with same superscripts are not significantly different (P>0.05)

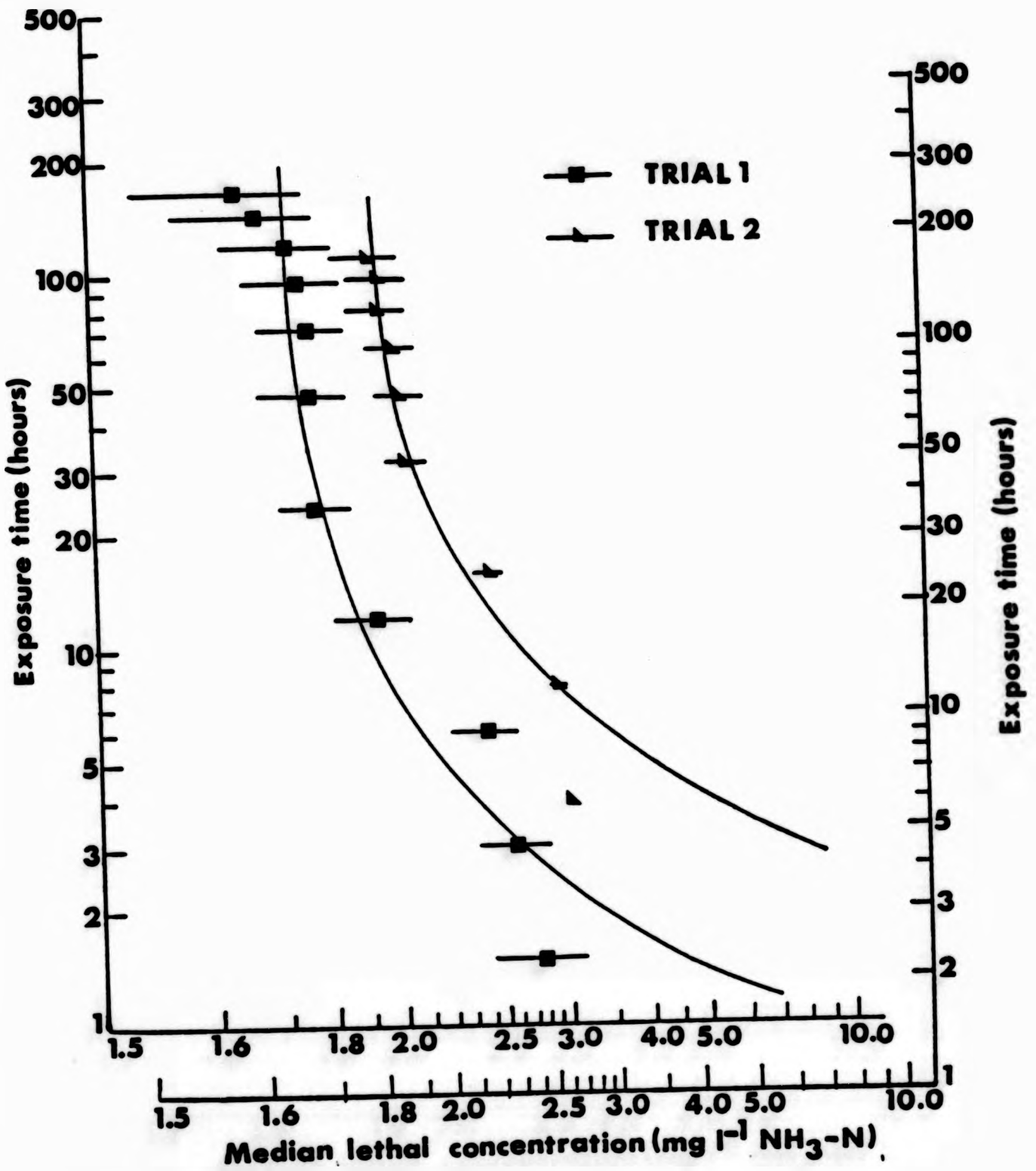


Fig. 3. 2 Toxicity curves of un-ionized ammonia for carp fry from trials 1 and 2 Bars indicate 95% confidence limits.

total ammonia-N and those to NH_3 were 1.87, 1.84 and 1.78 mgl^{-1} NH_3-N respectively. In both trials the actue toxicity of NH_3 ceased within 168h, which is indicated by the toxicity curve (Fig. 3.2) becoming asymptotic with the time axis (Sprague, 1969). These asymptotes mark the approximate values of lethal threshold concentration (= incipient LC50) which are given in Table 3.7 Since these LC50's are subjective, no mathematic confidence limits have been placed on them. The test of significance between median lethal concentrations of different exposure times shows no significant differences ($P > 0.05$) between LC50's after 24 and 48h in the first and second trials respectively (Table 3.6). There was also no signficant difference ($P > 0.05$) between the 96-h LC50 values obtained in the first and second trials.

3.2.4. Discussion

In the present investigation the acute toxicity of un-ionized ammonia (NH_3) ceased, and the lethal threshold concentration was reached, within 168 hours of exposure. This is similar to the observation made by several authors (Ball, 1967; Armstrong et al., 1978; Buckley, 1978; Redner and Stickney, 1979). Ball (1967) obtained lethal threshold concentrations for NH_3 within 24 to 96h for trout, bream, roach and rudd. He, however, observed that the acute toxic action of ammonia on rainbow trout was rapid and the lethal threshold concentration could be determined relatively earlier than for other coarse fish, studied. In this investigation, the median lethal concentration for different exposure time had no significant

differences (P>0.05) after 24 and 48 h respectively in the first and second trial. Similarly Buckley (1978) observed that the median lethal concentration of NH_3 remained unchanged between 14 and 94 hours exposure period in the case of coho salmon. For chinook salmon, Harader and Allen (1983) proposed that a 24-hour test should be sufficient to produce adequate ammonia toxicity results. In contrast Colt and Tchobanoglous (1976) observed no threshold for ammonia toxicity on channel catfish even after expsoure for more than 168 hours. Sprague (1969), in his review on pollutant toxicity on fish, observed that out of 375 acute toxicity studies only in 42 cases were lethal threshold levels reached after 96 hours. He recommended that a 96-h LC50 is, therefore, a useful substitute and is often equivalent to the incipient LC 50, though he suggested that tests should be continued until the shape of the toxicity curve is clearly established.

The present investigation was conducted in a completely recirculatory water system (i.e. with no change in water). Static bioassays are reported to be reliable for non-volatile toxicants (Alabaster and Lloyd, 1980; American Public Health Association et al. 1980). Moreover, Rubin and Elmaraghy (1977) reported that static toxicity tests proved to be reliable for the determination of ammonia and nitrate toxicities. In the present investigation changes in the ammonia concentration over a 168-h exposure period were minimal (Table 3.4 and 3.5). Similarly Rubin and Elmaraghy (1977) observed that change in ammonia concentration over a 4-day period were minimal. A slight increase in value may be attributed to water

evaporation losses.

In contrast, Colt and Tchobanoglous (1976, 1978) observed variation in 96-h LC50 values of NH_3 for channel catfish when bioassays were conducted under different test conditions (Table 3.1) They observed channel catfish were more resistant to NH_3 when the test was conducted in a static system compared to a water flow through system. They postulated that variation in fish size in the two tests or handling stress in the static test, could have been responsible for the observed variation in susceptibility. Differential NH_3 tolerance to different fish species due to variation in size has been demonstrated by Rice and Stokes (1975), Holt and Arnold (1983) and Thurston and Russo (1983). In static systems, the fish were handled prior to their introduction into the test solution. Lloyd and Orr (1969) observed that rainbow trout were less resistant to ammonia in a flow-through system than in a static system. Handling causes a diuretic response (Lloyd and Orr, 1969) and the increased urine flow induced by handling may aid in adjustment to the high urine flow required when fish are exposed to ammonia (Colt and Tchobanoglous, 1976).

In the present investigation, fish were acclimated for 24 hours in the test system before introducing the chemicals and therefore, handling stress, which may have increased their resistance to ammonia, were avoided.

Background or acclimation concentrations of ammonia have marked influence on the tolerance of ammonia to fish. The exposure of fish

to sublethal levels of ammonia increases their subsequent resistance to lethal concentrations (Vamos, 1963; Lloyd and Orr, 1969; Redner and Stickney, 1979). Redner and Stickney (1979) observed that when tilapia (Oreochromis aureus) were exposed to sublethal concentration of NH_3 (0.43-0.53 mgl^{-1} NH_3-N) for 35 days, subsequent concentrationas high as 3.40$mgl^{-1}NH_3$-N produced no mortality within 48 hours, whereas under the same condition for unexposed fish the 48-h median lethal concentration was 2.40$mgl^{-1}NH_3$-N. Two mechanisms for the observed subsequent increase in resistance to ammonia by fish after initial exposure to sublethal levels of ammonia have been identified. One involves changes in the permeability of the cell membrane (Vamos, 1963, Lloyd and Orr, 1969) and the other involves excretion or natural detoxification of ammonia (Olsen and Fromm, 1971; Mehrle and Bloomfield, 1974, cited by Buckley, 1978).

Ammonia increases the permeability of fish to water probably by changing the tissue permeability, which in turn resulted in the increase of the rate of urine excretion (Lloyd and Orr, 1969) and is thought to result in death when the increase in permeability exceeds the maximum sustainable rate of urine production. However, after an initial exposure to sublethal concentration of ammonia, a reduction in the permeability of cell membrane occurs which subsequently reduced the entry of ammonia into the fish body. Vamos (1963) recorded a reduction in the cell membrane permeability caused by the drug Suprastin, which reduced the toxic effect of NH_3 to a certain extent in common carp. Similarly, elevated environmental calcium has been reported to increase the tolerance of channel catfish to ammonia, probably by decreasing

gill membrane permeability to the toxin (Tomasso et al. 1980).

Olsen and Fromm (1971) reported that when exposed to sublethal ammonia levels, goldfish (Carassius auratus) were able to increase urea production and excretion and maintain a normal nitrogen excretion rate. Since fish lack a complete ornithine cycle, Olsen and Fromm (1971) postulated that goldfish are capable of synthesizing urea from ammonia through purine synthesis and catabolism. A sublethal exposure to ammonia may induce the production of the enymes required for this pathway and result in increased resistance when fish are exposed to lethal levels of ammonia. The ability to utilize this pathway may offer a partial explanation of why some species are more resistant than others to ammonia. Background concentrations of NH_3 in the present investigation were less than 0.001 $mgl^{-1}NH_3$-N, so this low background concentration of NH_3 was unlikely to have had any acclimation effect on the tolerance of the carp fry to ammonia.

Fluctuations in the pH values may cause significant variations in the proportion of NH_3 present in total ammonia resulting in the variation in susceptibility. Phosphate buffers were used in the present investigation to maintain a constant pH and the observed variation in pH was minimal (Table 3.4 and 3.5). In static bioassays, buffers have been used by several authors (Rice and Stokes, 1975; Rubin and Elmaraghy, 1977; Buckley, 1978; Tomasso et al., 1980; Harader and allen, 1983) to maintain a constant pH. However, Albaster and Lloyd (1980) opinioned after referring to the experimental procedure of Rice and Stokes(1975) that the addition of tris buffer might have affected the toxicity of ammonia. Several preliminary trials were conducted to examine the effect of phosphate

buffer and it was found that buffer levels of 0.05m or less did not cause any mortality or change in the behaviour over the 168-h observation period. However, it is not known if phosphate has any antagonistic or additive effect on ammonia toxicity. The concentration of sodium ions in the test solution was higher than that of the dilution water due to the addition of di-sodium hydrogen phosphate and sodium di-hydrogen phosphate (buffer). Effects of sodium ions on NH_3 toxicity have not been reported so far, though low sodium ion concentrations have reportedly increased the toxicity of NH_4^+ at low pH (Shaw, 1960; Armstrong et al., 1978).

The 96-h median lethal concentration of NH_3 to carp fry ranges between 1.74 to 1.85mgl^{-1} NH_3 and there are very little changes following further exposure periods. These LC50 values are considerably higher than those reported for salmonids and some species of coarse fish (Table 3.1). Ball (1967) observed little difference between the four different species in terms of the asymptotic LC50 values though clearly some of the species were more sensitive in the early period of exposure. However, this is not the case for all species as susceptibility to ammonia varies markedly among different species (see Table 3.1). Thurston et al. (1984) reported that acute toxicity values in 96-h tests ranged from 0.07 to 0.91 mgl^{-1} NH_3-N for salmonids and 0.12 to 3.79 mgl^{-} NH_3-N for non-salmonids. The 96-h median lethal concentration for NH_3 values obtained for carp fry fall within the range of LC50 values reported for non-salmonids. The reported 96-h LC50 values of NH_3 for channel catfish (1.60-3.13 mgl^{-1} NH_3-tilapia, O. aureus (72-h LC50 2.35 mgl^{-1} NH_3-N) and fathead minnows (1.52-2.83 mgl^{-1} NH_3-N) (see Table 3.1) are

comparatively similar or higher than those obtained for carp fry (1.74-1.84mgl^{-1} NH_3-N) in the present investigation (Table 3.7). The 96-h LC50 values obtained in the present are higher than the lethal values of NH_3 for carp reported by Danecker (1964, cited by Alabaster and Lloyd, 1980) (See Table 3.2). Alabaster and Lloyd (1980) however noted that a replotting of the data of Danecker indicated that a threshold concentration might not have been reached within the time period used by Danecker. The toxicity values for NH_3 reported by Vamos (1963) and Flis (1968a, b) cannot be compared to the present observed values, as loss of equilibrium, instead of mortality, was used by Vamos (1963) as a criterion for toxicity, while Flis (1968a, b) did not determine the median lethal concentration but rather the partial lethal concentration.

The 96-h TLm (=LC 50) value for NH_3 for common carp has been reported by Rao et al.(1975) to be 0.95 mgl^{-1} NH_3-N which is lower than the present observed value as well as values reported by Flis (1968a). Flis (1968a) reported 16-18% mortality of carp at an NH_3 concentration of 0.71-0.98 mgl^{-1} NH_3 (Table 3.2) as opposed to 50% mortality at 0.92 mgl^{-1} NH_3 recorded by Rao et al. (1975). However, the results reported by Rao et al.(1975) were somewhat incomplete. TLm value was reported in terms of ammonium chloride concentration and it was never mentioned how the TLm value was estimated from the concentration - mortality data, which were also not given in the results. An approximate estimate of TLm value in terms of NH_3 has been calculated from the TLm value

of ammonium chloride, mean temperature and pH, and again pH value had considerable fluctuations (7.2-7.8) (Table 3.2). However, the lower LC50 value reported by Rao et al. (1975) may be attributable to the variation in the size of fry used. The fry used by Rao et al. (1975) weighed approximately 1-2g compared to the 0.2 to 0.3g fry used in this study.

It has been demonstrated by several authors (Rice and Stokes, 1975; Alabaster and Lloyd, 1980; Holt and Arnold, 1983, Thurston and Russo, 1983; see section 3.2.1) that the susceptibility of fish to NH_3 varies with age. Variation in the lethal concentration of NH_3 may also be attributed to the variation in the dissolved oxygen contents of test waters (Downing and Merkens, 1955; Alabaster et al., 1979; Thurston et al., 1981b). A DO content of 7.0 mgl^{-1} or more was maintained in the present study, compared to the DO content of 5.0 mgl^{-1} or more maintained in the test water used by Rao et al (1975).

The conclusion derived from this study is that carp fry are fairly tolerant to un-ionized ammonia. In both trials, only concentrations greater than 1.0mgl^{-1} NH_3 caused mortalities higher than 10%, therefore, it is unlikely that the concentration of NH_3 would increase to such an extent to cause significant mortality in a recirculatory system unless the water pH increased considerably or the biological filter ceased to function.

Under normal circumstances, ammonia does not occur at sufficiently high levels in natural water to cause any significant mortality in fish populations. Alderson (1979), from his work on sole and turbot, noted that the tolerance to total ammonia is much greater than that used for the calculation of the capacity of biological filters required to support fish stocks in recirculatory systems. Similarly, Wickins (1980), from a review of the literature concerning the toxicity of ammonia to fish, concluded that ammonia does not pose the threat in intensive aquaculture that was once assumed. However, sublethal levels of NH_3 may lead to suppression of growth and histopathological changes as discussed earlier. Therefore adequate consideration should be given to these aspects when evaluating the toxicity of NH_3 to common carp fry.

3.3 Experiment 3.2

Estimation of median lethal concentration of nitrite to carp fry.

3.3.1. Introduction

Nitrite occurs naturally in lakes and rivers as a result of the nitrification of ammonia and denitrification of nitrate. Usually values for nitrite (NO_2) are in the range of 0.002-0.01mgl^{-1} nitrite-nitrogen (NO_2-N) in surface waters; however in stagnant lakes and ponds much higher NO_2 levels occur particularly in anoxic regions (EIFAC, 1984). Nitrite can increase to a very high concentration in streams receiving effluents from sewage treatment plants (Russo et al, 1974). Klinger (1957) reported nitrite concentrations in excess of 30mgl^{-1} NO_2-N for waters receiving effluents from metal, dye and celluloid industries.

Nitrite occurs in water recirculation systems as an intermediate product of bacterial nitrification of ammonia to nitrate. In newly constructed recirculation systems where the rate of bacterial nitrification is initially slow, nitrite concentrations could rapidly increase to very high levels (Liao and Mayo, 1974; Collins et al, 1975; Colt and Tchobanoglous, 1976).

Nitrite has been shown to be toxic to fish (Gillette et al, 1952; Wallen et al, 1957; Smith and Williams, 1974) and its toxicity varies greatly between cultured species (Table 3.8). For example, nitrite concentrations of 0.19mgl^{-1} NO_2 were shown to be lethal to rainbow trout, Salmo gairdneri whereas mottled sculpin, Cottus bairdi could tolerate levels in excess of 65.0mgl^{-1} NO_2-N when exposed for 154 hours (Russo and Thurston, 1977).

Table 3.8 A summary of published values for the toxicity of nitrite to various species of fish

Fish species	Weight (g)	Temp. (^{o}C)	pH	Total Hardness (mgl^{-1} as $CaCO_3$)	Total Alkalinity (mgl^{-1} as $CaCO_3$)	Conc. of chloride (mgl^{-1})	Conc. of NO_2^--N (mgl^{-1})	Effect	Author
Rainbow trout *Salmo gairdneri*	Post-alevin	12.0	6.4-6.7	3-9	2-8	N.R.	0.23	96-h LC50	Brown & McLeay(1975)
	24.3-188.0	9.8-10.4	7.7-8.1	188-207	171-191	0.35	0.19-0.28	96-h LC50	Russo & Thurston (1977)
	86.4	10.5	7.8	188-207	171-191	20.2	6.69	96-h LC50	Russo & Thurston(1977)
Steelhead Trout *S. gairdneri*	5.0	10.0	7.0	25	N.R.	1.9	1.5	96-h LC50	Wedemeyer & Yasutake (1978)
Cut-throat trout *Salmo clarkii*	3.1	11.8-12.1	7.8-7.9	199	176	0.44	0.48-0.56	96-h LC50	Thurston *et al* (1978)
Chinook salmon *Oncorhynchus tshawtscha*	1.5 - 10.6	13.6-15.6	6.8-7.2*	N.R.	N.R.	N.R.	0.61-1.28	96-h LC50	Westin (1974)
Channel catfish *Ictalurus punctatus*	40.0	21.0	7.4-7.8	N.R.	60-70	N.R.	7.55	96-h LC50	Konikoff (1975)
	3-4**	22.0	8.6-8.8	102	220	22.0	12.78	96-h LC50	Colt & Tchobanoglous (1976)
	3.0-4.0**	30.0	8.6-8.8	102	220	22.0	13.09	96-h LC50	Colt & Tchobanoglous (1976)
	3.0	23.0	7.7-8.2	202-255	166-223	22.0	7.1	96-h LC50	Palachek & Tomasso (1984a)

contd....

Table 3.8(contd) A summary of published values for the toxicity of nitrite to various species of fish

Fish species	Weight (g)	Temp. (°C)	pH	Total Hardness (mgl^{-1} as $CaCO_3$)	Total Alkalinity (mgl^{-1} as $CaCO_3$)	Conc. of chloride (mgl^{-1})	Conc. of NO_2^--N (mgl^{-1})	Effect	Author
Tilapia Oreochromis aureus	3.4	23.0	7.7-8.2	202-255	166-223	22.0	16.2	96-h LC50	Palachek & Tomasso (1984a)
Large mouth bass Micropterus salmoides	2.8	23.0	7.7-8.2	202-255	166-223	22.0	140.2	96-h LC50	Palachek & Tomasso (1984a)
Fathead minnow Pimephales promelas	2.3	12.7-13.0	8.1	188-207	171-191	0.35	2.99-	96-h LC50	Russo & Thurston (1977)
	0.9-3.3	23.0	7.7-8.2	191-268	153-250	22.0	45.3***	96-h LC50	Palachek & Tomasso (1984b)
Common carp Cyprinus carpio	5.6cm (2.0g approx)	14.0	7.6	260	N.R.	19.0	40.0	96-h LC50	Solbé et al (1981) +
Roach Rutilus rutilus	6.9cm	16.0	7.4	261	N.R.	20.0	12.0	96-h LC50	Cooper & Solbé (1980)+

N.R. Not reported

* Personal communication referred to by Russo & Thurston (1977)
** Reported by Colt & Tchobanoglous (1978) *** Obtained from toxicity curve given
\+ Unpublished date referred to by EIFAC (1984)

Information on the effects of exposure of fish to sublethal concentrations of nitrite are scanty, Wedemeyer and Yasutake (1978) observed that steelhead trout, Salmo gairdneri, stocked for six months in soft water with a concentration of 0.03-0.06mgl^{-1} NO_2-N caused no significant reduction in growth, or changes in gill histology. With juvenile channel catfish, NO_2-N concentrations of 1.60mgl^{-1} and above have been reported to cause reduced growth rate, but with no change in gill histology or body composition (Colt et al, 1981). Exposure to sublethal concentration of nitrite has also been observed to reduce the growth rate of crustaceans (Wickins, 1976; Armstrong et al, 1978).

The exact mechanism by which nitrite toxicity occurs is not clearly understood. It is known that nitrite oxidizes haemoglobin (Hb) in the blood to methaemoglobin (Met-Hb). Methaemoglobin is incapable of carrying oxygen and thus accumulation of excess methaemoglobin in the blood of fish may be fatal. Nitrite induced methaemoglobin formation has been studied experimentally in salmonids (Brown and McLeay, 1975; Smith and Russo, 1975) and channel catfish, Ictalurus punctatus (Huey et al, 1980; Bowser et al, 1983). However, nitrite-induced methaemoglobinaemia may not be necessarily the prime cause of death since fish poisoned with carbon monoxide are known to survive many hours, particularly at low temperatures, with little or no functional haemoglobin, while the ice fish has no haemoglobin at all (Eddy et al, 1983). Nitrite toxicity also occurs in crustaceans most of which have no haemoglobin.

Recent investigation has shown that different environmental ions e.g. chloride, calcium, bicarbonate, nitrate have some protective effect against nitrite toxicity. The protective effect of chloride has been well documented for salmonids (Perrone and Meade, 1977; Russo and Thurston, 1977; Bath and Eddy, 1980; Meade and Perrone, 1980; Russo et al, 1981; Eddy et al, 1983) and channel catfish (Tomasso et al, 1979, 1980; Huey et al, 1980; Bowser et al, 1983) (see Table 3.9). At 9.8mgl^{-1} NO_2-N and undetectable levels of Cl^-, Bath and Eddy (1980) observed 90% mortality of rainbow trout within 24 hours, whereas, when Cl^- levels were increased to 21mgl^{-1} only 50% mortality occurred within 42 hours. Similarly, Perrone and Meade (1977) reported that an ionic ratio of about 1:17 (NO_2-N:Cl^-) could prevent complete mortality in yearling coho salmon, Oncorhynchus kisutch exposed for 72 hours to 9.0-15.0mgl^{-1} NO_2-N. With channel catfish, Tomasso et al.(1979) reported that an ionic ratio 1:16 (NO_2-N:Cl^-) was capable of complete suppression of nitrite-induced methaemoglobin formation, when the maximum nitrite concentration tested was 20mgl^{-1}NO_2.

The protective effect of calcium has been documented only for salmonids (Crawford and Allen, 1977; Wedemeyer and Yasutake, 1978; Bath and Eddy, 1980). However, Tomasso et al.(1980) observed little or no protective effect of calcium against nitrite toxicity in the case of channel catfish. Similarly Bowser et al.(1983) who also worked with channel catfish reported that when chloride was calculated on a percentage by weight basis, NaCl and $CaCl_2$ were

Table 3.9 A summary of published values for the toxicity of nitrite to fish - effect of chloride concentration.

Fish species	Weight (g)	Temp. (^{o}C)	pH	Total Hardness (mgl^{-1} as $CaCO_3$)	Total Alkalinity (mgl^{-1} as $CaCO_3$)	Conc. of chloride (mgl^{-1})	Conc. of NO_2^--N (mgl^{-1})	Effect	Author
Rainbow trout	69.5	10.4	7.9	188-207	171-191	1.2	0.46	95-h LC50	Russo & Thurston(1977)
Salmo gairdneri	69.1	10.4	8.0	188-207	171-191	5.1	2.36	96-h LC50	Russo & Thurston(1977)
	113	10.4	7.7	188-207	171-191	40.8	12.6	96-h LC50	Russo & Thurston(1977)
	9.3	8.9-14.7	7.6	178-209	160	0 -0.47	0.18	96-h LC50	Russo et al (1981)
	28.2	9.9-10.7	7.5	199-206	174	10.9	3.74	96-h LC50	Russo et al(1981)
Steelhead trout S. gairdneri	5.0	10.0	6.8	50	30-35	1.4	0.56	96-h LC50	Wedemeyer & Yasutake(1978)
	5.0	10.0	8.4	300	225-245	8.4	10.3	96-h LC50	Wedemeyer & Yasutake(1978)
Coho salmon Oncorhynchus Kisutch	12.9-22.2	11.2	7.2	32.3	19.7	19.6	9.2	50%mortality in 24-h exposure	Perrone & Meade(1977)
	12.9-22.2	11.2	7.2	32.3	19.7	148.0	8.9	No mortality in 72-h exposure	Perrone & Meade(1977)
	0.65	11.2	7.2	32.3	19.7	33.2	8.9	No mortality in 72-h exposure	Perrone & Meade(1977)

contd...

Table 3.9 (contd.) A summary of published values for the toxicity of nitrite to fish - effect of chloride concentration

Fish species	Weight (g)	Temp. (°C)	pH	Total Hardness (mgl^{-1} as $CaCO_3$)	Total Alkalinity (mgl^{-1} as $CaCO_3$)	Conc. of chloride (mgl^{-1})	Conc. of NO_2^--N (mgl^{-1})	Effect	Author
Channel Catfish *Ictalurus punctatus*	7-13cm	21.0-24.0	7.0	40	47	4.0	4.99	24-h LC50	Tomasso *et al*(1980b)
	7-13cm	21.0-24.0	7.0	40	47	60.0	98.0	24-h LC50	Tomasso *et al*(1980)
Fathead minnow	2.3	12.7-13.0	8.1	188-207	171-191	0.3	2.30-2.99	96-h LC50	Russo & Thurston(1977)
Pimephales promelas	0.9-3.3	23.0	7.7-8.2	191-268	153-250	22.0	45.3*	96-h LC50	Palachek & Tomasso (1984b)

*Obtained from toxicity curve given

found to be equivalent as protective compounds, indicating that calcium did not have any protective effect against nitrite toxicity.

An antagonistic effect between the presence of bicarbonate ions and nitrite toxicity has been reported for rainbow trout (Bath and Eddy, 1980; Eddy et al, 1983) and channel catfish (Huey et al, 1980; Bowser et al, 1983). However, Bowser et al (1983) noted that bicarbonate ions were comparatively less effective than chloride ions in preventing nitrite induced methaemoglobinemia in channel catfish. A similar observation was reported by Eddy et al (1983) for rainbow trout who compared the increases in survival of rainbow trout due to the protective effects of chloride and bicarbonate (HCO_3).

The protective effect of nitrate (NO_3) in the form of HNO_3 was reported by Russo et al (1981) for rainbow trout. pH in all test solutions was maintained within $\pm$ 0.02 unit. However, a 70-90 times increase in nitrate concentration resulted in a corresponding increase in the 96-h LC50 of NO_2-N only by 2-2.5 times. Therefore it may be concluded that nitrate has only a very minor protective effect compared to chloride as discussed earlier

Variations in the pH of water has also been reported to affect the toxicity of nitrite (Colt and Tchobanoglous, 1976; Wedemeyer and Yasutake, 1978; Meade and Perrone, 1980; Russo et al, 1981) (see Table 3.11). The hypothesis that pH affects nitrite toxicity was reported for the first time by Colt and Tchobanoglous (1976). While comparing the mortality curves for exposure of channel catfish to

nitrite and nitrous acid, these authors found that a better fit could be obtained for nitrous acid than for nitrite. On the basis of this result, they suggested that nitrous acid may be the toxic form. This supports the hypothesis that the unionized molecule can move across biological membranes much faster than its ionized counterpart (Forster and Goldstein, 1969).

The proportion of nitrous acid present in freshwater is dependent largely upon pH according to the equilibrium $HNO_2 \rightleftharpoons H^+ + NO_2$. The proportion of nitrous acid will be higher at lower pH and therefore a decrease in pH should increase the toxicity of nitrite to animals. Subsequent experimentation by several authors (Wedemeyer and Yasutake, 1978; Meade and Perrone, 1980; Russo et al, 1981) have supported the hypothesis that the toxicity of nitrite is pH dependent and that nitrous acid may be the toxic form. However, Russo et al (1981) hypothesised that both of the nitrite forms, NO_2 and HNO_2 are toxic, although not necessarily equally so. In contrast, Russo and Thurston (1977) found that pH within the range of 7.5-8.5 had no effect on the nitrite tolerance of rainbow trout.

Size is also thought to be a factor influencing the susceptibility of fish to nitrite (Table 3.11). Tolerance to nitrite has been reported to decrease with increasing fish size. This has been demonstrated for rainbow trout (Russo et al, 1974; Smith and Williams, 1974), Coho salmon (Perrone and Meade, 1977), sea bass (Saroglia et al, 1981) and fathead minnow (Palachek and Tomasso, 1984b). In contrast, Wedemeyer

Table 3.10 A summary of published values for the toxicity to fish - effect of size

Fish species	Weight (g)	Temp. (^{o}C)	pH	Total Hardness (mgl^{-1} as $CaCO_3$)	Total Alkalinity (mgl^{-1} as $CaCO_3$)	Conc. of chloride (mgl^{-1})	Conc. of NO_2^--N (mgl^{-1})	Effect	Author
Rainbow trout	2.3	10.8	7.9	199	176	N.R.	0.39	96-h LC50	Russo et al (1974)
Salmo gairdneri	11.9-14.0	11.6-12.6	7.9	199	176	N.R.	0.19-0.27	96-h LC50	Russo et al (1974)
	235	9.5	7.9	199	176	N.R.	0.20	96-h LC50	Russo et al (1974)
Steelhead trout S. gairdneri	5.0	10.0	6.8	50	30 - 35	1.4	0.5	96-h LC50	Wedemeyer & Yasutake (1978)
	10.0	10.0	6.8	50	30 - 35	1.4	1.9	96-h LC50	Wedemeyer & Yasutake (1978)
Fathead minnow Pimephales promelas	0.9-3.3	23.0	7.7-8.2	191-268	153 -250	22.0	45.3*	96-h LC50	Palachek & Tomasso (1984b)
	0.3-0.8	23.0	7.7-8.2	191-268	153 -250	22.0	69.2*	96-h LC50	Palachek & Tomasso (1984b)
Seabass Dicentrarchus labrax	1.2	17.0	8.1-8.4	N.R.	N.R.	Seawater	309.0	96-h LC50	Saroglia et al(1981)
	1.5	17.0	8.1-8.4	N.R.	N.R.	Seawater	211.0	96-h LC50	Saroglia et al(1981)
	5.5	17.0	8.1-8.4	N.R.	N.R.	Seawater	274.0	96-h LC50	Saroglia et al(1981)
	5.5	23.0	8.1-8.4	N.R.	N.R.	Seawater	220.0	96-h LC50	Saroglia et al(1981)
	15.0	23.0	8.4-	N.R.	N.R.	Seawater	170.0	96-h LC50	Saroglia et al(1981)

* Obtained from toxicity curve given N.R. not reported

Table 3.11 A summary of the reported effect of pH on the toxicity of nitrite to fish

Fish species	Weight (g)	Temp (^{o}C)	pH	total hardness (mgl^{-1} as $CaCO_3$)	total alkalinity (mgl^{-1} as as $CaCO_3$)	Conc. of Chloride (mgl^{-1})	Conc. of NO_2^- N (mgl^{-1})	Effect	Author
Rainbow trout *Salmo gairdneri*	10 - 15.3	8.9 - 14.7	7.0 - 7.4	178-209	120-150	0-0.47	0.11 - 0.19	96 h LC50	Russo *et al* (1981)
	3.1 - 13.2	8.9 - 14.7	7.60 - 8.0	178-209	160-188	0-0.47	0.17 - 0.40	96 h LC50	Russo *et al* (1981)
	8.2 - 15.5	8.9 - 14.7	8.3 - 8.7	178-209	178-188	0-0.47	0.45 - 0.71	96 h LC50	Russo *et al* (1981)
	7.0 - 10.4	8.9 - 14.7	8.8 - 9.0	178-209	188-202	0-0.47	0.61 - 1.17	96 h LC50	Russo *et al* (1981)
Steelhead trout *S. gairdneri*	5.0	10.0	6.0	25	N.R.	1.9	0.3	96 h LC50	Wedemeyer & Yasutake (1978)
	5.0	10.0	7.0	25	N.R.	1.9	1.5	96 h LC50	Wedemeyer & Yasutake (1978)
	5.0	10.0	8.0	25	N.R.	1.9	2.5	96 h LC50	Wedemeyer & Yasutake (1978)
Coho salmon *Oncorhynchus Kisutch*	175	11.2	6.5	32.3	19.7	15	10.0	33.4% Met-Hb	Meade & Perrone (1980)
	175	11.2	8.0	32.3	19.7	15	10.0	18.9% Met-Hb	Meade & Perrone (1980)

and Yasutake (1978) observed that 10g size steelhead trout were somewhat more resistant to nitrite than smaller (5g) fish. However, Thurston et al (1978) studying cut-throat trout, observed no significant difference between two size groups (1.0 and 3.1g) tested for their susceptibility to nitrite. Similarly, Russo (1980), who summarized the results from twenty 96-h nitrite bioassays on rainbow trout over the size range from 2 to 387g, observed no relationship between fish size and susceptibility to nitrite. However, he noted a certain tendency for higher resistance existed for smaller sizes.

On the basis of the information discussed above it can be concluded that the presence of certain mineral salts especially chloride has a large influence on the toxicity of nitrite to fish. However, the experimental evidence for the effect of pH and fish size on nitrite toxicity is still equivocal. A thorough search of the literature revealed only one published report on the toxic effect of nitrite to carp. McCoy (1972) cited by Russo and Thurston (1977) reported no mortality over a 45 hour period for carp when exposed to $40mgl^{-1}$ NO_2-N, although a concentration of $100mgl^{-1}$ NO_2-N resulted in mortality over a 45 hour exposure period. Unfortunately the physico-chemical characteristics of the experimental water and the levels of mortality were not reported.

In view of the potential significance of nitrite toxicity to carp under hatchery and nursery conditions and the general lack of information on the toxicity of this ion to carp,the following study

was made to determine the acute toxicity of nitrite to carp fry and to evaluate the effect of chloride, pH and fish size on nitrite toxicity.

3.3.2. Materials and Methods

Three sets of toxicity bioassays were conducted to study the effect of chloride, pH and fish size on the toxicity of nitrite to carp fry. While evaluating the effect of pH on nitrite toxicity, a preliminary study was undertaken prior to the main study.

The source of the experimental animal, their quarantine and acclimation procedures are described in Section 2.2.2.2-3 and 3.2.2.1.

3.3.2.1. Experimental system

The recirculation system described in Section 3.2.2.2, was used to determine the acute nitrite toxicity for different sizes of carp fry. For the rest of the nitrite toxicity bioassay experiments, a static water system was used because the recirculation system was unavailable. 10 litre capacity glass aquaria containing 8l of water were used as static bioassay tanks. The individual tanks were used for replicate concentrations and two replicates were used for each nitrite concentration tested unless stated otherwise. In each tank a

200-W 'Nimrod' microtonic thermostatic heater (R. Aitchisons Co., Edinburgh) with a dial temperature control was used to maintain the required temperature and a stone aerator was operated in each tank to maintain adequate level of dissolved oxygen concentration. All tanks were kept in a 1 m high wooden platform to facilitate better observation and accessibility.

3.3.2.2. Test Water

The test water used was prepared according to the procedure described in Section 3.2.2.3. The chemical characteristics of the test dilution water are presented in Table 3.12. The water prepared had a chloride concentration of about 30 mgl^{-1}. To study the effect of chloride concentration on the nitrite toxicity, various concentrations of chloride were needed. High chloride concentrations were prepared by adding extra sodium chloride to the dilution water as this did not affect water hardness. When lower chloride concentrations were required the calcium chloride used in the dilution water was replaced with an appropriate amount of sodium chloride, and the calcium concentration in the dilution water was adjusted to give the desired level of hardness by the addition of calcium sulphate.

3.3.2.3. Experimental procedure

The experimental procedures used were the same as those described in

Table 3.12 Chemical characteristics of the dilution water used in the nitrite toxicity bioassays*

	Mean	Range
Total oxidised nitrogen	0.5	0.3-0.9
Conductivity	222.0	194-245.0 μScm^{-1}
Suspended solids	2.0	1-3.0
Chloride	30.0	26-32.0
Total ammonia-nitrogen	0.02	0.01-0.02
Total alkalinity	42.5	40.0-45.0
Total hardness(as $CaCo_3$)	57.0	55.0-60.0
Orthophosphate (as P)	0.01	<0.01-0.02
Calcium	6.85	4.5-8.8
Magnesium	4.0	3.2-4.7
Potassium	0.55	0.1-1.0
Sodium	26.5	24.6-28.3
Copper	0.009	0.006-0.01
Iron	0.02	<0.01-0.02
Lead	<0.005	-
Manganese	<0.005	-
Zinc	0.007	<0.005-0.01

* All values are in mgl^{-1} unless otherwise noted

Section 3.2.2.4, except for some minor modifications which are stated below. All the tests were conducted with five to seven concentrations of toxicants and one control. Sodium nitrite was used as a source of nitrite in the test solution. Phosphate buffer was not used in any of the tests except for the evaluation of the effect of pH on nitrite toxicity. In this case, 0.01 M phosphate buffer (see Section 3.2.2.4a for details) was used to maintain the desired pH. Phosphate buffer has been used widely to maintain pH in static nitrite toxicity bioassays (Wedemeyer and Yasutake, 1978; Tomasso et al, 1979, 1980; Huey et al, 1980). Twenty or more fish were used per replicate tank in all the tests. All tests were conducted for 168 hours unless mentioned otherwise. The incidence of feeding by the test fish and delayed mortality were observed in both trials to study the effect of chloride and fish size on nitrite toxicity. However, no observation was made on the incidence of feeding and delayed mortality in these trials as in both of the other two trials, active feeding was observed and little or no delayed mortality occurred.

3.3.2.4. Measurement and analysis of water quality

This was carried out as described in Section 2.2.2.9. Analytical procedures for the determination of calcium, magnesium and chloride levels were as follows:

Calcium and Magnesium:- Calcium and magnesium concentrations were

measured in an atomic absorption spectophotometer (UNICAM Sp 90) following the method described by Golterman et al (1978).

Chloride:- Chloride concentration was measured by the argenometric method (American Public Health Association et al, 1980).

The temperature, dissolved oxygen and pH of the test solutions were measured every day from each toxicant concentration. Nitrite concentrations in each test solution including the control were measured once in every two days. Total ammonia was measure two to three times during the experiments. Total alkalinity, total hardness chloride, calcium and magnesium concentrations were measured once during each test.

The fraction of nitrous acid (NHO_2) present in the total nitrite (NO_2^-) concentration was calculated using the following formula:

$$F_{HNO_2} = \frac{1}{1 + 10^{(pH - pKHNO_2)}}$$ (after Colt and Tchobanoglous, 1976)

where,

F_{HNO_2} = the fraction of nitrous acid present in total nitrite concentration

pH = the measured pH of the solution

$pKHNO_2$ = the dissociation constant of nitrous acid

The value of $pKHNO_2$ as a function of temperature T(^oC) was calculated using the following equation:

$$pKHNO_2 = \frac{655.586}{T + 273.16} + 1.148$$ (after Colt and Tchobanoglous, 1976)

The concentrations of both nitrite and nitrous acid presented in the result are expressed on a nitrogen basis.

3.3.2.5. Analysis of experimental data

Calculation of median lethal concentration (LC50) values and tests for significant differences between different LC50's were carried out according to the methods described in Section 3.2.2.6. LC50 values in terms of total NO_2-N were calculated using the measured total NO_2-N concentrations and mortality observations. For calculation of LC50 values in terms of HNO_2-N, the calculated HNO_2-N concentrations, from the measured NO_2-N, concentration and pH were used. Correlation coefficients and regression equations relating fish size, chloride concentration and pH to the median lethal concentration of nitrite and nitrous acid were calculated according to Sokal and Rohlf (1981). The percentages of mortality between different replicates and treatments were tested using X^2(Chi^2) test (Parker, 1979).

3.3.3. Results

3.3.3.1 Effect of chloride concentration

The physico-chemical characteristics of the bioassay test solutions containing chloride concentrations of 1.0, 5.0, 10.5, 27.5 and 45.0 mgl^{-1} are presented in Table 3.13, 3.14, 3.15, 3.16 and 3.17 respectively. The median lethal concentrations of nitrite, with 95% confidence limits, one shown for various exposure times in Table 3.18 and graphically in Fig. 3.3. The values of median lethal concentration vary markedly at different chloride ion concentration. The 96-h LC50's at chloride concentration of 1.0, 5.0, 10.5, 27.5 and 45.0 mgl^{-1} are 2.55, 5.77, 14.41, 27.26 and 48.70 mgl^{-1} NO_2-N respectively. A summary of the results is presented in Table 3.19.

The acute toxicity of nitrite at all levels of chloride ceased towards the end of 96-h as indicated by the curves becoming asymptotic with the time axis (Fig. 3.3). These asymptotes mark the approximate values of lethal threshold concentrations. These values are presented in section 3.19. The LC50' between 96 and 168 hours were not significantly different (P 0.05) for chloride concentrations of 1.0, 27.5 and 45.0 mgl^{-1} (Table 3.18). Similarly no significant differences were observed in the LC50's between 72 and 168 and 120 and 168 hours for chloride concentrations of 5.0 and 10.5 mgl^{-1} (Table 3.18). There was a highly significant correlation (r = 0.996; d.f. = 3; P 0.001) between Cl^- and the 96-h LC50 of NO_2-N; as the chloride concentration increased,

Table 3.13 Physico-chemical characteristics of the test solutions during the period of bioassy for nitrite toxicity at a chloride concentration of 1.0 mgl^{-1}

Solution No.	Nitrite -N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control **	N.A.	7.30 (7.20-7.40)	7.68 (7.62-7.74)
1 **	0.72 (0.66-0.78)	7.50 (7.40-7.60)	7.60 (7.54-7.66)
2 **	1.39 (1.39-1.39)	6.70 (6.40-7.00)	7.54 (7.43-7.62)
3	3.22 (2.84-3.77)	7.00 (6.80-7.40)	7.70 (7.59-7.82)
4	6.70 (5.68-7.65)	7.35 (7.10-7.60)	7.74 (7.68-7.82)
5	13.09 (11.90-15.04)	7.40 (6.80-7.80)	7.70 (7.59-7.78)
6	24.98 (22.20-28.20)	7.20 (6.90-7.40)	7.74 (7.64-7.80)

* Other measured variables were: temperature 27.5 - 28.5°C; total hardness (as $CaCO_3$) 52.0 mgl^{-1}; total alkalinity (as $CaCO_3$) 43.0 mgl^{-1}; calcium 8.5 mgl^{-1}; magnesium 4.55 mgl^{-1}; total ammonia 0.10 - 0.68 mgl^{-1} chloride 1.0 - 1.0 mgl^{-1}

** One replicate per test solution

NA Not analysed

Table 3.14 Physico-chemical characteristics of test solution during the period of bioassay for nitrite toxicity at chloride concentration of 5.0 mgl^{-1}.

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.01 (0.00(4)-0.01)	7.20 (7.00-7.40)	7.53 (7.46-7.59)
1	1.44 (1.28-1.62)	7.20 (7.00-7.40)	7.57 (7.52-7.62)
2	3.35 (3.14-3.52)	7.25 (7.00-7.60)	7.58 (7.53-7.63)
3	6.39 (5.87-6.78)	7.25 (7.10-7.50)	7.61 (7.57-7.67)
4	9.18 (8.73-9.43)	7.35 (7.10-7.60)	7.60 (7.56-7.65)
5	12.21 (11.66-12.65)	7.40 (7.20-7.60)	7.63 (7.57-7.66)
6	22.93 (21.99-23.85)	7.10 (6.80-7.30)	7.65 (7.60-7.68)

* Other measured variables were: temperature 27.5-28.50°C total hardness (as $CaCO_3$)57.0 mgl^{-1}; total alkalinity (as $CaCO_3$) 48.50 mgl^{-1}; Calcium 10.38 mgl^{-1}; magnesium 7.11 mgl^{-1} total ammonia-N 0.21-0.56 mgl^{-1}; chloride 4.5-5.5 mgl^{-1}

Table 3.15 Physico-chemical characteristics of the test solutions during the period of bioassay for nitrite toxicity at a chloride concentration of 10.5 mgl^{-1}

Solution	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.00(7) (0.00(5)-0.00(8))	7.10 (6.70-7.50)	7.57 (7.51-7.64)
1	1.70 (1.60-1.81)	7.25 (6.90-7.60)	7.87 (7.77-7.95)
2	3.67 (3.51-3.91)	7.10 (6.80-7.50)	7.78 (7.67-7.91)
3	6.87 (6.38-7.34)	7.25 (7.00-7.50)	7.72 (7.67-7.78)
4	11.04 (10.46-11.35)	7.15 (6.80-7.50)	7.86 (7.76-7.95)
5	14.42 (13.84-14.92)	7.15 (6.80-7.60)	7.78 (7.71-7.86)
6	28.63 27.20-29.74)	7.25 (6.90-7.60)	7.86 (7.73-8.00)
7	54.55 (53.06-56.12)	7.30 (7.10-7.60)	7.86 (7.76-7.95)

* Other measured variables were: temperature 27.5-28.5°C; total hardness (as $CaCO_3$) 53.0 mgl^{-1}; total alkalinity (as $CaCO_3$) 40.5 mgl^{-1}; calcium 9.44 mgl^{-1}; magnesium 2.23 mgl^{-1}; total ammonia-N 0.31-0.56 mgl^{-1}; chloride 10.0 - 11.0 mgl^{-1}

Table 3.16 Physico-chemical characteristics of the test solutions during the period of bioassay for nitrite toxicity at a chloride concentration of 27.5 mgl^{-1}*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control**	N.A.	7.10 (7.00 - 7.20)	7.65 (7.58 - 7.72)
1	9.81 (8.84 - 10.53)	7.35 (7.10 - 7.60)	7.74 (7.68 - 7.78)
2	13.32 (12.01 - 14.03)	7.00 (6.70 - 7.50)	7.72 (7.60 - 7.78)
3	19.41 (18.24 - 20.37)	7.25 (6.60 - 7.60)	7.70 (7.63 - 7.76)
4	25.67 (24.43 - 28.17)	7.10 (6.90 - 7.40)	7.69 (7.60 - 7.76)
5	40.40 (38.78 - 41.87)	7.40 (7.10 - 7.60)	7.69 (7.56 - 7.78)
6	53.72 (50.82 - 56.16)	7.10 (6.80 - 7.40)	7.68 (7.55 - 7.82)

* Other measured variables were: temperature 28.0 - 28.5 °C; total hardness (as $CaCO_3$) 58.5 mgl^{-1}; total alkalinity (as $CaCO_3$) 45.0 mgl^{-1}; calcium 7.90 mgl^{-1}; magnesium 3.7 mgl^{-1}; total ammonia-N 0.11 - 0.96 mgl^{-1}; chloride 26.5 - 28.5 mgl^{-1}

** One replicate for control solution

N.A. Not analysed

Table 3.17 Physico-chemical characteristics of the test solutions during the period of bioassay for nitrite toxicity at a chloride concentration of 45.0 mgl^{-1} *

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.01(2) (0.01 - 0.01(4))	7.30 (7.00 - 7.60)	7.71 (7.63 - 7.76)
1	27.08 (25.51 - 28.19)	7.25 (6.90 - 7.60)	7.71 (7.61 - 7.78)
2	40.06 (38.91 - 41.54)	7.20 (6.80 - 7.60)	7.70 (7.62 - 7.81)
3	50.20 (48.74 - 51.04)	7.30 7.00 - 7.60)	7.72 (7.65 - 7.79)
4	82.07 (79.52 - 83.84)	7.35 (7.10 - 7.60)	7.73 (7.65 - 7.81)
5	106.56 (104.33 - 108.91)	7.15 (6180 - 7.60)	7.77 (7.71 - 7.83)

* Other measured variables were: temperature 27.5 - 28.5 °C; total hardness (as $CaCO_3$) 56.5 mgl^{-1}; total alkalinity (as $CaCO_3$) 47.0 mgl^{-1}; calcium 8.54 mgl^{-1}; magnesium 3.65 mgl^{-1}; total ammonia-N 0.09 - 0.97 mgl^{-1}; chloride 44.0 - 46.5 mgl^{-1}

Table 3.18 Median lethal concentration and 95% confidence limits of nitrite-nitrogen for various exposure times at different chloride ion concentrations

Exposure Time (h)	chloride 1.0mgl^{-1} LC50 ($mgl^{-1}NO_2^-$-N)	95% CL	chloride 5.0mgl^{-1} LC50 ($mgl^{-1}NO_2^-$-N)	95% CL	chloride 10.5mgl^{-1} LC50 ($mgl^{-1}NO_2^-$-N)	95% CL	chloride 27.5mgl^{-1} LC50 ($mgl^{-1}NO_2^-$-N)	95% CL	chloride 45.0mgl^{-1} LC50 ($mgl^{-1}NO_2^-$-N)	95% CL
6	22.49[a]*	16.78-30.14	-	-	-	-	-	-	-	-
12	19.21[a]	14.81-24.92	15.88[a]	13.56-18.59	53.21[a]	30.83-91.81	-	-	-	-
24	11.25[b]	7.87-16.07	9.24[b]	8.19-10.56	43.63[a]	36.32-52.41	49.78[a]	42.04-58.95	-	-
48	6.46[c]	4.89-8.53	7.16[c]	6.51-7.87	20.79[b]	18.37-23.52	42.37[a]	35.79-50.16	92.85[a]	84.09-102.54
72	4.22[d]	3.28-5.42	5.97[d]	5.47-6.51	15.87[c]	14.32-17.59	30.18[b]	26.61-34.24	64.17[b]	57.10-72.11
96	2.55[e]	2.09-3.23	5.77[d]	5.30-6.28	14.41[cd]	13.10-15.85	27.26[bc]	24.40-30.44	48.70[c]	43.40-54.65
120	2.26[e]	1.79-2.86	5.77[d]	5.30-6.28	13.36[de]	12.16-14.67	26.38[bc]	23.64-29.43	45.18[c]	39.73-51.37
144	2.17[e]	1.72-2.73	5.70[d]	5.23-6.20	12.64[e]	11.56-13.81	24.81[c]	22.46-27.41	43.89[c]	38.37-50.21
168	2.17[e]	1.72-2.73	5.70[d]	5.23-6.20	12.04[e]	11.01-13.16	24.54[c]	22.24-27.08	43.89[c]	38.37-50.21

* Figures in the same column with same superscripts are not significantly different ($P > 0.05$)

Table 3.19 A summary of the bioassay results for nitrite toxicity at different chloride ion concentrations*

Weight of fry (mg) Mean (± SE)	Conc. of chloride (mgl^{-1})	Conc. range tested (mgl^{-1} NO_2^--N)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)	LC50(95% CL) (mgl^{-1} NO_2^--N) 96 h	168 h	Incipient
216 (± 9.2)	1.0	0.72 - 24.98	7.20 (6.70 - 7.50)	7.67 (7.54 - 7.74)	2.55 (2.09 - 3.23)	2.17 (1.72 - 2.73)	2.20
226 (± 5.4)	5.0	1.44 - 22.93	7.25 (7.10 - 7.40)	7.60 (7.53 - 7.65)	5.77 (5.30 - 6.28)	5.70 (5.23 - 6.20)	5.80
217 (± 8.1)	10.5	1.70 - 54.55	7.20 (7.10 - 7.30)	7.79 (7.57 - 7.87)	14.41 (13.10 - 15.85)	12.04 (11.01 - 13.16)	13.00
251 (± 9.4)	27.5	9.81 - 53.72	7.20 (7.00 - 7.40)	7.70 (7.65 - 7.74)	27.26 (24.40 - 30.44)	24.54 (22.24 - 27.08)	25.00
291 (± 11.5)	45.0	27.08 - 106.56	7.25 (7.15 - 7.35)	7.72 (7.70 - 7.77)	48.70 (43.40 - 54.65)	43.89 (38.37 - 50.21)	45.00

* Temperature 27.5 - 28.5 °C

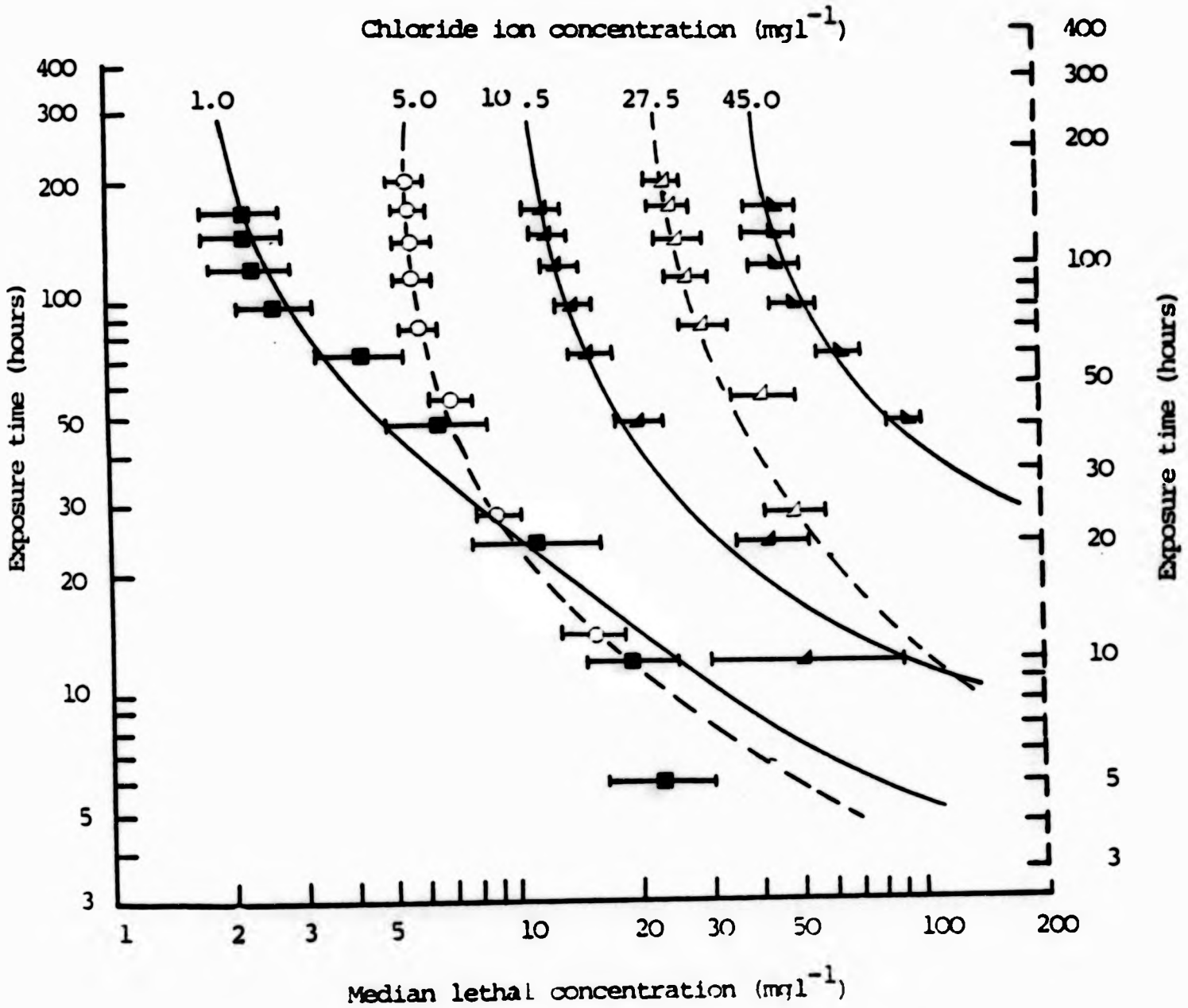


Fig. 3.3 Toxicity curve of nitite-nitrogen for carp fry at different chloride ion concentrations. Bars indicate 95% confidence limits.

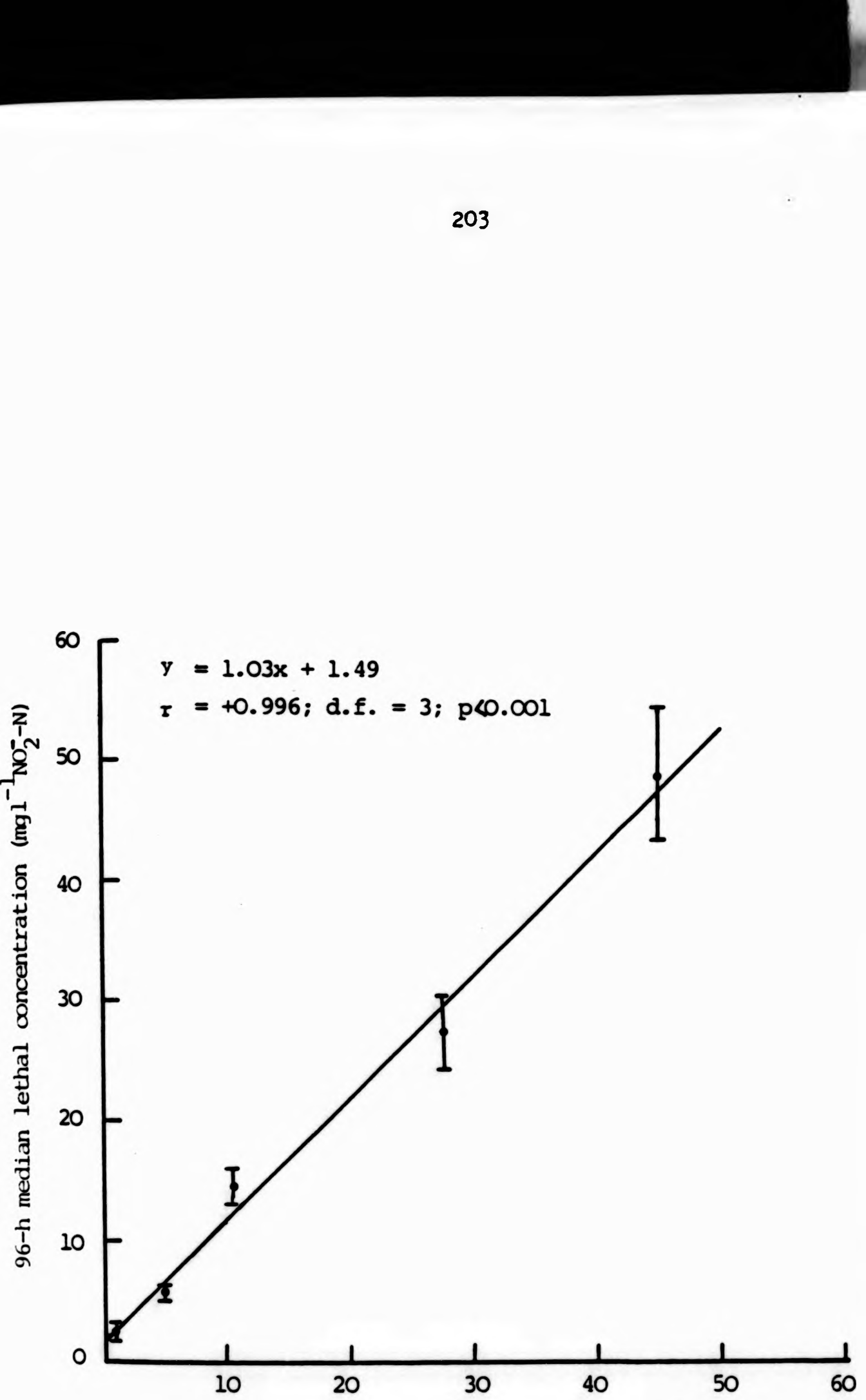


Fig. 3.4 Effect of chloride ion concentration on the acute toxicity of nitrite to carp fry: 96-h LC50 vs. Cl^-. Bars indicate 95% confidence limits.

Table 3.20 Highest levels of nitrite tested that resulted in no mortality of common carp fry over a 168-h exposure period at different chloride ion concentrations.

Conc. of chloride (mgl^{-1})	Highest concentrations tested with no mortality (NO_2^--N mgl^{-1})	Ratio of NO_2^--N to Cl^- that caused no mortality
1.0	0.72*	-
5.0	3.35	1:1.49
10.5	7.09	1:1.48
27.5	10.07	1:2.73
45.0	26.50**	-

* Lowest concentration tested with 5% mortality

** Lowest concentrations tested with 15% mortality

nitrite toxicity decreased (Fig. 3.4). This linear relationship is best described by the equation: $y = 1.03x + 1.49$ (d.f. = 3; $p < 0.01$) where, y = 96-h LC50 and x = concentration of Cl^-. The highest nitrite concentrations not resulting in mortality at each Cl^-level over the 168h period are presented in Table 3.20. An NO_2-N to Cl^- ratio of about 1:1.5-3.0 prevented complete mortality over a 168-h period. No mortality occurred in the control and there was no delayed mortality in any of the test tanks. Active feeding was observed in all the surviving fish.

3.3.3.2. Effect of fish size

Due to a lack of sufficient number of different sizes of fish fry available for the tests, only three sizes of fish could be used to investigate the effect of fish size on the acute toxicity of nitrite to common carp fry. Initially 75 mg (SE $\pm$ 3.1) of carp fry were used and subsequently two other sizes (449 mg; SE $\pm$ 13.7 and 1780 mg; SE $\pm$ 103.5) were tested.

The physico-chemical characteristics of the test solutions during the nitrite toxicity bioassay for different fish sizes are presented in Tables 3.21 - 3.22. Median lethal concentrations and their 95% confidence limits of NO_2-N for different exposure periods and for different sizes of fry are presented in Table 3.23 and shown graphically in Fig. 3.5

Table 3.21 Physico-chemical characteristics of the test solutions during the period of bioassay for nitrite toxicity to fish of mean weight 75 mg (SE ± 3.1)*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.02 (0.01 - 0.03)	6.60 (6.20 - 7.10)	7.53 (7.44 - 7.68)
1	5.99 (5.75 - 6.23)	6.55 (6.20 - 6.90)	7.64 (7.57 - 7.73)
2	12.71 (12.71 - 12.71)	7.00 (6.80 - 7.20)	7.65 (7.60 - 7.71)
3	25.56 (25.28 - 25.93)	6.80 (6.50 - 7.00)	7.60 (7.55 - 7.68)
4	31.44 (30.17 - 32.10)	6.65 (6.40 - 7.00)	7.62 (7.56 - 7.69)
5	36.74 (36.15 - 37.12)	6.75 (6.50 - 7.00)	7.74 (7.65 - 7.85)
6	46.07 (45.67 - 46.70)	6.70 (6.30 - 7.10)	7.69 (7.60 - 7.82)

* Other measured variables were: temperature 28 - 28.5 °C; total hardness (as $CaCO_3$) 53.75 - 56.50 mgl^{-1} ; total alkalinity (as $CaCO_3$) 50.0 - 54.5 mgl^{-1}; total ammonia-N 0.29 - 0.86 mgl^{-1}

Table 3.22 Physico-chemical characteristics of the test solutions during the period of bioassay for nitrite toxicity to fish of mean weight 1780 mg (SE ± 103.5) and 449 mg (SE ± 13.7)*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control	0.06 (0.05 - 0.08)	7.20 (7.10 - 7.20)	7.64 (7.55 - 7.75)
1	13.03 (12.5 - 13.5)	7.10 (7.00 - 7.20)	7.63 (7.53 - 7.74)
2	20.83 (20.60 - 21.00)	7.20 (7.10 - 7.40)	7.66 (7.55 - 7.78)
3	27.90 (27.40 - 28.50)	7.20 (7.10 - 7.30)	7.64 (7.58 - 7.80)
4	42.33 (41.50 - 43.50)	7.15 (7.00 - 7.30)	7.70 (7.65 - 7.70)
5	55.20 (53.40 - 57.10)	7.20 (7.10 - 7.30)	7.67 (7.50 - 7.75)
6	73.47 (71.40 - 76.20)	7.10 (7.00 - 7.40)	7.57 (7.50 - 7.65)
7	85.80 (84.60 - 87.00)	7.30 (7.20 - 7.40)	7.65 (7.60 - 7.70)

* Other measured variables were: temperature 27.5 - 28.5°C; total ammonia-N 0.70 - 1.55 mgl^{-1}

Table 3.23 Median lethal concentrations and 95% confidence limits for three weight classes of carp fry exposed to nitrite-nitrogen for various time periods.

Exposure time (h)	Mean weight 75mg(SE±31) LC 50*** (mgl^{-1} NO_2^--N)	95% CL	Mean weight 449 mg(SE± 13.7)* LC 50 (mgl^{-1} NO_2^--N)	95% CL	Mean weight 1780 mg(SE± 103.5)** LC50*** (mgl^{-1} NO_2^--N)	95% CL
12	35.44[a†]	31.99-39.26	75.15[a]	66.72-84.64	-	-
24	31.73[a]	28.36-35.50	38.69[b]	34.06-43.95	63.78[a]	54.17-75.09
48	27.46[b]	25.10-30.04	33.99[bc]	30.49-37.89	54.92[a]	45.97-65.60
72	27.35[b]	25.01-29.92	31.75[cd]	28.89-34.88	-	-
96	27.20[b]	24.87-29.75	31.09[cd]	28.27-34.20	39.15[c]	34.15-44.31
120	-	-	30.03[cd]	27.44-32.87	-	-
144	-	-	29.53[d]	27.14-32.13	-	-
168	-	-	29.53[d]	27.14-32.13	-	-

* 10 fish per replications

** 6 fish per replications

*** Test conducted for 96 hours

† Figures in the same column with same superscripts are not significantly different (P>0.05)

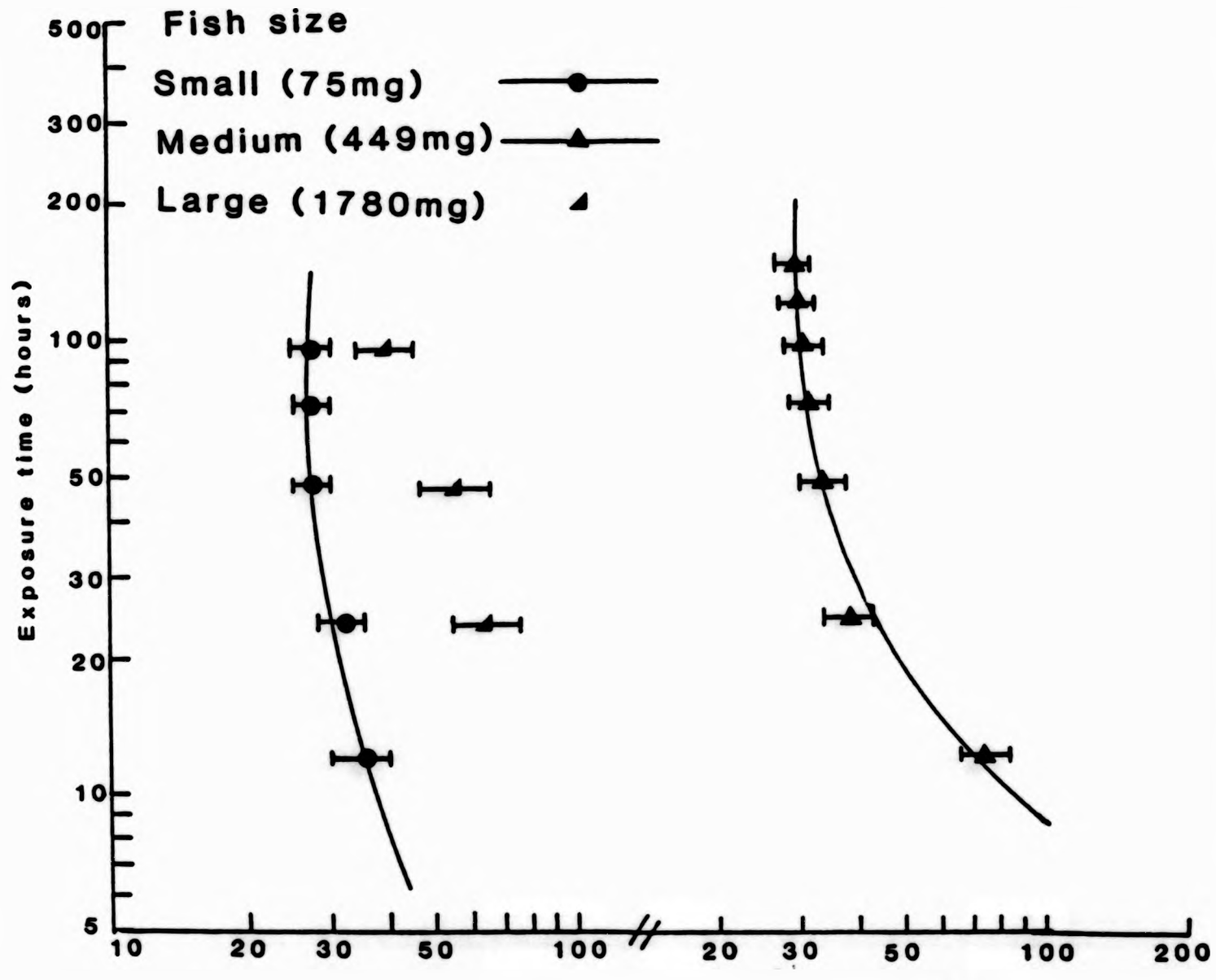


Fig. 3.5 Toxicity curves of nitrite-nitrogen for different sized carp fry. Bars indicate 95% confidence limits.

The first trial was conducted for 96-h as it was observed that the acute toxicity ceased within this exposure period (Table 3.23 and Fig. 3.5). For medium sized fry the trial was conducted for 168h but for large sized fry, the trial was terminated after 96-h to avoid water quality deterioration. The acute toxicity of nitrite for both small and medium sized fry, ceased within the exposure period as indicated by the toxicity curve becoming asymptotic with the time axis (Fig. 3.5). For large sized fry only three LC50 values were available and therefore, evidence for asymptotic line is limited in the toxicity curve (Fig. 3.5). Nevertheless, results of all other nitrite toxicity bioassay showed that 96-h exposure should be sufficient to obtain a lethal threshold concentration and it is unlikely that a significant change in LC50 would have occurred after 96-h exposure.

The LC50's between 48 and 96-h were not significantly different ($P > 0.05$) for small sized fry (Table 3.23). Similarly, the LC50's between 72 and 168-h and 96 and 168-h were not significantly different ($P > 0.05$) for medium and large sized fry respectively (Table 3.23). A summary of the toxicity bioassay of NO_2-N for different sizes of carp fry is presented in Table 3.24. For comparison, data have been included for another size of fry (251 mg; SE $\pm$ 9.4) from the chloride and nitrite toxicity bioassay which had a chloride concentration (27.5 mgl^{-1} Cl^-) similar to that in the present investigation. The 96-h LC50's for 75, 251 and 449 mg size fry were 27.20, 27.26 and 31.09 mgl^{-1} NO_2-N respectively and were not significantly different ($P > 0.05$) from

Table 3.24 A summary of the bioassay results for nitrite toxicity for differnt weight classes of carp fry*

Weight of fry (mg) Mean (± SE)	Conc. range tested (mgl^{-1} NO_2-N	Conc. of chloride (mgl^{-1})	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)	LC50 (95% CL) (mgl^{-1} NO_2^--N) 96-h	Incipient
75 (± 3.1)	5.99 - 46.07	30.0	6.70 (6.55 - 7.00)	7.64 (7.53 - 7.74)	27.20^{a**} (24.87 - 29.75)	27.00
251 (± 9.4)***	9.81 - 53.72	27.5	7.20 (7.00 - 7.40)	7.70 (7.65 - 7.74)	27.26^{a} (24.40 - 30.44)	25.0
449 (± 13.7)†	13.03 - 85.80	30.0	7.20 (7.10 - 7.30)	7.65 (7.57 - 7.70)	31.09^{a} (28.27 - 34.20)	31.00
1780 (± 103.5)†	13.03 - 85.80	30.0	7.20 (7.10 - 7.30)	7.65 (7.57 - 7.70)	39.15 (34.15 - 44.31)	40.0

* Temperature 27.5 - 28.5°C

** Figures in the same colum with same superscripts are not significantly different ($P > 0.05$)

*** Results of toxixity bioassay of nitrite at chloride conc. of 27.5 mgl^{-1} used for comparison

† Tests conducted in different tanks in a single recirculation system

Table 3.24a A comparison of the median lethal concentration of nitrite-nitrogen of three weight classes of carp fry for different exposure time.

weight of fry (mg)	LC50 (95% CL) (mg l^{-1} NO_2^--N) 12 h	24 h	48 h	72 h
75	35.44[a]* (31.99-39.26)	31.73[a] (28.36-35.50)	27.46[a] (25.10-30.04)	27.35[a] (25.01-29.92)
449	75.15[b] (66.72-84.64)	38.69[b] (34.06-43.95)	33.99[b] (30.49-37.89)	31.75[b] (28.89-34.88)
1780	-	63.78 (54.17-75.09)	54.92 (45.97-65.60)	-

* Figures in the same column with different superscripts are significantly different ($P<0.05$)

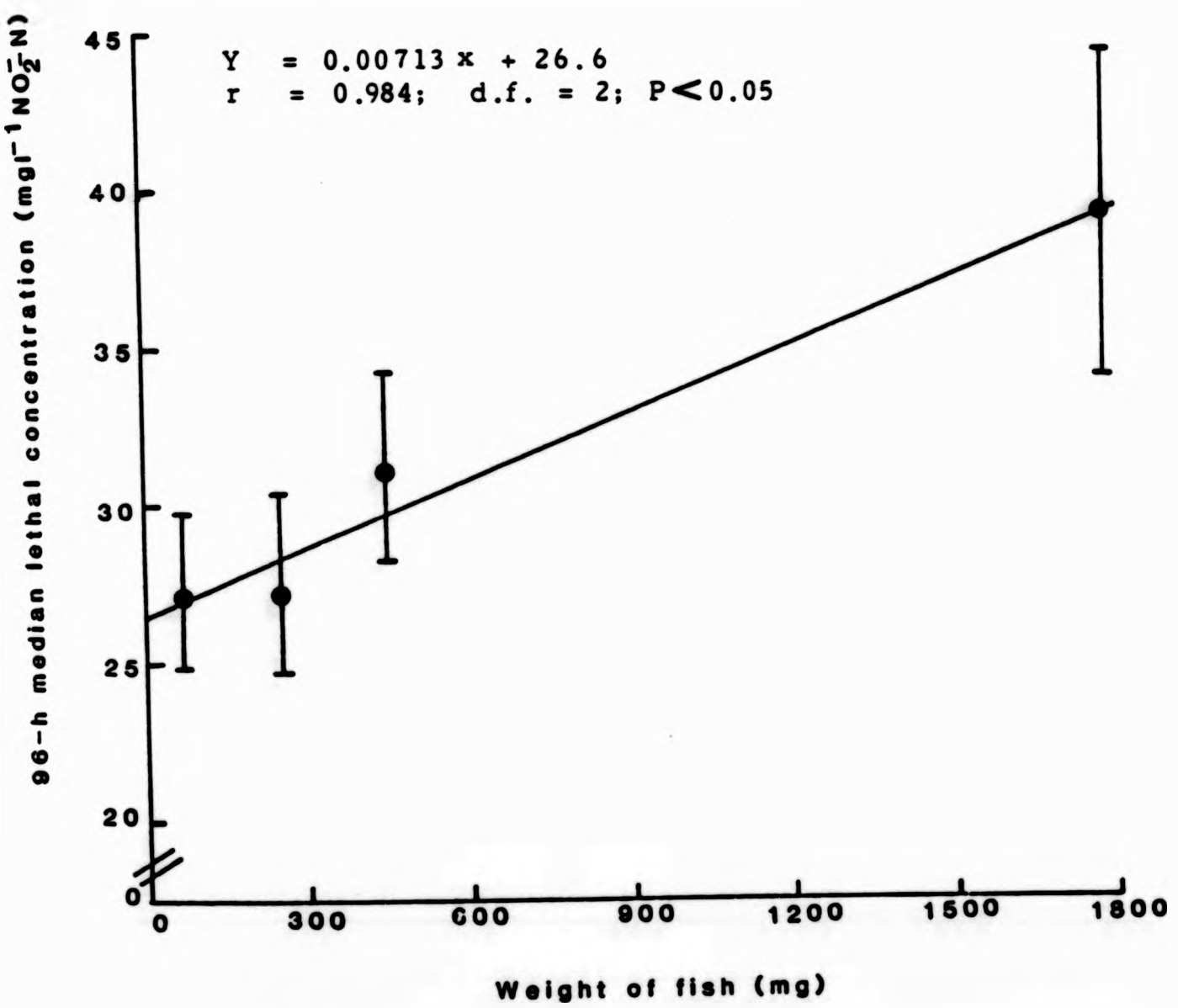


Fig. 3.6 Effect of fish size on the acute toxicity of nitrite to carp fry: 96-h LC50 vs. fish size. Bars indicate 95% confidence limits.

Table 3.25 Delayed mortality of small carp fry ($\bar{x}$ size 75 mg, SE ± 3.1) after exposure to test levels of nitrite-nitrogen

Conc. of nitrite ($mgl^{-1} NO_2^-$-N)	No. of fish kept for delayed mortality	No. died				
		24h	48h	72h	96h	Total
Control	20	-	1	-	-	1
5.99	20	-	-	-	-	0
12.71	20	-	1	-	-	1
25.56	12	1	-	-	1	2
31.44	10	-	-	-	-	0
36.74	8	-	1	-	-	1

each other. The 96h LC50 for large sized fry (1780mg) was 39.15 mgl^{-1} NO_2-N and was significantly different ($P<0.05$) from the rest of the fry used.

The relationship between fish size and 96-h LC50's have been presented graphically in Fig. 3.6. There was a significant positive correlation ($r = 0.984$; d.f. = 2; $P<0.05$) between fish size and 96-h LC50 of NO_2-N. This linear relationship is best described by the equation: $y = 0.00713 + 26.6$ (d.f. = 2; $P<0.05$), where y = 96h LC50 in mgl^{-1} and x = fish size in mg.

No control mortality was observed in any of the trials. The incidence of feeding and any delayed mortalities were monitored after termination of the tests. Out of three fish sizes tested, no delayed mortality was observed in case of medium and large sized fry, whereas some delayed mortalities were recorded in the case of small sized fry as shown in Table 3.25. Active feeding was observed in all the surviving fish.

3.3.3.3 Effect of pH

Preliminary study

A preliminary trial was conducted prior to the main study to evaluate the effect of pH on the toxicity of nitrite to carp fry (mean weight 30 mg; SE $\pm$ 1.1). Fish were exposed to three similar concentration of nitrite (23.28 - 24.03 mgl^{-1} NO_2-N) maintained at three

Table 3.26 Results of the preliminary study to evaluate the effect of pH on the toxicity of nitrite to carp fry*

Treatment No.	Conc. of nitrite-N (mgl^{-1}) Mean (range)	Conc. of HNO_2-N (μgl^{-1}) Mean (range)	pH Mean (range)	Temperature (^oC) Mean (range)	% mortality 96-h exposure	% mortality 168-h exposure
1	23.90 (22.25 - 25.39)	71.19 (63.15 - 81.96)	5.88 (5.82 - 5.91)	25.1 (24.0 - 26.2)	51.56^{a}**	90.63^{a}
2	24.03 (23.37 - 25.17)	4.84 (4.43 - 5.07)	7.06 (7.05 - 7.06)	25.4 (24.0 - 26.5)	23.44^{b}	46.88^{b}
3	23.28 (21.90 - 24.25)	0.56 (0.46 - 0.70)	7.95 (7.88 - 8.04)	25.7 (24.5 - 26.5)	6.25^{c}	31.25^{b}

* Dissolved oxygen concentration 7.20 - 7.60 mgl^{-1}

** Figures in the same column with different superscripts are significantly different ($P<0.05$)

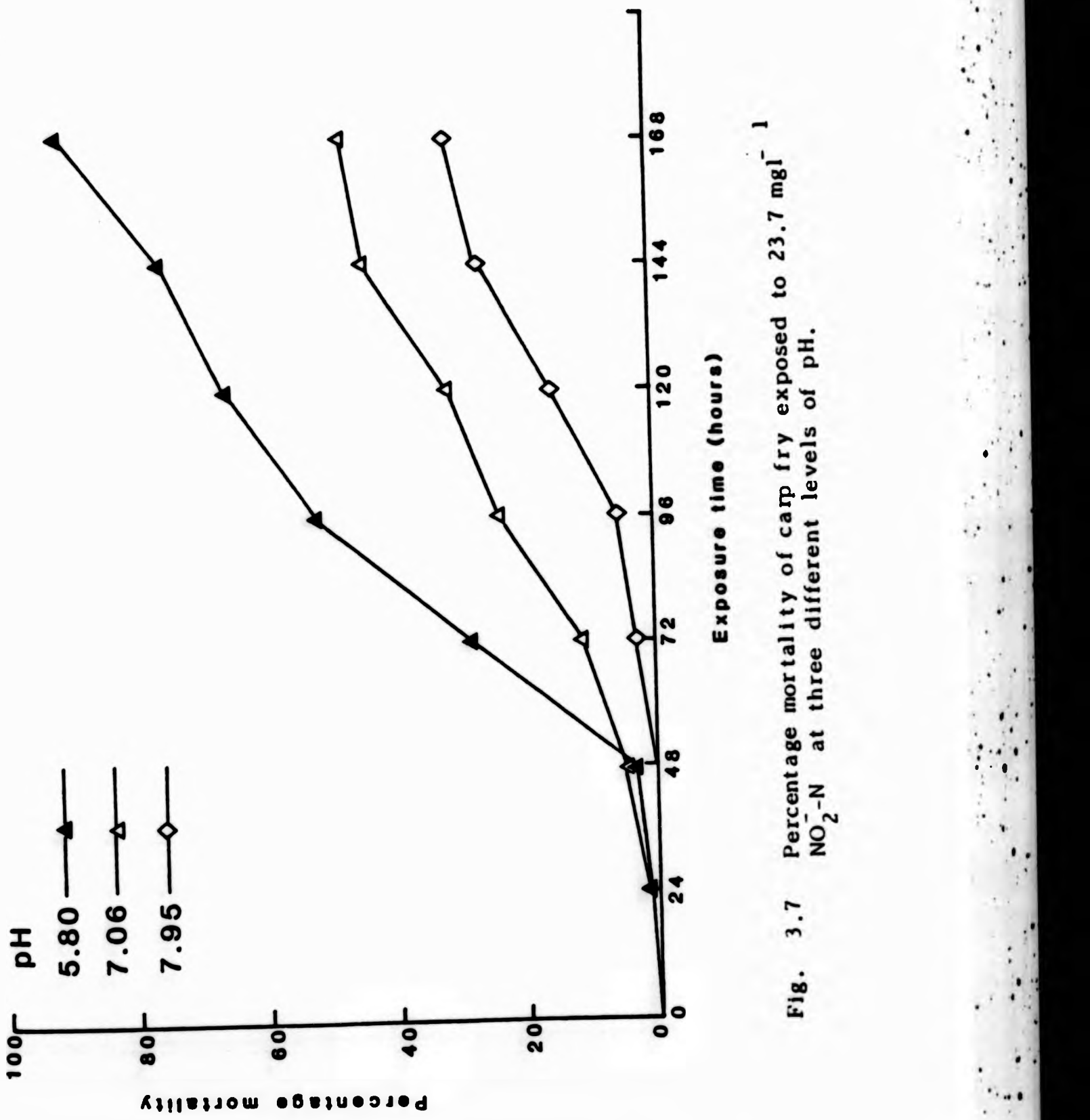


Fig. 3.7 Percentage mortality of carp fry exposed to 23.7 mg l^{-1} NO_2^--N at three different levels of pH.

levels of pH (5.88, 7.06 and 7.95). The results of this preliminary study are presented in Table 3.26. The percentage mortality at different exposure times for three levels of pH are presented graphically in Fig.3.7. The precentage mortalities recorded after 96-h exposure were 51.56, 23.44 and 6.25% at pH 5.88, 7.06 and 7.95 respectively and differed significantly (x^2test, $P<0.05$) from each other. After 168-h exposure, 90.63% mortality was recorded at pH 5.88 which was significantly greater (x^2test, $P<0.001$) than those (46.88 and 31.25%) recorded at other two pH levels, between which there was no significant difference ($P>0.05$).

Main study

Five 144-168-h toxicity bioassays were conducted over the pH range 5.92 to 7.81. The physico-chemical characteristics of the bioassay test solutions at pH 5.92, 6.46, 6.81, 7.06 and 7.81 are presented in Table 3.27 - 3.31. At each level of pH, five to seven concentrations of nitrite and one control were maintained. The present investigation was carried out with a pH range of 5.80 to 8.00, as this range of pH is most likely to be maintained in a fish culture system. The size ranges of carp fry used in all five toxicity bioassay trials were similar (230 - 275 mg; Table 3.35) to avoid any possible effect due to variation in size.

Median lethal concentration values for NO_2-N and HNO_2-N and their 95% confidence limits for different exposure periods and pH levels are presented in Table 3.32 - 3.34. The LC50 values for NO_2-N

Table 3.27 Physico-chemical characteristics of the test solutions during the period of bioassay to determine the effect of pH on nitrite toxcity (pH 5.92)*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	HNO_2-N** (ugl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control***	0.02[7] (0.00[3] - 0.07)	0.01[7] (0.01[1]0.02[7])	7.30 (6.90 - 7.50)	5.95 (5.77 - 6.04)
1	3.30 (2.95 - 3.76)	8.88 (5.73 -13.22)	7.40 (6.80 - 7.80)	5.96 (5.76 - 6.05)
2	6.55 (5.90 - 7.21)	18.02 (15.33 - 22.70)	7.00 (6.20 - 7.50)	5.93 (5.81 - 5.99)
3	12.72 (11.38 - 14.12)	33.35 (24.94 - 46.83)	7.30 (6.00 - 7.80)	5.96 (5.81 - 6.02)
4	18.77 (17.01 - 20.56)	51.28 (39.32 - 65.54)	6.90 (6.20 - 7.60)	5.93 (5.82 - 5.99)
5	28.77 (24.39 - 28.12)	78.92 (67.74 - 91.72)	6.90 (6.20 - 7.60)	5.88 (5.80 - 5.99)
6	41.33 (39.48 - 43.56)	116.10 (94.55 - 138.86)	7.00 (6.40 - 7.60)	5.92 (5.82 - 6.01)
7	56.19 (54.28 - 58.68)	177.02 (173.03 - 182.81)	7.20 (6.70 - 7.60)	5.83 (5.82 - 5.83)

* Other measured variables were: temperature 27.0 - 29.0 °C; total ammonia-nitrogen 0.43 - 1.56 mgl^{-1}

** Calculated from nitrite, pH and temperature

*** One replicate for control solution

Table 328 Physico-chemical characteristics of the test solutions during the period of bioassy to determine the effect of pH on nitrite toxicity (pH 6.46)*

Solution No.	Nitrite - N (mgl^{-1}) Mean (range)	HNO_2-N** (μgl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control ***	0.02 (0.01[1]-0.02[9])	0.01[7] (0.00[9]-0.02[4])	7.10 (6.60-7.60)	6.40 (6.38-6.42)
1	3.54 (3.10-4.02)	2.77 (2.27-3.12)	6.85 (6.40-7.40)	6.44 (6.46-6.46)
2	6.26 (5.95-7.10)	4.62 (3.88-5.83)	7.30 (7.10-7.80)	6.46 (6.40-6.52)
3	13.03 (11.92-14.02)	9.22 (7.43-11.25)	7.30 (6.40-7.60)	6.48 (6.42-6.54)
4	19.07 (17.15-21.31)	13.38 (11.83-16.34)	7.20 (6.90-7.60)	6.48 (6.42-6.54)
5	27.02 (25.40-29.39)	19.12 (16.57-22.53)	7.00 (6.60-7.60)	6.48 (6.44-6.53)
6	39.95 (38.10-43.01)	28.58 (24.30-30.69)	7.10 (6.60-7.60)	6.47 (6.43-6.52)
7	54.58 (52.40-58.12)	39.38 (36.30-44.55)	7.20 (7.00-7.40)	6.46 (6.43-6.49)

* Other measured variables were : temperature 27.5 - 28.5°C; total ammonia-nitrogen 0.23-0.83 mgl^{-1}

** Calculated from nitrite, pH and temperature

*** One replicate per control solution

Table 3.29 Physico-chemical characteristics of the test solutions during the period of bioassay to determine the effect of pH on nitrite toxicity (pH 6.81)*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	HNO_2-N** (ugl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control***	0.06 (0.00[1] - 0.09)	0.02 (0.00(0 3) - 0.03)	7.40 (7.30 - 7.60)	6.79 (6.73 - 6.89)
1	13.18 (11.88 - 14.87)	4.37 (3.42 - 5.21)	7.00 (6.50 - 7.50)	6.78 (6.73 - 6.89)
2	18.43 (17.22 - 20.56)	6.06 (5.23 - 6.58)	7.05 (6.30 - 7.60)	6.79 (6.75 - 6.87)
3	22.70 (21.12 - 24.03)	7.18 (6.27 - 8.23)	6.90 (6.40 - 7.50)	6.81 (6.75 - 6.91)
4	27.26 (25.31 - 29.15)	8.39 (7.77 - 9.28)	7.30 (6.90 - 7.60)	6.82 (6.75 - 6.91)
5	32.28 (31.03 - 34.56)	9.26 (8.33 - 9.83)	7.20 (6.80 - 7.50)	6.84 (6.79 - 6.90)

* Other measured variables : temperature 27.5 - 28.5 ^{o}C; total ammonia-nitrogen 0.34 - 0.87 mgl^{-1}

** Calculated from nitrite, pH and temperature

*** One replicate per control solution

Table 3.30 Physico-chemical characteristics of the test solutions during the period of bioassay to determine the effect of pH on nitrite toxicity (pH 7.06)*

Solution No.	Nitirite-N (mgl^{-1}) Mean (range)	HNO_2-N** (ugl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control***	0.00(8) (0.00(5) - 0.01)	0.00[15] (0.00[1] - 0.00[2])	7.10 (6.10 - 7.70)	7.03 (6.96 - 7.06)
1	6.59 (6.09 - 7.30)	1.34 (1.11 - 1.72)	6.85 (6.30 - 7.50)	7.04 (6.96 - 7.07)
2	12.72 (11.56 - 13.80)	2.41 (2.01 - 2.92)	7.50 (6.10 - 7.90)	7.05 (6.99 - 7.09)
3	19.37 (17.10 - 21.05)	3.62 (3.03 - 4.38)	7.35 (6.00 - 7.80)	7.06 (7.01 - 7.10)
4	25.53 (24.05 - 27.15)	4.67 (4.16 - 5.48)	6.50 (6.10 - 7.10)	7.07 (7.01 - 7.10)
5	33.49 (31.85 - 35.15)	6.13 (5.64 - 7.26)	6.45 (6.00 - 7.00)	7.07 (7.01 - 7.09)
6	38.99 (37.36 - 41.00)	7.11 (6.56 - 8.27)	6.70 (6.40 - 7.10)	7.06 (7.02 - 7.08)
7	57.10 (55.00 - 59.31)	10.24 (9.23 - 11.78)	7.20 (6.90 - 7.40)	7.07 (7.02 - 7.10)

* Other measured variables were : temperature 27.0 - 28.0 ^{o}C; total ammonia-nitrogen 0.56 - 1.08 mgl^{-1}

** Calculated from nitrite, pH and temperature

*** One replicate for control solution

Table 3.31 Physico-chemical characteristics of the test solutions during the period of bioassay to determine the effect of pH on nitrite toxicity (pH 7.81)*

Solution No.	Nitrite-N (mgl^{-1}) Mean (range)	HNO_2-N** (ugl^{-1}) Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)
Control***	0.01[7] (0.00[8] - 0.03)	0.00[06] (0.00[03] - 0.00[1])	7.10 (6.30 - 7.60)	7.82 (7.80 - 7.84)
1	12.70 (11.43 - 14.31)	0.42 (0.39 - 0.45)	6.90 (6.10 - 7.60)	7.81 (7.79 - 7.84)
2	18.38 (17.06 - 20.32)	0.62 (0.57 - 0.67)	7.00 (6.40 - 7.40)	7.80 (7.77 - 7.83)
3	25.83 (23.56 - 28.01)	0.84 (0.81 - 0.91)	7.30 (6.90 - 7.60)	7.81 (7.76 - 7.85)
4	39.10 (37.36 - 40.82)	1.27 (1.17 - 1.37)	7.20 (6.50 - 7.60)	7.81 (7.78 - 7.85)
5	54.77 (52.13 - 59.12)	1.80 (1.71 - 1.91)	7.10 (6.30 - 7.50)	7.81 (7.77 - 7.85)
6	72.02 (68.44 - 76.40)	2.26 (2.14 - 2.40)	7.00 (6.20 - 7.50)	7.82 (7.79 - 7.84)

* Other measured variables were : temperature 27.5 - 28.5 ^{o}C; total ammonia-nitrogen 0.17 - 0.4 mgl^{-1}

** Calculated from nitrite, pH and temperature

*** One replicate for control solution

Table 3.32 Median lethal concentrations and 95% confidence limits of nitrite and nitrous acid for various exposure time at different levels of pH

Exposure Time (h)	pH 5.92*				pH 6.46			
	NO_2^--N (mgl^{-1})		HNO_2-N (μgl^{-1})		NO_2^--N (mgl^{-1})		HNO_2-N (μgl^{-1})	
	LC50	95% CL	LC50	95% CL	LC50	95% CL	LC50	95% CL
6	43.82^{a**}	41.37-46.41	128.13^{a}	120.42-136.35	-	-	-	-
12	29.52^{b}	27.20-32.04	85.84^{b}	78.83-93.48	44.46^{a}	40.04-49.36	31.31^{a}	28.40-34.51
24	21.28^{c}	19.80-22.87	60.17^{c}	55.55-65.16	33.58^{b}	31.14-36.22	23.82^{b}	22.00-25.79
48	17.26^{d}	16.23-18.36	47.92^{d}	44.57-51.52	27.84^{c}	26.00-29.81	19.69^{c}	18.37-21.11
72	15.88^{de}	14.94-16.88	43.28^{e}	40.45-46.30	25.89^{cd}	24.24-27.65	18.43^{cd}	17.23-19.72
96	15.43^{e}	14.59-16.33	41.79^{e}	39.31-44.42	23.95^{de}	25.58-25.40	16.94^{de}	15.97-17.97
120	15.10^{e}	14.34-15.90	40.74^{e}	38-57-43.03	22.85^{e}	21.60-24.17	16.15^{e}	15.27-17.09
144	15.10^{e}	14.34-15.90	40.74^{e}	38.57-43.03	22.85^{e}	21.60-24.17	16.15^{e}	15.27-17.09
168	-	-	-	-	22.85^{e}	21.60-24.17	16.15^{e}	15.27-17.09

* Test conducted for 144 hours

** Figures in the same column with same superscripts are not significantly different ($P>0.05$)

Table 3.33 Median lethal concentrations and 95% confidence limits of nitrite and nitrous acid for various expsoure time at different levels of pH

Exposure time (h)	pH 6.81				pH 7.06 *			
	NO_2^--N (mgl^{-1})		HNO_2-N (μgl^{-1})		NO_2^--N (mgl^{-1})		HNO_2-N (μgl^{-1})	
	LC50	95% CL	LC50	95% CL	LC50	95% CL	LC50	95% CL
12	-	-	-	-	45.05[a]	40.68-49.89	8.15[a]	7.40-8.97
24	-	-	-	-	30.96[b]	29.05-32.99	5.68[b]	5.34-6.04
48	26.70[a**]	24.92-28.61	8.22[a]	7.89-8.57	24.30[c]	23.13-25.53	4.49[c]	4.28-4.71
72	23.16[b]	22.22-24.13	7.53[b]	7.22-7.84	23.00[cd]	22.08-23.95	4.25[cd]	4.09-4.42
96	21.90[bc]	20.95-22.90	7.00[c]	6.73-7.29	22.07[d]	21.26-22.91	4.09[d]	3.94-4.23
120	20.71[cd]	19.77-21.70	6.66[cd]	6.39-6.95	22.07[d]	21.26-22.91	4.09[d]	3.94-4.23
144	19.47[d]	18.59-20.40	6.30[d]	6.04-6.57	22.07[d]	21.26-22.91	4.09[d]	3.94-4.23
168	19.37[d]	18.50-20.29	6.26[d]	6.01-6.53	-	-	-	-

* Test conducted for 144 hours

** Figures in the same column with same superscripts are not significantly different ($P>0.05$)

Table 3.34 Median lethal concentrations and 95% confidence limits of nitrite and nitrous acid for various exposure time at pH 7.81

Exposure time (h)	NO_2^--N (mgl^{-1}) LC 50	95% CL	HNO_2-N (μgl^{-1}) LC 50	95% CL
24	45.02^{a*}	41.31-49.07	1.45^{a}	1.33-1.57
48	37.66^{b}	34.57-41.02	1.23^{b}	1.13-1.34
72	34.16^{bc}	31.42-37.14	1.11^{bc}	1.02-1.21
96	31.42^{cd}	29.03-34.01	1.03^{cd}	0.95-1.11
120	30.25^{d}	28.02-32.65	0.99^{cd}	0.92-1.07
144	29.97^{d}	27.80-32.32	0.98^{d}	0.92-1.06
168	29.97^{d}	27.80-32.32	0.98^{d}	0.92-1.06

*Figures in the same column with same superscripts are not significantly different ($P>0.05$)

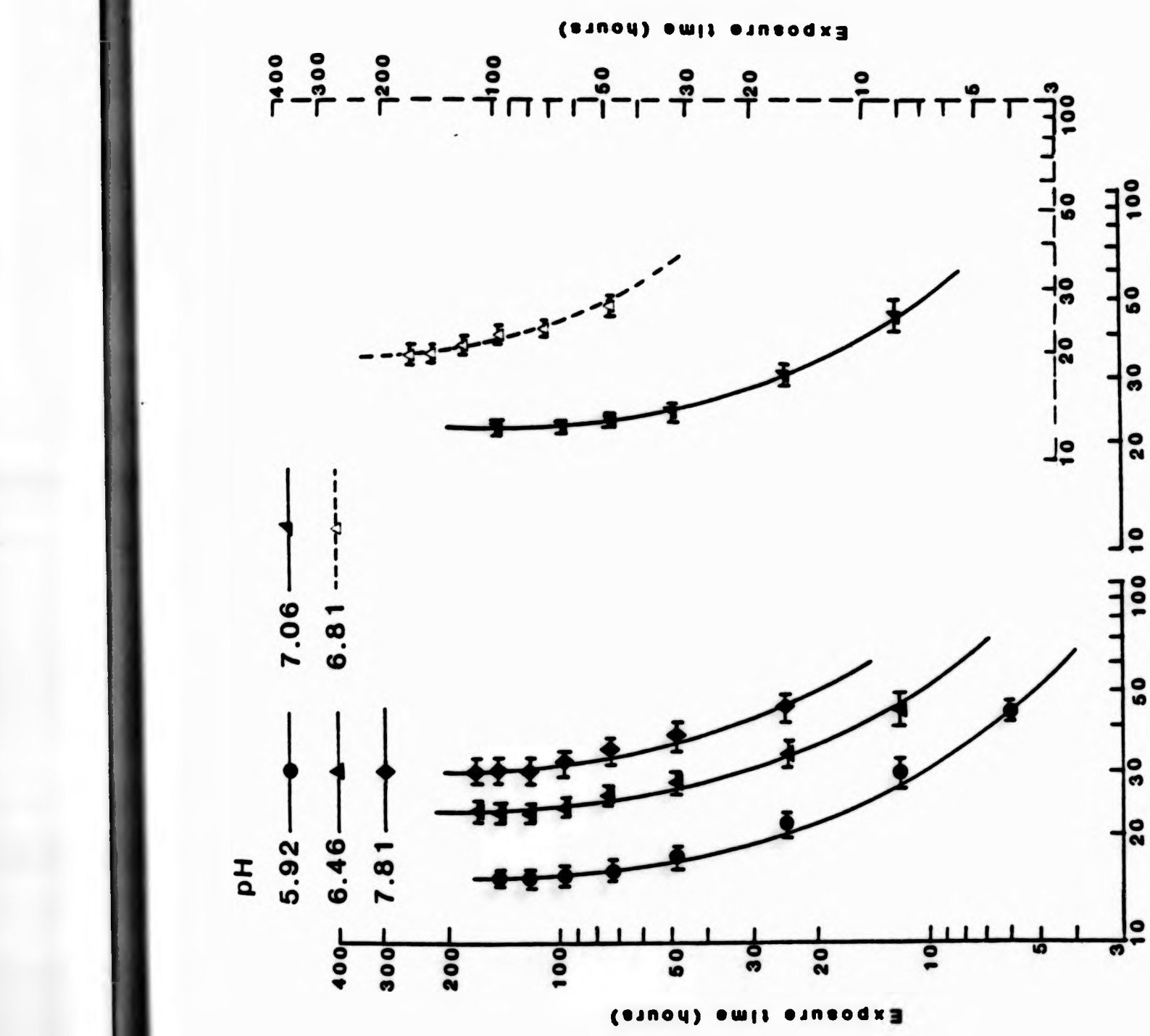


Fig. 3.8 Toxicity curves of nitrite-nitrogen for carp fry at different pH. Bars indicate 95% confidence limits.

are shown graphically in Fig. 3.8. The values for NO_2-N were the measured concentrations of total nitrite-nitrogen and those for HNO_2-N were the calculated values as described in section 3.3.2.4. The 96-h LC50's ranged from 15.43 mgl^{-1} NO_2-N (41.79 μgl^{-1} HNO_2-N) at pH 5.92 to 31.42 mgl^{-1} NO_2-N (1.03 μgl^{-1} HNO_2-N) at pH 7.81. All the trials were conducted for 168 hours except those at pH 5.92 nd 7.06. Both of these were terminated after 144 hours as at pH 7.06, no mortality was recorded after 96 hours and at pH 5.92 very little mortality was recorded after 96 hours and none after 120 hours. A summary of these toxicity bioassays is presented in Table 3.35. The LC50 values for nitrite increased with increase in pH level. The 96-h LC50's are 15.43, 23.95, 21.90, 22.07 and 31.42 mgl^{-1} NO_2-N at pH 5.92, 6.46, 6.81, 7.06 and 7.81 respectively. In contrast, the LC50 values for nitrous acid decreased with increase in pH. The 96-h LC50's are 41.79, 16.94, 6.30, 4.09 and 1.03 μgl^{-1} HNO_2 at pH 5.92, 6.46, 6.81, 7.06 and 7.81 respectively.

The acute toxicity of nitrite at all pH ceased towards the end of 96-h as indicated by the toxicity curves becoming asymptotic with the time axis (Fig. 3.8). Approximate values for the lethal threshold concentration of NO_2-N were obtained from the toxicity curves and these are presented in Table 3.35. The lethal threshold concentration values ranged from 15.20 mgl^{-1} NO_2-N at pH 5.92 to 30.20 mgl^{-1} NO_2-N at pH 7.81.

Table 3.35 A summary of the bioassay results for nitrite and nitrous acid toxcity at different levels of pH

Weight of fry (mg) Mean (± S.E.)	pH Mean (range)	Conc. range tested $mgl^{-1}NO_2^-$-N	Conc. range tested $ugl^{-1}HNO_2^-$-N	Dissolved oxygen (mgl^{-1}) Mean (range)	LC50 (95% C L) $mgl^{-1}NO_2^-$-N 96-h	LC50 (95% C L) $mgl^{-1}NO_2^-$-N Incipient LC50	LC50 (95% C L) $ugl^{-1}HNO_2$-N 96-h
243(± 8.1)	5.92 (5.83 - 5.96)	3.30 - 56.19	8.88 - 177.02	7.10 (6.90 - 7.40)	15.43^{a*} (14.59 - 16.33)	15.20	41.79^{a} (39.31 - 44.42)
230(± 5.7)	6.46 (6.40 - 6.48)	3.54 - 54.58	2.77 - 39.38	7.10 (6.85 - 7.30)	23.95^{b} (22.58 - 25.40)	23.80	16.94^{b} (15.97 - 17.97)
239(±8.6)	6.81 (6.78 - 6.84)	13.18 - 32.28	4.37 - 9.26	7.15 (6.90 - 7.40)	21.90^{c} (20.95 - 22.90)	19.50	6.30^{c} (6.04 - 6.57)
240(±9.0)	7.06 (7.03 - 7.07)	6.59 - 57.10	1.34 - 10.24	6.95 (6.45 - 7.50)	22.07^{c} (21.26 - 22.91)	22.10	4.09^{d} (3.94 - 4.23)
275(± 13.1)	7.81 (7.80 - 7.82)	12.70 - 72.02	0.42 - 2.26	7.10 (7.00 - 7.30)	31.42^{d} (29.03 - 34.01)	30.20	1.03^{e} (0.95 - 1.11)

Temperature 27 - 29 °C

* Figures in the same colum with same superscripts are not significantly different ($P>0.05$)

The relationships between pH and the 96-h LC50's for nitrite and nitrous acid are presented graphically in Figs. 3.9 and 3.10 respectively. There was a significant positive correlation ($r = 0.906$; d.f. = 3; $P<0.05$) between pH and the 96-h LC50 for NO_2-N, whereas a significant negative correlation ($r = -0.88$; d.f. = 3; $P<0.05$) between pH and the 96-h LC50 for HNO_2-N. These linear relationships are best described by the following equations:

96-h LC50 for NO_2-N = 7.38 pH - 27.3 (d.f. = 3; $P<0.05$)

96-h LC50 for HNO_2-N = - 20.8 pH + 156 (d.f. = 3; $P<0.05$)

No mortality was observed in any of the control populations used in these trials.

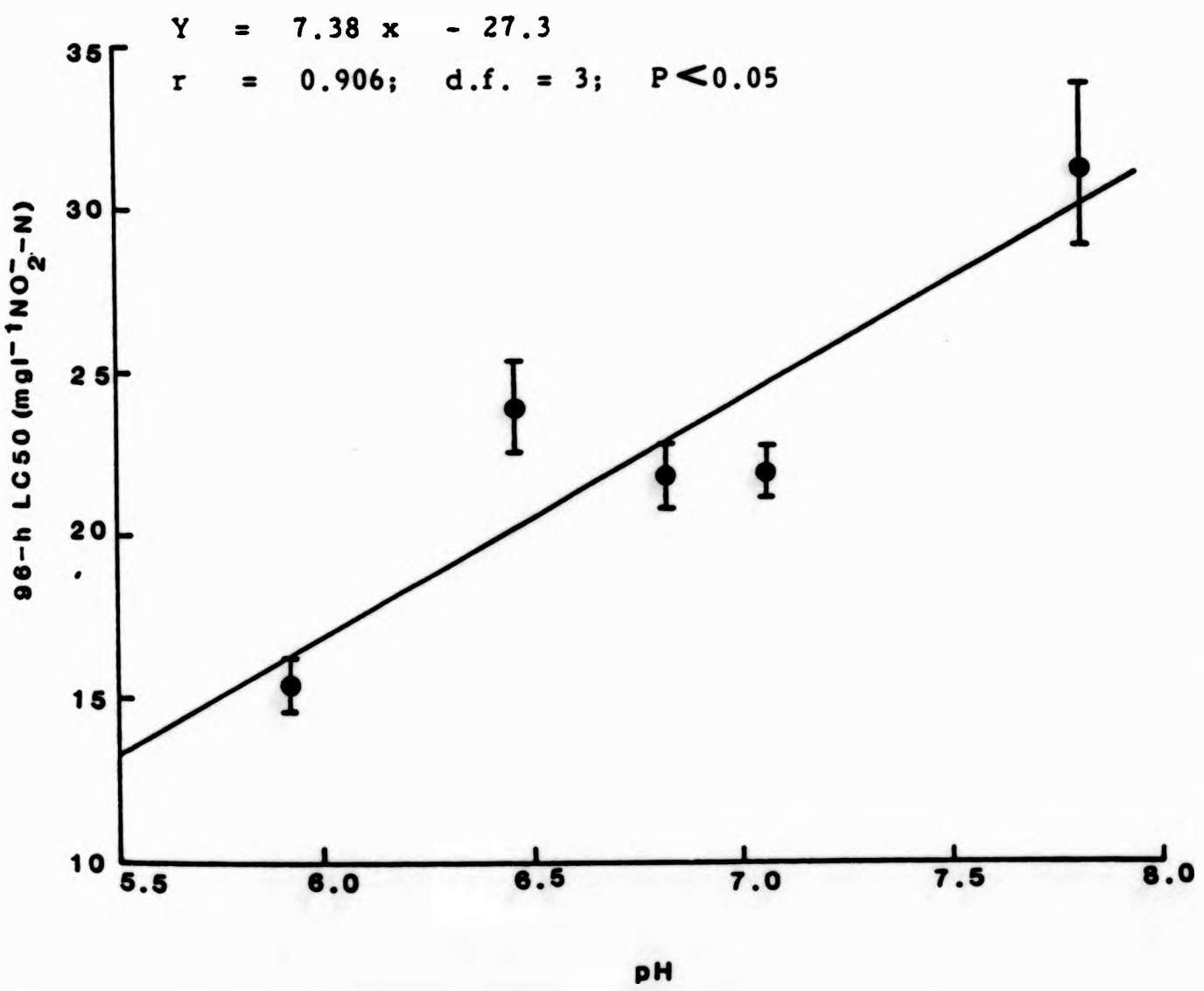


Fig. 3.9 Effect of pH on the acute toxicity of nitrite to carp fry: 96-h LC50 of NO_2^--N vs. pH.
Bars indicate 95% confidence limits.

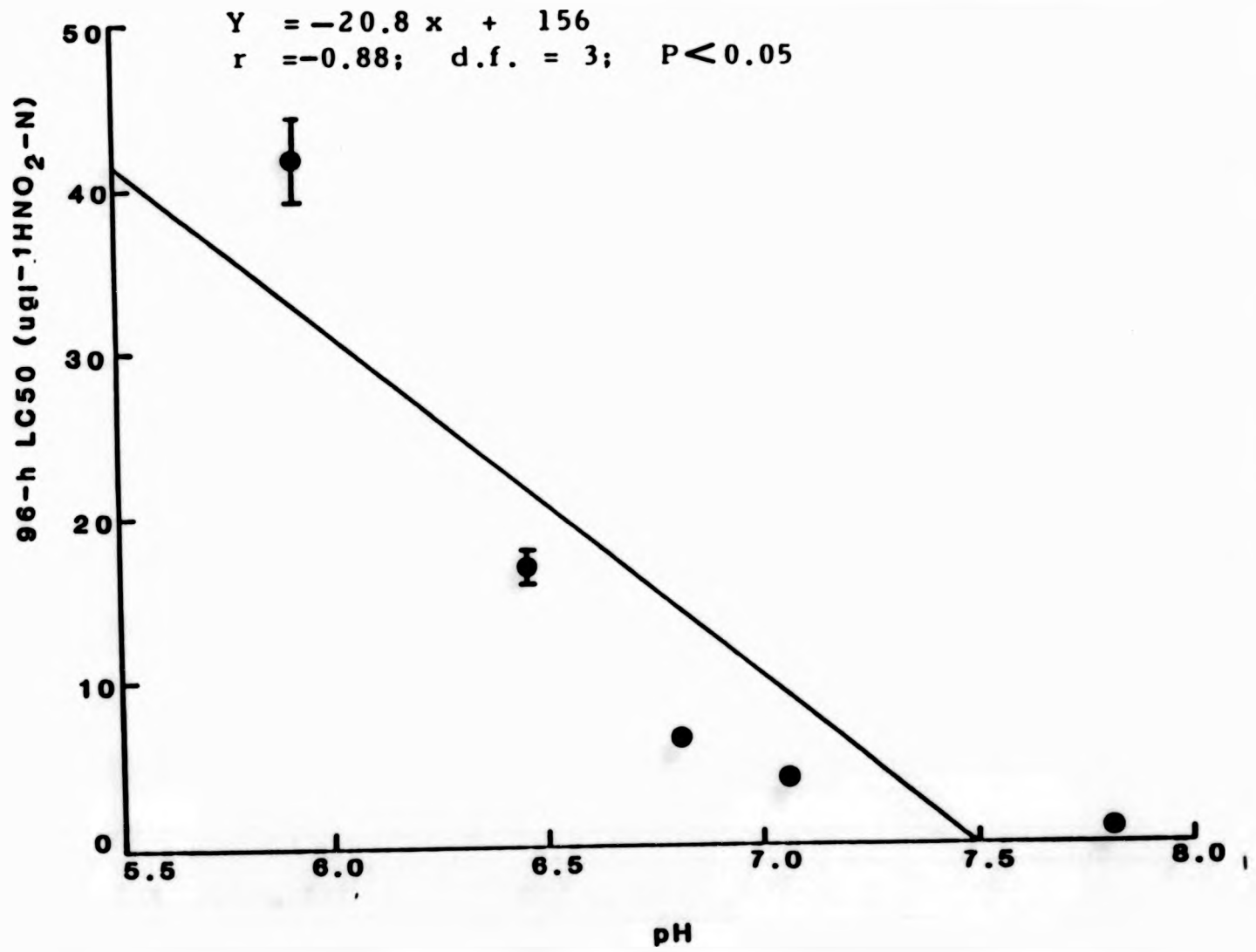


Fig. 3.10 Effect of pH on the acute toxicity of nitrous acid to carp fry: 96-h LC50 of HNO_2-N vs pH. Bars indicate 95% confidence limits.

3.3.4. Discussion

3.3.4.1. Effect of Chloride Concentration

The present nitrite toxicity bioassays were conducted in a static system. Static bioassay are reported to be reliable for non-volatile toxicants (see Section 3.2.4.) and have been used by several authors (Konikoff, 1975; Colt and Tchobanoglous, 1976; Tomasso et al., 1979; Bath and Eddy, 1980; Huey et al., 1980; Bowser et al., 1983) to determine the nitrite toxicity to fish. A preliminary trial conducted to study the stability of nitrite in water did not show any significant change in its concentration over a period of seven days in a static water system. Similar observations were also reported by Konikoff (1975), Armstrong et al., (1976) and Bath and Eddy (1980). Moreover, it has been demonstrated that endogenous ammonia production of unfed fish is not sufficient to cause any mortality or measurable stress to the experimental animals(Westin, 1974; Colt and Tchobanoglous, 1976; Tomasso et al., 1979; Huey et al., 1980; Tomasso et al., 1980; Bowsen et al., 1983; personal observation).

The background or acclimation concentrations of nitrite in the rearing water before the tests, which might have had an influence on nitrite tolerance of the fish (Tucker and Schwedler, 1983), was less than $0.01 mgl^{-1}$ No_2-N in the present investigation. So this background concentration of nitrite was unlikely to have had any acclimation effect on the tolerance of nitrite to carp fry.

In the present investigation the acute toxicity of nitrite ceased towards the end of the 96 hours which is in agreement with observations made by several authors (Russo et al., 1974; Armstrong et al., 1976; Thurston et al., 1978). However, in contrast, Colt and Tchobanoglous (1976) observed no threshold for nirite toxicity to channel catfish even after an exposure of 168 hours. The chemical characteristics of the test solution used in the different trials were similar (Table 3.12 and 3.13-17) except for the concentrations of sodium and sulphate ions. These varied in the different trials because of the addition of sodium chloride as a source of chloride and calcium sulphate as a source of calcium respectively (Section 3.3.2.2.). A preliminary trial conducted on the toxicity of carp fry showed that there was no protective effect associated with the addition of sodium sulphate. A similar observation has also been reported by Russo and Thurston (1977) and Huey et al.(1980).

Since the water quality in all trials was similar the higher LC50's for carp fry were clearly due to the antagonistic effect of Cl^- towards nitrite toxicity. The protective effect of chloride ion against nitrite toxicity has been demonstrated by several authors (Table 3.9). The protective effect of Cl^- is probably due to its competition with NO_2 for transportation across the gill at the chloride uptake sites (Perrone and Meade, 1977; Tomasso et al.,1979, Huey et al., 1980; Bath and Eddy, 1980). Huey et al.(1980) reported that monovalent cations and divalent anions had no impact on nitrite toxicity. They concluded that nitrite, being a charged anion, must compete for entrance sites in the gill with common anions like chloride and

bicarbonate and therefore these monovalent anions might reduce the entrance of the nitrite when present in water. Bath and Eddy (1980) suggested that the gills have the ability to transport nitrite against a concentation gradient by means of a branchial anion (Cl^-/HCO_3) exchange mechanism. They proposed that the Cl^- uptake mechanism present in freshwater fish gills has a low affinity for NO_2 ions and thereby NO_2 uptake may be competitively inhibited by external Cl^- ions.

The 96-h LC50's for nitrite-nitrogen for carp fry are considerably higher than those reported for salmonids (Table 3.8-9). At chloride concentrations of 1.0 and 5.0 mgl^{-1} the 96-h LC50's for carp fry were 2.55 and 5.70 mgl^{-1} (Table 3.19) compared to 0.46 and 2.36 mgl^{-1} NO_2-N for rainbow trout (Table 3.9). At a chloride concentration of 22.0 mgl^{-1} the 96-h LC650's for channel catfish, tilapia, Oreochromis aureaus, fathead minnow, Pimephales Promelas and largemouth bass, Micropterus salmoides are reported as 7.10 - 13.09, 16.2, 45.3 and 140.2 mgl^{-1} NO_2-N respectively (Table 3.8). By comparison the value obtained for common carp was 24.11 mgl^{-1} NO_2-N (calculated from the regression equation). These comparisons, however, should be viewed with caution since there were variations in the water chemistry and fish size between individual test conditions. Solbe et al. (1981, unpublished data referred to by EIFAC, 1984) reported the 96-h LC50 of carp (5.6cm; approx. weight 2.0g) to be 40mgl^{-1} NO_2-N at a chloride concentration of 19.0 mgl^{-1} which is higher than the value obtained in the present investigation. However,

the water temperature, hardness and fish size (Table 3.8) used by Solbe et al. were different to those used for common carp fry.

The ratio of nitrite to chloride preventing mortality or suppressing complete methaemoglobin (Met-Hb) formation in fish has been reported by several authors (Perrone and Meade, 1977; Tomasso et al., 1979, 1980; Bath and Eddy, 1980; Bowser et al., 1983).In this investigation, the NO_2-N : Cl^- ratio of about 1:1.5-3.0 prevented complete mortality of carp fry over 168-h period, which is similar to the ratio of 1:4 reported by Perone and Meade (1977) for coho salmon fry. Tomasso et al. (1980) observed that a ratio of about 1 NO_2-N: 20 Cl^- produced Met-Hb levels similar to those of control channel catfish. Bowser et al.(1983) reported that a NO_2-N to Cl^- ratio of 1:3 prevented Met-Hb from reaching 50% levels during 48-h laboratory trial with channel catfish. However, it has been suggested that elevated levels of Met-Hb do not necessarily result in mortality (Smith & Williams, 1974; Crawford & Allen, 1977; Perrone & Meade, 1977; Palachek & Tomasso, 1984a).

The above findings have been substantiated by a recent study by Arillo et al. (1984)which suggests that liver hypoxia, not tissue hypoxia, caused by nitrite-induced high methaemoglobinemia, is at the root of the actue toxicity mechanism in rainbow trout. Similarly Bowser et al. (1983) reported that Met-Hb level only in excess of 50% may be lethal to channel catfish and recommended a NO_2-N:Cl^- ratio of 1:3 for preventive management is production ponds. EIFAC (1984) recommended a NO_2-N to Cl^- ratio of 1:17

for rainbow trout and about 1:8 for coarse fish for maximum protection against nitrite toxicity.

The physiological basis for the NO_2: Cl^- ratio which prevents mortality, or completely suppresses Met-Hb formation, has yet to be investigated. This apparent nitrite to chloride ratio in the water might be affected by the presence of other ions as calcium (Crawford & Allen, 1977; Wademeyer & Yasutake, 1978), nitrate (Russo et al., 1981) and bicarbonate (Bath & Eddy, 1980; Huey et al., 1980; Bowser et al., 1983) have some protective effect against nitrite toxicity. In addition, variation in the nitrite to chloride ratios preventing mortality may also be attributable to the variations in the tolerance of Met-Hb level among different fish species (Perrone & Meade, 1977; Russo, 1980); to the presence of an active Met-Hb reductase system (Cameron, 1971; Huey & Beitinger, 1982); or to variation in the numbers, proliferation and differential selectivity of chloride cells to nitrite or chloride ions (Laurent, 1984; Palachek & Tomasso, 1984a).

From the present investigation, it can be concluded that carp fry are highly susceptible to nitrite toxicity in water containing very low levels of Cl^- (1.0-5.0 mgl^{-1}). However, the lethal levels of nitrite in culture systems could be rendered harmless if an adequate concentration of Cl^- is present in the water. The fish farmer, may, therefore, avert nitrite mortalities of carp fry by the addition of Cl^- in the water. Based on the results of the present study an NO_2-N to Cl^- ratio of 1:5

(i.e. safely above the level of 1:3 observed in this study) can be recommended for protection of carp fry against nitrite mortality.

3.3.4.2. Effect of fish size.

The results of the present study suggest that the nitrite tolerance of carp fry increases with increasing fish size (Table 3.24) and the 96-h LC50 for nitrite is lineally correlated to fish size (Fig. 3.6). The above findings are similar to those of Wademeyer and Yasutake (1978) who observed that 10g steelhead trout were more tolerant to nitrite toxicity than 5g fish. However, these findings are contrary to the observations reported by several other authors (Russo et al., 1974; Smith and Williams, 1974; Perrone and Meade, 1977; Saroglia et al., 1981; Palachek and Tomasso, 1984b), who observed a tendency for higher resistance among smaller sized fish. The physiological basis for the size effect on nitrite susceptibility is not clearly understood. Russo et al. (1974) postulated that the higher tolerance to nitrite by young trout may be attributable to the higher oxygen affinity of haemoglobin in the larval and possibly in the juvenile stages of rainbow trout when compared with adults. Spicer and Reynolds (1949) cited by Perrone and Meade (1977) reported that under certain conditions, red cells from weaning rabbits reduce Met-Hb more rapidly than cells from adult rabbits. Similarly, Kiese (1974) proposed that there might be a variation in the activity of the Met-Hb reductase system between young and adult individuals. Perrone and Meade (1977), citing Spicer and Reynold

(1949), and Kiese (1974), therefore suggested that if piscine erythrocytes function in a manner similar to mammalian erythrocytes, younger fish may be able to cope with nitrite exposure for longer periods because they have a more efficient Met-Hb reduction process. However, the present experimental findings do not support the above hypothesis.

Although there seems to be an increase in nitrite tolerance, for carp fry as their size increased, the relationship between median lethal concentration and fish size is not conclusive (see Table 3.24). The 96-h LC50's for large sized carp fry were significantly different from the three smaller groups, whereas 96-h LC50's of the latter were not significantly different. But comparisons of the LC50's for 12, 24, 48 and 72-h exposure periods for small and medium sized fry do show significant differences ($P<0.05$; Table 3.24a). Similarly Palachek and Tomasso (1984b) recorded significant differences in the tolerance of nitrite at 48, 72 and 96 hours exposure for two weight classes (0.3 - 0.8 and 0.9 - 3.3g) of the fathead minnow. But after examining the slopes of the toxicity curves for both size groups, the authors opinioned that the differences in the tolerance between two size groups possibly would not have been significant, had the study been extended to 120 or 144-h.

The experimental evidence on the effect of fish size on the toxicity of nitrite to fish is not very conclusive. Saroglia et al. (1981) observed a derease in the median lethal concentration of nitrite with increasing fish size in the case of sea bass, Dicentrarchus

labrax. However, he noted that the relationship between fish size and LC50's was not clearly demonstrated. Russo et al. (1974) reported that 2g trout and yolk sac fry exhibited greater tolerance to nitrite than the larger (12, 14 and 235g) fish. But again the size-tolerance relationship was not demonstrated conclusively. Fingerling (4.5g) rainbow trout were also reported to be less susceptive than yearlings (100g) (Smith and William, 1974). In contrast, however, Russo (1980) observed no relationship between tolerance to nitrite and fish size in rainbow trout while studying the results from twenty 96-h nitrite toxicity bioassays on this species over the size range of 2 to 387g. Similarly no relationship between susceptibility to nitrite and fish size was obtained for cut-throat trout fry (Thurston et al., 1978).

The present study indicates that at chloride concentrations of 27.5 - 30.0 mgl^{-1}, the 96-h LC50's of carp fry (75 - 450 mg weight) range between 27.20 and 31.09 mgl^{-1} NO_2-N. However, within the size-range used, larger sized fish show higher tolerance for short exposure periods than the smaller ones. The 96-h LC50 of nitrite for large sized carp fry (1780 mg) is 39.15 mgl^{-1} NO_2-N which is similar to the 96-h LC50 (40 mgl^{-1} NO_2-N) reported by Solbe et al. (1981) cited by EIFAC (1984) for 5.6 cm(approx. 2g) carp fry (Table 3.8).

From the present investigation it can be concluded that large sized carp fry within the size range, are somewhat more resistant than smaller ones. However, the size-range of fish used in this investigation was probably too small to show conclusively the effect of size on the nitrite tolerance for common carp.

3.3.4.3. Effect of pH

The result of the preliminary trial and that of the bioassay for nitrite toxicity at different pH levels demonstrates clearly that the toxicity of nitrite is influenced by pH of the water. Experimental evidence on the pH dependent toxicity of nitrite has been reported by several authors. Wademeyer and Yasutake (1978) observed that for juvenile steelhead trout an increase in pH from 6.8 to 8.0 reduced the toxicity of nitrite by a factor of about 8 for small (5g) fish and by a factor of about 3 for larger (10g) fish. Similarly coho salmon exposed to 3 mgl^{-1} NO_2-N showed significantly lower levels of blood plasma nitrite at pH 8.0 compared to pH 6.5 (Meade and Perrone, 1980). In the preliminary trial, similar concentrations of nitrite resulted in significantly different mortalities of carp fry in the different pH levels. This indicates that the variation in mortality is probably due to the effect of pH on the dissociation of nitrite.

For each exposure period a higher concentration of nitrous acid-nitrogen resulted in a higher percentage mortality in conditions where the total nitrite concentration was similar (Table 3.26.). The experimental evidence, therefore, suggest that nitrous acid is a toxic form of nitrite, although it does not show if nitrous acid is the only toxic form of nitrite. It has been suggested that HNO_2, being the unionized molecule of nitrite, can move across

the biological membrane much faster than its ionized counterpart (NO_2^-) and therefore it may be the principal toxic form of nitrite (Colt and Tchobanoglous, 1976; Wedemeyer and Yasutake, 1978). Evidence for this hypothesis is not satisfactory, however, as it does not explain the protective effect of Cl^- (Section 3.3.3.1.) and it is unlikely that chloride ion would compete effectively with gaseous nitrous acid (Perrone and Meade, 1980).

The results of the present investigation indicate that both unionized (HNO_2) and ionized (NO_2^-) forms of nitrite are toxic. If all the toxicity resided in one or the other form, the LC50 values for that form would be the same at all pH levels, i.e. be independent of pH and a plot of LC50 values against pH for that form would give a horizontal straight line. However, it has been shown that the 96-h LC50 for NO_2^--N is positively correlated with pH; i.e. as pH increases, apparently the toxicity of NO_2^--N decreases (Fig. 3.9) The fraction of total nitrite present as nitrous acid is greatly reduced at higher pH. For example at 28°C the calculated percent nitrite present as nitrous acid at pH 5.92 and 7.81, are 0.25 and 0.0033% respectively. Therefore the reduced toxicity of NO_2^--N at higher pH may partially be explained by the fact that the formation of HNO_2 is suppressed at higher pH. On the contrary, the 96-h LC50 of HNO_2 is negatively correlated with pH i.e., as pH increases, apparently the toxicity of HNO_2-N increases (Fig. 3.10). Therefore, the findings of the present investigation demonstrates conclusively that both forms of nitrite are toxic,

although not necessarily to the same extent. Similar observations have also been reported by Russo et al. (1981), who from an extensive series of toxicity tests on rainbow trout over the pH range of 6.4 - 9.1, found that as pH increased, the toxicity of NO_2^- decreased while that of HNO_2 increased suggesting that both forms of nitrite are toxic. However an alternative cause for pH dependent toxicity of nitrite has been put forward by Meade and Perrone (1980). The authors proposed that since nitrite ions are being actively pumped across the gill epithelia, a rise in pH may adversely affect the pumping mechanism.

In the present investigation, the 96-h LC50 values obtained at the medium range of pH (6.81 - 7.06) are not significantly different ($P > 0.05$) (Table 3.35). A possible explanation for this insignificant difference recorded at LC50 values is that the differences in pH were quite small and therefore the amount of HNO_2 present in the total nitrite-N concentration with this range of pH did not vary sufficiently to influence the result. The calculated percent nitrite present as nitrous acid at pH 6.81 and 7.06, and 0.033 and 0.018% respectively. The reason for the higher LC50's at pH 6.46, however, is not apparent.

Based on the results of the present study it can be concluded that both NO_2^- and HNO_2 are toxic and therefore the toxicity of nitrite to fish is influenced by the variation in pH. It can also be suggested that both forms of nitrite contribute significantly to

the total toxicity. However, as HNO_2 is present in amounts ranging from about one part in 300 to 60000 of the total nitrite level within the pH range tested, it is further concluded that at equal nitrogen level, toxicity of HNO_2 must be higher than that of NO_2.

It is therefore recommended that pH of water should be carefully monitored to evaluate the toxicity of nitrite in intensive fish culture systems. EIFAC (1984) also suggested, that the pH of water appears to be an important variable when evaluating the toxicity of nitrite and second only to chloride in importance.

3.4 Experiment 3.3

Estimation of median lethal concentration of nitrate to carp fry.

3.4.1 Introduction

Generally, in natural waters, nitrate occurs at a very low level ($< 1.0 mgl^{-1}$ NO_3^--N). High levels of nitrate, however, can accumulate in recirculated fish culture systems as an end product of bacterial nitrification of ammonia. Nitrate levels of 45-70mgl^{-1} NO_3^--N have been reported in the recirculation system at the University of Rhode Island (Westin, 1974) and 310 mgl^{-1} NO_3^--N in the Zoological Society of London Aquarium (Oliver, 1957). However nitrate is reported to be the least toxic of the inorganic nitrogen compounds (Colt and Tchobanoglous, 1976; Wickins, 1976; Colt and Armstrong, 1981). The toxicity of this compound is due to its effect on osmoregulation (Colt and Tchobanoglous, 1976; Brownell, 1980; Colt and Armstrong, 1981) and possibly on oxygen transport (Oliver, 1957; Colt and Armstrong, 1981). The toxicity of nitrate differs from species to species of fish (Table 3.36). The 96-h LC50 for bluegills was found to be 1840 mgl^{-1} NO_3-N (Trama, 1954, cited by Westin, 1974), whereas the 24-h LC 50 for first feeding larvae of a sparid, Lithognathus mormyrus, was 779 mgl^{-1} NO_3-N (Brownell, 1980).

Published information on the nitrate tolerance of carp fry is not available. In the present research project the following experiment was conducted with three sizes of carp fry (134mg, SE± 6.8; 452mg, SE± 28.6; 2281mg, SE± 230) to estimate the lethal level of nitrate and to evaluate the effect of fish size, if any, on nitrate toxicity.

Table 3.36 A summary of the tolerance of different species of fish to nitrate.

Fish species	Size/age (weight/length)	NO_3 Conc (mgl^{-1} NO_3-N)	Effect	Author
Rainbow trout	1-5g	1355	96-h LC50	Westin (1974)
Salmo gairdneri	1-5g	1062	168-h LC50	Westin (1974)
Chinook salmon	1-10g	1310	96-h LC50	Westin (1974)
Oncorhynchus tshawtyscha	1-10g	1084	168-h LC50	Westin (1974)
Channel catfish Ictalurus punctatus	3-4*g	1335-1423	Incipient LC50	Colt & Tchobanoglous (1976)
Bluegills Lepomis macrochirus	-	1840	96-h LC50	Trama(1954 cited by Westin,1974)
	-	416**	96-h LC50	Trama(1954, cited by Westin, 1974)
Gadid, G. capensis	First feeding larvae	>900	24-h LC50	Brownell (1980)
Sparid, D. sargus	First feeding larvae	804	24-h LC50	Brownell (1980)
Sparid, L. mormyrus	First feeding larvae	779	24-h LC50	Brownell (1980)
Soleid, Heteromycteris capensis	First feeding larvae	1141	24-h LC50	Brownell (1980)
Guppy, P. reticulatus	7.8-8.5mm	266.5*	24-h LC50	Rubin & Elmaraghy (1979)
		191.3**	96-h LC50	Rubin & Elmaraghy (1979)
Penaeid prawn	0.5-1.5g	3400	48-h LC50	Wickins (1976)

* Weight reported by Colt & Tchobanoglous (1978)

** KNO_3 was used as a source of nitrate

3.4.2. Materials and Methods

The source of experimental animals, their quarantine and acclimation procedures were as described in sections 2.2.2.2., 2.2.2.3 and 3.2.2.1. respectively.

10l glass aquaria were used as experimental tanks, details of which are described in section 3.3.2.1. Synthetic dilution water was used as the test water (section 3.2.2.3.). The experimental procedures were as described in section 3.2.2.4. except for some minor modifications which are explained below.

All three trials were conducted with five concentrations of toxicants and one control. Prior to the main study, a preliminary trial was conducted with concentrations ranging between 125 and 2000 mgl^{-1} NO_3-N. In the preliminary trial, no mortality was observed at concentrations of 1000 mgl^{-1} NO_3-N and below, whereas 90-100% mortalities were recorded at a concentration of 2000 mgl^{-1} NO_3-N. Therefore on the basis of the observed mortality in the preliminary trial, a series of closely spaced concentrations within the range of 1000 - 2000 mgl^{-1} NO_3-N were used for all three trials in the main study. Sodium nitrate was used as a source of nitrate in the test solution. For both small (mean weight 134 mg,SE $\pm$ 6.8) and medium (mean weight 452 mg SE $\pm$ 28.6) sized fry, each concentration was tested in duplicate; a single control test was conducted. Due to a shortage of large fish, only a single test was possible for large sized fry at each nitrate concentration. A single control test was again performed. 24, 26 and 10 fish were

used per tank for small, medium and large sized fry respectively.

All tests were conducted for 168 hours; observations were made on the incidence of feeding and delayed mortality in each test tank.

Measurement and analysis of water quality of the test water were carried out as described in section 2.2.2.5. The temperature, dissolved oxygen and pH of test solutions were measured everyday. Total hardness and total alkalinity were measured at least two to three times during each trial. Nitrate concentration was not measured during the trial as such high levels of NO_3-N concentration (1000 - 2000 mgl^{-1} NO_3-N) used in the bioassay were not expected to vary significantly. Therefore, calculated concentrations of NO_3-N were used in the analysis of data. The experimental data were analysed according to methods described in section 3.2.2.6.

3.4.3. Results

The results of the nitrate toxicity bioassy for small, medium and large sized carp fry are presented in Table 3.37. The median lethal concentrations for nitrate, and their 95% confidence limits, for various exposure time are presented in Table 3.38 for the three sizes of carp fry tested. The 96-h LC50's for small and medium sized fry were 1565 (95% CL 1504-1628) and 1895 (95% CL 1842-1950) mgl^{-1} NO_3-N respectively. The 96-h LC50 for large sized fry could not be calculated due to insufficient mortality during that

Table 3.37 Results of the nitrate toxicity bioassay for three sizes of carp fry*

Weight (mg) Mean (± S.E.)	Conc. range tested ($mgl^{-1}NO_3$-N)	Dissolved oxygen (mgl^{-1}) Mean (range)	pH Mean (range)	LC50 (95% CL) ($mgl^{-1}NO_3$-N) 96-h	168-h
134(± 6.8)	1000 - 2000	7.25 (7.00 - 7.40)	7.74 (7.63 - 7.87)	1565^{a}** (1504 - 1628)	1337^{a} (1274 - 1404)
452(± 28.6)	1000 - 2000	7.20 (7.00 - 7.40)	7.68 (7.58 - 7.83)	1895^{b} (1842 - 1950)	1620^{b} (1571 - 1671)
2281(± 230)	1000 - 2000	7.00 (6.70 - 7.30)	7.70 (7.60 - 7.83)	N.C.	1844^{c} (1794 - 1895)

* Other measured water quality variables were: temperature 27.5 - 28.5 °C; total alkalinity 40 - 52 mgl^{-1} as $CaCO_3$; total hardness 47 - 55 mgl^{-1} as $CaCO_3$

** Figures in the same colum with different superscripts are significantly different ($P<0.05$)

N.C. Not calculable

Table 3.38 Median lethal concentrations and 95% confidence limits for three sizes of carp fry exposed to nitrate - nitrogen for various time period

Exposure time	Mean weight 134mg(S.E.±6.8)		Mean weight 452(S.E.±28.6)		Mean weight 2281mg(S.E.±230)	
	LC 50 (mgl^{-1} NO_3-N)	95% CL	LC 50 (mgl^{-1} NO_3-N)	95% CL	LC50 (mgl^{-1} NO_3-N)	95% CL
48	1999^{a*}	1544-2590	-	-	-	-
72	1766^{a}	1644-1896	1953^{a}	1856-2056	-	-
96	1565^{b}	1504-1628	1895^{ab}	1842-1950	-	-
120	1461^{c}	1400-1526	1841^{b}	1803-1880	-	-
144	1397^{cd}	1332-1465	1751^{c}	1697-1806	-	-
168	1337^{d}	1274-1404	1620^{d}	1571-1671	1844	1794-1895

* Figures in the same column with same superscripts are not significantly different (P> 0.05)

period even at the highest concentration tested. The 168-h LC50's for small, medium and large sized fry were 1337 (95% CL 1274-1404), 1620(1571-1671) and 1844 (1794-1895) mgl^{-1} NO_3-N respectively.

The LC50's for various exposure times for small and medium sized carp fry are shown graphically in Fig. 3.11. A toxicity curve for the large sized fry could not be drawn due to insufficient data (Table 3.38). The acute toxicity of nitrate for both small and medium sized fry had not ceased within 168 hours of the experimental period as shown by the toxicity curves (Fig. 3.11). Toxicity curves for both groups are linear and no indication of a threshold value for toxicity was observed. The 96-h LC50's of small and medium sized fry and 168-h LC50's of all these groups were significantly different ($P<0.05$) with smaller fish having lower LC50's than the larger ones. However, no significant relationship between median lethal concentration and fish size could be established due to the limited number of trials conducted.

In all these trials the fish seemed to be sluggish, lying on the bottom of the tanks from the second day of exposure and appeared to be stressed. For small sized fry, fish were stressed even at the minimum concentration (1000 mgl^{-1} NO_3-N) used in the trial. For medium sized fry, fish seemed stressed at concentrations of 1500 mgl^{-1} NO_3-N and more and for large sized fry at 1750 mgl^{-1} NO_3-N and above during the experimental period.

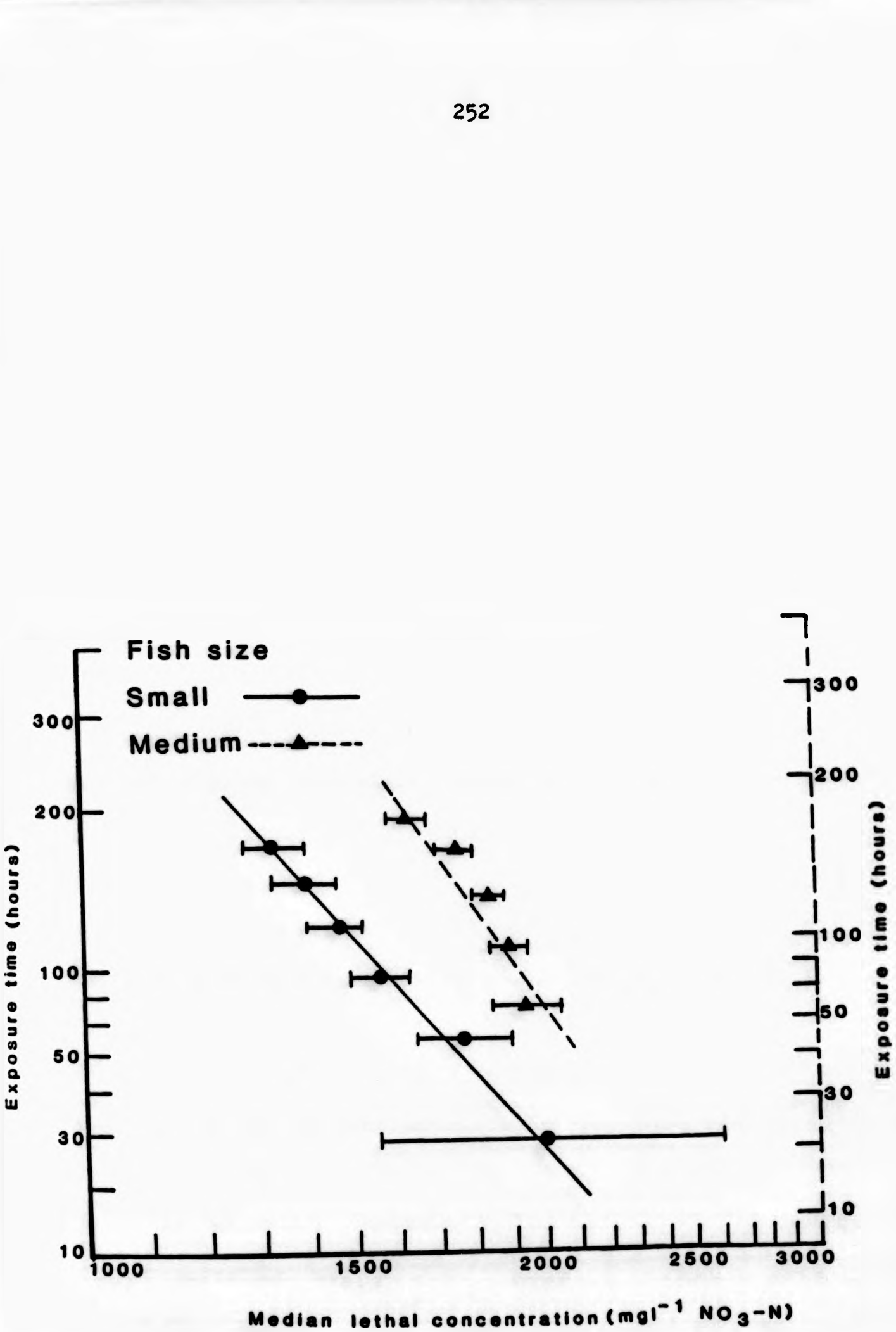


Fig. 3.11 Toxicity curves of nitrate-nitrogen for small and medium size carp fry. Bars indicate 95% confidence limit.

Observations on the incidence of feeding and delayed mortality were recorded after terminating the tests and are presented in Table 3.39. For small sized fry no incidence of feeding was observed. The fry were so stressed that they were unable to feed. For medium sized fry, some degree of feeding was recorded, but the incidence of feeding (1.2 percentage of fish observed to feed) was inversely proportional to the concentration of nitrate to which they were previously exposed. For example, none of the fish exposed to 1750 mgl^{-1} NO_3-N were observed feeding, whereas feeding was shown by 73% of fish exposed to 1000 mgl^{-1} NO_3-N.

For large sized fry visual observation of incidence of feeding was recorded and no microscopic examination of gut was done since the same fish had to be used for determining delayed mortality. Active feeding was recorded at all concentrations except a few fish exposed to concentations of 1500 and 1750 mgl^{-1} NO_3-N, where incidence of feeding were 80 and 60% respectively (Table 3.39).

38-50% delayed mortality were recorded for small sized fry with exception of no delayed mortality for fish previously exposed 1500 mgl^{-1} NO_3-N (Table 3.39). For medium and large sized fry little or no (0-7%) delayed mortality was recorded except that 40% delayed mortality was recorded at large sized fry of those exposed to 1750 mgl^{-1} NO_3-N.

Table 3.39 Incidence of feeding and delayed mortality of three sizes of carp fry after 168-h exposure to test levels of nitrate

Conc. of NO_3 (mgl^{-1} NO_3-N)	Incidence of feeding (%fish fed)			Delayed mortality after 4 days(% mortality)		
	134mg fry	452mg fry	2281mg fry	134mg fry	452mg fry	2281mg fry
Control	83	100	100	8	0	0
1000	5	73	100	50	0	0
1250	0	56	100	38	7	0
1500	0	21	80	0	0	0
1750	-*	0	60	-*	0	40

* No surviving fish left after 168-h exposure

3.4.4. Discussion

All three sizes of carp fry used in the present investigation proved to be tolerant to very high nitrate levels. However, larger sized fry showed a higher tolerance to nitrate than smaller fry. The 168-h LC50's for carp fry ranged between 1337-1844 mgl^{-1} NO_3-N, values comparable to the lethal concentrations reported for bluegills, channel catfish, chinook salmon and rainbow trout (See Table 3.36). For both small and medium sized fry lethal threshold concentration had not been reached within 168 hours which is contrary to the observations made by Colt and Tchobanoglous (1976) for channel catfish and Rubin and Elmaraghy (1977) for guppy fry. In both of the above cases the lethal threshold concentration was obtained within 96 hours of exposure. However, Rubin and Elmaraghy (1977) used potassium nitrate as a source of nitrate as opposed to the more commonly used sodium nitrate. The toxicity of nitrate depends greatly on the cationic composition of the solution. Potassium nitrate solutions are reported to be more toxic than sodium nitrate solutions (Trama, 1954, cited by Westin, 1974; Dowden and Bennet, 1964) and consequently the lethal threshold concentrations for nitrate obtained by Rubin and Elmaraghy (1977) were probably a function of the toxicity of potassium as well as nitrate. However, as in the present investigation, Westin (1974) observed significant differences between the 96 and 168-h LC50 values for rainbow trout and chinook salmon, which indicate threshold concentrations might not have been reached within the 168h exposure period.

Observation on the incidence of feeding indicates that small fry were greatly stressed and unable to feed even at the lowest concentration used. Observation of delayed mortality demonstrates that large numbers of the stressed fish did not recover and eventually died. For medium sized fry some degree of feeding was recorded. However, the incidence of feeding was proportional to the concentration of nitrate solution to which they were exposed. At a concentration of 1500 mgl^{-1} only 21.4% of fish was found feeding while, at a concentration of 1750 mgl^{-1} no fish were observed to feed. For larger fish, 40% of those exposed to a concentration of 1750 mgl^{-1}, died during the period of observation for delayed mortality. Therefore it may be concluded that a significant number of fish would have died had the exposure time extended beyond 168 hours - i.e. damage was deep rooted and permanent. It is further probable that even the lowest concentration used would have been lethal (1000 gml^{-1} NO_3-N) for small sized carp fry had the exposure period been longer. The acute behavioural signs of carp fry exposed to lethal concentrations of nitrate are similar to those reported by Westin (1974), for chinook salmon and rainbow trout. These include an inability to swim upright, laboured respiration, and erratic swimming alternated with periods of little movement.

The 168-h LC50 for carp fry ranged between 1337-1844 mgl^{-1} NO_3-N. Because nitrate was added in the form of sodium nitrate, these values can be multiplied by 6.07 to obtain the total solute concentration of the test solutions, i.e. 8.11-11.19g $NaNO_3$/1.

Therefore, at these high levels, the toxicity of $NaNO_3$ may be due in part to the Na^+ level (Colt and Armstrong, 1981). However, 8.1 to 11.2g $NaNO_3$/1, which is equivalent to a salinity of 8-11 ppt is very high for freshwater fish. Grabda *et al*. (1974) found that low levels of nitrate (5-6mgl^{-1} NO_3-N) caused a significant increase in the methaemoglobin content of the blood of rainbow trout. Nevertheless, such a high tolerance and the slow death recorded in the lethal concentrations demonstrates that the toxicity of nitrate to carp fry are probably related to the failure of the animal to maintain osmoregulation as postulated by Colt and Tchobanoglous (1976), Brownell (1980) and Colt and Armstrong (1981).

The conclusions derived from this study are that a nitrate concentration of 1000 mgl^{-1} NO_3-N or 6.1gl^{-1} $NaNO_3$ is lethal to carp fry for a relatively short exposure period (168 hrs).

In natural water, under normal conditions nitrate (concentration ranging between <1-6mgl^{-1} NO_3-N) do not constitute a problem for aquatic animals, where the dynamics of the nitrogen cycle maintain a balance between different nitrogenous compounds. Nitrate level in recirculated water systems vary widely. High levels of NO_3 (>100mgl^{-1} NO_3-N) may build up in an aged recirculation system. However, periodic changes of water can prevent accumulation of nitrate. Therefore a concentration as high as 1000 mgl^{-1} need not occur in a recirculation system. The toxicity of nitrate, therefore, should pose no hazard to carp fry in a recirculatory hatchery or nursery system.

3.5 Experiment 3.4

Determination of the upper incipient lethal temperature for carp fry acclimated to different rearing temperatures

3.5.1. Introduction

Lethal temperature is known to be greatly influenced by the acclimation temperature or the previous thermal history of the species in question. Acclimation is the physiological adaptation of an animal to some selected environmental condition including any adverse stimulus involved (Alabaster and Lloyd, 1980). Temperature may act as a lethal factor as well as constantly conditioning the fish through acclimation while influencing the scope of the metabolic rate (Brett, 1956). Since acclimation temperature has a profound influence on the lethal temperature limits of fish, lethal temperature should always be defined in terms of its acclimation temperature. Such acclimation temperature may be fixed or cyclical depending on the circumstances. However, a common practice is to maintain fish at a given constant temperature for acclimation.

For any particular acclimation temperature every species of fish has a temperature range within which they can survive for an indefinite period. The range has an upper and a lower limit, the thermal death point or incipient lethal temperature. The incipient lethal temperature has been defined by Fry et al (1946) as that level at which 50% of the population is dead after indefinite exposure. As long as the resistance times continue to be finite, the fish are considered to be in a zone of resistance and beyond this lies the zone of tolerance (Brett, 1952). Above the incipient lethal temperature the resistance time shortens with progressive rise or fall in temperature until a point is reached at which the animal

is killed instantaneously (Jones, 1964). This is often termed the zone of instantaneous death (Cocking, 1959). Typically most fish show an increase of about 1°C in their upper incipient lethal temperature for about a 3°C change in their acclimation temperature (Fry et al., 1942; Brett, 1944; Doudoroff, 1942; 1945; Cocking, 1959). Thus the lethal temperature limit of fish may be extended to a certain extent by increasing the acclimation temperature. The temperature beyond which no increase in lethal temperature results following an increase in acclimation temperature is called the ultimate upper incipient lethal temperature (Fry et al., 1946).

The incipient lethal temperature of fish is usually determined by a thermal shock method (Fry et al., 1942; 1946; Brett, 1952) which involves use of a series of water baths preset at different lethal temperatures. The test fish are subjected to each bath abruptly and the time to death for all fish is recorded. The lethal temperature of fish may also be determined by the critical thermal maximum (CTM) technique (Hutchinson, 1961) in which test fish are subjected to a steadily increasing temperature, e.g. one degree every one to a few minutes. The temperature at which a test fish dies, or is incapacitated or losses equilibrium, is recorded and the temperature at which 50 per cent of a sample of fish die or lose equilibrium is considered to be the critical thermal maximum.

Although for CTM determination, the most commonly used rate of heating is $1^{0}C$ per minute, this has not been universally adopted. Because various heating rates have been used in determinating CTM, there is considerable variation in the values of CTM for given species (Jobling, 1981b). Moreover, death from extremes of temperature is not just dependent on the threshold level below or above which an organism either lives or dies, but may be considered resultant of both temperature and exposure time (Brett, 1952). In CTM, extremes of temperature has been used as the only factor to determine the lethal level, the effect of exposure time has not been given adequate consideration. Fry (1971) pointed out that in practice when rates of heating are of the order of minutes per degree change, only the most acute cause of death will be displayed and there is no time for resultant effect of temperature and exposure time. Similarly Hodson et al (1981) observed that once the critical temperature is reached, it may take several minutes for the fish to completely succumb; thus, the fish appears to be more tolerant than they actually are.

Conversely the thermal shock method integrates time as a factor enabling the full effects of a specific temperature to become manifest in the test temperature (Hodson et al., 1981). Therefore, as pointed out by Becker and Genoway (1979) and Jobling(1981b), values of CTM are likely to be of value in assessing temperature tolerances of fish subjected to abrupt changes in water temperature induced by periodic thermal discharges, whereas when fish are subjected to long term temperature changes, determination of upper incipient lethal temperature appears to be more realistic.

Table 3.40 A summary of lethal temperatures of some commercially important fish species expressed as upper incipient lethal temperature or, when marked with asterisk (*) as critical thermal maxima

Species	Size/age (Weight/length)	Acclimation temp. (°C)	Lethal temp (°C)	Author
Rainbow trout (Salmo gairdneri)	-	20	25.8	Charlon et al(1970, cited by Alabaster & Lloyd 1980)
	-	24	26.3	Charlon et al (1970, cited by Alabaster & Lloyd 1980)
Brown trout Salmo trutta	-	20	26.4	Alabaster & Downing (1966)
Speckled trout Salvenilus fontinalis	2 - 24g	20,24 & 25	25.3(UULT)**	Fry et al (1946)
Chum salmon Oncorhynchus keta	1.6g	23	23.8(UULT)	Brett (1952)
Coho salmon O. kisutch	1.4g	20 & 23	25.0(UULT)	Brett (1952)
Sockeye salmon O. nerka	0.9g	23	24.3(UULT)	Brett (1952)
Spring salmon	1.0g	15.0	25.0	Brett (1952)
O. tshawtyscha	1.0g	20 & 24	25.1(UULT)	Brett (1952)
White fish Coregonus clupeoformis	Young of the year 3.8-5.2cm	20 & 25	26.65(UULT)	Edsall & Rottiers (1976)

Table 3.40(contd.) A summary of lethal temperatures of some commercially important fish species expressed as upper incipient lethal temperature or, when marked with asterick(*) as critical thermal maxima

Species	Size/age (Weight/length)	Acclimation temp. (°C)	Lethal temp. (°C)	Author
Alewife *Alosa pseudoharengus*	Young of the year	20	30.3	Otto *et al* (1976)
	Young of the year	25	32.1	Otto *et al* (1976)
	Adult	20	24.5	Otto *et al* (1976)
	Adult	27	28.2	McCauley & Binkowski (1982)
	Adult	30	31 - 34(UULT)	McCauley & Binkowski (1982)
Bream *Abramis brama*	-	20	30.2	Alabaster & Downing (1966)
Goldfish *Carassius auratus*	Young	32	39.2	Fry *et al* (1942)
	Young	38	41.0(UULT)	Fry *et al* (1942)
Golden carp *Carassius carassius*	3.5-8.5cm	26.5-27.8	38.3-38.7*	Horoszewicz (1973)
Grass carp *Ctenopharyngodon idella*	Fry (1.8-2.9g)	22.5	41*	Opuszynski (1967)
	Yearling(47-48g)	20.4	36 - 37*	Opuszyuski (1967)
Largemouth bass *Micropterus salmoides*	52.2g	20-21	28.9	Black (1953)
Major carp *Catla catla*	Fry(2.5-3.5cm)	22	36	Balani (1973)

Table 3.40(contd). A summary of lethal temperatures of some commercially important fish species expressed as upper incipient lethal temperature or, when marked with asterisk (*) as critical thermal maxima.

Species	Size/age (Weight/length)	Acclimation temp. (°C)	Lethal temp (°C)	Author
Mullet *Mugil cephalus*	18g	21	27-29.6	Sylvester *et al* (1974)
Northern Black catfish *Ameiurus melas melas*	51.9g	23	35	Black (1953)
Roach	2-15g	26	31	Cocking (1959)
Rutilus rutilus	2-15g	30	32.5	Cocking (1959)
	2-15g	32,33	33.5(UULT)	Cocking (1959)
Silver carp *Hypophthalmichthys molitrix*	Fry (1.3-2.0g)	22.5	37-40*	Opuszynski (1967)
	Yearling (11 - 23g)	20.4	34-36*	Opuszynski (1967)
Spot *Leiostomus xanthurus*	Juvenile 3.9-4.9cm (standard length)	25-35	35.2 (UULT)	Hodson *et al* (1981)
Tilapia *Tilapia zilli*	7.3cm	20-29	40.2*	Hauser (1977)
Yellow perch *Perca flavescens*	8.6g	18	26.5	Black (1953)
	9.8g	22-24	29.2	Black(1953)

** Ultimate upper lethal temp.

Lethal temperature values vary markedly between different fish species (Table 3.40). The tropical and warmwater fish species tend to show higher upper incipient lethal temperature compared to temperate species. Young goldfish, Carassius auratus,are reported to be the most eurythermal fish known (ultimate upper incipient lethal temperature 41^0C) (Fry et al., 1942) and chum salmon, Oncorhynchus keta the most stenothermal fish known (ultimate upper incipient lethal temperature 23.8^0C) (Brett, 1952). However, the variations in lethal temperature between and within fish species may sometimes be attributable to the different test methods used or due to the variations in the temperatures to which they were previously acclimated.

Considerable amounts of published information are available on the temperature tolerance of common carp fry and fingerlings(Table 3.41). However, no upper incipient lethal temperatures are available for common carp acclimated to temperature below 20^0C and above 28^0C.

On the basis of the information discussed above the present investigation was conducted to determine the upper incipient lethal temperature of carp fry at different acclimation temperatures ranging from 16 to 34^0C.

Table 3.41 A summary of available temperature tolerance data of common carp

Size (weight/length)	Acclimation temp. (° C)	Test method used	Lethal temp. (° C)	Author
12.8g	20	TSM*	31 - 34	Black (1953)
28.2g	26	TSM	35.7	Black (1953)
Fry	-	-	35.1 - 36.3	Shkorbatov (1954, cited by Opuszynski, 1967)
Fry 0.5 - 1.3g	22.5	CTM**	38 - 39	Opuszynski (1967)
Fry 1.7 - 7.2 cm length	24.5 - 28	CTM	40.2 - 40.9	Horoszewicz (1973)

* Thermal shock method

** Critical thermal maxima

3.5.2. Materials and Method

3.5.2.1. Experimental animals and acclimation

The source of the experimental animals and their quarantine procedures were the same as those described in section 2.2.2.1.2.

Prior to exposing the carp fry to acclimation temperature, they were maintained at 28°C in a 1501 stocking tanks (see section 3.2.2.1 for detail).

A static experimental system similar to that used for the nitrite and nitrate toxicity trials was used to determine the temperature tolerance. Details of the static water system are given in 3.3.2.1.

3.5.2.2. Acclimation to different rearing temperatures

Five trials were conducted to determine the incipient upper lethal temperature of carp fry acclimated to different rearing temperatures. The acclimation temperatures used were 16, 20, 25, 28 and 34°C; these temperatures were controlled to within $\pm$0.5°C. Carp fry were acclimated to a particular rearing temperature for a specific time period (15-20 days) before sujecting them to the test temperatures. For acclimation to the upper temperature of 34°C,

the tank water temperature was increased at a rate of 1°C per day until the desired acclimation level was reached. The acclimation period was at least 15 days. For acclimation to the lower temperatures (25, 20 and 16°C). the temperature was decreased at a rate of 0.5°C per day until the desired acclimation level was reached. Acclimation to lower temperature is much slower than upward acclimation(Brett, 1952). Therefore for downward acclimation, temperature lowering of 0.5°C per day was used instead of 1°C per day. Acclimation for 15-30 days was allowed before the test.

801 glass aquaria were used as acclimation tanks. These were serviced by an 'Eheim' combined water pump and filter through which water was continuously circulated in order to maintain good water quality. The desired water temperature was maintained by a 'Nimrod' 200W microtronic thermostatic heater (R. Aitchisens Ltd., Edinburgh) with a dial temperature control. Stone aerators connected to a compressed air supply were used to maintain dissolved oxygen concentrations near saturation. During the holding and acclimation period the test fish were fed with a ground commercial trout pellet (see section 3.2.2.1. for details). All the acclimation tanks were kept in a constant temperature room maintained at 16°C, except for the acclimation tanks at 25°C, which were kept in he Tropical Aquarium Building. A photoperiod of 12 hours light: 12 hours dark was maintained in both constant temperature room and Tropical Aquarium. The pH of the acclimation water was within the range of 6.90 - 8.00. However, the range of variation

in pH was similar in all acclimation tanks and therefore this apparent large variation did not influence any of the treatments unequally.

3.5.2.3. Test Water

Synthetic dilution water was used as the test water (see Section 3.2.2.3 for details).

3.5.2.4. Experimental procedure

a) Selection of test method

The thermal shock method (Fry et al., 1942; 1946; Brett, 1952) was used to determine the upper lethal temperature for carp fry. It involves use of a series of baths preset at different test temperatures (32.5 - 41.4°C). The test fish are subjected to each test temperature abruptly and the time to death for individual fish is recorded.

Carp fry were transferred abruptly from their acclimation tank to the preset temperature baths. They were starved for 24 hours while in the acclimation tank before subjecting them to test temperatures. The time to death for each fish was recorded to the nearest minute during the first six-hour period and thereafter mortality was recorded regularly at intervals of 3-6 hours. Additional observations were made whenever possible. Cessation of opercular

movements by fish was used as the criterion for death as recommended by Cocking (1959). Fish which died during the test, and the survivors, were weighed individually after each test.

b) Test temperatures used.

The fish from each acclimation temperature were subjected to seven or eight test temperatures and one control temperature. Test temperatures were selected on the basis of preliminary trials conducted for each acclimation temperature. As a control for each trial, one group of fish was placed in a test tank at the same temperature at which they were acclimated. Test temperatures were spaced at 0.5°C as recommended by Brett (1952). Temperatures in the experimental tanks were selected to cover a range that would cause rapid, complete mortality in some groups and slow and incomplete mortality in others. A single test was used for each temperature and 20 fish were used per test temperature in all the trials.

c) Duration of the tests

Each trial was conducted for a period of 96 hours to determine the incipient lethal temperature. In several preliminary trials it was observed that most of the mortalities occurred within a period of 12 to 24 hours. As a result it was decided that a 96-h exposure period would be adequate for lethal temperature determination for carp fry.

Moreover, in most of the previous temperature tolerance studies, the duration of the exposure period used was 96 hours or less (Fry et al., 1942; 1946; Cocking, 1959; Symons et al., 1976; Hodson et al., 1981).

d) Incidence of feeding and delayed mortality

Incidence of feeding and delayed mortality was observed in all trials as described in section 3.2.2.4. Feeding incidence was determined only by visual observation. Active feeding was observed in all the surviving fish at all test temperatures and no delayed mortality was recorded in any of the tests.

3.5.2.5. Measurement and analysis of water quality

The physical and chemical characteristics of the test water were measured as described in section 2.2.2.6. The temperature and dissolved oxygen concentration were measured daily in each test tank; pH was measured at least once during the trial period. Specific temperature of test water was maintained with maximum variation of $\pm 0.1^{o}C$. pH of test water was within the range of 7.55 to 8.03 and dissolved oxygen concentration was maintained near saturation in all test tanks.

3.5.2.6. Analysis of experimental data

Median survival time at each test temperature was estimated from the geometric mean of individual survival time after all experimental animals had died. This procedure is recommended by Fry et al. (1946) and has been used by several authors (Brett, 1952; Gibson, 1954; Cocking, 1959; McCauley and Binkowski, 1982). In cases where some of the fish survived after exposure to test temperature or where the exact time of death of some fish was not recorded, the median survival time at each test temperature was estimated graphically by the method of Litchfield (1949). Using this method of analysis, the percentage mortality resulting from a particular test temperature is plotted in probability units and the time to death (survival time) in log units. In this way, a linear progression of points is obtained. A line is fitted by eye through the series of points for each test temperature. The median survival time at each test temperature is read directly at the probit 5.0 (=probability 50%) level.

The application of time-mortality curves originally proposed by Bliss (1937) and modified by Litchfield (1949) has been widely used and found to be quite appropriate in lethal temperature experiments with fish by several authors (Fry et al., 1946; Brett, 1952; Gibson, 1954; Otto et al.,1976; Hodson et al., 1981). For each acclimation temperature, a series of median survival times was plotted on a logarithmic scale against the corresponding test temperatures (linear scale). Straight lines were fitted to the

data points by the least squares method of linear regression as recommended by Fry et al. (1946). A series of lines were drawn for different acclimation temperatures. These straight lines were terminated at temperatures below which there was less than 50% mortality.

Incipient lethal temperature values at different level of thermal acclimation and their 95% confidence limits were calculated by the trimmed Spearman-Karber method (Hamilton et al.,1977). Tests of significance were carried out between LC50 values using the method of APHA et al. (1980).

3.5.3. Results

The percentage survival of carp fry at different test temperatures following their acclimation at 16, 20, 25, 28 and 34°C are presented in Table 3.42. The median survival (resistance) time at different test temperatures for carp fry acclimated to different temperatures are presented in Table 3.43. A series of resistance curves drawn by plotting median survival time on a logarithmic scale against the corresponding test temperatures for each level of thermal acclimation are presented in Fig. 3.12. These resistance curves were extrapolated to the Y-axis and the temperature at which instantaneous death would result at each thermal acclimation were obtained from the points at which the line would intersect Y-axis. Equations of the straight lines describing the relationship between median survival time and the test temperature at each acclimation temperature are given in Table 3.44 and the temperatures for instantaneaous death at each acclimation temperature are given in Table 3.45.

For any acclimation temperature, the survival time decreased as the test temperature increased (Table 3.43). For example, at 16°C acclimation temp., the median survival time at 33.5°C was 110 minutes, whereas, at 35.5, it was only 2.5 minutes. Similarly, at an acclimation temp., of 34°C the median survival time was 525 minutes at 39°C test temp., whereas it was only 12 minutes at 41.4°C. The resistance to any given test temperatures also

Table 3.42 Percentage survival at different test temperatures for carp fry acclimated to different temperatures.

Test temperature (°C)	Acclimation temperature (°C) and survival (%) at the test temperature				
	16	20	25	28	34
32.5	100	100	-	-	-
33.0	80	80	-	-	-
33.5	40	85	-	-	-
34.0	20	65	-	-	-
34.5	5	60	-	-	-
35.0	0	35	-	-	-
35.5	0	5	100	-	-
36.0	-	0	85	-	-
36.5	-	-	75	100	-
37.0	-	-	50	85	-
37.5	-	-	5	90	-
38.0	-	-	0	0	100
38.5	-	-	0	-	80
38.6	-	-	-	0	-
39.0	-	-	0	0	20
39.5	-	-	-	0	0
40.0	-	-	-	-	0
40.1	-	-	-	0	-
40.5	-	-	-	-	0
41.0	-	-	-	-	0
41.4	-	-	-	-	0

Table 3.43 Median survival time at different test temperatures for carp fry acclimated to different temperatures

Test temperature (°C)	Acclimation temperature (°C) and median survival time (minutes) at the test temperature.				
	16	20	25	28	34
33.5	110*	-	-	-	-
34.0	47*	-	-	-	-
34.5	19*	-	-	-	-
35.0	7	120*	-	-	-
35.5	2.5	7*	-	-	-
36.0	-	4	-	-	-
36.5	-	-	-	-	-
37.0	-	-	400*	-	-
37.5	-	-	175*	-	-
38.0	-	-	32	605*	-
38.5	-	-	20	-	-
38.6	-	-	-	77	-
39.0	-	-	7	60	525*
39.5	-	-	-	27	111*
40.0	-	-	-	-	99
40.1	-	-	-	12	-
40.5	-	-	-	-	29
41.0	-	-	-	-	17
41.4	-	-	-	-	12

* Estimated from log-probit analysis

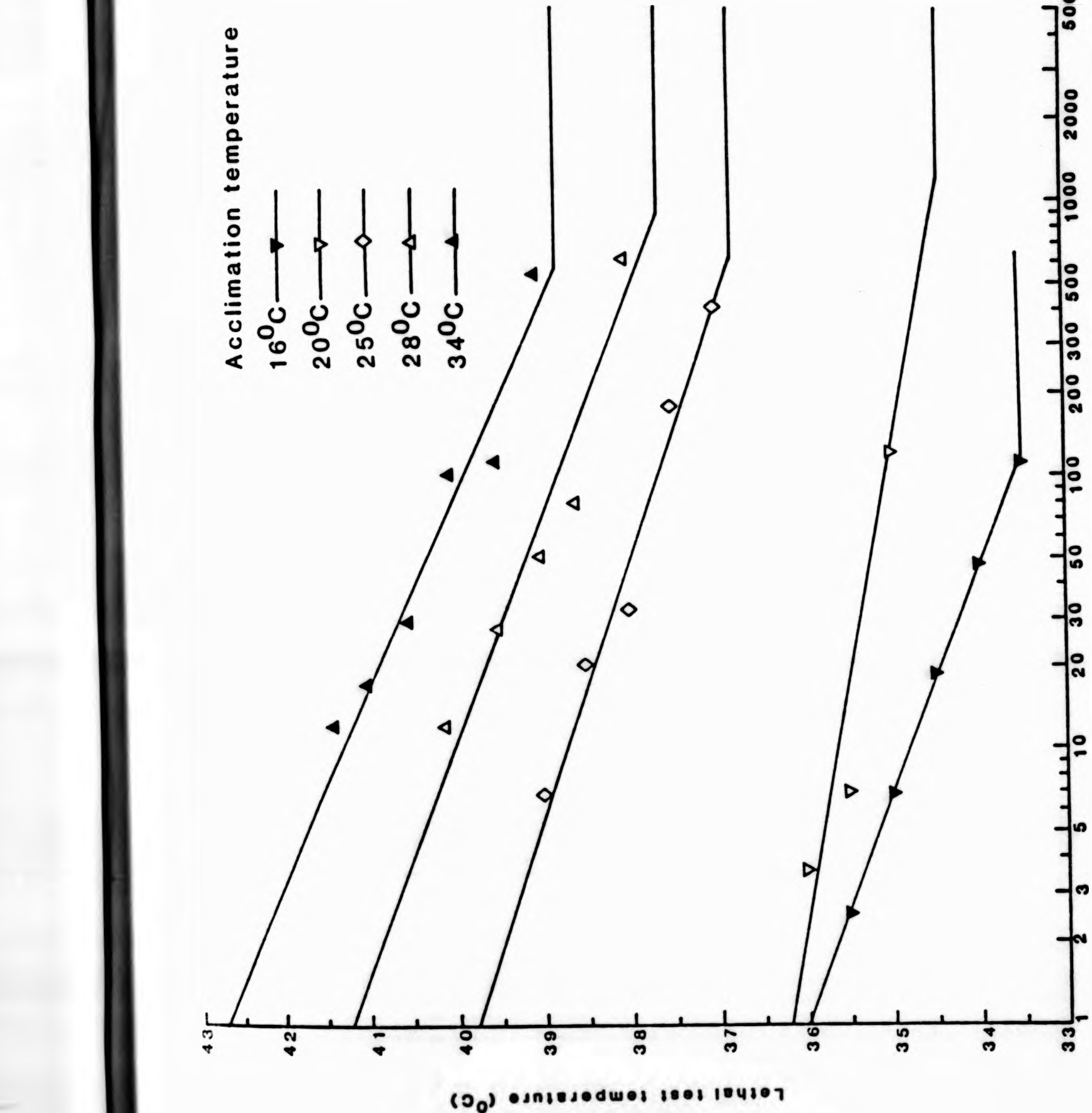


Fig. 3.12 Median survival times for various temperatures in the lethal range plotted for various levels of thermal acclimation

Table 3.44 Linear regression equations describing relationships between test temperature (y) and loge median survival time (x) at different acclimation temperatures.

Acclimation temperature (°C)	Regression equation
16	$y = 36.0 - 0.525\ x$ ($r = -0.999$; d.f. = 3; $P<0.001$)
20	$y = 36.2 - 0.256\ x$ ($r = -0.932$; d.f. = 1; $P>0.1$)
25	$y = 39.8 - 0.472\ x$ ($r = -0.988$; d.f. = 3; $P<0.01$)
28	$y = 41.2 - 0.530\ x$ ($r = -0.967$; d.f. = 3; $P<0.01$)
34	$y = 42.7 - 0.623\ x$ ($r = -0.976$; d.f. = 4; $P<0.001$)

increased with a rise in acclimation temperature. For example, at 35°C the median survival time was 7 minutes for carp fry acclimated to 16°C, whereas at the same test temperature the median survival time was 120 minutes with a rise of acclimation temperature to 20°C. Similarly, at 28°C acclimation temperature the median survival time was 60 minutes when exposed to a test temperature of 39°C whereas it was 525 minutes when fish were acclimated to 34°C.

The lethal test temperature had a marked effect on the carp fry as demonstrated by the highly significant (P 0.01) negative correlations between test temperature and $\log_e$ median survival time at all levels of thermal acclimation, except at 20°C. (Table 3.44). For acclimation at 20°C the relationship was not significant (P 0.1). However, even at this acclimation, the percentage of mortality and median survival times at different test temperatures were considerably different (Table 3.42 and 3.43). For example the median survival time was 120 minutes at 35°C compared to 7 minutes at 35.5°C. Therefore the reason for this insignificant relationship may be explained by the lower number of observations (n = 3) available in this case. In Fig. 3.12 the lower ends of the regression lines (resistance curves) were terminated abruptly by boundary lines. The regression lines do not carry much meaning beyond these boundaries because in the temperature range beyond them, 50% mortality does not occur no matter how prolonged the exposure may be. This shows that temperature induced mortality had ceased. The boundary lines thus separate the zone of resistance from the zone of tolerance, and delineate

Table 3.45 Upper incipient lethal temperatures and temperatures of instantaneous death for carp fry acclimated to different temperatures.

Weight of fry (mg) Mean (± SE)	Acclimation temperature (°C)	Upper incipient lethal temp. (°C)	95% CL	Temperature of instantaneous death (°C)
404(±12.1)	16	33.5*	33.3-33.6	36.0
412(± 9.8)	20	34.4	34.2-34.6	36.2
381(±10.5)	25	36.8	36.6-37.0	39.8
355(±10.2)	28	37.6	37.5-37.7	41.2
278(±14.6)	34	38.8	38.6-38.9	42.7

Incipient lethal temperature values are significantly different ($P < 0.05$) from each other

the upper incipient lethal temperatures for the various levels of thermal acclimation.

The upper incipient lethal temperatures (with 95% confidence limits) at different acclimation temperatures are presented in Table 3.45. The incipient lethal temperatures at acclimation temperatures of 16, 20, 25, 28 and 34°C are 33.5, 34.4, 36.8, 37.6 and 38.8°C respectively. The incipient lethal temperature values are significantly different ($P<0.05$) from each other. There is a highly significant positive correlation ($r = 0.983$; $df = 3$; $P<0.01$) between acclimation temperature and incipient lethal temperature. This linear relationship is best described by the equation: $y = 0.312x + 28.5$ (d.f. = 3; $P<0.01$) where, y = incipient lethal temperature and x = acclimation temperature.

From Table 3.45 it can be seen that there was an increase of about 1°C in the incipient lethal temperature for every 4°C. rise in acclimation temperature. However, there was a sharp increase of incipient lethal temperature (2.4°C) for a corresponding increase of 5°C in acclimation temperature (20 and 25°C). The upper incipient lethal temperatures at different levels of thermal acclimation are plotted in Fig. 3.13 to show the upper lethal temperature limits. The straight line was fitted to the data points by the linear regression equation given above. This line, which shows the upper temperature tolerance at different acclimation temperatures, demarks the zone of thermal tolerance from the zone of thermal resistance. The temperatures causing instantaneous death are plotted against the acclimation temperature and a straight

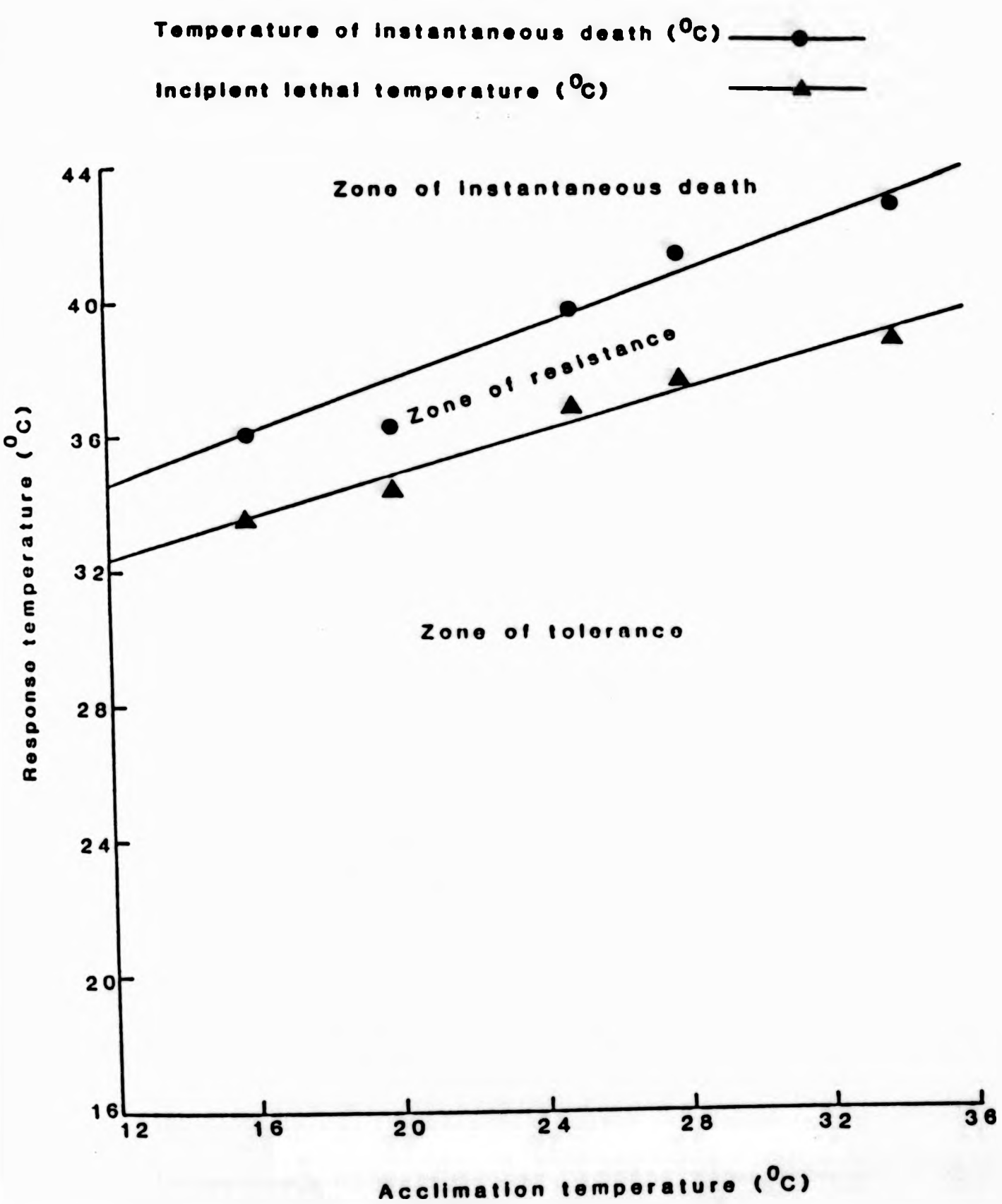


Fig. 3.13 Upper temperature limit for carp fry in relation to acclimation temperature.

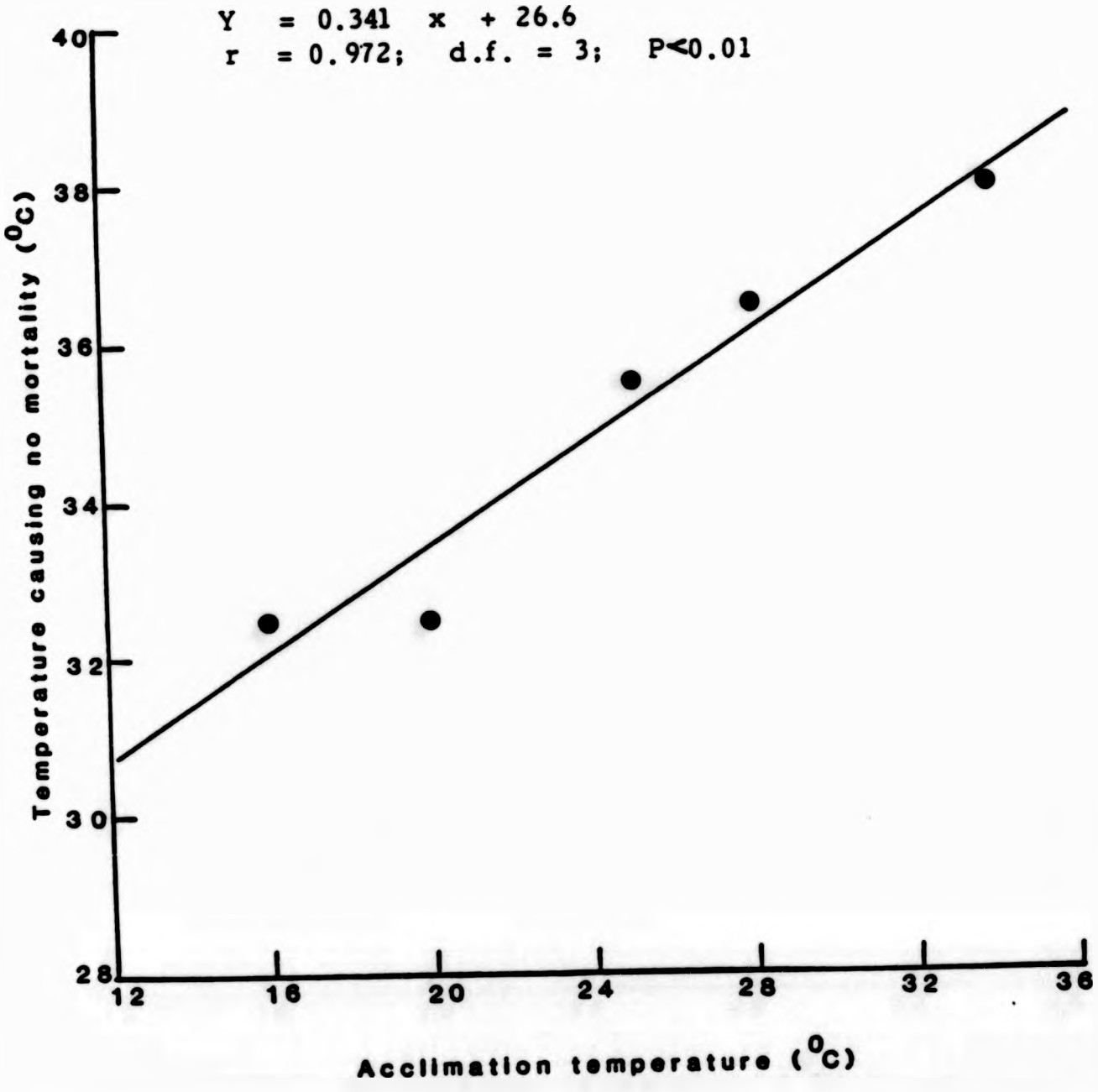


Fig. 3.14 Thermal tolerance limit of carp fry (up to which no mortality occurs) in relation to acclimation temperature.

line drawn by the least squares method of linear regression is shown which demarks the zone of thermal resistance from the zone of instantaneous death. A relationship between acclimation temperature and the test temperature which resulted in no mortality after continued exposure was also established using the data given in Table 3.42. This is shown graphically in Fig. 3.14. The relationship between these two is highly significant ($r = 0.972$; $d.f. = 3$; $P<0.01$) and the regression equation is : $y = 26.6 + 0.341\ x$ ($d.f. = 3$; $P<0.01$) where, y = temperature which caused no mortality after continued exposure and x = acclimation temperature.

3.5.4. Discussion

The results show that the upper incipient lethal temperature limit for carp fry is positively correlated with acclimation temperature. The lethal temperature limit increased by 1°C for every 4°C. rise in acclimation temperature. A significant positive relationship between lethal temperature and acclimation temperature has been reported for other fish species by several investigations (Fry et al., 1942; Brett, 1944; Doudoroff, 1942; 1945; Cocking, 1959; Edsall and Rottiers, 1976; Hodson et al., 1981). An increase of about 1°C in incipient lethal temperature for every 3°C increase in acclimation temperature has been reported for goldfish (Fry et al., 1942), green fish, Girella nigricans (Doudoroff, 1942), bullhead, Ameiurus nebulosus (Brett, 1944), Fundulus parvipinnis (Doudoroff, 1945) and roach (Cocking, 1959). Considerably higher increases in lethal temperature with acclimation temperature were found for lake whitefish, Coregonas clupeoformis (2.5°C increase for every 5°C incrase in acclimation temperture, Edsall and Rottiers, 1976) and spot, Leiostomus xanthurus (0.75°C increase for every 1°C in acclimation temperature, Hodson et al., 1981). However, a lack of marked change in the upper lethal temperature in response to changes in acclimation temperature was displayed in speckled trout, Salvenilus fontinalis (1°C increase for every 7°C increase in acclimation temperature, Fry et al., 1946) and in Pacific salmon, Oncorhynchus spp. (0.5 - 1.0°C for 5°C, Brett, 1952).

The incipient lethal temperature of carp fry ranged between 33.5 and 38.8°C at acclimation temperatures ranging from 16 to 34°C. These values are higher than those for salmonids and other temperate species (Table 3.40). However, these values are similar to the values reported for goldfish, golden carp, grass carp, major carp, black catfish, silver carp, spot and tilapia (Table 3.40). This comparison indicates that species belonging to the family cyprinidae are more tolerant to higher temperature than members of the other families.

A considerable number of investigations have been carried out to determine the lethal temperature of carp. However some of the information available is limited in its scope. Black (1953) reported the median lethal temperature (= incipient lethal temperature) of carp fingerling to be 31-34°C and 35.7°C at acclimation temperatures of 20 and 26°C respectively. These values are lower than incipient lethal temperature values obtained for carp fry in the present investigation at similar acclimation temperature (34.4°C at 20°C and 36.8°C at 25°C) (Table 3.45). These lower values may be attributable to the comparatively large size (6.9 - 64.6g) of fish used by Black compared to the small fish (0.38g) in the present investigation. Lethal temperatures do vary with size in some cases (Huntsman and Sparks, 1924, cited by Fry et al. 1946; Opuszynski, 1967; Hogman, 1971, cited by Edsall and Rottiers, 1976; Edsall and Rottiers, 1976). However, the low and wide range of lethal temperature values at 20°C reported by Black may also be attributable to the fact that fish acclimated to 20°C were kept at 11°C for 4-15 days before the trial was conducted;

therefore it is probable that a partial downward acclimation occurred during that period.

The critical thermal maxima values for carp fry reported by Opuszynski (1967) and Horoszewicz (1973) are 38-39^{o} and 40.2-40.9^{o}C at acclimation temperatures of 22.5 and 24.5-28^{o}C respectively (see Table 3.40), which are higher than the incipient lethal temperature values obtained in the present investigation. Critical thermal maxima values are always reported to be higher than incipient lethal temperature values (Fry, 1971; Hodson *et al.*, 1981) Moreover, Horoszewicz (1973) demonstrated that even the disturbing temperature (temperature at which animals show signs of increased activity or changes in behaviour) determined by CTM technique could be lethal for 100% fish, if they are exposed to the same temperature for a longer duration.

At all levels of acclimation and in most of the test temperatures used, the median survival time was less than 100 minutes (Table 3.43 and Fig. 3.12) which suggests that carp fry have a short zone of resistance. But the incipient lethal temperatures were higher (Table 3.45). Similar responses to lethal temperature have been noted in the case of young of the year alewife, *Alosa pseudoharengus* (Otto *et al.*, 1976), goldfish (Fry *et al.*, 1942) and roach (Cocking, 1959). In contrast, adult alewife (Otto *et al.*, 1976;McCauley and Binkowski, 1982) speckled trout (Fry *et al.*, 1946), Pacific salmon (Brett, 1952) have high zones of resistance and low incipient lethal temperatures.

There appear to be two main types of responses to heat resistance in fish: (a) a short zone of resistance and a high upper lethal limit, or (b) a wider zone of resistance and a low upper lethal limit. However, deviations from these general patterns have been reported. For example bullhead (Brett, 1944) and spot (Hodson et al., 1981) had normal zones of resistance with high incipient lethal temperature, and lake white fish had a short noze of resistance with a low incipient lethal temperture (Edsall and Rotiers, 1976).

Since carp fry had a high incipient lethal temperture, their zone of tolerance with respect to thermal acclimtion is fairly large. Therefore in nature carp fry would not be killed by a sudden rise in temperature, provided that the increase in temperature was not large enough to exceed the zone of tolerance for the particular acclimation temperature. The capacity of carp fry to withstand large temperature changes, is advantageous to their culture in shallow nursery ponds in tropical and subtropical regions. However, as the difference between their acclimation and lethal temperatures becomes progressively reduced with incrrease in acclimation temperature, carp fry would be more susceptible to a sharp rise in temperature if they are reared at higher temperature. Furthermore, carp fry have a short zone of resistance, so that a sudden rise in temperature close to their lethal level even for a short time would kill them. However, fish kills in nature are not always correlated with temperature, but may be due to the effects of temperature dependent facotrs (Cocking, 1959).

Fig. 3.14 shows the thermal tolerance limit of carp fry at or below which no mortality would occur even after continued exposure and may, therefore, be termed as the 'safe' zone. Delineation of this tolerance limit would be most useful from the practical aspects of fish culture. However, in this investigation, the effect of temperature has been evaluated only in the context of its lethal action and it should not be concluded that fully acclimated fish can live indefinitely at high sub-lethal temperatures The life span of a poikilotherm becomes progressively shorter as temperature increases beyond a specific range, since increasing temperature speeds their rate of metabolism (Fry, 1971).

CHAPTER 4 Experiment 4.1

Evaluation of some dietary ingredients commercially available in Bangladesh for the formulation of a complete carp fry diet

4.1. Introduction

Various oilseed cakes and meals are produced in Bangladesh on a large scale as by-products of the edible oils industry. They include mustard, linseed, sesame, groundnut and coconut. These oil cakes and meals are traditional and highly valued feeds for farm animals. Two other dietary ingredients for farm animals, rice bran and wheat bran are also available in large quantities in Bangladesh. These cereal by-products are moderately rich in protein, but are used primarily as dietary carbohydrate sources in animal feeds.

In recent years, intensification of carp fry production in Bangladesh has made it essential to develop suitable complete and supplemental diets for use in hatcheries and nursery ponds. Traditionally, fish meal has been the major source of dietary protein for fish fry elsewhere in the world. However, the use of fish meal for fry diets is not feasible in Bangladesh because it is not widely available in the country and is in any case prohibitively expensive. Therefore, it is a matter of urgency that alternate protein sources for carp fry diets are developed to boost the aquaculture industry.

The efficiency of various alternative protein sources has been evaluated as a partial or complete dietary replacement for fish meal in carp diets, e.g., soybean meal (Viola, 1975; Jauncey, 1979; Viola *et al.*, 1982), rapeseed meal (Dabrowski and Kozlowska, 1981),

leaf protein concentrate (Ogino et al., 1978), coffee pulp (Christensen, 1981) and single cell protein (Ohame et al., 1979; Sandbank and Hepher, 1978; Atack et al., 1979; Anwar et al., 1982). But information on the efficiency of mustard, linseed, sesame, groundnut and coconut (Copra) oil cake or meals as alternate protein sources and rice bran and wheat bran as carbohydrate sources in carp feeds is scanty. Nutritional evaluation of some of these ingredients (e.g. mustard and groundnut meal, rice bran and wheat bran) has been carried out by several researchers (Lakshmanan et al., 1966; Chakrabarty et al., 1973, Sharma and Kulshreshtha, 1975; Chowdhary et al., 1978; 1978; Capper et al., 1982). However, with one exception (Capper et al., 1982), all of these studies have been conducted in earthern rearing ponds where natural food was available to the fish. Therefore from the results it is impossible to evaluate the contribution of these ingredients to the observed fish production. Besides, a proportion of these ingredients may have acted as a fertilizer in the ponds i.e. providing nutrients for plankton production rather than being consumed directly by the fish.

Mustard oil cake, rice bran and wheat bran are commonly used as supplemental feed in carp hatcheries and nurseries in Bangladesh. Therefore, there is a need to evaluate the quality and suitability of these ingredients as carp feed. Furthermore, copra and groundnut meal which are also available in Bangladesh have proved successful alternate protein sources in diets for tilapia (Cruz and Laudencia, 1978; Jackson et al., 1982)

Leucaena a leguminous plant, is also grown in Bangladesh and used principally as cattle and poultry feed. It has been recommended by the National Academy of Science (NAS) (1977) as a useful animal feed. It also has been used as fish feed with variable success (Cruz and Landencia, 1978; Pantastico and Baldia, 1977).

A study was undertaken to determine the nutritive value of mustard, linseed, groundnut, sesame, copra and leucaena meal and rice bran and wheat bran fed to common carp in the form of a compounded pelleted diet. The study was conducted in a laboratory recirculated water system.

Many of the above ingredients contain various toxic factors (see Table 4.1). Although different processing methods/treatment(see Table 4.1 for detail)are available to detoxify them, no attempt was made to do so in the present investigation as most are impractical to apply under field conditions or expensive to operate.

Two consecutive trials each of 35 days duration were conducted. In the first trial various plant protein sources were used at different dietary inclusion levels depending on their protein content The aim of the first trial was to determine maximum inclusion levels for different plant protein sources and to identify the limitations of their use arising from palatibility or apparent toxicity factors.

Table 4.1 Toxic constituents of the dietary ingredients used in Experiment 4.1

Source	Toxic Factor	Effect	Prevention/treatment	References
Indian mustard	Glucosinolates - Allyl (Sinigrin) and 3-Butenyl (Gluconapin)	Glucosinolates upon hydrolysis by thioglucosidase present in the seeds, release growth inhibitory, potentially goitrogenic isothiocyanates - which prevent thyroid from accumulating iodine.	Partially detoxified by cold autolysis followed by removal of allyl isothiocyanates by volatization with steam gave satisfactory meal. Supplementing with iodine is effective with isothiocyanates.	Tookey et al (1980); Jauncey and Ross (1982)
		Thioglucosidase also acts on sinigrin to produce highly irritant mustard gas or intestinal irritant.	Two hours of steaming or steeping in water at 85°C for 1 hour and washing residue 5 times to remove water soluble sinigrin.	Gohl (1981); Jauncey and Ross (1982)
Linseed	Immature linseed contain linamarin (Cyanogenetic glucosides)	Cyanide poisoning with wet meal. Linamarin, in presence of an associated enzyme, lynase, liberates hydrogen cyanide (HCN) on hyrolysis. Linseed may liberate up to 53 mg of HCN per 100g	Solvent extracted meals non-toxic. Linase destroyed by 10 min boiling or processing heat.	Montgomery (1980); Gohl (1981); McDonald et al. (1981)
Leucaena leaf	Mimosine	Adverse effect on growth in case of ruminants due to the under production of thyroxine	Detoxified by soaking in water and them drying or steeping one week with $FeSo_4$ or storing at 70°C in presence of moisture or addition of $FeSO_4$ in diet	National Academy of Science (1977); Liener (1980); Gohl (1981)

On the basis of the results of the first trial, the second trial was confined to the study of linseed, sesame, groundnut and mustard meal. These ingredients were combined in various proportions and used at different inclusion levels to substitute for fish meal up to a maximum of 75% of the protein level. Besides the above four plant protein sources, rice bran and wheat bran were used as dietary carbohydrate sources to replace dextrin and corn starch.

4.2. Materials and Methods

The source of experimental animals and their quarantine procedure are described in section 2.2.2.2.-3.

4.2.1. Experimental system

The experimental system described in section 2.2.2.1 was used for the present experiment but modified, as described in section 2.3.2.1. to operate as a single unit. Each experimental tank was supplied initially with about 300 ml of water per minute. The flow rates were increased after each weighing in proportion to the increase in fish weight. The same rate of water flow was, however, maintained for all treatments.

4.2.2. Diet formulation and preparation

Trial 1

Twelve semi-purified isonitrogenous diets were formulated to evaluate the following sources of plant protein available commercially in Bangladesh: -

Indian mustard (Brassica juncea)

- expeller cake; origin - Bangladesh

Sesame (Sesamum indicum)

- hexane extracted meal; locally obtained and processed in Stirling, but similar to sesame meal available in Bangladesh

Linseed (Linum usitatissimum)

- expeller cake; origin - Indonesia

Leucaena (Leucaena leucocaphala)

- dried leaf meal; origin - Malawi

Copra (Cocos nucifera)

- expeller cake; origin - Indonesia

Groundnut (Arachis hypogaea)

- solvent extracted meal; origin - unknown

aflatoxin level - 30 ppb

Mustard oilcake was brought directly from Bangladesh. The other ingredients could not be obtained in the same way for logistic reasons and had to be collected from elsewhere. Linseed and copra cake were obtained from Protein Supplement Ltd., Brakes Farm, Suffolk, England and leucaena leaf and groundnut meal were obtained from the Tropical Development Research Institute (TDRI), 56/62 Gray's Inn Road, London. Information on the aflatoxin level in the groundnut meal was provided by TDRI.

The dietary protein sources and herring fish meal were analysed for proximate composition and amino acid content. Results of the proximate analysis and amino acid analysis are presented in Table 4.3. All diets were formulated to contain 40% protein, 11% lipid, 6% crude fibre and 25% digestible carbohydrate. The level of protein used in the present trial was below the optimum level recommended by Bryant (1980) and the National Research Council (NRC) (1983) for carp fry. However, the optimum dietary protein level for advanced carp fry and fingerlings has been reported to be below 40% (Ogino and Saito, 1970; Jauncey, 1981). The dietary protein level of 40% was used because the protein content of one of the plant ingredients was only 24.6% (Table 4.3). The diets were also formulated to be isoenergetic. However, the diets cannot be

Table 4.2. A summary of the methodology used to evaluate some dietary ingredients commercially available in Bangladesh for the formulation of a carp fry diet.

Experimental duration	:	35 days
Initial weight of fry	:	50mg (SE ± 1.6) - trial 1 106mg (SE ± 2.95) - trial 2
Treatments	:	Evaluation of mustard, sesame, linseed, groundnut, copra and lencaena leaf meal as dietary protein source and rice bran and wheat bran as dietary carbohydrate source for common carp fry
Replication	:	3/treatment
Feeding rates	:	20 - 25% BW/day
Water temperature	:	27 - 29°C
Volume of water in each tank	:	61
Stocking density	:	40/tank
Water flow rate to each tank	:	300 ml/min with corresponding increase at each subsequent sampling
Physico-chemical characteristics monitored	:	Temperatue, pH, dissolved oxygen total ammonia and nitrite

Table 4.3. Results of proximate analysis (% dry matter) and amino acid contents (% crude protein) of dietary ingredients used in trial 1 and 2, Experiment 4.1

Components	Ingredients									
	Herringmeal	Mustard	Sesame 1	Sesame 2	Linseed	Groundnut	Copra	Leucaena	Ricebran	Wheatbran
Dry matter	93.61	92.53	95.34	90.83	90.52	88.05	90.04	92.39	90.32	92.65
Crude protein	74.95	34.71	50.72	46.34	38.08	59.01	24.60	30.22	17.74	16.62
Crude lipid	10.89	6.34	11.84	9.82	8.31	0.18	1.73	2.27	1.16	3.25
Ash	13.03	11.26	9.43	14.00	5.98	9.44	9.66	7.62	17.33	8.82
Crude fibre	1.18	13.40	7.44	7.65	10.05	12.06	13.95	21.92	10.10	11.33
N.F.E.	-	34.28	20.57	22.19	37.58	19.31	50.06	37.97	53.67	59.98
Amino acid content										
Arginine	7.79	5.85	10.24	13.55	7.45	11.31	9.18	4.53	7.84	5.02
Histidine	2.56	2.30	2.06	2.54	1.79	2.61	1.56	1.49	2.92	2.35
Isoleucine	4.05	3.25	2.60	3.27	3.10	3.24	2.80	6.53	3.66	2.39
Leucine	7.63	6.16	5.34	6.55	5.07	6.88	6.40	6.09	8.75	5.38
Lysine	7.84	4.25	2.25	2.88	3.22	3.49	2.45	4.82	5.13	3.42
Methionine	2.99	1.12	1.87	2.25	0.89	0.87	1.36	0.35	0.82	0.68
Phenylalanine	3.91	3.77	3.58	4.96	3.64	5.17	4.23	4.35	5.43	3.31
Threonine	4.64	3.51	2.68	3.76	2.99	2.82	2.78	3.70	4.50	2.79
Tryptophan *	1.08	1.19	1.61	1.61	1.41	0.96	0.96	-	0.79	1.88
Valine	5.87	3.96	3.37	4.10	3.76	3.86	4.70	4.03	5.93	3.73
Cystine	1.14	1.25	0.85	1.78	1.15	1.60	0.89	0.36	1.31	1.13
Tyrosine	3.34	2.50	2.82	3.88	2.06	3.93	3.03	3.47	3.60	2.63

* Tryptophan values were obtained from NAS (1983) except that of mustard cake, which was obtained from Capper et al. (1982)

considered completely isoenergetic in relation to metabolizable energy due to the different nature and digestibilities of the protein and carbohydrate contents of the diets. Where possible, each plant protein was tested at three different inclusion levels (25, 50 and 75% of total protein). The levels of inclusion of test protein sources in the experimental diets are shown in Table 4.4

One hundred percent replacement of fish meal protein was not possible as none of the ingredients tested contained a high enough protein content to supply 40% protein in the diet.

The control diet was prepared with fish meal as the sole source of protein. Each diet was formulated to contain 0.5% chromic oxide to enable a study of protein digestibility. The composition of the experimental diets are presented in Table 4.5.

All prepared diets were analysed for proximate composition to check the formulation (Table 4.6). Their amino acid contents were not analysed, but were calculated using the amino acid content values for the individual dietary protein sources (Table 4.3). The calculated levels of amino acid contents and the chemical score of each diet are presented in Table 4.7.

The detailed precedures for the diet formulation and preparation were similar to those described in section 2.2.2.4 and 2.2.2.5 respectively.

Table 4.4. Level of inclusion of different test proteins (as % of total protein) in the experimental diets used in trial 1, Experiment 4.1

Test protein source	Diet No.											
	1 (control)	2	3	4	5	6	7	8	9	10	11	12
Herring meal	100	75	50	75	50	25	75	50	75	75	75	25
Mustard	-	25	50	-	-	-	-	-	-	-	-	-
Sesame	-	-	-	25	50	75	-	-	-	-	-	-
Linseed	-	-	-	-	-	-	25	50	-	-	-	-
Leucaena	-	-	-	-	-	-	-	-	25	-	-	-
Copra	-	-	-	-	-	-	-	-	-	25	-	-
Groundnut	-	-	-	-	-	-	-	-	-	-	25	75

Table 4.5 Composition of the experimental diets used in trial 1, Experiment 4.1 (% dry weight basis)

Ingredients	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Mustard	-	28.81	57.62	-	-	-	-	-	-	-	-	-
Sesame	-	-	-	19.72	39.44	59.15	-	-	-	-	-	-
Linssed	-	-	-	-	-	-	26.26	52.52	-	-	-	-
Leucaena	-	-	-	-	-	-	-	-	33.09	-	-	-
Copra	-	-	-	-	-	-	-	-	-	40.65	-	-
Groundnut	-	-	-	-	-	-	-	-	-	-	16.95	50.84
Herring meal	53.37	40.03	26.68	40.03	26.68	13.34	40.03	26.68	40.03	40.03	40.03	13.34
Cod liver oil	1.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00
Corn oil	3.50	3.00	3.00	3.00	2.50	2.00	3.00	3.00	4.00	4.00	4.00	4.50
Mineral mix**	4.00	3.00	3.00	4.00	3.50	3.50	4.00	4.00	4.00	3.00	4.00	4.00
Vitamin mix**	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder *	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
∝-cellulose	6.00	2.00	-	4.50	3.00	2.00	3.00	1.00	-	-	4.00	-
Dextrin	16.50	10.00	2.50	13.75	11.00	8.50	10.00	4.00	8.00	4.00	15.00	12.00
Corn starch	11.13	7.16	1.20	9.00	7.88	5.51	7.71	2.80	4.88	2.32	10.02	7.32

* Sodium carboxymethyl cellulose (high viscosity)

** See table 2.5 and 2.6 for composition of mineral and vitamin mix respectively

Table 4.6 Proximate composition of the experimental diets used in trial 1, Experiment 4.1 (% dry matter basis)

Components	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Crude protein (%)	42.53	40.83	41.37	40.91	40.67	40.30	41.25	41.39	41.05	39.56	40.51	39.77
Crude lipid (%)	10.76	10.95	10.39	11.72	12.31	13.06	10.84	11.87	11.56	11.69	10.78	9.89
Ash (%)	10.70	11.96	13.02	11.01	11.36	10.85	10.34	10.27	11.70	12.75	10.68	10.61
Crude fibre (%)*	6.63	6.33	8.03	6.44	6.25	6.56	6.11	6.59	7.72	6.14	6.51	6.29
N.F.E..(%)**	27.63	27.04	23.46	26.81	27.00	26.18	27.58	26.54	25.44	26.67	28.29	29.14
Chromic oxide (%)	0.49	0.50	0.50	0.47	0.50	0.47	0.48	0.52	0.45	0.45	0.50	0.48
Gross energy (Kcal/g)	4.45	4.35	4.18	4.42	4.46	4.48	4.39	4.44	4.35	4.33	4.37	4.28
Metabolizable energy (ME)(Kcal/g)	3.79	3.71	3.56	3.77	3.82	3.84	3.74	3.80	3.72	3.71	3.73	3.65
PE ratio***	112.1	110.0	116.1	108.4	106.5	105.0	110.3	109.0	110.4	106.8	108.7	109.0

* calculated as crude fibre derived from test protein source and α-cellulose

** Nitrogen free extractives, calculated as NFE derived from test protein source, corn starch and dextrin

*** Protein to energy ratio in mg protein/Kcal of M.E.

Table 4.7 Calculated level of amino acids in diets used in trial 1 as % crude protein and the dietary amino acid requirement for carp in a 38.5% protein diet (Nose, 1979) (Experiment 4.1)

Amino acid	Requirement for carp	Diet No. 1	2	3	4	5	6	7	8	9	10	11	12
Arginine	4.3	7.79	7.31	6.82	8.40	9.02	9.63	7.71	7.62	6.98	8.14	8.67	10.43
Histidine	2.1	2.56	2.50	2.43	2.44	2.31	2.19	2.37	2.18	2.29	2.31	2.57	2.60
Isoleucine	2.5	4.05	3.85	3.65	3.69	3.33	2.96	3.81	3.58	4.67	3.74	3.85	3.44
Leucine	3.3	7.63	7.26	6.90	7.06	6.49	5.91	6.99	6.35	7.25	7.32	7.44	7.07
Lysine	5.7	7.84	6.94	6.05	6.44	5.05	3.65	6.69	5.53	7.09	6.49	6.75	4.58
Methionine	2.1*	2.99	3.52	2.06	2.71	2.43	2.15	2.47	1.94	2.33	2.58	2.47	1.40
Phenylalanine	3.4*	3.91	3.88	3.84	3.83	3.75	3.66	3.84	3.78	4.02	3.99	4.23	4.86
Threonine	3.9	4.64	4.36	4.08	4.15	3.66	3.17	4.23	3.82	4.41	4.18	4.19	3.28
Tryptophan	0.8	1.08	1.11	1.14	1.22	1.35	1.48	1.16	1.25	0.81	1.05	1.05	0.99
Valine	3.6	5.87	5.39	4.92	5.25	4.62	4.00	5.34	4.82	5.41	5.58	5.37	4.36
Cystine	-	1.14	1.17	1.20	1.07	1.00	0.92	1.14	1.15	0.95	1.08	1.26	1.49
Tyrosine	-	3.34	3.13	2.92	3.21	3.08	2.95	3.02	2.70	3.37	3.26	3.49	3.78
Chemical score (%)**		115.0	111.8	98.1	106.4	88.6	64.0	108.5	92.4	101.3	107.2	107.4	66.7

* The values of methionine and phenylalanine are the requirements in the presence of 2% cystine and 1% tyrosine of the diet respectively

** Chemical score calculated based on the amino acid requirement of carp (Nose, 1979)

Trial 2

Out of the six plant protein sources tested in the first trial, linseed fish fed linseed and groundnut followed by sesame meal, when used at low inclusion levels, showed good growth and food conversion. Even though mustard meal did not produce growth comparable to that for linseed, groundnut or sesame, its performance was not as poor as leucaena or copra meal. Therefore, considering the abundance of mustard oilcake in Bangladesh and its very good amino acid profile especially lysine and theomine, it was decided to use this oilcake along with linseed, groundnut and sesame meal as dietary protein sources in trial 2. Fish meal protein was substituted up to a level of 75%. Different dietary ingredients were combined and four inclusion levels of the plant protein were used 40, 50, 60 and 75% levels as shown in Table 4.6. A combination of different ingredients was used, this has been reported to increase palatability of the diet (K. Jauncey, pers. comm.) and helps to balance limiting essential amino avids of the plant proteins.

In addition to the above plant protein sources, rice bran (obtained from Protein Suppl. Ltd., Suffolk) and wheat bran (origin - Bangladesh) were used in two diets mainly as a dietary carbohydrate sources to replace dextrin and corn starch. The sources and origins of linseed, groundnut and mustard were the same as those of trial 1. A different batch of sesame was used for trial 2 which was purchased locally and processed as described for trial 1. The composition of

Table 4.8 Level of inclusion of different plant proteins (as % of total protein) in the experimental diets used in trial 2, Experiment 4.1

Plant protein source	Diet No.											
	1 (control)	2	3	4	5	6	7	8	9	10	11	12
Linseed	-	20	20	20	25	25	20	20	20	20	15	20
Groundnut	-	20	-	10	25	-	10	20	-	20	-	20
Sesame	-	-	20	10	-	25	20	20	20	15	13	10
Mustard	-	-	-	-	-	-	-	-	20	20	-	-
Rice bran	-	-	-	-	-	-	-	-	-	-	5.8	5.6
Wheat bran	-	-	-	-	-	-	-	-	-	-	6.6	4.8
Total inclusion (% of total protein)	0	40	40	40	50	50	50	60	60	75	40.4	60.4

Table 4.9 Composition of the experimental diets (% dry weight basis) used in trial 2, Experiment 4.1

Ingredients	Diet No.											
	1 (control)	2	3	4	5	6	7	8	9	10	11	12
Linseed	-	21.01	21.01	21.01	26.26	26.26	21.01	21.01	21.01	21.01	15.76	21.01
Groundnut	-	13.56	-	6.78	16.95	-	6.78	13.56	-	13.56	-	13.56
Sesame	-	-	17.26	8.63	-	21.58	17.26	17.26	17.26	12.95	11.22	8.63
Mustard	-	-	-	-	-	-	-	-	23.05	23.05	-	-
Rice bran	-	-	-	-	-	-	-	-	-	-	13.04	12.78
Wheat bran	-	-	-	-	-	-	-	-	-	-	15.96	11.67
Herring meal	53.37	32.02	32.02	32.02	26.68	26.68	26.68	21.35	21.35	13.34	32.02	21.35
Cod liver oil	1.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Corn oil	3.50	3.50	2.50	3.00	4.00	2.50	3.00	3.00	2.00	3.00	3.00	3.00
Mineral mix	4.00	4.00	3.00	4.00	4.00	3.00	3.00	3.00	3.00	2.50	2.00	2.00
Vitamin mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder *	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-Cellulose	6.00	2.50	3.00	3.00	1.90	2.25	2.50	1.00	-	-	1.00	-
Dextrin	16.50	10.00	9.00	9.00	8.00	6.73	8.00	7.50	3.83	2.59	-	-
Corn starch	11.13	7.41	6.21	7.05	6.21	5.00	5.77	5.82	2.50	2.00	-	-

* Sodium carboxymethyl cellulose

Table 4. 10 Proximate composition of the experimental diets used in trial 2, Experiment 4.1 (% dry matter basis)

Components	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Crude protein (%)	42.06	41.95	41.55	40.37	40.62	39.79	40.72	39.41	40.96	40.62	40.60	39.40
Crude lipid (%)	10.62	10.65	11.01	11.34	10.90	11.50	10.90	10.72	10.46	10.50	10.86	10.42
Ash (%)	10.79	11.69	11.71	11.78	11.32	11.12	11.89	11.91	12.80	12.48	13.25	12.61
Crude fibre (%) *	6.63	6.63	6.81	6.97	6.89	6.85	7.06	6.82	6.77	7.99	6.95	7.30
N.F.E. (%) **	27.63	27.93	26.94	27.78	27.35	26.39	26.81	27.67	25.96	26.06	24.98	26.29
Chromic oxide (%)	0.44	0.46	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.49	0.50	0.47
Gross energy(Kcal/g)	4.41	4.42	4.39	4.39	4.35	4.32	4.33	4.28	4.26	4.26	4.25	4.19
Metabolizable energy(ME)(Kcal/g)	3.76	3.77	3.75	3.75	3.71	3.69	3.7	3.65	3.64	3.63	3.62	3.58
PE ratio ***	111.8	111.3	110.9	106.6	109.5	107.8	110.2	107.9	112.5	111.9	112.1	110.1

* Calculated as crude fibre derived from test protein source and ∝-cellulose

** Nitrogen free extractives, calculated as NFE derived from test protein source, corn starch and dextrin

*** Protein to energy ratio in mg protein/Kcal of M.E.

Table 4.11 Calculated level of amino acids in diets used in trial 2 as % crude protein (Experiment 4.1)

Amino acid	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Arginine	7.79	8.43	8.87	8.65	8.59	9.15	9.23	9.58	8.49	8.90	8.31	8.87
Histidine	2.56	2.42	2.40	2.41	2.38	2.36	2.41	2.41	2.35	2.36	2.45	2.42
Isoleucine	4.05	3.70	3.70	3.70	3.61	3.62	3.62	3.54	3.54	3.42	3.67	3.52
Leucine	7.63	6.97	6.90	6.94	6.80	6.72	6.83	6.75	6.61	6.51	7.02	6.81
Lysine	7.84	6.05	5.92	5.99	5.60	5.45	5.49	5.05	5.21	4.58	6.05	5.19
Methionine	2.99	2.12	2.42	2.28	1.94	2.28	2.21	2.00	2.05	1.66	2.30	1.84
Phenylalanine	3.91	4.11	4.07	4.09	4.16	4.11	4.19	4.32	4.04	4.24	4.05	4.27
Threonine	4.64	3.95	4.13	4.04	3.77	4.01	3.95	3.77	3.91	3.59	4.15	3.76
Tryptophan	1.08	1.12	1.25	1.08	1.13	1.30	1.24	1.23	1.27	1.22	1.23	1.20
Valine	5.87	5.05	5.09	5.07	4.84	4.90	4.89	4.69	4.71	4.40	5.19	4.77
Cystine	1.14	1.23	1.27	1.25	1.26	1.30	1.32	1.36	1.29	1.35	1.23	1.31
Tryosine	3.34	3.20	3.19	3.20	3.17	3.16	3.25	3.31	3.02	3.12	3.19	3.24
Chemical score*	115.0	101.0	103.9	103.6	92.4	95.6	96.3	88.6	91.4	79.05	106.1	87.6

* Chemical score calculated based on the amino acid requirement of carp (Nose, 1979)

all twelve experimental diets used in trial 2 are presented in Table 4.9.

In the formulation of all diets, efforts were made to balance the four limiting essential amino acids - lysine, methionine, threonine and tryptophan. All twelve diets were prepared to contain 40% protein, 11% lipid, 11% ash, 6% crude fibre and 25% digestible carbohydrate and were formulated to be isoenergetic. Diets were analysed for proximate composition and the results are presented in Table 4.10. The calculated amino acid contents and chemical score of each diet are given in Table 4.11.

Other general procedures employed in the formulation and preparation of the diets were similar to those described for trial 1.

4.2.3. General experimental procedure

In both trials, there were three replications for each treatment with 40 fish fry in each replicate. Fish fry were acclimated to the experimental system three days before the start of the experiment. The weighing and acclimation procedures were similar to those described in section 2.2.2.7. Weighing of the fish during the experiment was carried out every seven days.

The fish were fed four times a day at three hourly intervals between

09.00 and 18.00 hours. Initially, fish were fed at a feeding rate of 25% BW/day in both trials. This was reduced to 20% BW after three and two weeks in the first and second trials respectively. The details of administration of food and feeding are described in section 2.2.2.8.

During the last week of each trial, faecal samples were collected from the culture tanks for determination of protein digestibility. One hour after feed had been administered, any feed and faeces present in the tanks were removed. Fresh faeces produced by the fish after this period and before the second feed was given, were siphoned out, filtered on to a filter paper and dried at 105^{o}C for 12 hours. The faecal samples of each replicate treatment tank were pooled, and stored in air tight bottles for subsequent chemical analysis.

Both trials were conducted for a period of 35 days. A summary of the methodology used in the present investigation are given in Table 4.2.

4.2.4. Analytical technique

Measurements and analysis of water quality were carried out as described in section 2.2.2.8. Temperature, pH, dissolved oxygen, total ammonia and nitrite levels of the water were monitored at

weekly intervals in both trials. These ranged between 27-29°C, 5.87-7.80, 6.20-7.50 mgl^{-1}, 0.08-0.84 mgl^{-1} TA-N, and 0.007-0.28 mgl^{-1} NO_2-N for temperature, pH, dissolved oxygen, total ammonia and nitrite respectively.

Proximate composition analysis was carried out on the dietary ingredients, prepared diet and samples of the experimental fish as described in section 2.2.2.6.

Amino acid analysis

The amino acid content of the dietary protein sources, including those in the rice bran and wheat bran, were measured according to the precedure described below.

Sample preparation: - Samples for amino acid analysis were hydrolysed with 6 N HCl for 24 hours at 110°C in Vacuo, following the procedure given in the LKB 4151 Alpha-plus Instruction mannual (1983).

Analysis: - All analyses were carried out using a LKB 4151 Alpha-plus amino acid analyser (LKB Biochrom Ltd., Cambridge). The amino acid tryptophan could not be analysed due to its destruction during acid hydrolysis. Therefore, tryptophan values for all ingredients were obtained from NRC (1983) except that for mustard oilcake, which was obtained from Capper et al., (1982). The Chemical score of the

essential amino acid profile of each diet was calculated after Jauncey and Ross (1982).

Histological technique.

At the end of the first trial, fish samples from each dietary treatment were fixed in 10% buffered formalin for histological study. Serial transverse and longitudinal sections from the control and test fish were fixed in 10% buffered formalin, processed by routine methods, embedded in paraffin wax and sectioned by a microtome set at 5 microns. The tissue sections were stained with haemotoxylin and eosin, and examined under a light microscope to assess the general histopathological condition of the gills, liver, muscle, kidney, pancreas and intestine. Formalin fixed fish samples from treatment 3 (50% mustard) were lost accidentally from the laboratory and therefore histological studies on this group of fish could not be done.

4.2.5. Analysis of experimental data

The experimental data were analysed by the methods described in section 2.2.2.10. The method of digestibility determination which has not been described previously in this thesis is given below.

Digestibility determination: chromic oxide was used as an external dietary marker in the study of protein digestibility. The chromic oxide content of the experimental diets and faeces was determined by the acid digestion method of furukawa and Tsukahara (1966). An estate of apparent protein digestibility was derived from the following equation (Maynard and Loosli, 1969).

Apparent nutrient digestibility (%) =

100-(100 x indicator content in feed% ÷ indicator content in faeces %

x nutrient content in faeces ÷ nutrient content in feed %)

Statistical analyses

These were performed as described in section 2.2.2.10.

4.3. Results

4.3.1. Trial 1

4.3.1.1. Acceptability of diets

All fish become acclimated to the experimental diets within two to three days of the start of feeding. However, the acceptability of all diets was not equal. The control diet, and diets containing linseed and groundnut meal were generally quite acceptable, but diets with higher inclusion levels of plant protein (50-75% of total protein) showed poor acceptability. Diets containing leucaena meal, even at a low inclusion level (25% of protein), showed the poorest acceptability. An account of the observations on the acceptability of the different diets used in trial 1 are presented in Table 4.12.

4.3.2.2. Mortality

Significantly different fry mortality rates were recorded between some of the dietary treatments during the experimental period. Total mortality of carp fry at each dietary treatment are presented in Table 4.13 and the cumulative percentage of mortality (recorded daily) at different time intervals are shown graphically in Fig. 4.1. Mortality values ranged from 0.8% for diet 1 (control) to 56.7% for diet 3 (50% mustard). Diet 9 containing leucaena meal resulted in very high mortality (40%) second only to diet 3. It is important to note that the diets containing 50% levels of linseed and sesame did not produce high mortalities (3.3 and 8.3% respectively) whereas diets containing

Table 4.12 Observations on the acceptability of different plant protein diets fed to carp fry in trial 1, Experiment 4.1

Diet No.	Specification of diet	Observations on acceptability
1	Control (fish meal base))	Active feeding observed through -
7	25% linseed)	out; uneaten food rarely found
8	50% linseed)	at any time in the tanks
9	25% groundnut)	
2	25% mustard	Active feeding observed through- out, but sometimes uneaten food present in tank; large amounts of faeces produced immediately after feeding
10	25% copra	Moderate feeding activity observed throughout the trial
12	75% ground nut	Initially fish were seen feeding actively but towards the end of trial acceptability of the diet was poor largely due to fish's poor health
4	25% sesame)	Uneaten food observed frequently
5	50% sesame)	in the tanks after administration of food
3	50% mustard	Initially feeding was good, but from week 2 onwards, feeding activity deteriorated and frequently large amounts of uneatean food accumulated.
6	75% sesame	Feeding was poor throughout the experimental period
9	25% leucaena	Acceptability of diet was poor from the beginning; large amounts of faeces were found after administration of diet

Table 4.13 Mortality, growth and food utilization of common carp fry reared for 35 days in trial 1, Experiment 4.1

Mean initial weight 50 mg (SE ± 1.6)

Mean values	Diet No.												
	1	2	3	4	5	6	7	8	9	10	11	12	±SE**
Final weight (mg)	612^{a*}	400^{d}	233^{f}	466^{c}	400^{d}	216^{f}	569^{b}	323^{e}	173^{g}	326^{e}	545^{b}	263^{f}	14.41
% mortality	0.8^{e}	14.2^{cd}	56.7^{a}	3.3^{e}	8.3^{de}	26.7^{c}	0.8^{e}	3.3^{e}	40.0^{b}	15.0^{cd}	1.7^{e}	20.0^{cd}	2.68
SGR	7.15^{a}	5.94^{c}	4.40^{ef}	6.38^{b}	5.93^{c}	4.17^{f}	6.95^{a}	5.32^{d}	3.53^{g}	5.35^{d}	6.82^{a}	4.74^{e}	0.14
SGR as % of control	100	83.1	61.5	89.2	82.9	58.3	97.2	74.4	49.4	74.8	95.4	66.3	-
FCR	2.26^{a}	2.92^{bc}	6.61^{g}	2.82^{abc}	3.24^{c}	5.77^{f}	2.33^{ab}	3.13^{c}	8.13^{h}	3.80^{d}	2.63^{abc}	4.52^{e}	0.15
PER	1.06^{a}	0.84^{c}	0.37^{h}	0.87^{c}	0.76^{d}	0.43^{g}	1.04^{a}	0.77^{d}	0.30^{j}	0.67^{e}	0.94^{b}	0.56^{f}	0.01
ANPU (%)	13.71^{a}	10.24^{cd}	4.53^{h}	10.77^{bc}	9.22^{de}	5.04^{g}	13.06^{a}	9.84^{cd}	3.87^{h}	8.31^{e}	11.60^{b}	6.31^{f}	0.32
APD (%)	77.8	85.8	86.2	78.7	86.1	88.9	83.1	82.4	63.3	81.0	84.4	89.5	-

* Figures in the same row with same superscripts are not significantly different ($P > 0.05$)

** Standard error of treatment, calculated from the residual mean square in the analysis of variance

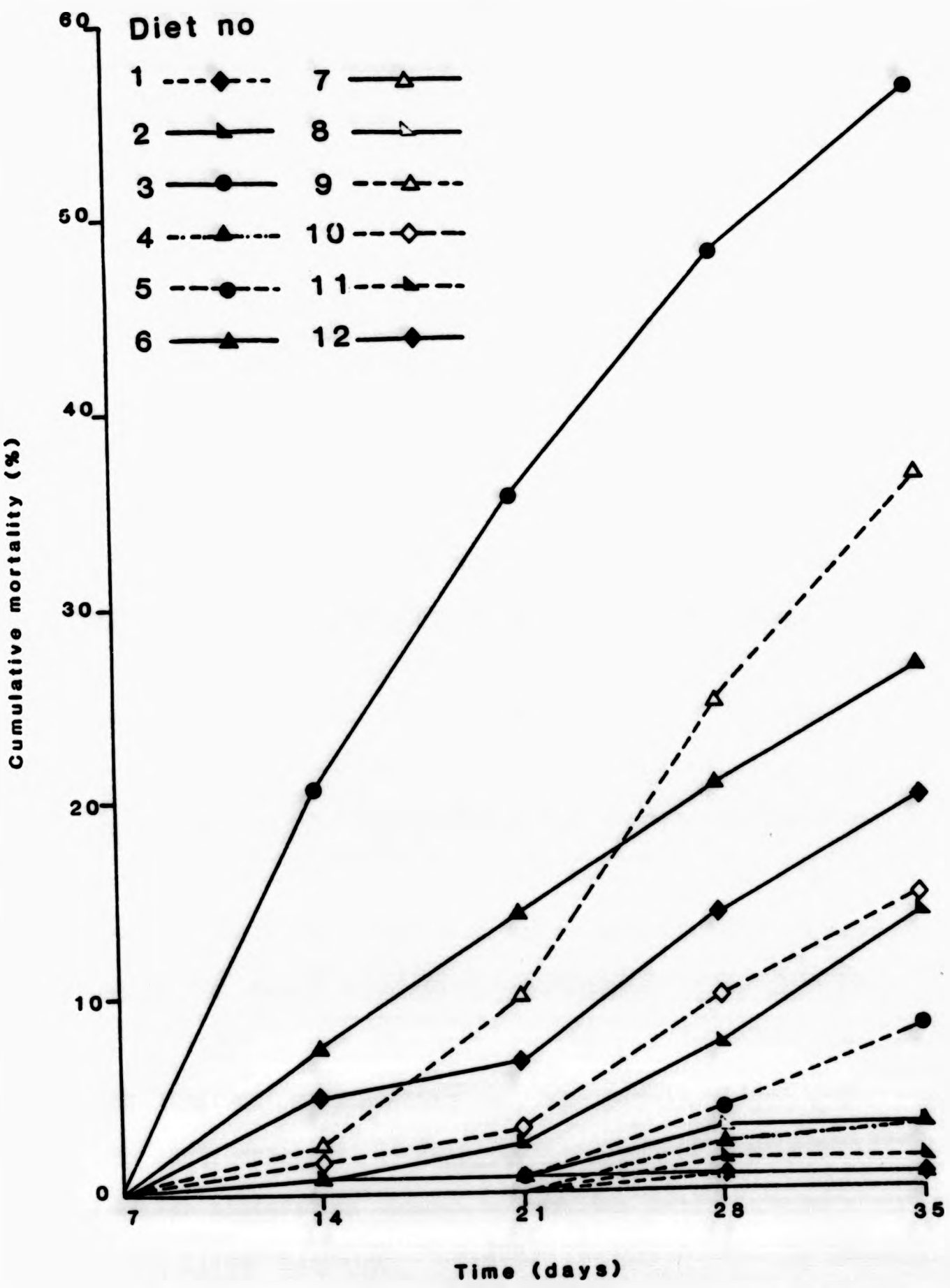


Fig. 4.1 Cumulative percentage mortality of carp fry at different dietary treatments in trial 1, Experiment 4.1

mustard oilcake even at a 25% inclusion level produced substantial mortality (14.2%). Similarly 15% mortality was recorded at diet with 25% copra meal. Both sesame and groundnut at a higher inclusion level (75%) caused significantly higher mortality (26.7 and 20.0% respectively).

4.3.1.3. General health and histopathological examination

A large proportion of fish fed on diet 12 (75% protein from groundnut) showed severe deformations of the body (Plate 4.1-4.2). A small proportion of the fish fed on diet 6 (75% sesame) and 9 (25% leucaena) also showed malformations (Plate 4.3-4.4). These physical deformities were probably caused by underfeeding or by nutritional imbalances in the diet. Meske and Pfeffer (1978) reported that with superoptimal concentrations of algae in the feed, the growth of carp and grass carp fry was reduced and severe malformations occurred, especially in grass carp. The authors postulated that malformations of the fish body were probably due to nutritional imbalances in diets containing superoptimal levels of algae.

Histopathological examinations of the gills, liver, muscle, kidney, pancreas and intestine of fish reared under different dietary regimes revealed no significant changes except for the diet containing mustard meal. Liver histology revealed a higher level of intracellular

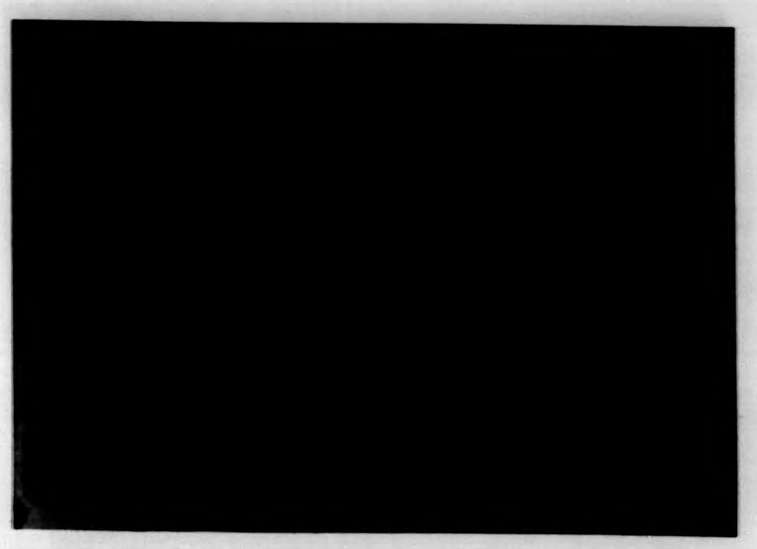


Plate 4.1a

Plate 4.1b

Plate 4.1 Dorsal (a) and lateral (b) views of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing severe deformations of the body. Fish on the right are the normal fish from fish meal based control diet.

Plate 4.1 Dorsal (a) and lateral (b) views of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing severe deformations of the body. Fish on the right are the normal fish from fish meal based control diet.

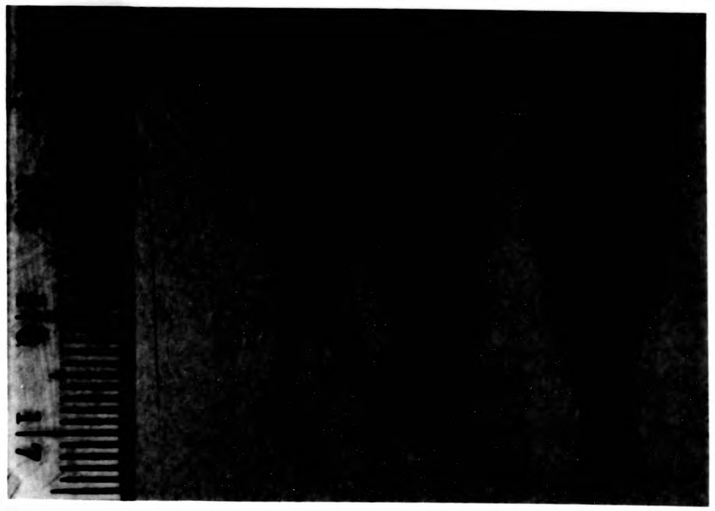


Plate 4.1a

Plate 4.1b

Plate 4.1 Dorsal (a) and lateral (b) views of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing severe deformations of the body. Fish on the right are the normal fish from fish meal based control diet.

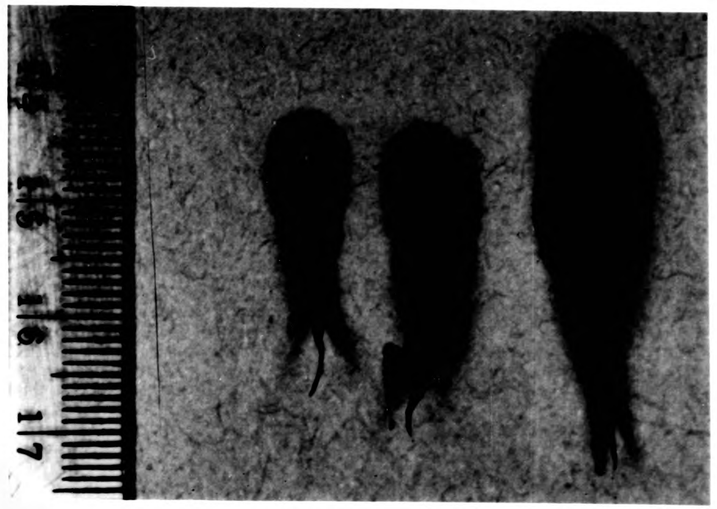


Plate 4.1a

Plate 4.1b

Plate 4.2 Longitudinal section of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing curvature of the vertebral column (X 26)

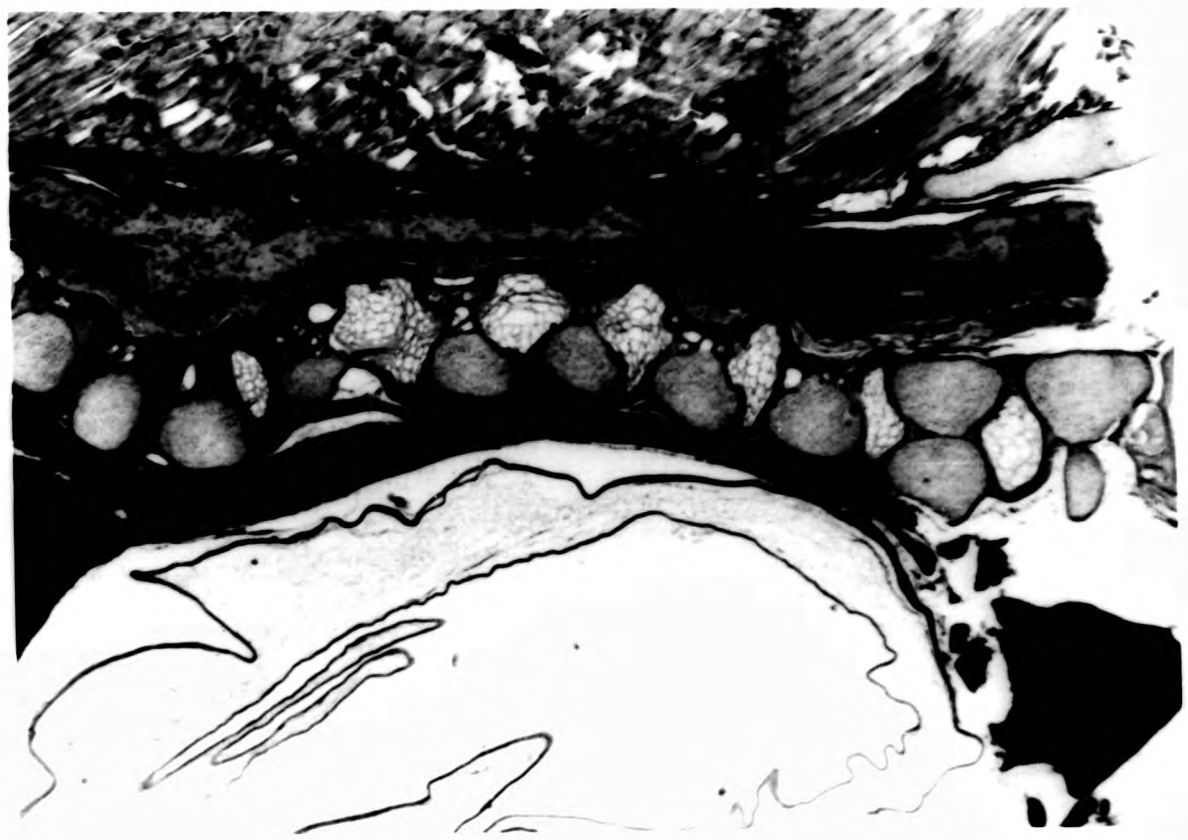


Plate 4.2 Longitudinal section of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing curvature of the vertebral column (X 26)

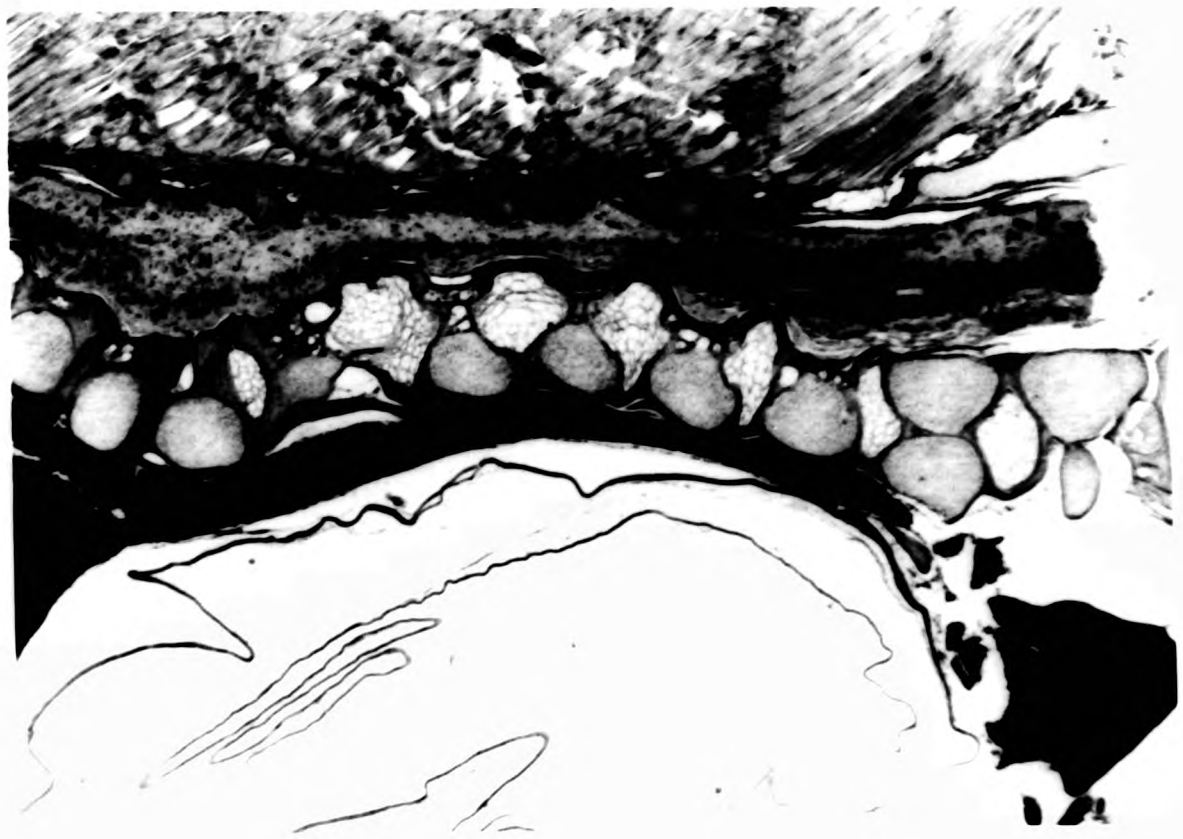


Plate 4.2 Longitudinal section of carp fry fed diet 12 (75% groundnut) from trial 1, Expt. 4.1 showing curvature of the vertebral column (X 26)

Plate 4.3 Dorsal (a) and lateral (b) views of carp fry fed diet 6 (75% sesame) from trial 1, Expt. 4.1 showing malformations. Fish on the top are the normal fish from fish meal based control diet.

Plate 4.3a

Plate 4.3b

Plate 4.3 Dorsal (a) and lateral (b) views of carp fry fed diet 6 (75% sesame) from trial 1, Expt. 4.1 showing malformations. Fish on the top are the normal fish from fish meal based control diet.

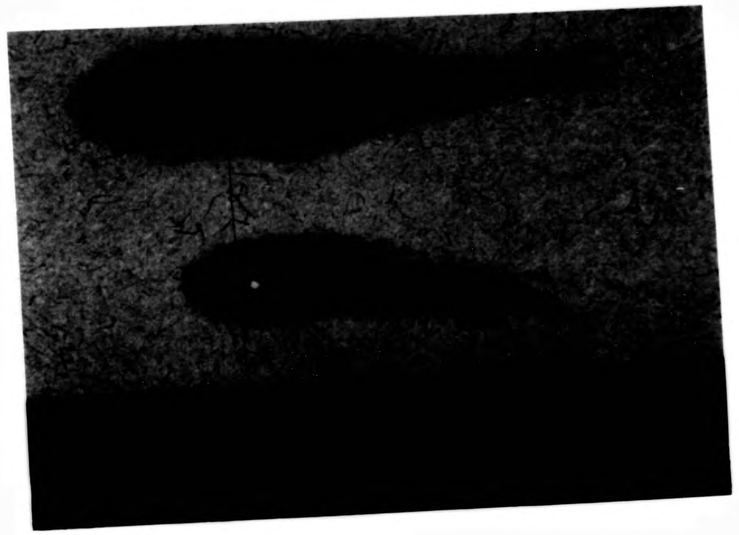


Plate 4.3a

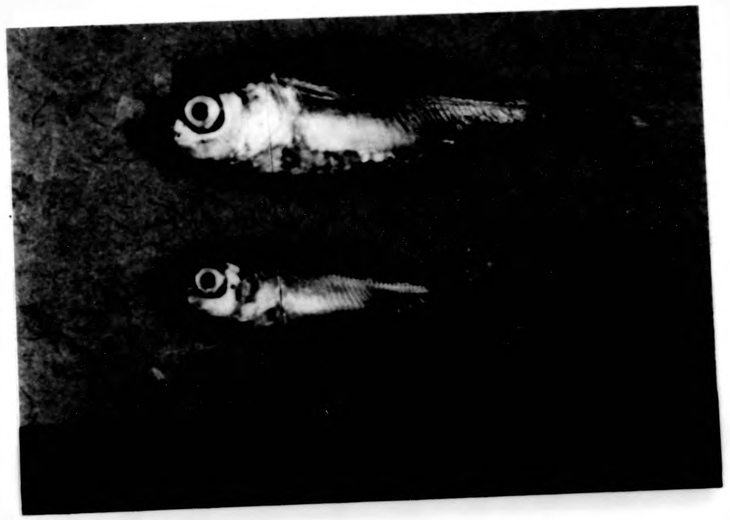


Plate 4.3b

Plate 4.3 Dorsal (a) and lateral (b) views of carp fry fed diet 6 (75% sesame) from trial 1, Expt. 4.1 showing malformations. Fish on the top are the normal fish from fish meal based control diet.

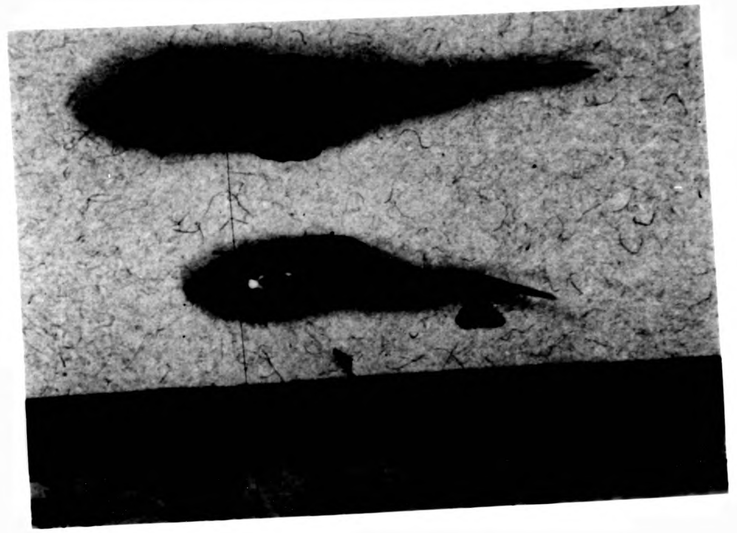


Plate 4.3a

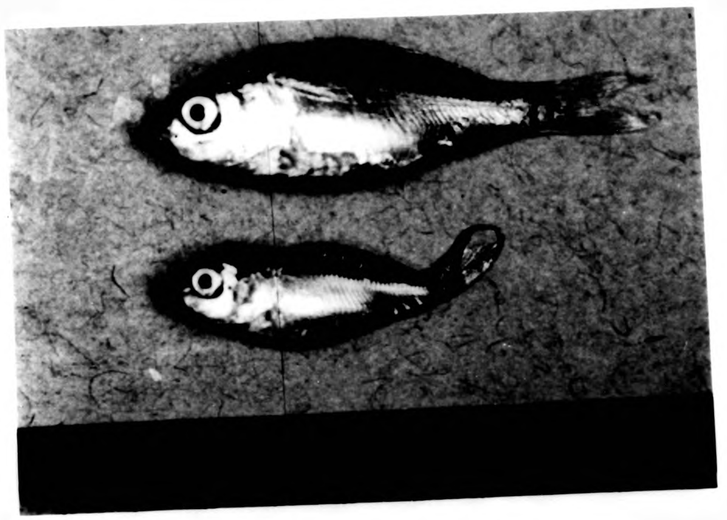


Plate 4.3b

Plate 4.4 Lateral view of carp fry fed diet 9 (25% Leucaena) from trial 1, Expt. 4.1 showing malformation. fish in the top is the normal fish from fish meal based control diet

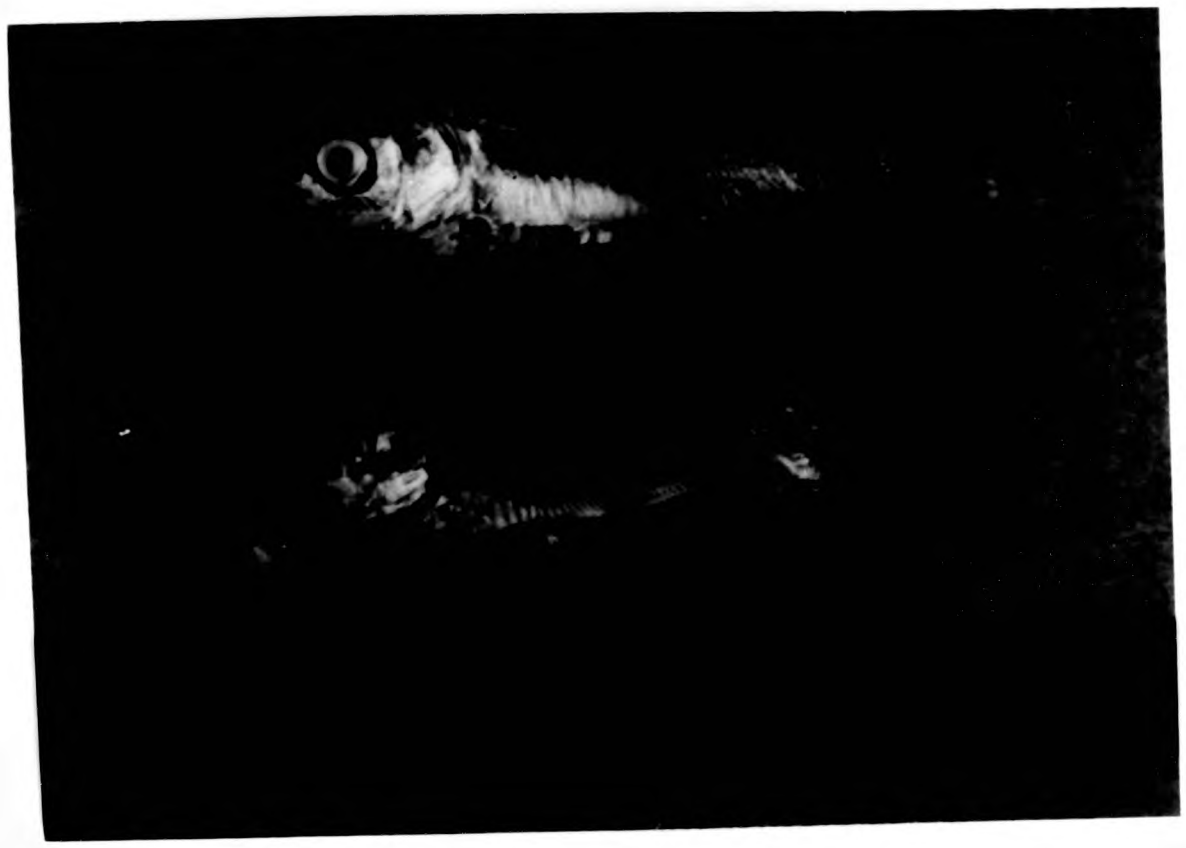


Plate 4.4 Lateral view of carp fry fed diet 9 (25% Leucaena) from trial 1, Expt. 4.1 showing malformation. fish in the top is the normal fish from fish meal based control diet

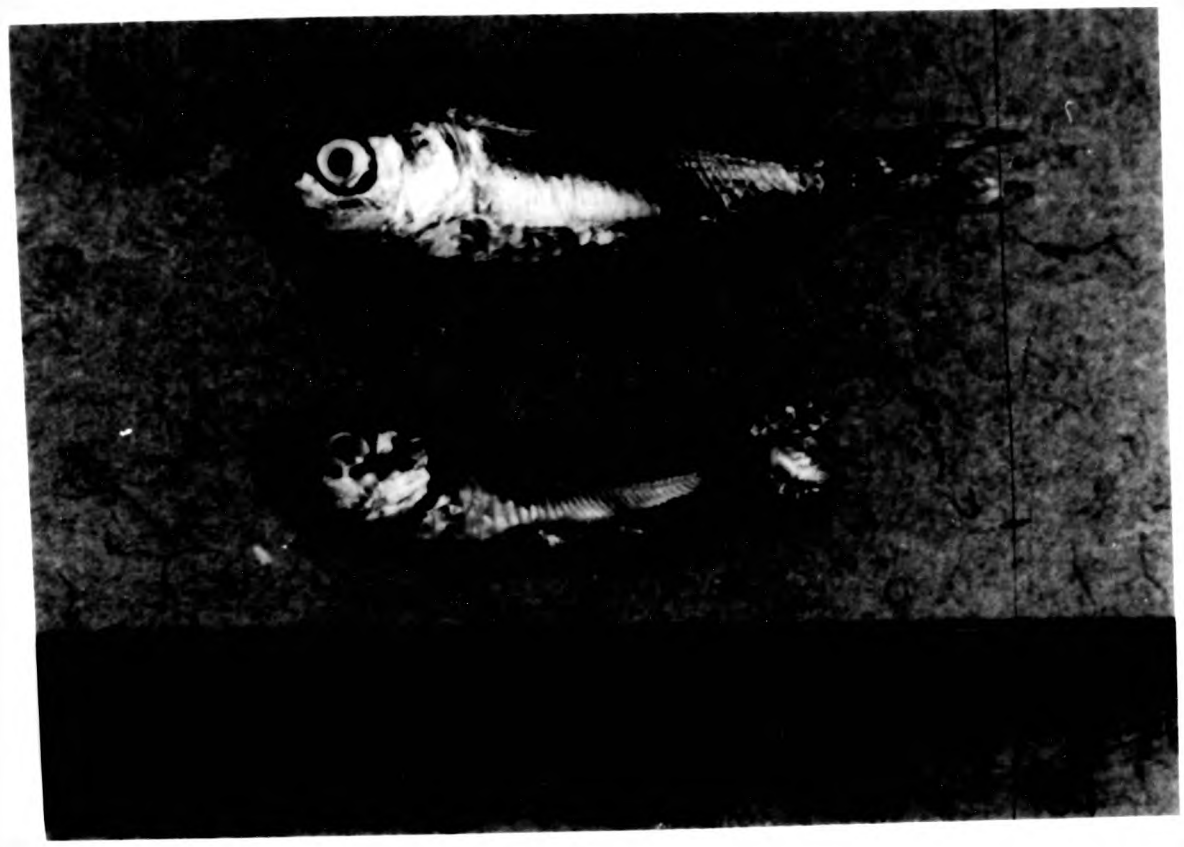


Plate 4.4 Lateral view of carp fry fed diet 9 (25% Leucaena) from trial 1, Expt. 4.1 showing malformation. fish in the top is the normal fish from fish meal based control diet

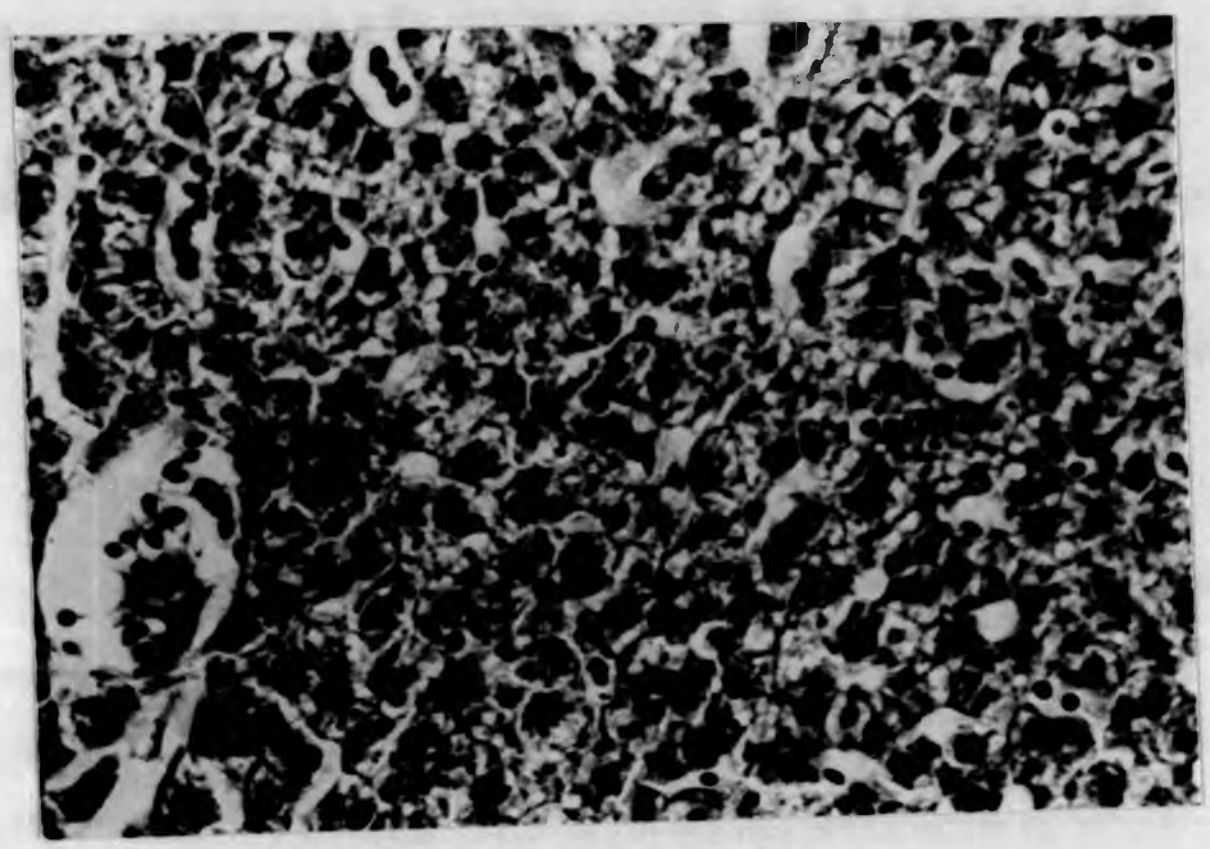


Plate 4.5 Section of liver from carp fry fed fish meal based control diet from trial 1, Expt. 4. 1. Note no visible intracellular lipid deposition (X 400)

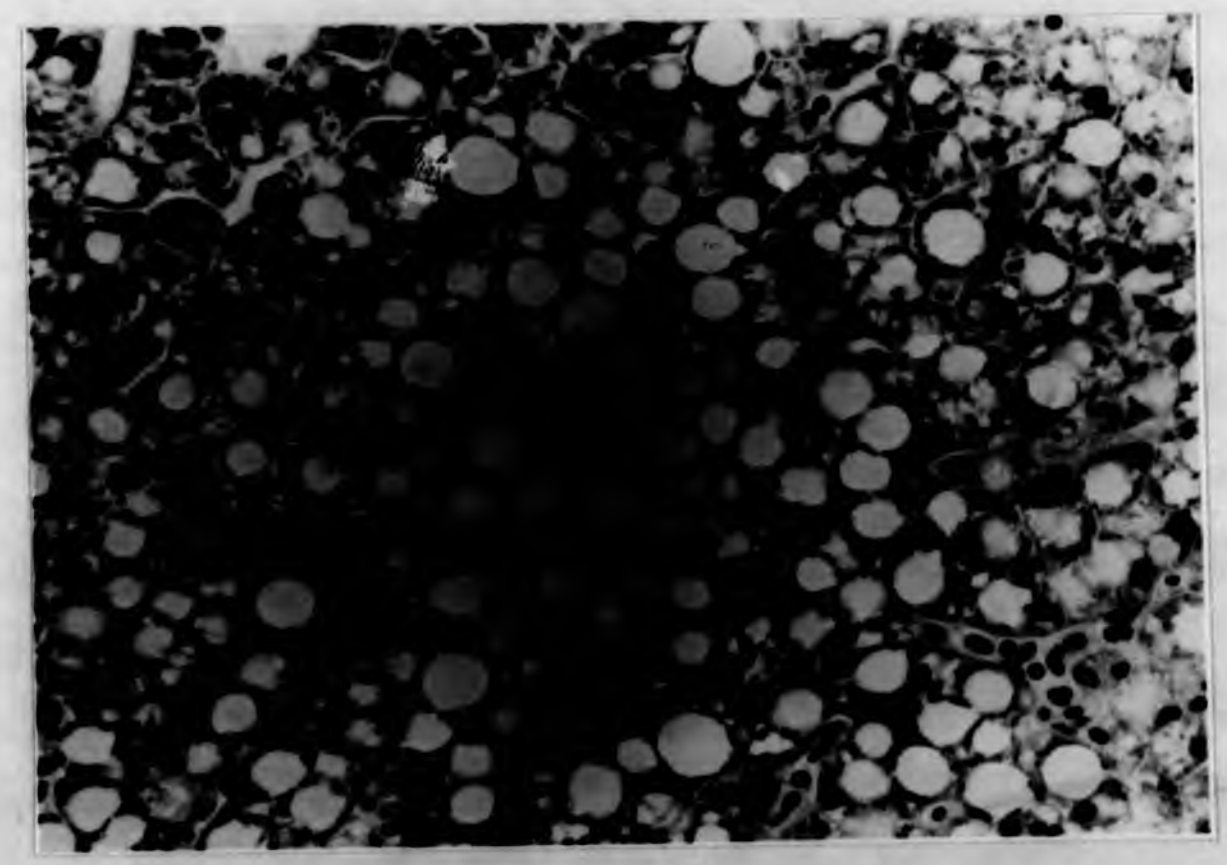


Plate 4.6 Section of liver from carp fry fed diet 2 (25% mustard) from trial 1, Expt. 4.1 showing severe intracellular lipid deposition (X 400)

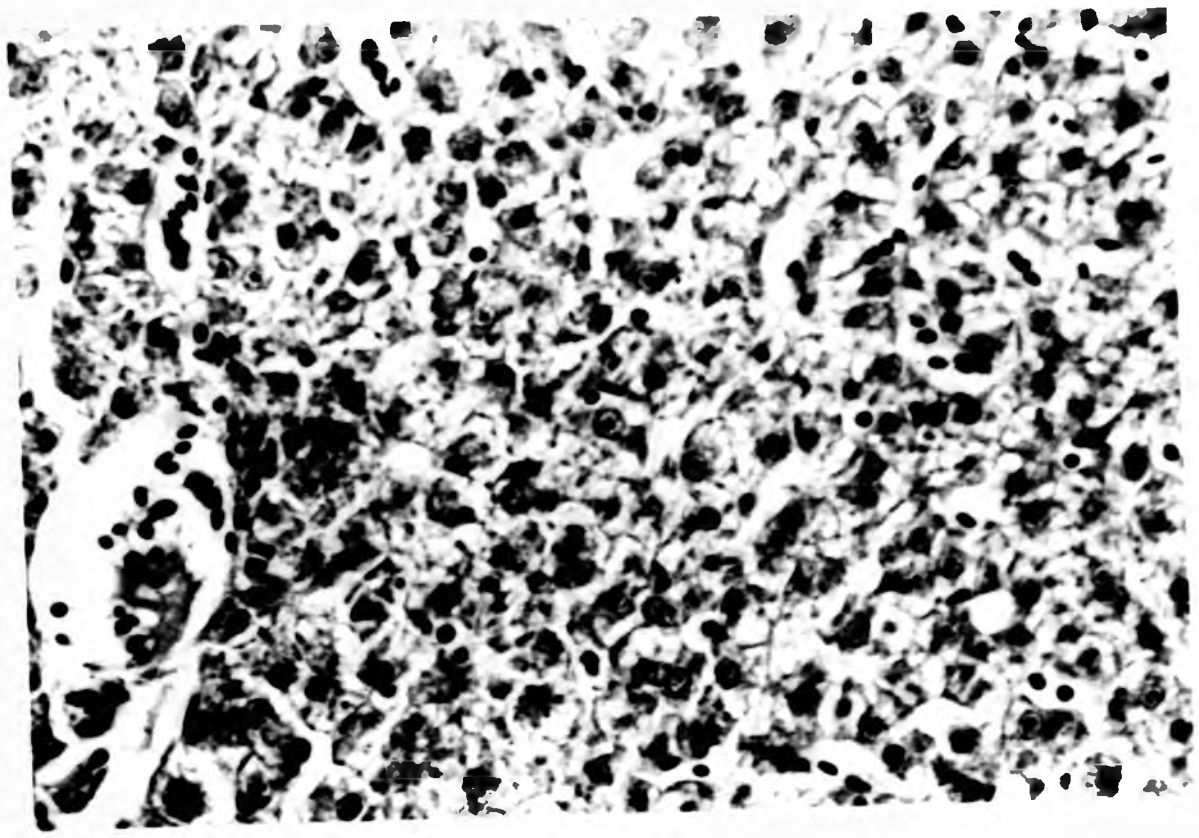


Plate 4.5 Section of liver from carp fry fed fish meal based control diet from trial 1, Expt. 4. 1. Note no visible intracellular lipid deposition (X 400)

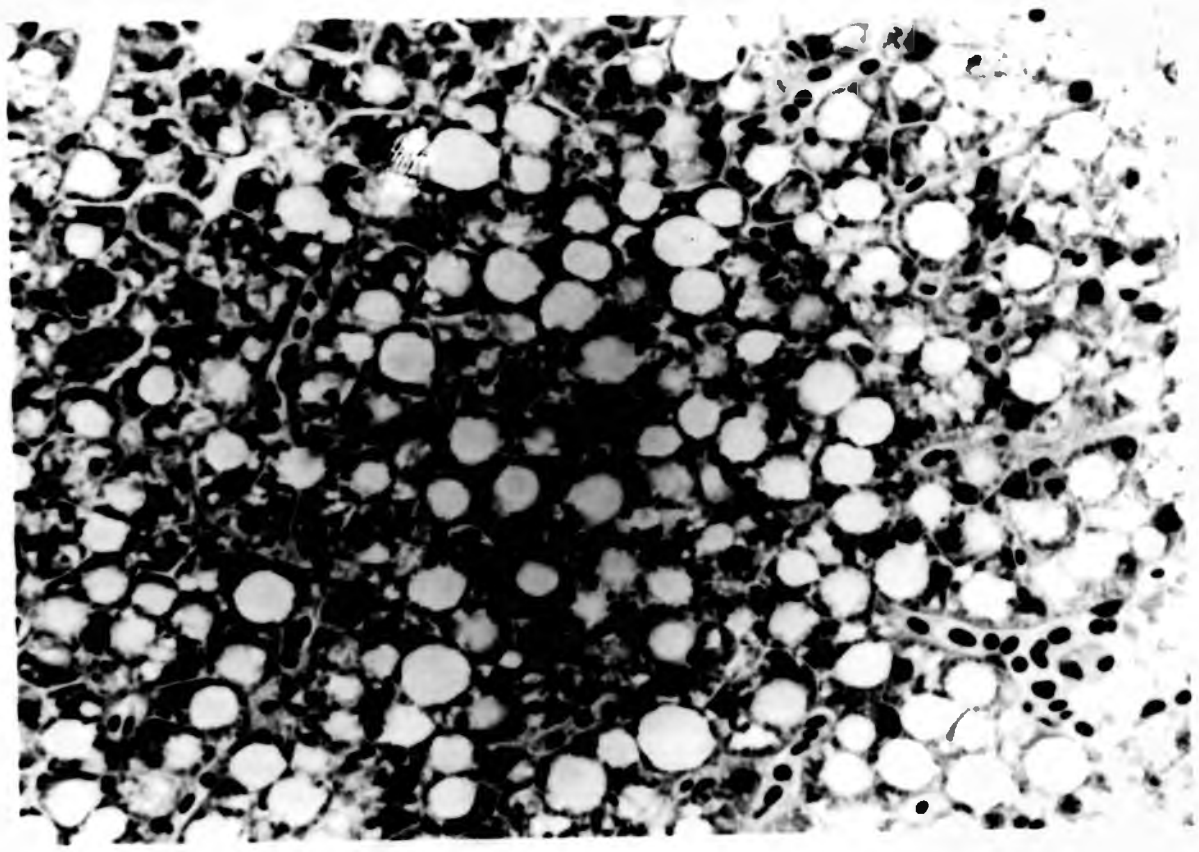
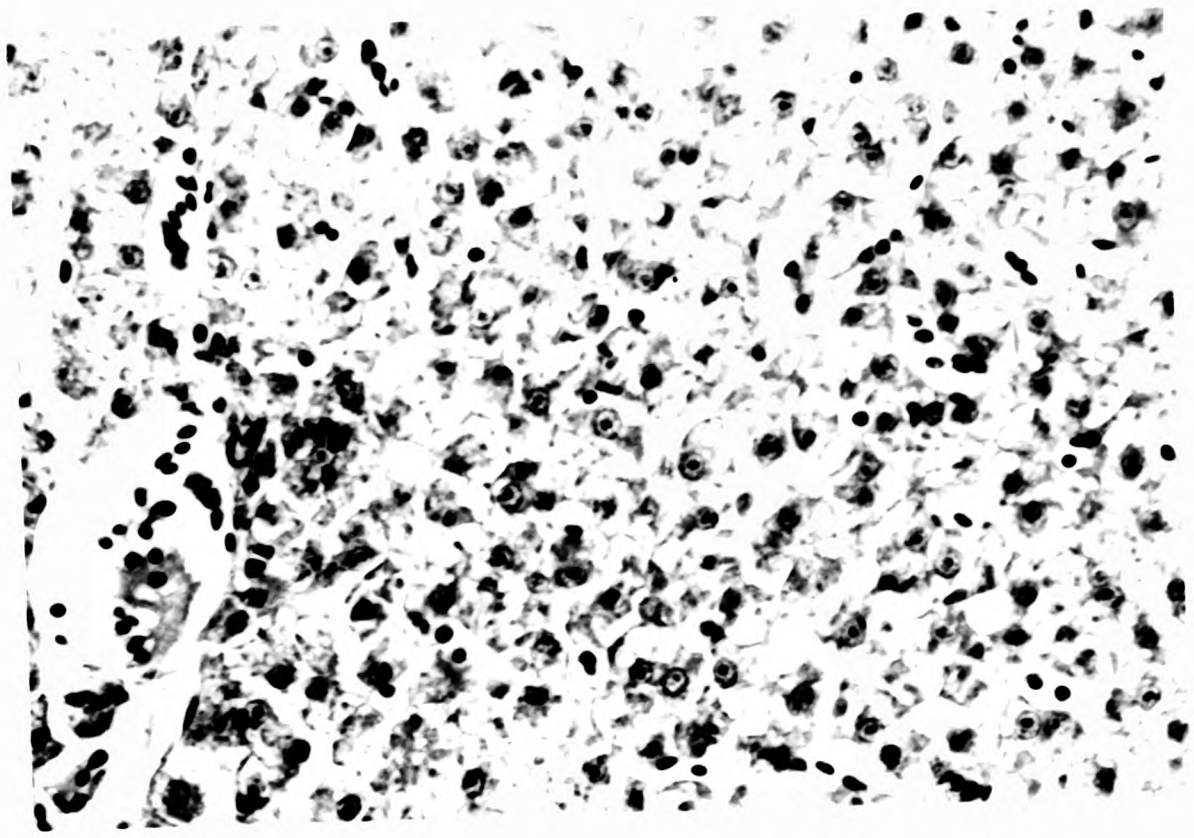


Plate 4.6 Section of liver from carp fry fed diet 2 (25% mustard) from trial 1, Expt. 4.1 showing severe intracellular lipid deposition (X 400)



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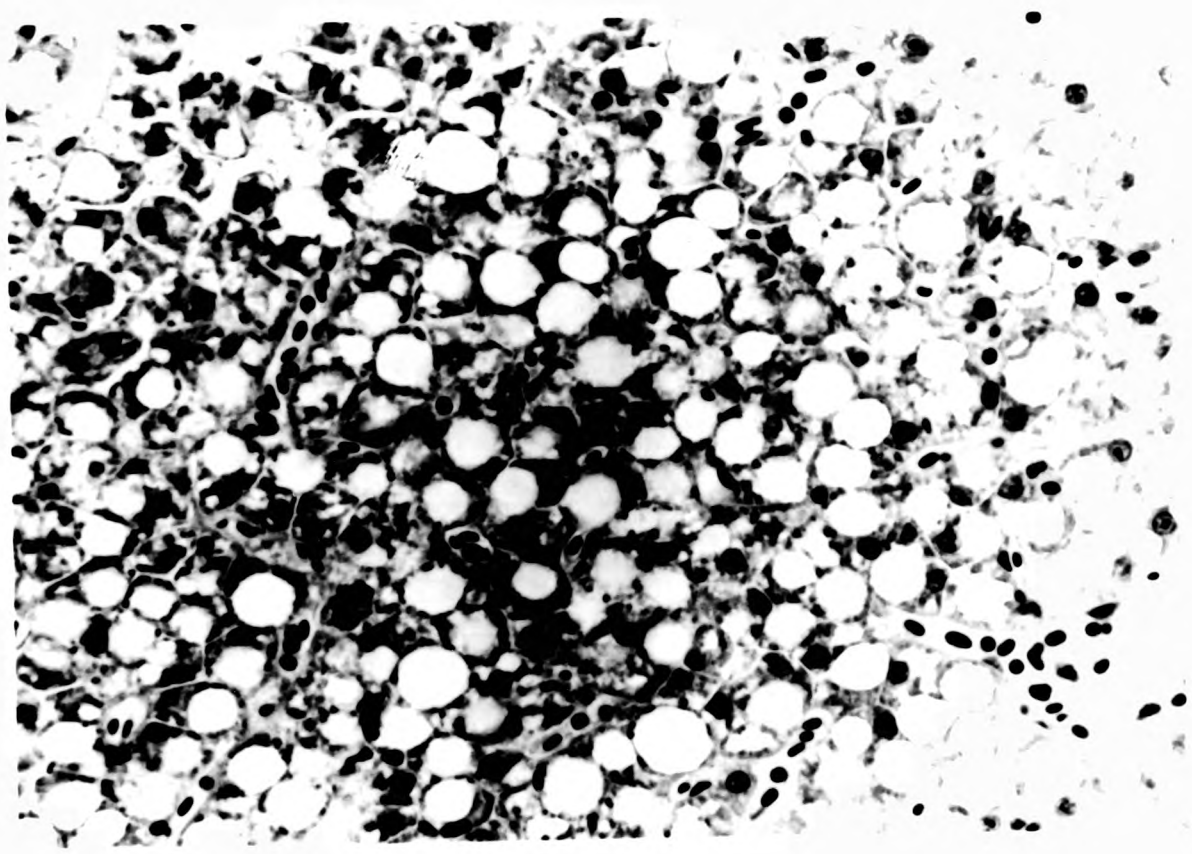

Plate 4.6 Section of liver from carp fry fed diet 2 (25% mustard) from trial 1, Expt. 4.1 showing severe intracellular lipid deposition (X 400)

lipid deposition in fish fed diet containing 25% mustard meal (diet 2) compared to control fish (Plate 4.5-4.6). Due to the loss of the sample, histological examination of fish on diet 3 (50% mustard) could not be done.

Increased lipid deposition in the liver of rats fed rapeseed meal containing low and high level of glucosinolates has been reported by Oliver *et al*. (1971). Similarly, Higgs *et al*. (1979)reported increased lipid deposition in the liver of coho salmon fed various diets containing poultry by-products meal and rapeseed meal.

4.3.1.4. Growth

The growth responses of carp fry fed different diets are presented in Table 4.13 and shown graphically in Fig. 4.2 From the above table and figure it can be seen that growth responses were significantly affected by the type of plant protein as well as its level of inclusion. The SGR values ranged from 3.53 for diet 9 (25% leucaena) to 7.15 for diet 1 (control). Out of several plant protein sources tested, the diets containing linseed and groundnut meal at 25% inclusion level resulted in comparable growth to that of the control. The SGR's for diet 1 (control), 7 (25% linseed) and 11 (25% groundnut) were not significantly different ($P > 0.05$) from each other, but these were all significantly higher ($P < 0.05$) than the SGR's recorded for the other diets.

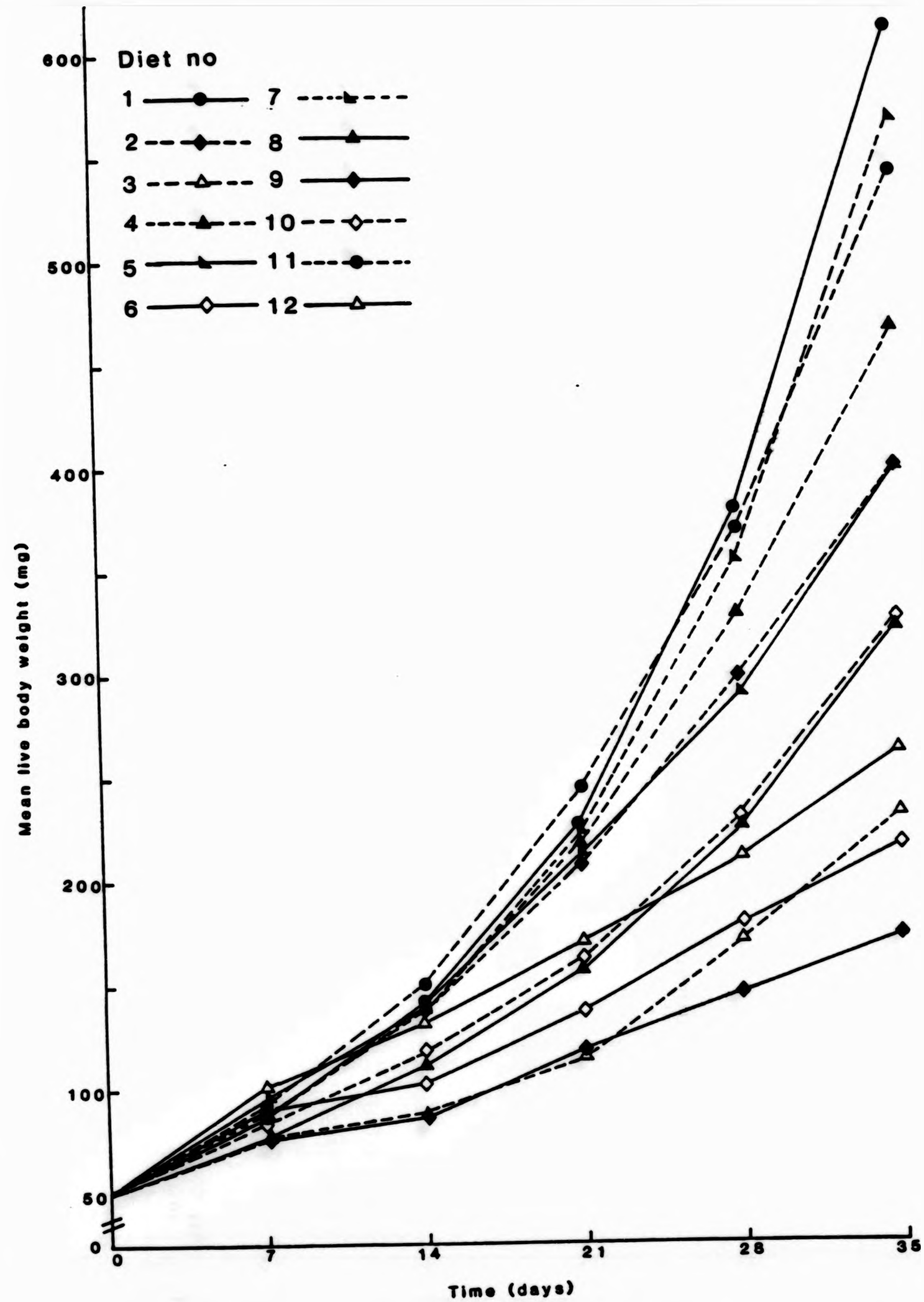

Fig. 4.2 Growth responses of carp fry at different dietary treatments in trial 1. Experiment 4.1

Table 4.14 Proximate carcass composition analysis (% fresh weight) of fish samples at the start and end of trial 1, Experiment 4.1

Components	Initial (all fish)	Final Diet No.												
		1	2	3	4	5	6	7	8	9	10	11	12	± SE**
Moisture	82.70	80.38^{b*}	77.52^{d}	77.90^{d}	78.30^{d}	77.94^{d}	78.18^{d}	79.57^{c}	79.14^{c}	82.21^{a}	79.42^{c}	79.02^{c}	78.24^{d}	0.19
Crude protein	11.55	12.90^{a}	12.35^{abc}	12.23^{bc}	12.44^{abc}	12.26^{bc}	11.86^{cd}	12.45^{abc}	12.54^{ab}	11.59^{d}	12.37^{abc}	12.38^{abc}	11.92^{bcd}	0.13
Crude lipid	2.02	4.03^{g}	7.52^{a}	6.61^{c}	6.31^{d}	7.09^{b}	6.72^{c}	5.42^{f}	5.52^{f}	3.17^{h}	5.55^{f}	6.09^{e}	7 14^{b}	0.07
Ash	2.21	2.05	1.71	1.92	1.84	1.68	1.68	1.87	1.88	2.11	1.89	1.76	1.41	-
Total	98.48	99.36	99.10	98.66	98.89	98.97	98.42	99.14	99.08	99.08	99.23	99.25	98.73	

* Figures in the same row with superscripts are not significantly different ($P>0.05$)

** Standard errors of treatment means calculated from the residual mean square in the analysis of variance

4.3.1.5. Food conversion

Food conversion ratios (FCRs) for the various diets tested are presented in Table 4.13. The fish meal based control diet gave the lowest FCR (2.26), although this was not significantly different ($P > 0.05$) from the diets containing 25% inclusion of linseed (FCR 2.33) groundnut (FCR 2.63) and sesame (FCR 2.82) meal protein. FCR's were significantly higher ($P < 0.05$) for diets containing 50% inclusion of mustard and 75% inclusion of sesame or groundnut meal protein. Diets containing leucaena meal even at 25% inclusion showed the poorest FCR (8.13).

4.3.1.6. Protein utilization

The level of protein utilization of the different dietary protein sources fed to carp fry were evaluated in terms of (a) protein efficiency ratio (PER) and (b) apparent net protein utilization (ANPU%) The PER and ANPU(%) values for each dietary treatment are presented in Table 4.13

The PER values of the fish meal based control diet (1.06) and 25% linseed diet (1.04) were not significantly different ($P > 0.05$); both of the PER values were significantly higher ($P < 0.05$) than those of all other diets. Diet 12 (75% groundnut), 6 (75% sesame), 3 (50% mustard) and 9 (25% leucaena) gave very poor PERs with diet 9 being significantly the poorest ($P < 0.05$).

Since the carcass protein content of all the experiment fish groups was similar, the ANPU(%) values tended to reflect the PER values. The ANPU values ranged from 3.87% (diet 9) to 13.71% (control diet).

4.3.1.7. Protein digestibility

Apparent protein digestibility (APD) values for different dietary treatments are presented in Table 4.13. The control diet with herring fish meal as the sole protein source had fairly poor digestibility (77.8%). But apart from diet 9 (25% leucaena meal; digestibility 63.3%) and diet 4 (25% sesame; digestibility 78.7%), all the plant protein sources in combination with fish meal protein showed very good digestibility; the values ranging between 81.0-89.5%.

4.3.1.8 Carcass composition

The proximate carcass composition of fish at the start and end of the trial are presented in Table 4.14. With the exception of fish fed diet 1 and 9, the carcass composition of the experimental fish was relatively unaffected by different dietary treatments. The carcass moisture content was highest ($P<0.05$) for diet 9 (82.9%) followed by diet 1 (80.38%); the values for other dietary treatment groups ranged between 77.52% and 79.57%. The carcass lipid content was the lowest ($P<0.05$) for diet 9 (3.17%) followed by diet 1 (4.03%); the values for other treatment groups ranged from 5.42% to 7.52%. The carcass crude

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protein and ash contents were similar for all dietary treatments and varied between 11.59-12.90% and 1.41-2.11% respectively.

4.3.2. Trial 2

During trial 2 experimental fish survived well on all diets and mortalities between different treatment were not significantly different ($P>0.05$) (Table 4.15).

4.3.2.1. Acceptability of diets

Acceptability of the different diets did not vary greatly during trial 2. However, the acceptability was influenced by the level of inclusion of plant protein as well as by the type of ingredients used. In general, diets containing linseed and groundnut meal were more acceptable than the other diets. Diets containing mustard meal, rice bran and wheat bran and or 75% plant protein showed the poorest acceptability. On the basis of the general observation on feeding, the acceptability of the diets may be placed in the following order: diet 1 and 2>4, 5, 6 and 8>3 and 7>9>10, 11 and 12.

4.3.2.2. Growth

The growth responses of carp fry in trial 2 are presented in Table 4.15 and illustrated in Fig. 4.3. Of the twelve diets tested in this trial,

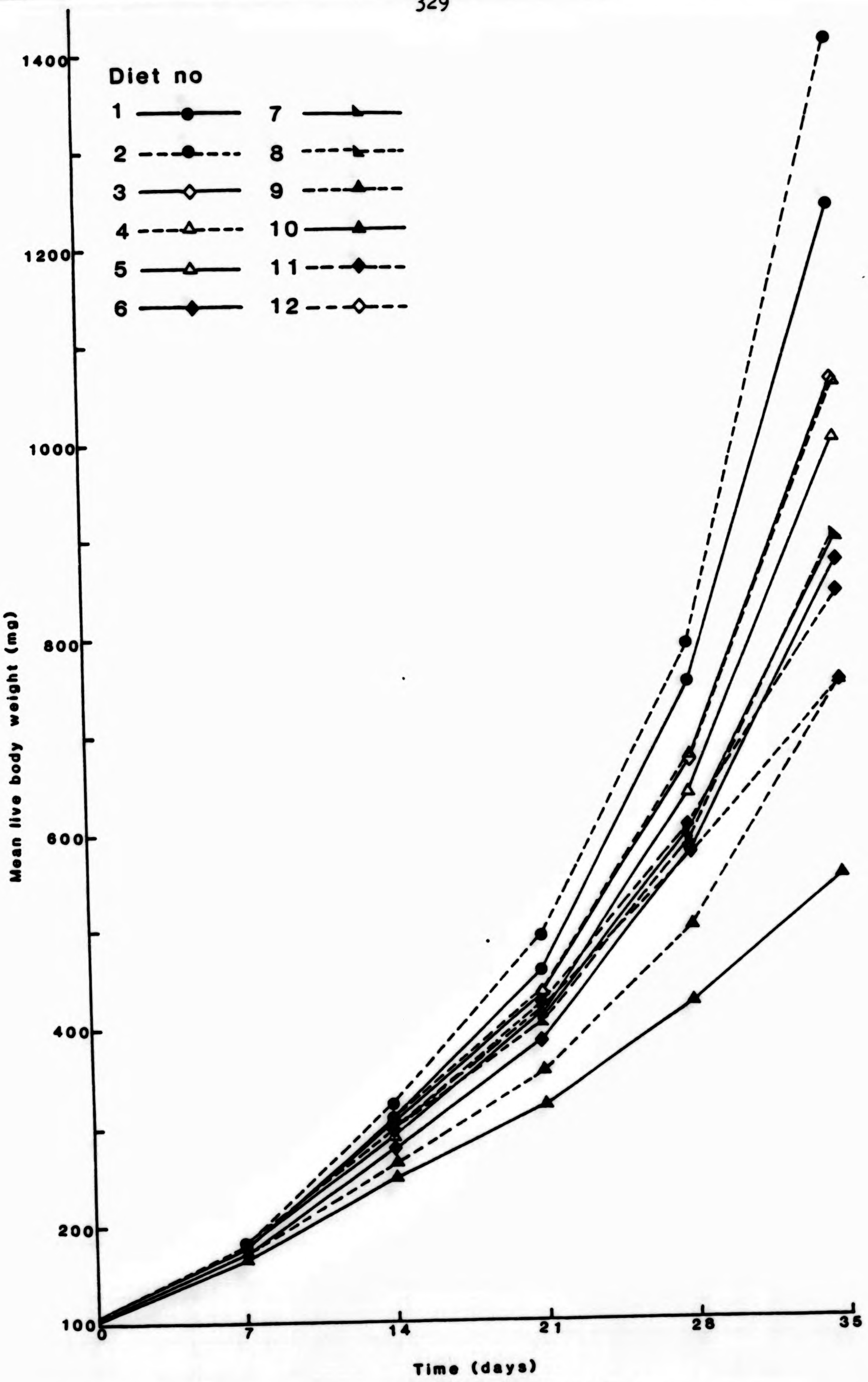

Fig. 4.3 Growth responses of carp fry at different dietary treatments in trial 2, Experiment 4.1

Table 4.15 Mortality, growth and food utilization of common carp fry reared for 35 days in trial 2 , Experiment 4.1

Mean initial weight 106 mg (± SE 2.95)

Mean values	Diet No.												
	1	2	3	4	5	6	7	8	9	10	11	12	±SE **
Final weight (mg)	1240^{b} *	1411^{a}	1062^{c}	1058^{c}	1000^{c}	874^{d}	897^{d}	899^{d}	752^{e}	559^{f}	844^{d}	747^{d}	20.46
Mortality (%)***	0	0	0.8	0	0	0	0	0	1.7	0.8	1.7	4.1	2.56
SGR	7.03^{b}	7.40^{a}	6.59^{c}	6.57^{c}	6.41^{c}	6.03^{d}	6.10^{d}	6.11^{d}	5.60^{e}	5.08^{f}	5.92^{d}	5.58^{f}	0.10
SGR as % of control	100	105.3	93.7	93.5	91.2	85.8	86.8	86.9	79.7	72.3	84.2	79.4	-
FCR	2.14^{b}	1.70^{a}	2.14^{b}	2.30^{c}	2.35^{c}	2.45^{cd}	2.54^{de}	2.60^{def}	2.76^{f}	3.69^{h}	2.70^{ef}	2.98^{g}	0.05
PER	1.11^{b}	1.40^{a}	1.13^{b}	1.08^{bc}	1.05^{bc}	1.03^{cd}	0.97^{d}	0.98^{d}	0.89^{ef}	0.67^{g}	0.91^{e}	0.85^{f}	0.02
ANPU (%)	15.08^{bc}	19.52^{a}	15.62^{b}	15.32^{bc}	13.95^{d}	14.36^{cd}	12.94^{e}	12.95^{e}	11.88^{ef}	9.17^{g}	12.58^{e}	11.35^{f}	0.31
APD (%)	80.6	85.6	87.0	87.8	88.5	87.3	85.8	86.1	85.6	84.5	83.3	84.6	-

* Figures in the same row with same superscripts are not significantly different (P>0.05)

** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

*** Mortality in different treatment are not significantly different (P>0.05)

Table 4.16 Proximate carcass composition analysis(% fresh weight) of fish samples at the start and end of trial 2 , Experiment 4.1

Components	Initial (all fish)	Final Diet No.												
		1	2	3	4	5	6	7	8	9	10	11	12	± SE**
Moisture	82.17	79.52^{a} *	77.88^{c}	78.21^{cc}	78.26^{bc}	78.08^{bc}	78.19^{bc}	78.44^{bc}	78.38^{bc}	78.67^{b}	78.57^{b}	77.96^{c}	78.65^{b}	0.13
Crude protein	12.07	13.45^{ab}	13.76^{a}	13.67^{ab}	13.52^{ab}	13.16^{b}	13.78^{a}	13.24^{b}	13.13^{b}	13.21^{b}	13.37^{ab}	13.57^{ab}	13.47^{ab}	0.11
Crude lipid	2.72	4.47^{d}	5.85^{abc}	5.76^{abc}	5.81^{abc}	5.72^{abc}	5.65^{abc}	5.94^{ab}	5.98^{a}	5.51^{c}	5.77^{abc}	5.90^{ab}	5.61^{bc}	0.08
Ash	2.03	2.06^{a}	2.01^{abc}	2.00^{abc}	2.02^{ab}	2.02^{abc}	2.04^{ab}	1.94^{bc}	1.94^{bc}	2.09^{a}	1.91^{c}	2.07^{a}	1.81^{d}	0.02
Total	98.99	99.50	99.50	99.64	99.61	98.98	99.66	99.56	99.43	99.48	99.62	99.50	99.54	-

* Figures in the same row with same superscripts are not significantly different (P>0.05)

** Standard error of treatment mean, calculated from the residual mean square in the analysis of variance

diet 2 (20% linseed and 20% groundnut) produced the best growth response throughout the experimental period (Fig. 4.3), while diet 10 (75% inclusion of plant protein) resulted in the poorest growth. The SGRs at different dietary treatments varied between 5.58 and 7.40.

In general, a combination of linseed and groundnut meal resulted in better growth responses than the other combinations used. For example, diet 2, where 20% linseed and 20% groundnut meal protein were used, produced an SGR of 7.40 whereas diet 3, where 20% linseed and 20% sesame meal were used, gave an SGR of only 6.59. Similarly, diet 5 (25% linseed and 25% groundnut)produced a significantly higher ($P < 0.05$) SGR (6.41) than diet 6 (25% linseed and 25% sesame) (SGR 6.03). Growth rate also decreased with increase in the level of inclusion of plant protein. For example, diet 2 (40% plant protein) gave a SGR of 7.40 compared to a SGR of 6.41 produced by diet 5 (50% plant protein).

The inclusion of rice bran and wheat bran (diet 11 and 12) as dietary carbohydrate resulted in reduced growth in comparison to the diets containing similar levels of plant protein but with dextrin and corn starch as the carbohydrate source.

4.3.2.3. Food conversion and protein utilization

The food conversion ratios (FCRs), protein efficiency ratios (PERs) and apparent net protein utilization (ANPU %) for the different diets

diet 2 (20% linseed and 20% groundnut) produced the best growth response throughout the experimental period (Fig. 4.3), while diet 10 (75% inclusion of plant protein) resulted in the poorest growth. The SGRs at different dietary treatments varied between 5.58 and 7.40.

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4.3.2.3. Food conversion and protein utilization

The food conversion ratios (FCRs), protein efficiency ratios (PERs) and apparent net protein utilization (ANPU %) for the different diets

tested in trial 2 are presented in Table 4.14. The FCR, PER and ANPU(%) all followed closely the trend observed in the growth responses under different dietary treatments. In all three cases, diet 2 gave significantly the best (P 0.05) responses and diet 10 the poorest (P 0.05). The FCR, PER and ANPU(%) values observed in this trial ranged between 1.70-3.69, 0.67-1.40 and 9.17-19.52% respectively.

4.3.2.4. Protein digestibility

All the diets showed very good digestibility of protein except the fish meal based control diet, which had a lower digestibility similar to that of the control diet in trial 1. The apparent protein digestibility (APD) value for control diet was 80.6% and those for all other diets varied between 83.3 and 88.5%.

4.3.2.5. Carcass composition

The proximate carcass composition of initial and final samples of fish from trial 2 are presented in Table 4.15. Apart from fish fed diet 1, the carcass composition of the experimental fish was little affected by dietary regimes. The fish fed diet 1 had a significantly higher (P 0.05) moisture content and lower (P 0.05) lipid content. The values for carcass moisture, crude protein, crude lipid and ash content varied between 77.88-79.52%, 13.13-13.76%, 4.47-5.8% and 1.81-2.07% respectively.

4.4. Discussion

The results from trial 1 of the present investigation indicate that linseed and groundnut meal at low inclusion levels (25% of protein) are very good dietary protein sources for carp fry. Although the performance of sesame meal was not as good as those of linseed or groundnut, it proved to be a moderately good protein source for carp fry even at an inclusion level of 50%. Leucaena and copra meal proved to be unsuitable even at low inclusion level. Mustard meal was unsuitable at a high inclusion level (50% of protein), while at a low inclusion level, it did not produce growth comparable to linseed, groundnut or even sesame. Nevertheless its performance was not as poor as that of leucaena or copra meal.

There are several factors which might have contributed to the observed variation in the growth responses of carp fry.

(1) Variations in the acceptability of diets;

(2) Presence of various toxic and antinutritional factors such as glucosinolates in mustard oilcake, phytic acid in sesame meal, linamarin in linseed oilcake, mimosine in leucaena leaf meal and aflatoxin in groundnut meal;

(3) Imbalance of limiting essential amino acids in the diets containing higher inclusion of plant protein source;

(4) Variations in the digestibility of plant protein and carbohydrates.

Despite none of the essential amino acid being limiting and with moderately good acceptability, diets containing mustard meal even at low inclusion level (25% of protein) showed poor growth responses and high mortality, in comparison to fish meal based control diet.

The above observations support the finding of Capper et al. (1982) The authors observed that untreated mustard meal (black mustard from Nepal) included at 20% in the diet of fingerling common carp for 50 days resulted in depression of live-weight gain and adverse change in the rate of food conversion.

The poor growth responses of carp fry fed diets containing mustard meal were presumably due to the presence of toxic components in the mustard seeds. Potentially the most toxic components are glucosinolates, which upon hydrolysis by an enzyme thioglucosidase usually present in the seed, release highly toxic isothiocyanates (Tookey et al., 1980). Moreover, thioglucosidase may also act on glucosinolate to produce highly irritant mustard gas or intestinal irritants (Gohl, 1981).

Experimental evidence for the toxic action of isothiocyanates is well documented in rodents and poultry. Feeding rodents with rapeseed containing high levels of glucosinolates, has been reported to cause depression of growth; marked enlargement of the thyroid, liver, adrenals and kidneys; delay in ovary development and histopathological changes in pituitary (see review by Tookey et al., 1980).

The mustard meal used in the present trial was in the form of expeller cake produced in Bangladesh where it is not commercially heat treated (section 4.2.2.) and presumably, contains high levels of glucosinolates and the enzymatic breakdown products isothiocyanates.

The performance of carp fry fed 25% linseed meal protein (diet 7) was not significantly different (P 0.05) to that of fish receiving the control diet. In respect of growth, food conversion, protein utilization and survival, the good performance of linseed meal may be attributed to its excellent acceptability to carp fry (Table 4.12). Although the linseed meal used in the present investigation was deficient in lysine, methionine and threonine, the prepared diets did not have a deficiency in any of the essential amino acids because of the amino acid profile contributed by their fish meal component.

However, the diet with higher inclusion of linseed meal (50% of protein; diet 8) did not achieve comparable growth and food conversion to the control diet or diet 7, although the survival of fish was comparable. The high survival of fish on this diet reflects the fact that the fish fed actively throughout the experimental period, while their poor growth implies the presence of some nutritional deficiency or growth inhibitory substance in the linseed meal.

Published information on the suitability of linseed meal as a dietary protein source for fish is not available. However, the efficiency

of linseed meal as a dietary protein supplement for domestic farm animals has been investigated fairly extensively (Montgomery, 1980; Gohl, 1981; McDonald et al., 1981). Immature linseed contains a small amount of the cyanogenetic glucoside, linamarin, which in the presence of an associated enzyme, linase, liberates hydrogen cyanide (HCN) on hydrolysis. Unprocessed whole seeds, and linseed meal processed under low temperature, can be toxic to animals especially if wetted before being fed (Gohl, 1981; McDonald et al., 1981). Normal processing conditions involving high temperature treatment, however, destroys linase and most of the linamarin, and the resultant meal are quite safe (Gohl, 1981; McDonald et al., 1981; Aquaculture Development Coordination Programme (ADCP), 1983).

The linseed meal used in the present trial was in the form of expeller cake and therefore presumably contained some linamarin. However, the excellent growth performance as well as the absence of histopathological changes (section 4.3.1.3.) for carp fry fed a low dietary inclusion of linseed meal demonstrates that they can tolerate a lower level of linamarin or hydrogen cyanide. Similarly McDonald et al. (1981) reported that linseed has a very good reputation as a feed for ruminants. They also observed that linseed meal is an excellent protein food for pigs, provided it is given with an animal protein supplement to make good its deficiency in methionine, lysine and calcium.

Another positive aspect of linseed meal as a fish feed component is

its high pelletability (ADCP, 1983) and diets incorporating linseed have better water stability which in turn reduces nutrient leaching.

The groundnut meal at low dietary inclusion level (25% of protein) produced very good growth comparable to that of the fish meal based control diet. However, when a higher inclusion of groundnut meal (75% of protein; diet 12) was used, the growth rate of carp fry was 36% lower than that for the control diet, while food conversion and protein utilisation were correspondingly poor.

There are a number of factors which might have contributed to the poor growth responses of carp fry fed the diet containing a high level of groundnut meal. The groundnut meal used in the present trial was deficient in three essential amino acids - lysine, methionine and threonine. Consequently a high inclusion of groundnut meal protein resulted in amino acid deficiency - especially of methionine, giving a chemical score as low as 66.7% (Table 4.7).

Similarly, severely depressed growth responses have been reported for Oreochromis aureus (Wu and Jan, 1977), O. mossambicus (Jackson et al., 1982) and O. niloticus (Kamara, 1982) when fed diets containing high inclusion (50% or more) of protein from groundnut meal. Wu and Jan (1977) and Jackson et al. (1982) attributed this growth depletion to an essential amino acid deficiency in the diet.

The groundnut meal used in the present trial contained 30 ppb of

aflatoxin (section 4.2.2.) Aflatoxins are toxic metabolites of Aspergillus flavus Link, a mutant variety of the common bluegreen mold. They are found commonly in groundnut oilcake as a result of improper storage. Aflatoxins are powerful hepatocarcinogens and mortality among afflicted animals and fish can result from severe liver damage (Ashley, 1970; Wales, 1974; Austwick, 1975). However, the level of aflatoxins present in the groundnut meal used in this investigation is unlikely to have affected the survival or growth of carp fry during their short exposure to the diet (see review of nutritional pathology by Ashley, 1970, 1972).

Histopathological examination of the liver and other organs of carp fry fed a high dietary level of groundnut meal also did not show any significant changes (section 4.3.1.3.). Ashley (1970) has reported that when 20 ppb of crude, or 0.5 - 8.0 ppb or crystalline B_1 aflatoxin was fed in the daily ration to trout for 3-12 months, chronic aflatoxicosis induced hepatoma occurred. However, coho salmon and channel catfish were refractive to aflatoxin and none had hepatoma even after two years when fed crude or crystalline B_1 aflatoxin. Therefore long term growth studies would be required to fully evaluate the effect of aflatoxins on carp fry.

Unlike linseed and groundnut meal, sesame meal even at a low incluson level (25% of protein) did not produce comparable growth to that of the control diet despite its good amino acid profile and absence of any known toxic factor. However, sesame seed has a high phytic acid

content (Gohl, 1981). Phytic acid - a phosphoric acid derivative (myo inositol-hexadihydrogen phosphate) has the capacity to bind, di- and trivalent metal ions such as calcium, magnesium, zinc and iron to form insoluble salts (Liener, 1977; McDonald et al., 1981) and therefore rendering them unavailable during digestion (Liener, 1977; smith, 1977).

The ability of phytic acid to bind metal ions is lost when the phosphate groups are hydrolysed through the action of the enzyme phytase (Liener, 1977). Although phytase activity has been shown to be present in ruminants (McDonald et al., 1981), animals like fish with simple stomachs lack this enzyme in their gastrointestinal tracts (Lall, 1979). Therefore fish cannot utilize phytate bound phosphorus, or other metal ions. Unless phytic acid is already saturated with metal ions naturally present in the plant and sufficient mineral supplementation has not been used in the diet, mineral deficiency may occur. The requirements of carp and other fin fish for phosphorus, magnesium and zinc are well documented (Lall, 1979; Jauncey, 1982). Therefore, the presence of a high level of phytic acid in the sesame meal diet may have been a factor responsible for the observed poor growth response of carp fry.

Sesame meal also has poor palatability which may have reduced the fish's food intake. Gohl (1981) reported that when sesame meal is wetted, it acquires an unpleasant taste and recommended that it should be kept unwetted when fed to animals.

Essential amino acid deficiency may have been another contributory factor explaining the lower growth responses of carp fry that received a high dietary level of sesame meal protein. Diet 5 (50% inclusion) had a lysine content of 5.05% (of crude protein) and diet 6 (75% inclusion) 3.65%, whereas the lysine requirement for carp is 5.70% (after Nose, 1979). Moreover, during the processing of feedstuffs, the E-amino group of the lysine molecule may react with non-protein molecules present in the feedstuffs to form additional compounds that render lysine biologically unavailable although chemically measurable (Cowey 1979). Diet 6 also had a deficiency in threonine, its concentration being only 3.17% (of crude protein), whereas the requirement for carp is 3.9%.

Leucaena leaf meal proved to be a very poor source of protein for carp fry even at a low dietary inclusion level (25% of protein). The acceptability of the diet was also very poor and consequently there was high wastage of food and correspondingly a high FCR value.

Leucaena leaf contains an unusual amino acid, mimosine, which comprises about 3-5% of the dry weight of its protein content (NAS, 1977). An adverse effect on the growth of cattle has been observed when leucaena constitutes more than 50% of the diet. This is due to the under production of thyroxine presumably because the rumen bacteria convert mimosine to 3, 4-dihydroxy-pyridine, which acts as a goitrogenic agent (Hegarty et al. 1976; Jones et al., 1976). Although the goitrogenic effect of mimosine in ruminants seems to be well

established, the precise mechanism of toxicity in other animals remains obscure (Liener, 1980).

Contrary to the widespread utilization of leucaena in the rations of cattle and other terrestrial farm animals (NAS, 1977), feeding of fish with leucaena leaf has not proved very successful. In tests with copra, groundnut, soybean, sunflower, rapeseed, cottonseed and leucaena as a dietary protein source, Jackson et al. (1982) obtained the poorest growth rate and food conversion ratio for fingerling O. mossambicus from a diet containing 30.4% leucaena leaf meal (25% of total protein). Similarly reduced growth and food conversion has been reported for fingerling O. niloticus fed a ration containing rice bran, fish meal and leucaena leaf meal (33:44:33) compared to a diet containing only ricebran and fish meal (63.4: 36.6) (Cruz and Laudencia, 1978). Madraisan and Bryan (1976) cited by Cruz and Laudencia (1978) also found that weight gain in rabbitfish, Siganus canaliculatus, fed with leucaena leaf meal was negligible. Poor performances in all the above three species has been attributed to the presence of mimosine in leucaena leaf.

Although copra meal is not known to contain any toxic components (Jauncey and Ross, 1982), it did not prove to be an acceptable protein source for carp fry. The test diet containing 40.65% copra meal (25% of protein) resulted in significantly lower growth rate compared to the control diet. Published information regarding the efficiency of copra meal as a dietary protein source for fish is scarce. Cruz and

Laudencia (1978) observed that complete or partial substitution of rice bran by copra meal did not improve the weight gain or food conversion efficiency of fingerling O. niloticus. On the contary, Jackson et al. (1982), in a study with fingerling O. mossambicus used 34.1% copra meal to replace 25% of fish meal protein in a 30% protein diet and observed significantly better growth of fish when compared to fish meal based control diet. They attributed the improved growth performances of copra meal diet to its higher level of arginine content compared to argine content of control diet. However, when the authors used a higher inclusion level (68.2% - 50% of protein), the growth rate and food conversion efficiency of the tilapias were reduced significantly.

Despite a high protein digestibility (81%) a good amino acid profile and the absence of any known toxic factor in the diet containing copra,it did not result in growth comparable to that of the control diet.

Copra meal has a high crude fibre and carbohydrate content (Table 4.3) and therefore no additional dietary fibre (in the form of -cellulose) was added to the copra meal diet. Generally different dietary carbohydrate sources have wide variation in their digestibility and the digestibility decreases with the complexity of its molecular structure (Chow and Halver, 1980; NRC, 1983). The presence of certain complex carbohydrates in the plant product may have an adverse effect on growth (Friedman and Shibko, 1972).

It is possible that comparatively low digestible energy was available

from the dietary carbohydrate source in the copra meal, which ultimately reduced the protein sparing action of digestible carbohydrate (Jauncey, 1982; NRC, 1983), thereby effectively reducing the growth rate of the fish.

The poor pelletability of copra meal may be another contributing factor affecting its suitability as a component in fish feed. Due to the inclusion of a high proportion (40.65% of diet) of copra meal in the test diet, the desired feed particle size could not be maintained while feeding. Feed particle size has been shown to be an important parameter affecting the food intake and growth of carp fry and other fin fish (see section 2.4). Moreover, the administered food particles tended to absorb a large amount of water causing the feed particle to disintegrate quickly, which inevitably led to nutrient leaching.

In the present trial, the control diet with herring meal showed somewhat lower digestibility. Although fish meal protein generally has digestibility in excess of 95% (Jauncey and Ross, 1982), that of herring meal protein is reported to be lower than white fish meal for carp and channel catfish (see Table 4.17). Apart from the diet containing leucaena leaf meal (diet 9) all the test protein sources in combination with fish meal protein showed very good digestibility. Generally oilseed meal proteins have digestibility of 80-90% for fish (Jauncey and Ross, 1982) and carp are reported to be able to digest the proteins of plants as well as, or slightly better than monogastric mammals (NRC, 1983) (see Table 4.17).

Table 4.17 Protein digestion coefficient (%) of various dietary ingredients for carp, catfish, rainbow trout and two terrestrial farm animals

Dietary ingredients	TEST ANIMAL				
	Carp[a]	Channel catfish[a]	Rainbow trout[b]	cattle[c]	sheep[d]
Alfalfa hay meal	-	13	-	68-74	74-76
Copra meal	-	-	-	79	91
Corn gluten meal	91*	80	-	85	80
Cotton seed meal	-	76-93	73-84	77-86	75-83
Fish meal, herring	80[e]*	80	-	-	-
Fish meal, white fish	95*	85	81-94	90	93
Groundnut meal	85	-	-	89-92	94
Linseed oilcake	-	-	-	86	87
Leucaena, fresh browse	-	-	-	65	-
Rapeseed meal	-	-	-	84	-
Rice bran	-	71	-	-	-
Sesame meal	-	-	-	-	91
Silk worm pupae meal	93	-	80-88	-	88
Soybean meal	81-96	72-84	71-88	90	85-92
Wheat bran	-	-	-	74	-

[a] NRC (1983) [b] Hastings (1969) [c] McDonald et al.(1981) [d] Gohl (1981) [e] Data from Atack et al.(1979)

* True digestion coefficients

The diet containing leucaena leaf meal gave a very poor protein digestibility (63.3%). No published information is available on the digestibility of leucaena leaf protein by fish except that Cruz and Laudencia (1978) mentioned that leucaena leaf meal is less digestible than mulberry leaf meal. The authors however did not present any data on digestibility. Channel catfish have reportedly a very low digestibility (13%) for protein from alfalfa hay meal (Table 4.17). Alfalfa leaf is comparable to leucaena both in terms of nutrients and roughage quantity (NAS, 1977). Dehydrated alfalfa meal, however, has a higher protein digestibility (66%) for goldfish, Carassius auratus (NRC, 1983).

The carcass protein contents of carp fry fed different diets did not show large variations, although some of the values are statistically different (Table 4.14). However, the carcass moisture and lipid contents did show large fluctuations. Carcass moisture and lipid contents of fish always tend to show greater fluctuations that other carcass components and they appear to be inversely related (Brett et al., 1969; Andrews and Stickney, 1972; Atack et al., 1979; Zeilter et al., 1984). The high carcass lipid content of carp fry fed diet containing leucaena leaf meal are similar to the observation reported by Appler and Jauncey (1983) with juvenile O. niloticus. The authors observed marked decrease in carcass lipid content with fish fed on diets containing filamentous green algae as a partial or sole source of dietary protein.

Trial 2 of the present investigation demonstrated that a combination

of different plant protein sources used as a single component partially substituting for fish meal was more acceptable to carp fry than plant protein materials used individually. However, the acceptability of the diet was influenced strongly by the inclusion of linseed and groundnut meal, both of which were found to be highly acceptable to carp fry in trial 1.

In comparison to trial 1, the better performance of carp fry fed the diets in trial 2 prepared with more than one plant protein may be attributed to the following: -

(1) comparatively better acceptability of the diets;

(2) maximum inclusion of plant protein from a single source was not more than 25% of total protein, which prevented a high inclusion level of any single toxic or antinutritional component;

(3) different plant protein sources were combined in such a way to make up for any essential amino acid deficiency in individual components of the diet.

A combination of different plant protein sources in diets for O. mossambicus has been advocated by Jackson et al.(1982) as a means of compensating for essential amino acid deficiency in any single protein source. Several authors have reported comparatively better performances by fish fed diets containing combinations of plant protein sources (Olukunle, 1982; Richards, 1983). Olukunle (1982) observed better performance by O. mossambicus fry when fed on a diet containing

groundnut and sunflower seed meal as partial substitutes for fish meal protein compared with diets containing groundnut or sunflower meal alone. Richards (1983) also noted that a combination of sunflower and sesame meals resulted in better growth of O. mossambicus when these ingredients were tested singly and together as partial substitutes for fish meal protein.

The best growth, food conversion and protein utilization by carp fry was achieved with diet 2 (20% ground and 20% linseed). This further demonstrates that both groundnut and linseed are good protein sources for carp fry at low inclusion levels, either as single components, or in combination. The significantly better performances of carp fry fed diet 2 compared to the fish meal based control diet was probably due to the variations in the protein digestibility of both diets. Diet 2 had a APD of 85.60% in comparison to 80.6% for diet 1.

In all combinations tested in the present study, lower growth responses were observed when sesame meal was used in place of groundnut meal. This is similar to the observation recorded in trial 1, where despite the absence of any known toxic factor and with an excellent amino acid profile, sesame meal resulted in comparatively lower growth responses than linseed and groundnut meal, even at a low inclusion level.

The compositions of diets 11 and 12 were similar to those of diets 3 and 8 respectively except that dietary carbohydrate as well as part

of the dietary protein were replaced by protein and carbohydrate from rice bran and wheat bran. The significantly lower growth response and food conversion of carp fry receiving diets 11 or 12 compared to diets 3 or 8 may have been due to lower diet acceptability, caused by the presence of rice bran and wheat bran. This may also be due to the lower digestibility of dietary carbohydrate from rice bran and wheat bran. The lower growth responses obtained from diets 11 and 12 may also have been due in part to the presence of phytic acid in the wheat bran (Lienen, 1977).

4.5. Economic evaluation

The primary purpose of the present investigation was to evaluate the nutritional qualities of various dietary ingredients available in Bangladesh for the formulation of an economically viable complete diet for carp fry. Therefore, economic evaluation is an essential prerequisite before a final recommendation is made on the use of these ingredients.

A simple economic analysis was performed to estimate the cost of feed to raise a unit biomass of fish fry. Cost of feed has been used as a single economic criterion on the assumption that all other operating costs for commercial fry production remain the same for all diets.

The approximate cost of each diet tested in trial 2 was first

calculated based on the cost of raw materials in Bangladesh (Dhaka Wholesale market price, 1985, Table 4.18). A comparison of comparative costs per unit of protein is also shown in Table 4.18 for information. The cost of dextrin and corn starch was assumed to be equal to the cost of refined wheatflour in Bangladesh. The cost of ∝-cellulose was assumed to be that of alkali digested rice straw which is available in Bangladesh. The cost of binder and chromic oxide has not been included in these estimations. An additional 7.5% on the top of the total raw material costs has been included towards manufacturing cost, marketing expenses and operating margin (ADCP, 1983).

The estimated total costs per kg of feed and the cost of feed to produce a kg weight gain by carp fry are shown in Table 4.19.

The cost anaylsis shows the control diet (fish meal based) to be the most expensive and diets 10 and 12 the cheapest. However, if the cost of feed per kg weight gain is considered, diet 2 would be the cheapest and diet 10 the most expensive. For other diets, the cost per kg decreases linearly with progressive substitution of fish meal, but there is a proportionate decrease in food conversion efficiency. Thus they are generally similar in terms of cost per kg of fish produced. Although higher inclusion (above 40%) of plant protein sources as a partial substitution for fish meal does not provide a significant economic advantage, the availability of fish meal would be an important factor in the ultimate selection of dietary ingredients.

Table 4.18 Cost of dietary ingredients and cost per unit protein from various dietary protein sources (Dhaka wholesale market price, 1985)

Ingredients	Price (Tk/kg ingredients)	Cost of protein (Tk/kg protein)
Fish meal*	16.00	22.81
Mustard oilcake	4.00	12.45
Sesame oilcake	2.75	5.70
Linseed oilcake	3.00	8.70
Groundnut oilcake	5.20	10.00
Rice bran	3.00	17.50
Wheat bran	2.25	14.61
Cod liver oil**	60.00	-
Corn oil	36.00	-
Mineral mix	56.00	-
Vitamin mix	60.00	-
Crude fibre	6.00	-
Starch	8.00	-

45 Bangladesh Taka = 1 U.K. Pound

* Prices of fishmeal and all other plant protein sources were obtained from Mr. M. A. Hussain, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

** Prices of cod liver oil and other additives were obtained from Dr. M. G. S. Alam, Faculty of Veterinary Science, Bangladesh Agricultural Sciences, Mymensingh, Bangladesh.

Table 4.19 An approximate estimate of the cost of feed used in trial 2 , Experiment 4.1 based on market prices in Bangladesh (1985)

Diet No.	Cost/kg feed (TK.)	Cost of feed/kg weight gain (Tk.)
1	17.96	38.44
2	14.94	25.40
3	13.55	28.98
4	14.53	33.43
5	14.19	33.35
6	12.60	30.86
7	13.06	33.17
8	12.38	32.20
9	11.57	31.93
10	10.75	39.67
11	12.04	32.51
12	10.88	32.42

The cost of the formulated diets in the present investigation could not be compared with a reference diet as there is no commercially available fish feed in Bangladesh (K. Khan, pers. comm.). However, a simple calculation is sufficient to make a comparison of feed costs to the total produce value. In Bangladesh, farm gate wholesale price of 1g fry would be about TK.10 per 100 fry; therefore 1kg of fry would be sold at about TK.100. The estimation shows the cost of feed to produce 1kg of carp fry to be about TK.25 to 33 if diets 1 and 10 are excluded from the estimation (Table 4.19). Therefore the feed cost represents about 30% of the total product value. ADCP (1983) in their studies on fish feed and feeding in developing countries, concluded that for a fish farm to be profitable feed costs should not exceed 20% of the farm-gate value of the product.

However, it should be pointed out that the costs of cod liver and corn oil and mineral and vitamin premixes account for about 40 to 50% of the total raw material cost. Therefore, the cost of these diets may be substantially reduced by using a cheaper source of lipid and by reducing the level of inclusion of mineral and vitamin premix.

Dietary lipid acts as a source of energy as well as source of essential fatty aacid. Carp require both the linolenic (W3) and linoleic (W6) series of fatty acid (Csengeri et al., 1979). Fish oil is a rich source of W3, while most of the oil seeds are generally rich in W6. However, among oilseed, linseed is a particularly good source of W3 (McDonald et al., 1981). In Bangladesh many of the vegetable

oils such as soybean, coconut, palm, sesame and linseed are cheaper than cod liver and corn oil. Therefore, use of soybean or any other vegetable oil and linseed oil along with saturated animal fat in the diet, will reduce the cost of the diet considerably, apart from fulfilling the requirement of energy and essential fatty acid for fish.

CHAPTER 5

General Discussion

5.1. General Discussion

The aim of this research project was to study some key husbandry and water quality factors known to affect the survival and growth of common carp fry. Several dietary ingredients from Bangladesh were also evaluated in an effort to develop a practical complete diet for carp fry.

All the experimental investigations were conducted in a laboratory recirculated water system, or in static bioassay tanks. Thus the experimental results are directly applicable to the culture of fish in tanks under conditions of controlled water exchange, but may have less relevance to fish culture in earth ponds.

Although there is widespread use of recirculated water for the intensive rearing of many species of fish fry, carp fry have traditionally been reared in shallow earthen nursery ponds in most developing countries. However, this situation is changing rapidly. In recent years in Bangladesh, for example, there has been a trend towards rearing of carp fry in indoor hatchery or nursery tanks in order to improve their survival rate and to maximise growth.

Jhingran and Pullin (1985), in reviewing carp farming in Asia, also emphasized the importance of early rearing of carp on defined artificial feeds under more controlled environmental conditions. The authors noted that various combinations of live and artificial feeds

and fertilization procedures presently used in earthen rearing ponds can cause unpredicatable consequences such as plankton blooms. Some species of plankton, e.g the bluegreen algae Microcystis, can be extremely harmful by being toxic to fish or by depleting oxygen in the pond (Jhi n gran, 1977; Jhingran and Pullin, 1985).

Lack of an adequate amount of food, the presence of predatory aquatic insects of fish, and other unwanted biota such as frogs and crabs, can cause severe damage to carp hatchling and fry population resulting in very poor survival and growth. Rothbard (1982) recalled the observations of Prugninin (1967) that frogs and tadpoles accounted for 80% of the mortality of carp fry in spawning ponds in Uganda.

Extreme variations of different water quality such as temperature, pH and dissolved oxygen in nursery ponds can also contribute to poor growth and high mortality of carp fry. Chakrabarty et al. (1973) reported levels of DO ranging between 1.6 and 12.4 mgl^{-1} in various nursery ponds in Orissa, India. A large variation in water pH (5.2 - 10.0) has been recorded in some rainfed nursery and production ponds in the Pabna District of Bangladesh because of a poor buffering capacity of the water (D.J. Macintosh, pers. comm.).

Indoor rearing of carp post larvae and fry for extended periods has been reported to improve their consequent survival in outdoor rearing ponds. Rothbard (1982) recommended rearing larvae for at least two weeks in indoor conditions, thus initiating outdoor nursing with strong and free swimming fry. Mires (1976) observed 90% survival of carp fry

in nursery ponds when they were stocked after an extended period (25-37 days) of nursing in indoor rearing tanks under controlled environmental conditions, whereas, 90-100% mortality of fry was recorded when they were transferred directly from the hatchery after 5 days of primary nursing.

Recirculated water indoor rearing systems for carp fry have been developed as a possible alternative to earth pond nurseries. Jhingran et al. (1979), in a preliminary study, showed that recirculatory rearing ponds can be economically viable for major carp fry in India. The authors concluded that their use in preference to conventional earthen rearing ponds would enable production of major carp fingerlings with considerable savings in time, space and water usage.

One of the most important aspects of rearing carp fry in nursery tanks is the provision of an adequate diet in the absence of natural food organisms. The most widely used starter feeds for carp larvae are rotifers or brine shrimp (Artemia) nauplii and/or a micro encapsulated egg diet. However, the provision of an adequate supply of live food is often difficult in a hatchery or nursery as the weekly output may be several million hatchlings. For example, the 3 largest Government hatcheries in Bangladesh, at Raipur, Mymensingh and Neemgachi produce about 8-10 million major and chinese carp hatchlings per week during the main carp spawning season from May to July (D. J. Macintosh pers. comm.).

"Microencapsulated" egg diet made by pouring vigorously beaten raw egg into boiling water, helps to enlarge the gut and make it function, but it is not a nutritionally complete food (Jhingran and Pullin, 1985). Natural zooplankton also can be easily exhausted when nursery ponds are heavily stocked. Therefore use of a complete artificial diet would constitute a significant development in the nursery management of carp.

In Experiment 2.1 an investigation was conducted to study the effect of temperature on survival, growth and food conversion of carp fry and to determine their optimum feeding rate in relation to temperature using a prepared artificial diet. The artificial diet was prepared to conform to the known nutritional requirements of carp fry using fish meal as the source of dietary protein. The results of the above experiment showed that feeding rates of 20-30% BW/day produced maximum growth at rearing temperatures in the range 24-35^{o}; the optimal rearing temperature for growth and food conversion was found to be 32^{o}C.

The food conversion ratio is frequently used as a measure of feeding efficiency. However, in terms of efficiency of food utilisation, the feeding levels producing maximum growth in the present investigation might not have been the optimum. The most commercially viable ration size is very much dependent on diet costs in relation to the cost of the culture system (Jauncey, 1982). The amounts of food needed to rear fry and fingerlings are relatively small compared to the feeding requirements of table fish and therefore it may be more economical (in terms of cost of biomass production) to

sacrifice optimum food ration to obtain maximum growth.

While studying the effect of stocking density and water flow rate (Experiment 2.2) it was observed that carp fry can grow as fast in crowded conditions (15 fish/litre) as in less crowded conditions (6 fish/litre), provided an adequate amount of food is availble and water quality parameters, especially dissolved oxygen, pH and ammonia, are within the optimum range for fish growth. Information on stocking density and water flow rate would be of significant importance as carp larvae and fry are kept in a highly crowded condition in hatchery and nursery tanks prior to their transfer to earthen rearing pond. Further, in Bangladesh water from underground sources is generally used to supply hatcheries and nurseries. This water, which is produced from tube-wells, is usually seriously deficient in dissolved oxygen (typical oxygen content 2-4mgl^{-1}).

However, in general nursery practice, it is the total production of fish per tank that provides the economic return. In Bangladesh and India carp hatchlings and young fingerlings are sold by weight. Thus it is the biomass production rather than the individual growth of the fish that hatchery producers are interested in. Therefore it may be advantageous from a practical point of view to increase the stocking density even at the sacrifice of high individual growth.

There are two important criteria for a successful artificial diet in intensive fish culture: (a) it must be pallatable and (b) it must be nutritionally complete (Westers, 1982). The first is determined by

particle size, texture, taste and/or odour. In Experiment 2.3 it was found that food particle preference varied markedly among different size classes of carp fry. This suggests that nursing success would be benefitted by the administration of proper food particle size. Jhingran and Pullin (1985) noted that efforts to improve feed presentation i.e, particle size and methods and frequency of feeding should form an important component of future applied research on rearing methods for carp fry.

Since feeding represents one of the major costs in intensive farming of fish, the development of nutritionally balanced practical diets is considered one of the major tasks of aquaculture research, (e.g. ADCP, 1983, Jhingran and Pullin, 1985). Use of fish meal as the only source of protein in formulated diets for carp fry has very limited applicability in most developing countries including Bangladesh, because fish meal is not widely available and is an expensive commodity. For example, in Bangladesh, the cost of protein using fish meal as the source is TK. 22.81/kg., whereas it is TK.5.70, TK.8.70 and TK.10.00 per kg protein from sesame, linseed and groundnut meal respectively (see Table 4.18). Therefore, use of alternative sources of protein in the formulation of diets for carp fry deserves special consideration.

Amongst several plant protein sources tested as potential partial substitutes for fish meal (Experiment 4.1, trial 1), linseed followed by groundnut and sesame meal gave the most promising results. Mustard oilcake is widely used as supplemental feed in fish ponds in Bangladesh

India and Nepal. Dry ground meal or oilcake is soaked in water and usually sprayed or sprinkled over the pond water surface. Frequently dry oilcake in a jute bag is dumped in the corner of the pond. Although the use of mustard oilcake in combination with ricebran has been reported to increase fish production significantly (Jhingran, 1977), its precise value as a fish feed has never been established. It is not unlikely that a significant proportion of these ingredients acts more as a fertilizer in the pond contributing to an increased plankton production. The results of the present investigation indicate that mustard oilcake is unsuitable as a dietary protein source for carp fry at a high inclusion level (50% of total protein).

Commercial heat processing of mustard meal or solvent extraction of oil from the seed, however, have been reported to increase the feeding value of mustard meal by destroying or reducing the enzyme thioglucosidase, thereby preventing enzymatic breakdown of glucosinolates to isothiocyanates (Tookey et al., 1980). Capper et al. (1982) observed that fingerling common carp fed a diet containing 20% roasted mustard meal did not suffer depressed growth or decreased food conversion. However, in Bangladesh the expeller method is most commonly used for oil extraction, while commercial heat treatment of expeller cake can be expensive or difficult to operate under field conditions.

Use of linseed, groundnut and sesame meal, which are locally available and relatively inexpensive in many developing countries, as an

alternative protein source for carp fry could reduce the feeding cost, perhaps by about 30%.

Several investigations were conducted to determine the tolerance of carp fry to different water quality parameters. Information on the tolerance of carp fry to these parameters particularly ammonia and nitrite would contribute greatly to the successful operation of carp hatcheries and nurseries. Carp fry as stated earlier, are usually reared in highly crowded conditions in indoor rearing tanks and water quality deterioration is commonly responsible for instances of large scale mortality. In Bangladesh, after harvesting from ponds, carp fry are usually stocked for 24 hrs at very high density (e.g. 101^{-}) in indoor rearing tanks to 'condition' them before they are sold or distributed to rearing ponds. Although precise monitoring of water quality conditions during this process has not been carried out, it is apparent from observation that rapid deterioration in water quality usually occurs and significant fish mortalities are not uncommon.

Variations in water quality especially of DO, pH and temperature between hatchery and nursery ponds are thought to be a major cause of fry mortality, as when fish are transferred directly from indoor rearing tanks to nursery ponds or vice-versa.

The build up of metabolites, principally ammonia, and accumulation of carbon dioxide during transportation can also be responsible for large scale mortalities of carp fry. Because the fish are

kept at high concentration during transportation, ammonia is likely to reach high levels from a contribution of ammonia excretion, bacterial decomposition of urea, other nitrogenous wastes, mucus production etc. Ramachardran (1969) cited by Jhingran (1977), reported 100% mortality of fish (species not specified) during transportation due to accumulation of ammonia above 20mgl^{-1} total ammonia -N although there was sufficient oxygen left in the sealed transportation container. However, the estimated tolerance of carp fry to un-ionized ammona (96-h LC50 1.74-1.84 mgl^{-} NH_3-N equivalent to 51.2 - 54.1 mgl^{-1} total ammonia -N at pH 7.70 and temp. 28^{0}C) indicates that a build up of ammonia during transportation may not cause mortality for carp fry unless the water pH is relatively high (> 8.0). In a sealed container accumulation of free CO_2will lower the water pH, which will increase the degree of ionization of ammonia thereby reducing the proportion of un-ionized aammonia in the water.

Sampson and Macintosh (1986) reported a build up of 15 mgl^{-1} of total ammonia during simulated 3 hour transportation of silver carp (Hypophthalmichthys molitrix) fry. However, due to the low level of pH (6.48-6.76), the level of un-ionized ammonia was extremely low and probably did not contribute to the observed fish mortality. Transportation times for fish are usually greater than 3 hours and it remains to be seen what level of ammonia build up would occur in sealed containers over longer time periods.

The tolerance of these water quality parameters, estimated by short term lethal tests, are the measure of acute toxicity. Sub-lethal levels of these water quality parameters especially ammonia and nitrite, on long term exposure, may lead to suppression of fish growth and histopathological changes. In the absence of any sub-lethal or chronic toxicity data, use of an appliction factor to estimate a 'safe' concentration from the lethal threshold concentration is frequently recommended. An application factor of 0.05 to 0.1 has been used for many toxicants (see section 3.1.). Available data on experimentally derived lethal concentration and no effect concentration of un-ionized ammonia and nitrite are compiled in Table 5.1. From the above table, it can be seen that a lethal to no effect concentration ratio of 1.0: 0.1-0.3 applies for several species of fish and crustaceans. Therefore use of an application factor of 0.1 to estimate a 'safe' level from 96-h or incipient LC50 would indicate a level to be maintained to prevent long term sub-lethal toxicity of the NH_3 and NO_2 in intensive rearing system for carp fry.

The upper temperature tolerance of carp fry reared at different acclimation temperatures were also investigated. The high incipient lethal temperature (33.5-38.8°C for acclimation temperatures of 16 to 34°C) of carp fry observed in this investigation indicates that carp fry are suitable for culture in shallow ponds or tanks in Bangladesh and other tropical and subtropical countries where large fluctuations of water temperature are commonplace. Edwards et al.(1984) recorded annual minimum and maximum temperatures of 27 and

Table 5.1 A comparison of experimentally derived lethal and no effect concentrations of un-ionized ammonia and nitrite to several species of fish and crustaceans

Un-ionized ammonia

Species	Lethal conc. (mg l^{-1} NH_3-N)	No effect conc. (mg l^{-1} NH_3-N)	Ratio of lethal conc:no effect conc.	Author
Rainbow trout	0.39 - lethal threshold conc.	0.047 - harmless conc.	1 : 0.12	Lloyd & Orr (1969)
	0.45 - 500 min LC50	0.13 - no effect on growth	1 : 0.29	Schulze-Weihenbrauck (1974, cited by Hampson, 1976)
	0.25 - 21 day incipient LC50 for sac fry	0.05 - some retardation of early growth and development when exposed for 42 days		Burkhalter & Kaya (1977)
Channel catfish	1.60 - 96 LC50	0.217 - reduced growth by 17% of control		Colt & Tchobanoglous (1978)
European eel	1.00 - 10 day LC50	0.12 - no effect on growth	1 : 0.12	Sadler (1981)
Red drum, Sciaenops acellatus	0.39 - 96-h LC50	0.11 - growth and survival comparable to control	1 : 0.28	Holt and Arnold (1983)
Penaeid prawn	1.29 - 48-h LC50	0.11 - maximum acceptable level - reduced growth rate by 1-2% of that of control in 3 weeks test	1 : 0.09	Wickins (1976)

continued . . .

Table 5.1 continued

Nitrite

Species	Lethal conc. (mg l^{-1} NO_2^--N)	No effect conc. (mg l^{-1} NO_2^--N)	Ratio of lethal conc:no effect conc.	Author
Channel catfish	12.79 - 13.09 96-h LC50			Colt & Tchobanoglous (1976)
		1.25 - no effect on growth after 31 days	1 : 0.10	Colt et al. (1981)
Freshwater prawn Macrobrachium rosenbergii	8.6 - 96-h LC50 5.0 - 168-h LC50 4.5 - 192-h LC50	1.8 - significantly reduced growth rate after 8 days		Armstrong et al.(1976)

36°C in fish ponds in Bangkok, Thailand. Diurnal variations in water temperature ranging between 27 and 37°C were recorded during the summer months in some shallow nursery ponds at Mymensingh, Bangladesh (unpublished data).

5.2. Suggestions for future work

The present investigation demonstrated that linseed, groundnut and sesame meals can be used as alternative sources of protein for partial replacement of fish meal in carp fry diets, but information on the possible effect of some toxic components (e.g. linamarin in linseed and aflatoxin in groundnut meal) on the long term growth and survivability of carp are lacking. As already discussed in section 4.4, long term exposure to these toxic factors can have a detrimental effect on animal growth. Therefore long term growth studies of carp should be undertaken before final recommendations on the use of these ingredients as dietary ingredients are given.

In the economic analysis, it was observed that a significant proportion of the cost of the diet was due to the high cost of corn oil, cod liver oil, vitamin mix and mineral mix. Therefore further investigation using alternate sources of dietary lipid such as linseed, soybean or other vegetable oil and saturated animal fat and using lower inclusion levels of vitamin and mineral mixes deserve consideration.

Although an attempt was made to predict the safe levels of NH_3 and

and NO_2 from experimentally derived lethal concentration values using an application factor, it would be more useful to conduct a long term growth study to evaluate the sub-lethal toxicity of these water quality parameters.

As pointed out in section 3.2.1, a reduction in the level of dissolved oxygen in water results in an increased toxicity of ammonia. Alabaster et al. (1979) demonstrated that a fall in dissolved oxygen concentration from 9.6 to 3.5 mgl^{-1} decreased the 24-h LC50 of NH_3 for Atlantic salmon smolts by about 1.7 times. The effect of dissolved oxygen on the toxicity of nitrite has not been studied experimentally. However, it is likely that low levels of DO would increase the toxicity of nitrite significantly, as nitrite is known to reduce the oxygen carrying capacity of haemoglobin by converting it to methaemoglobin (see section 3.3.1.). Even though simple aeration can maintain sufficiently high levels of DO in a hatchery or nursery, in Bangladesh, the lack of a regular supply of electricity in many cases prohibites the use of conventional aerators in fish tanks. Moreover, tube-well water obtained from underground sources used in hatcheries or nurseries is usually deficient in dissolved oxygen. Therefore it would be of practical value to study ammonia and nitrite toxicity to carp at both low and high levels of DO.

CHAPTER 6

Conclusions

(1) Investigations on the tolerance of carp fry to different water quality parameters showed that carp fry are fairly tolerant to un-ionized ammonia; the 96-h median lethal concentration (LC50) value ranged between 1.74 and 1.84 mgl^{-1} NH_3-N. This is equivalent to 51.2 - 54.1 mgl^{-1} total ammonia-N at pH 7.70 and temperature 28°C.

(2) Such high concentrations of ammonia are unlikely to occur in carp rearing systems under normal conditions, but could be approached during high density 'conditioning' or transportation of carp fry using the unsophisticated methods still practised in many developing countries.

(3) From an experimental study on the effect of fish size on the toxicity to nitrite, it was found that nitrite toxicity did not vary significantly for carp fry weighing between 75 and 450 mg.

(4) The toxicity of nitrite to carp fry was found to be dependent on the presence of chloride ions and pH of the water. The 96-h LC50 values of 1.0 mgl^{-1} to 48.70 mgl^{-1} NO_2-N at a chloride level of 45.0 mgl^{-1} (water pH 7.59 - 7.78). Similarly the 96-h LC50's for nitrite ranged from 15.43 mgl^{-1} NO_2-N at pH 5.92 to 31.42 mgl^{-1} NO_2-N at pH 7.81 (Cl^- level 30 mgl^{-1}).

(5) The 96-h LC50 of NO_2 was found to be positively correlated to the concentration of chloride and pH of water. These linear relationships are best described by the following equations:

(a) 96-h LC50 = 1.03 Cl^- + 1.49 ($r=0.996$; d.f. = 3; $p<0.01$).

(b) 96-h LC50 = 7.38pH - 27.3 ($r = 0.906$; d.f. = 3; $P<0.05$)

(6) A NO_2-N to Cl^- ratio of about 1:1.5 - 3.0 in water prevented complete fish mortality over a 158-h experimental period. Therefore a NO_2-N to Cl^- ratio of 1:5 is recommended for protection of carp fry against nitrite mortality.

(7) Carp fry are highly tolerant to nitrate toxicity. It was found that only concentrations of 1000 mgl^{-1} NO_3-N and above were lethal to carp fry.

(8) Such high concentrations of nitrate are unlikely to occur in natural or recirculated water systems and therefore nitrate toxicity should not be a problem in carp hatcheries and nurseries.

(9) The experimentally determined upper incipient lethal temperatures for carp fry were 33.5, 34.4, 36.8, 37.6 and 38.8°C at acclimation temperatures of 16, 20, 25, 28 and 34°C respectively. This study demonstrates that carp fry have a high incipient lethal temperature and can withstand large fluctuations in temperature.

(10) From an experimental study of the effect of temperature and feeding rate on fry growth, it was demonstrated that within the range 24 to 35°C, a rearing temperature of about 32°C was optimal for growth and food conversion of carp fry, provided their feeding rate was 20-30% BW/day. At lower feeding rates (10-25%), a rearing temperature of 28°C was more suitable.

(11) The potential for maximum growth of carp fry occurred when feeding rates of 20-25%, 25-30% and 30% BW/day were used at rearing temperatures of 24, 28 and 32°C respectively.

(12) Rearing temperatures up to 35°C did not inhibit growth of carp fry provided adequate feeding was provided.

However, temperatures as high as 35°C cannot be recommended for fry rearing as this is too close to their lethal temperature (37.6 - 38.3°C) and the saturation of oxygen in water is very much reduced at higher temperature.

(13) From an experimental study on the effect of stocking density and water flow rate, it is concluded that in a recirculated water system, a stocking density of 7-11g fry/litre can be maintained without any adverse effect on growth and food conversion under the following conditions:

(a) water temperatures is 28°C

(b) feeding rate is 15-25% BW/day

(c) water exchange time in the rearing system is 15 minutes

(d) reoxygenation capacity of the rearing system is 90-100% saturation.

(14) An investigation of the preferred size of food particles demonstrated that for carp larvae and fry, the preferred food particle size increases with increase in fish size.

(15) On the basis of the observations, it is concluded that food particle size ranging between 125-500 um, 300-790 um and 500-1000 um would be the most suitable for carp fry weighing 15-100, 100-250 and 250-500 mg respectively.

(16) The results of an evaluation of various plant protein sources for inclusion in a complete practical diet for carp fry showed that a combination of different plant protein sources proved more acceptable to carp fry than the same components used singly to partially substitute for fish meal.

(17) The best growth and economic return were achieved when linseed and ground nut meal were used in equal proportions to substitute 40% of the fish meal protein component in a diet containing 40% total protein.

(18) Linseed, groundnut and sesame meal in various combinations up to 60% replacement of fish meal protein proved suitable as dietary protein source in a complete diet for carp fry.

REFERENCES

Alabaster, J. S. and Downing, A. L., 1966. A field and laboratory investigation of the effect of heated effluents on fish. Fish. Invest., Minist. Agric. Fish., U.K. 6(4).

Alabaster, J. S. and Herbert, D. W. M., 1954. Influence of carbon dioxide on the toxicity of ammonia. Nature, Lond., 174: 404-405.

Alabaster, J. S. and Lloyd, R. (Editors) 1980. Water Quality Criteria for freshwater fish. Butterworth, London. 279pp.

Alabaster, J. S., Shurben, D. G. and Knowles, G., 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, Salmo salar L. J. Fish. Biol., 15: 705-712.

Alderson, R., 1979. The effect of ammonia on the growth of juvenile Dover sole, Solea solea (L.) and turbot, Scophthalmus maximus (L.). Aquaculture, 17: 291-309.

Alikunhi, K. H., 1966. Synopsis of biological data on common carp, Cyprinus carpio (Linnaeus, 1758) (Asia and the Far East). FAO Fish Synops. 31.1, 79pp.

Allen, J. R. M. and Wootton, R. J., 1982. The effect of ration and temperature on the growth of the three-spined stickleback, Gasterosteus aculeatus L. J. Fish Biol.,20: 409-422.

Allen, K. O., 1974. Effects of stocking density and water exchange rate on growth and survival of channel catfish, Ictalurus punctatus (Rafinesque) in circular tanks. Aquaculture, 4 : 29-39.

American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1980. Standard Methods for The Examination of Water and Wastewater. 15th edition. American Public Health Association, Washington, DC, 1134pp.

American Society for Testing and Materials, 1970. Standrad Method of Test for Evaluating Acute Toxicity of Industrial Water to Freshwater Fishes. ASTMB 1345-59, American Society for Testing and Materials, Philadelphia.

Andrews, J. W. and Stickney, R. R., 1972. Interaction of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. Trans. Am. Fish. Soc., 101: 94-99.

Andrews, J. W., Knight, L. H., Page, J. W., Matsuda, Y. and Brown, E. E., 1971. Interactions of stocking density and water turnover on growth and food conversions of channel catfish reared in intensively stocked tanks. Prog. Fish-cult., 33: 197-203.

Anwar, A., Ishak, M. M., El-Zeiny, M. and Hassanen, G. D. I., 1982. Activated sewage sludge as a replacement for bran-cotton seed meal mixture for carp, Cyprinus carpio L. Aquaculture, 28: 321-325.

Appelbaum, S., 1977. A possible substitute for live food of carp fry. Arch. Fischeriewiss, 28: 31-43.

Appelbaum, S. and Dor, U., 1978. Ten day experimental nursing of carp (Cyprinus carpio L.) larvae with dry feed. Bamidgeh, 30: 85-88.

Appler, H. N. and Jauncey, K., 1983. The utilization of a filamentous green algae (Cladophora glomerata (L) Kertzin) as a protein source in pelleted feeds for Sarotherodon (Tilapia) niloticus fingerlings. Aquaculture, 30: 21-30.

Aquaculture Development and Coordination Programme, 1983. Fish Feeds and Feeding in Developing Countries. ADCP/REP/83/18. UNDP of FAO, 97pp.

Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission, 1955. Aquatic life water quality criteria. First progress report. Sewage Ind. Wastes 27: 321-331.

Arillo, A., Margiocco, C., Melodia, F., Mensi, P. and Schenone, G., 1981. Ammonia toxicity mechanism in fish : studies in rainbow trout (Salmo gairdneri Rich.). Ecotoxicology and Environmental Safety, 5: 316-328.

Arillo, A., Gaino, E., Margiocco, C., Mesni, P. and Schenone, G., 1984. Biochemical and ultrastructural effects of nitrite in rainbow trout : Liver hypoxia as the root of the acute toxicity mechanism. Environmental Research, 34: 135-154.

Armstrong, D. A., Stephenson, M. J. and Knight, A. W., 1976. Acute toxicity of nitrite to larvae of the giant Malaysian prawn, Macrobrachium rosenbergii. Aquaculture, 9: 39-46.

Armstrong, D. A., Chippendale, D., Knight, A. W. and Colt, J. E., 1978. Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, Macrobrachium rosenbergii. Biol. Bull., 154: 15-31.

Ashley, L. A., 1970. pathology of fish fed aflatoxins and other antimetabolites. In : S. F. Sniesko (Editor), Proceedings of a symposium on Diseases of Fishes and Shellfishes. Special Pub. No. 5 Am. Fish. Soc., pp.366-379.

Ashley, L. M., 1972. Nutritional Pathology. In : J. E. Halver (Editor), Fish Nutrition, Academic Press, New York, N.Y., pp.337-348.

Askerov, T. A., 1975. Survival and oxygen consumption of young Kura carp under various keeping conditions. Gidrobiol Zh., 11: 76-77.

Association of Official Analytical Chemists., 1970. Official Methods of analysis of the association of official analytical chemists. AOAC, Washington, D.C., 1015pp.

Aston, R. J. and Brown, D. J. A., 1978. Fish farming in heated effluents. In : C. M. R. Pastakia (Editor), Proceedings of the conference on Fish Farming and Wastes, University College, London, Institute of Fisheries Management, pp.39-60.

Atack, T. H., Jauncey, K. and Matty, A. J., 1979. The utilization of some singel cell protein by fingerling mirror carp (Cyprinus carpio). Aquaculture, 18: 337-348.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13: 265-272.

Austwick, P. K. C., 1975. Mycotoxins. Br. Med. Bull., 31: 222-229.

Baird, R., Bottomley, J. and Taitz, H., 1979. Ammonia toxicity and pH control in fish toxicity bioassays of treated wastewaters. Water Res., 13: 181-184.

Balani, M. C., 1973. Thermal responses in Catla catla fry. Curr. Sci., 4: 601-602.

Ball, I. R., 1967. The relative susceptibility of some species of freshwater fish to poisons - 1. Ammonia. Water Res., 1: 767-775.

Bangladesh Times, 1986. Daily newspaper June, 1986.

Bardach, J. E., Ryther, J. H. and McLarney, W. C., 1972. Aquaculture. Wiley Inter Science, New York, NY, 868pp.

Bath, R. N. and Eddy, F. B., 1980. Transport of nitrite across fish gills. J. Exp. Zool., 214: 119-121.

Becker, C. D. and Genoway, R. G., 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Env. Biol. Fish, 4: 245-256.

Beitinger, T. L. and Fitzpatrick, L. C., 1979. Physiological and ecological correlates of preferred temperature in fish. Am. Zool., 19: 319-329.

Black, E. C., 1953. Upper lethal temperatures of some British Columbia freshwater fishes. J. Fish. Res. Bd. Can., 10: 196-206.

Bliss, C. L., 1937. Calculation of the time-mortality curve. Ann. appl. Biol., 24: 815-852.

Bowser, P. R., Falls, W. W., Van Zandt, J., Collier, N. and Phillips, J. D., 1983. Methemoglobinemia in channel catfish: Methods of prevention. Prog. Fish-Cult., 45: 154-158.

Brett, J. R., 1944. Some lethal temperature relations of Algonquin Park fishes. Univ. Toronto Stud. biol. Ser., 52. 63: 1-49.

Brett, J. R., 1952. Temperature tolerance in young Pacific salmon Oncorhynchus. Fish. Res. Board Can., 9: 265-323.

Brett, J. R., 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol., 31: 75-87.

Brett, J. R., 1970. Temperature : animals - fishes - functional responses. In : O. Kinne (Editor), Marine Ecology, Vol. 1. Environmental Factors, Part 1, Willey, Inter science, London, pp.515-560.

Brett, J. R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and fresh-water ecology of sockeye salmon (Oncorhynchus nerka) Am. Zool., 11: 99-113.

Brett, J. R., 1979. Environmental factors and growth. In : W. S. Hoar, D. J. Randall and J. R. Brett (Editors), Fish Physiology Vol. VIII, Bioenergetics and Growth. Academic Press, New York, NY, pp.599-675.

Brett, J. R. and Higgs, D. A., 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Board Can., 27: 1767-1779.

Brett, J. R., Shellbourne, J. E. and Shoop, C. T., 1969. Growth rate and body composition of fingerling sockeye salmon Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Bd. Can., 26: 2363-2394.

Brockway, D. R., 1950. Metabolic products and their effects. Prog. Fish-Cult., 12: 127-129.

Broussard, M. C. Jr. and Simco, B. A., 1976. High density culture of channel catfish in a recirculating system. Prog. Fish-Cult., 38: 138-141.

Brown, D. A. and McLeay, D. J., 1975. Effect of nitrite on methemoglobin and total hemoglobin of juvenile rainbow trout. Prog. Fish-Cult., 37: 36-38.

Brown, M. E., 1946a. The growth of brown trout (Salmo trutta Linn.) II The growth of two-year old trout at a constant temperature of 11.5°C. J. Exp. Biol., 22: 130-143.

Brown, M. E., 1946b. The growth of brown trout (Salmo trutta Linn.) III. The effect of temperature on the growth of two-year old trout. J. Exp. Bio., 22: 144-155.

Brown, M. E., 1957. Experimental studies on growth. In : M.E. Brown (Editor), The Physiology of Fishes. Vol. 1. Metabolism. Academic Press. Inc. New York. pp. 361-400.

Brownell, C. L., 1980. Water quality requirements for first-feeding in marine fish larvae I. Ammonia, nitrite, and nitrate. J. Mar. Bio. Ecol., 44: 269-283.

Bryant, P. L., 1980. The Husbandry and Nutrition of Carp Larvae and Fry . Ph.D. Thesis, University of Aston in Birmingham, 211pp.

Bryant, P. L. and Matty, A. J., 1980. Optimisation of Artemia feeding rate for carp larvae (Cyprinus carpio L.). Aquaculture, 11: 203-212.

Bryant, P. L. and Matty, A. J., 1981. Adaptation of carp (Cyprinus carpio) larvae to artifical diets : 1. Optimum feeding rate and adaptation age for a commercial diet. Aquaculture, 23: 275-286.

Bryant, P., Jauncey, K. and Atack, T., 1980. Backyard Fish Farming. Prism Press. Stable Court, Dorchester. U.K.,170p.

Buckley, J. A., 1978. Acute toxicity of un-ionized ammonia to fingerling coho salmon. Prog. Fish-Cult., 40: 30-32.

Burkhalter, D. E. and Kaya, C. M., 1977. Effects of prolonged exposure to ammonia in fertilized eggs and sacfry of rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 106: 470-475.

Burrows, R. E., 1964. Effects of accumulated excretory products on hatchery reared salmonids. Research report 66. United States Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, 12pp.

Cameron, J. N., 1971. Methemoglobin in erythrocytes of rainbow trout Comp. Biochem. Physiol., 40(3A): 743-749.

Campbell, J. W., 1953. Nitrogen excretion. In : C. L. Prosser (Editor), Comparative Animal Physiology. W. B. Saunders Company, Philadelphia, Pa., pp.279-316.

Capper, B. S., Wood, J. F. and Jackson, A. J., 1982. The feeding value for carp of two types of mustard seed cake from Nepal. Aquaculture, 29: 373-377.

Chakrabarty, R. D., Murty, D. S., Sen, R. R., and Chatterjee, D. K., 1971. Preliminary observations on the usefulness of silkworm pupae as feed for fingerlings of Indian major carps and common carp. J. Inland Fish. Soc., India, Barrackpore, 3: 117-118.

Chakrabarty, R. D., Sen, P. R., Chatterjee, D. K. and Kowtal, G. V., 1973. Observations on the relative usefulness of different feed for carp spawn and fry. J. Inland Fish Soc., India, 5: 182-188.

Chaudhuri, H., 1983. Status and problems of Carp hatchery and nursery management. In : A. Benedicto-Dormitorio (Editor), A compilation of SEAFDEC AQD Technical Papers on Milkfish and Other Finfishes Vol. I. Southeast Asian Fisheries Development Center, Iliolo, Philippines, pp.81-90.

Chaudhuri, H., Chakraborty, R. D., Sen, P. R., Rao, N. G. S. and Jana, S., 1975. A new high in fish production in India with record yield by composite fish culture in freshwater ponds. Aquaculture, 6: 343-355.

Chowdhary, S. K., Srivastava, J. B. and Iqbal, S. E., 1978. Observations on the relative usefulness of different feeds for common carp (Cyprinus carpio var. communis Linn.). Matsya, 4: 20-24.

Chiba, K., 1965. A study on the influence of oxygen concentration on the growth of juvenile Common Carp. Bull. Freshwater Fish. Res. Lab. (Tokyo), 15: 35-47.

Chiou, J. Y. and Ogino, C., 1975. Digestibility of starch in carp. Bull. Jpn. Soc. Sci. Fish., 41; 465-466.

Chow, K. W. and Halver, J. E., 1980. Carbohydrates. In : Fish Feed Technology, ADCP/REP/80/11, UNDP of FAO, pp.68-103.

Christensen, M. S., 1981. Preliminary test on the suitability of coffee pulp in the diets of common carp (Cyprinus carpio L.) and catfish (Clarias mossambicus Peters). Aquaculture, 25: 235-242.

Cocking, A. W., 1959. The effects of high temperatures on roach (Rutilus rutilus). I. The effects of constant high temperatures. J. Exp. Biol., 36: 203-216.

Collins, M. T., Gratzek, J. B., Shotts, E. B., Dawe, D. L. Jr., Campbell, L. M. and Senn, D. R., 1975. Nitrification in an aquatic recirculating system. J. Fish. Res. Board Can., 32: 2025-2031.

Colt, J. E. and Armstrong, D. A., 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. Bio-Engineering Symposium for Fish Culture (FCS Publ. 1), pp.34-47.

Colt, J. and Tchobanoglous, G., 1976. Evaluation of the short term toxicity of nitrogenous compounds to channel catfish, Ictalurus punctatus. Aquaculture, 8: 209-224.

Colt, J. and Tchobanoglous, G., 1978. Chronic exposure of channel catfish, Ictalurus punctatus, to ammonia: Effects on growth and survival. Aquaculture, 15: 353-372.

Colt, J., Ludwig, R., Tchobanoglous, G. and Cech, J., 1981. The effects of nitrite on the short-term growth and survival of channel catfish, Ictalurus punctatus. Aquaculture, 24: 111-122.

Coutant, C. C., 1977. Compilation of temperature preference data. J. Fish. Res. Board Can., 34: 739-745.

Cowey, C. B., 1979. Protein and amino acid requirement of finfish. In : J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fish feed Technology, Vol. 1, H. Heenemann Gm bH & Co, Berlin, pp.3-16.

Cowey, C. B. and Sargent, J. R., 1979. Nutrition. In : W. S. Hoar, D. J. Randall, and J. R. Brett (Editors). Fish Physiology, Vol. VIII, Bioenergetics and Growth. Academic Press. New York, N.Y, pp. 1-69.

Crawford, R. E., and Allen, G. H., 1977. Sea water inhition of nitrite toxicity to chinook salmon. Trans. Am. Fish. Soc., 106: 105-109.

Crawshaw, L. I., 1977. Physiological and behavioural reactions of fishes to temperature change. J. Fish. Res. Board Can., 34: 730-734.

Cruz, E. M. and Laudencia, I. L., 1978. Screening of feedstuffs as ingredients in the rations of Nile tilapia, Kalikasan, Phillip. J. Biol., 7: 159-164.

Csengeri, I., Majoros, F., Olah, J. and Farkas, T., 1979. Investigations on the essential fatty acid requirement of carp (Cyprinus carpio L.). In : J. E. Halver and K. Tiews (Editors) Finfish nutrition and Fish feed Technology Vol. 1, H. Heenemann GmbH, Berlin, pp.157-173.

Dabrowska, H., Grudniewski, C. and Dabrowski, K., 1979. Artificial diets for common carp: effect of the addition of enzyme extracts. Prog. Fish-Cult., 41: 196-200.

Dabrowski, K., and Bardega, R., 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. Aquaculture, 40: 41-46.

Dabrowski, K. and Kozlowska, H., 1981. Rapeseed meal in the diet of common carp reared in heated waters, 1. Growth of fish and utilization of diet. In : K. Tiews (Editor), Proc. World Symp. on Aquaculture in Heated Effluents and Recirculation Systems, Vol. II, pp.263-274.

Dabrowski, K., Bardega, R. and Przedwojski, R., 1983. Dry diet formulation study with common carp (Cyprinus carpio L.) larvae. Z. Tierphysiol. Tierernahr. Fuffermittelk, 50: 40-52.

Dabrowski, K., Dabrowska, H. and Grudniewski, C., 1978. A study of the feeding carp larvae with artificial food. Aquaculture, 13: 257-264.

Daoust, P. Y. and Ferguson, H. W., 1984. The pathology of chronic ammonia toxicity in rainbow trout, Salmo gairdneri Richardson. Journal of Fish Diseases, 7: 199-205.

Davis, J. C., 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board. Can., 32: 2295-2332.

Doudoroff, P., 1942. The resistance and acclimation of marine fishes to temperature changes. 1. Experiments with Girella nigricans (Ayes). Biol. Bull., 83: 219-244.

Doudoroff, P., 1945. The resistance and acclimatization of marine fishes to temperature changes. 2. Experiments with Fundulus and Atherinops. Biol. Bull., 88: 194-206.

Dowden, B. F. and Bennett, H. J., 1965. Toxicity of selected chemicals to certain chemicals. J. Wat. Pollut. Control Fed., 37: 1308-1316.

Downing, K. M. and Merkens, J. C., 1955. The influence of dissolved oxygen concentration on the toxicity of unionized ammonia to rainbow trout (Salmo gairdneri Richardson). Ann. appl. Biol., 43: 243-246.

Eddy, F. B., Kunzlik, P. A. and Bath, R. N., 1983. Uptake and loss of nitrite from the blood of rainbow trout, Salmo gairdneri Richardson, and Atlantic salmon, Salmo salar L. in fresh water and in dilute sea water. J. Fish Biol., 23: 105-116.

Edsall, T.A. and Rottiers, D.V., 1976. Temperature tolerance of young of the year lake whitefish, Coregonus clupeaformis J. Fish. Res. Board Can., 33: 117-180.

Edwards, P. Weber, K.E., McCoy, E.W., Chantachaeng, C., Pacharaprakili, C., Kaewpoitoun, K. and Nitsmen, S. 1983. Small-scale Fishery Project in Pathumthani Province, Central Thailand: A Socio-economic and Technological Assessment of Status and Potential. AIT Research Report No. 158. Agricultural and Food Engineering Division, Asian Institute of Technology, P.O Box 2754, Bangkok, Thailand, 256pp.

Edwards, P., Pacharaprakili, C., Kaewpaitoon, K., Rajput, V. S., Ruamthaveesub, P., Suthirawut, S. Yomjinda, M. and Chao, C-H., 1984. Re-use of cesspool slurry and cellulose agricultural residues for fish culture. AIT Research Report No. 166. Agricultural and Food Engineering Division, Asian Institute of Technology, P.O. Box 2754, Bangkok, Thailand. 338pp.

Elliott, J. M., 1975. The growth rate of brown trout (Salmo trutta L.) fed on reduced rations. J. Anim. Ecol., 44: 823-842.

Elliott, J. M., 1976. The energetics of feeding metabolism and growth of brown trout (Salmo trutta L.) in relation to body size, water temperature and ration size. J. Anim. Ecol., 45: 923-948.

Elliott, J. W., 1969. The oxygen requirements of chinook salmon. Prog. Fish-Cult., 31: 67-73.

Emerson, K., Russo, R. C., Lund, R. and Thurston, R. V., 1975. Aqueous ammonia equilibrium calculations: Effects of pH and temperature. J. Fish Res. Board Can., 32 : 2379-2383.

European Inland Fishery Advisory Commission, 1976. Workshop on controlled Reproduction of Cultivated Fishes - Report and Relevant Papers. EIFAC Tech. Pap. No. 25.

European Inland fisheries Adviosry Commission, 1984. Report on Nitrite and Freshwater Fish. EIFAC Tech. Pap., 46: 19pp.

FAO, 1985. 20 year Fisheries Development Plan (FDP) for Bangladesh, Food and Agricultural Organisation, 1985 (Unpublished).

FAO/UNDP, 1986. People's Republic of Bangladesh, Ministry of Fisheries and Livestock, Fisheries Research Institute, Report No. 2, Five year Masterplan, Part 1: Research Plan, FAO/UNDP TA Project BGD 83-010, 74pp.

Fijan, N., 1979. Health protection of fry and fingerlings in mass rearing operations. EIFAC Tech. Pap., 35 Suppl.1: 73-84.

Flis, J., 1968a. Anatomicohistopathological changes induced in carp (Cyprinus carpio L.) by ammonia water. Part 1. Effects of toxic concentrations.Acta. Hydrobiol., 10: 205-224.

Flis, J., 1968b. Anatomicohistopathological changes induced in carp (Cyrpinus carpio L.) by ammonia water. Part 2. Effects of subtoxic concentrations. Acta. Hydrobiol., 10: 225-238.

Forster, R. P. and Goldstein, L., 1969. Formation of excretory products. In : W. S. Hoar and D. J. Randall (Editors), Fish Physiology, Vol. 1. Academic Press, New York, N.Y., pp.313-350.

Friedman, L. and Shibko, S. I., 1972. Nonnutrient Components of the diet. In : J. E. Halver (Editor), Fish Nutrition, Academic Press, pp.181-264.

Fromm, P. O. and Gillette, J. R., 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (Salmo gairdnerii). Comp. Biochem. Physiol., 26: 887-896.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: W.S. Hoar and D.R. Randal (Editors) Fish Physiology, Vol. VI, Academic Press, New York, NY,pp1-98.

Fry, F. E. J., Brett, J. R. and Clawson, G. H., 1942. Lethal limits of temperature for young gold fish. Rev. Can. Biol., 1: 50-56.

Fry, F. E. J., Hart, J. S. and Walker, K. F., 1946. Lethal temperature relations for a sample of young speckled trout, Salvelinus fontinalis. University of Toronto Studies Biological Series 54, Publication of the Ontario Fisheries Research Laboratory, Toronto, Canada. 33pp.

Furuichi, M. and Yone, Y., 1980. Effect of dietary dextrin levels on growth and feed efficency, and chemical composition of liver and dorsal muscle, and the absorption of dietary protein and dextrin in fishes. Bull. Jpn. Soc. Sci. Fish., 46: 225-229.

Furukawa, A. and Tsukahara, H., 1966. On the acid digestion method for determination of chromic oxide as an indicator substance in the study of digestibility in fish. Bull. Jpn. Soc. Sci. Fish., 32: 502-506.

Ghittino, P., 1972. The diet and general fish husbandry. In: J. E. Halver (Editor), Fish Nutrition, Academic Press, pp. 540-650.

Gibson, M. B., 1954. Upper lethal temperature relations of the guppy, Lebistes reticulatus Can.J. Zool., 32: 393-407.

Gillette, L. A., Miller, D. L., and Redman, H. E. 1952. Appraisal of a chemical waste problem by fish toxicity tests. Sewage Ind. Wastes 24: 1397-1401.

Gohl, B., 1981. Tropical Feeds. Food and Agricultural Organisation of the United Nations, Rome. 528pp.

Golterman, H. L., Clymo, R. S. and Ohnstad, M. A. M., 1978. Methods for Physical and Chemical Analysis of Freshwaters. Blackwell Scientific Publications, Oxford, 214pp.

Goolish, E. M. and Adelman, I. R., 1984. Effects of ration size and temperature on the growth of juvenile common carp (Cyprinius carpio L.). Aquaculture, 36: 27-35.

Grabda, E., Einszporn-Orecka, T., Felinska, C. and Zbanysek, R., 1974. Experimental methemoglobinaemia in trout. Acta Ichthyol. Piscatoria, 4: 43-71.

Gribanov, L. V., Korneev, A. N. and Kovneeva, L. A., 1968. Use of thermal waters for commercial production of carps in floats in the USSR. FAO Fish. Rep., 44(5): 218-226.

Hamilton, M. A., Russo, R. C. and Thurston, R. V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol., II: 714-719.

Hampson, B. L., 1976. Ammonia concentrations in relation to ammonia toxicity during a rainbow trout rearing experiment in a closed freshwater-sea water system. Aquaculture, 9: 61-70.

Haque, K. A., 1974. Some observation on the induced spawning of major carps by pituitary hormone injections. Freshwater Fisheries Research Station Chaudpur Bull., 1: 19-33.

Harader, R. R. Jr. and Allen, G. H., 1983. Ammoia toxicity to chinook salmon parr: reduction in saline water. Trans. Am. Fish. Soc., 112: 834-837.

Hartman, G. F., 1958. Mouth size and food size in young rainbow trout, Salmo gairdneri. Copeia, 3: 233-234.

Haskell, D. C., 1955. Weight of fish per cubic foot of water in hatchery troughs and ponds. Prog. Fish-Cult., 17: 117-118.

Hastings, W. H., 1969. Nutritional score. In : O. W. Neuhaus and J. E. Halver (Editors), Fish in Research, Academic Press, New York, NY, pp.263-269.

Hastings, W. H. and Dickie, L. M. 1972. Feed formulation and evaluation. In: J.E. Halver (Editor), Fish Nutrition, New York: Academic Press, pp. 327-374.

Hauser, W. J., 1977. Temperature requirements of Tilapia zilli. Calif. Fish and Game, 63: 228-233.

Hecht, T. and Viljoen, J. H., 1982. Observations on the suitability of various dry feeds for the commercial rearing of carp, Cyprinius carpio larvae in South Africa. Water SA (Pretoria), 8: 58-65.

Hegarty, M. P., Court, R. D., Christie, G. S. and Lee, C. P., 1976. Mimosine in Leucaena leucocephala is metabolized to a goitrogen in ruminants. Australian Veterinary Journal, 52: 490.

Hepher, B. and Pruginin, Y., 1981. Commercial Fish Farming - with special reference to fish culture in Israel. John Wiley & Sons, Inc. 261pp.

Higgs, D. A., Marker, J. R., Macquarrie, D. W., McBride, J. R., Dosanjh, B. S., Nichols, C., Hoskins, G. 1979. Development of practical diets for coho salmon, Oncorhynchus kisutch using poultry by-product meal, feather meal, soybean meal and rapeseed meal as major protein sources. In : J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fish feed Technology, Vol. II, H. Heenemann Gm bH & Co., Berlin, pp. 191-218.

Hodson, R. G. and Fechhelm, R. G. and Monroe, R.J., 1981. Upper temperature tolerance of spot, Leiostomus xanthurus. Estuaries, 4: 345-356.

Holt, G. J. and Arnold, C. R., 1983. Effects of Ammonia and nitrite on growth and survival of red drum eggs and larvae. Trans. Am. Fish. Soc., 112: 314-18.

Horoszewicz, L., 1973. Lethal and 'disturbing' temperatures in some fish species from lakes with normal and artificially elevated temperature. J. Fish Biol., 5: 165-181.

Huey, D. W. and Beitinger, T. L., 1982. A methemoglobin reductase system in channel catfish Ictalurus puntatus. Can. J. Zool., 60: 1511-1513.

Huey, D. W., Simco, B. A. and Criswell, D. W., 1980. Nitrite-induced methemoglobin formation in channel catfish. Trans. Am. Fish. Soc., 109: 558-562.

Huisman, E. A., 1976a. Food conversion efficiencies at maintenance and production levels for carp (Cyprinus carpio L.) and rainbow trout (Salmo gairdneri Rich.). Aquaculture, 9: 259-73.

Huisman, E. A., 1976b. Hatchery and nursery operations. EIFAC Tech. Pap., (25): 101-110.

Huisman, E. A., 1979. The role of fry and fingerlings in fish culture. EIFAC Tech. Pap., (35) Suppl. 1: 3-6.

Huisman, E. A., Klein Breteler, J. G. P., Vismans, M. M. and Kanis, E., 1979. Retention of energy, protein, fat and ash in growing Carp (Cyprinus carpio L.) under different feeding and temperature regimes. In : Halver, J. E. and Tiews, K. (Editors) Finfish Nutrition and Fish feed Technology I: 175-188. H. Heenemann Gm bH & Co, Berlin 42.

Hutchinson, V. H., 1961. Critical thermal maxima in Salamanders. Physiol. Zool., 34: 92-125.

Hyatt, K. D., 1979. Feeding strategy. In : W. S. Hoar, D. J. Randall and J. R. Brett (Editors), Fish Physiology. Vol. VIII. Academic Press, London. pp.71-119.

Hysmith, B. T., Matlock, G. C. and Colura, R. L., 1983. Effects of stocking rate and food type on growth and survival of fingerling reddrum. In : R. R. Stickney and S. P. Meyers, (Editors) Special Publication No. 3. Louisiana State University, Division of Continuing Education, pp.133-141.

Itazawa, Y., 1971. An estimation of the minimum level of dissolved oxygen in water required for normal life of fish. Bull. Jap. Soc. Sci. Fish., 37: 273-276.

Jackson, A. J., Capper, B. S. and Matty, A. J., 1982. Evaluation of some plant proteins in complete diets for the tilapia Sarotherodon mossambicus. Aquaculture, 27: 97-109.

Jauncey, K., 1979. growth and nutrition of carp in heated effluents. Ph.D. Thesis, University of Aston in Birmingham, 202pp.

Jauncey, K., 1981. The effects of varying dietary composition on common carp (Cyprinus carpio) maintained in thermal effluents and laboratory recycling system. In : K. Tiews (Editor), Proceedings of World Symposium on Aquaculture in Heated Effluents and Recircultion Systems, Vol. II, pp.249-261.

Jauncey, K., 1982. Carp (Cyprinus carpio) Nutrition - a reveiw. In : J. F. Muir and R. J. Roberts (Editor), Recent Advances in Aquaculture. Croom Helm. London & Canbera, pp.215-263.

Jauncey, L. and Ross, B., 1982. A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture, University of Stirling, Scotland, 111pp.

Jensen, A. L., 1972. Standard error of LC50 and sample size in fish bioassays. Water Research, 6: 85-89.

Jezierska, B., Jowko, G. and Korwin-Kossakowski, M., 1979. Respiratory metabolism of carp larvae under constant and cyclically fluctuating temperature conditions. E. Stycsynska-Jurewicz, T. Backiel, E. Jaspers and G. Persoone (Editors). Cultivation of Fish Fry and its Live Food. European Mariculture Society - Special publication No. 4, pp.243-255.

Jhingran, V. G., 1977. Fish and Fisheries of India. Hindustan Publishing Corporation (India), Jawahar Nagen, Delhi, India, 954pp.

Jhingran, V. G. and Pullin, R. S. V., 1985. A hatchery manual for the common carp, Chinese and Indian major carps. ICLARM Studies and Reviews 11, 191p. Asian Development Bank, Manila, Philippines and International Centre for Living Aquatic Resources Management, Manila, Philippines.

Jhingran, V. G., Sehgal, K. L., Kumar, K. and Gosh, B. B., 1979. Rearing advanced fry of major Indian carp species in recirculatory-filtering ponds at Barrackpore, West Bengal, Aquaculture, 18: 45-49.

Jobling, M., 1981a. The influences of feeding on the metabolic rate of fishes: a short reveiw. J. Fish Biol., 18: 385-400.

Jobling, M., 1981b. Temperature tolerance and the final preferendum-rapid methods for the assessment of optimum growth temperatures. J. Fish Biol., 19: 439-455.

Jobling, M., 1983a. Growth studies with fish - overcoming the problems of size variation. J. Fish Biol., 22: 153-157.

Jobling, M., 1983b. Influence of body weight and temperature on growth rates of Arctic charr, Salvelinus alpinus (L.). J. Fish Biol., 22: 471-475.

Jobling, M. and Wandsvik, A., 1983. Effect of social interactions on growth rates and food conversion efficiency of Arctic charr Salvelinus alpinus L. J. Fish Biol., 22; 577-584.

Jones, J. R. E., 1964. Thermal pollution: The effect of heated effluents. In : Fish and River Pollution. Butterworth & Co (Publishers). London, pp.153-168.

Jones, R. J., Blunt, C. G. and Homes, J. H. G., 1976. Enlarged thyroid glands in cattle grazing leucaena pastures. Tropical Grasslands, 10: 113-116.

Jones, J. G., Downes, M. T. and Talling, I. B., 1980. The effect of sewage effluent on denitrification in Grasmere (English Lake District). Fresh water Biology, 10: 341-359.

Karim, M., 1978. Status and Potential of Bangladesh Fisheries. Ministry of Fisheries and Livestock, Govt. of the People's Republic of Bangladesh, 125pp.

Kamara, B.K.A., 1982. Evaluation of some oilseed meal protein in complete diets for tilapia Oreochromis niloticus. M.Sc. thesis University of Stirling, 49pp.

Kausch, H., 1969. The influence of spontaneous activity on the metabolic rate of starved and fed young carp (Cyprinus carpio L.). Vevhandle. Int. Ver. Limnol., 17: 669-679.

Kaushik, S.J., 1980. Influence of nutritional status on the daily patterns of nitrogen excretion in the Carp (Cyprinus carpio L.) and the rainbow trout (Salmo gairdneri R.) Reprod. Nutr. Dev., 20: 1751-1765.

Keast, A. and Webb, D., 1966. Mouth and body form relative to the feeding ecology in the fish fauna of a small lake, Lake Opinicon, Ontario. J. Fish Res. Board Can., 23: 1845-1867.

Kellog, R.L. and Gift, J.J., 1983. Relationship between optimum temperatures for growth and preferred temperatures for the young of four fish species. Trans. Am. Fish. Soc., 112: 424-430.

Kiese, M., 1974. Methemoglobinemia: A Comprehensive Treatise. CRC Press, Cleveland, Ohio, 260pp.

Kinne, O. 1970. Temperature: animals. invertebrates. In: O. Kinne (Editor), Marine Ecology. Vol. 1 Environmental factors. Part 1. Wiley-Interscience, London.

Klingler, K., 1957. Sodium nitrite, a slow-acting fish poison. Schweiz. Z. Hydrol., 19: 565-578.

Knight, B., 1983. Food particle-size preferences and feeding behaviour in warmwater aquaculture of European eel, Anguilla anguilla (L). Aquaculture, 30: 173-190.

Koebele, B.P., 1985. Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms: activity differences, disproportional food acquisition, physiological stress. Env. Biol. Fish. 12: 181-188.

Konikoff, M., 1975. Toxicity of nitrite to channel catfish. Prog. Fish -Cult., 37: 96-98.

Korn, S. and Macedo, D., 1983. Determination of fat content in fish with a nontoxic noninflammable solvent. J. Fish. Res. Board Can., 30: 1880-1881.

Korneyeva, L.A., 1969. Weight increase of carp in nurseries in relation to rearing conditions. Vop. 1 Jkhtiol., 9: 95-101.

Kossmann, H., 1976. Rearing of carp under laboratory conditions. EIFAC Tech. pap., (25): 127-129.

Kouvil, J., Matena, J., Skacelova, O. and Prikryl, I., 1981. Test feeding of carp early stages with zooplankton and the Ewos C10 Bul. Vyzk. Ustav Ryb. Hydrobiol., 17: 15-35.

Lakshmanan, M.A.V., Murty, D.S., Pillai, K.K. and Banerjee S.C., 1967. On a new artificial feed for carp fry. FAO Fish Rep., 44(3): 373-87.

Lall, S.P., 1979. Minerals in finfish nutrition. In: J.E. Halver and K. Tiews (Editors), Finfish Nutrition and Fish Feed Technology, Vol. I, H. Heenemann GmbH & Co, Berlin, pp. 85-97.

Laurent, P., 1984. Gill internal morphology, In: W.S. Hoar and and D.J. Randall (Editors), Fish Physiology, Vol. X, Gills Part A - Anatomy, Gas Transfer and Acid-base Regulation, Academic Press Inc. New York, N.Y., pp. 73-183.

Liao, P.B. and Mayo, R.D., 1974. Intensified fish culture combining water reconditioning with Pollution abatement. Aquaculture, 3: 61-85.

Liener, I.E., 1977. Removal of naturally occuring toxicants through enzymatic processing. Adv. Chem. Ser., 160: 283-300.

Liener, I.L., 1980. Miscellaneous toxic factors. In: I.L. Liener (Editor), Toxic Constituents of plant foodstuffs. Academic Press, New York, pp. 429-467.

Litchfield, J.T., 1949. A method for rapid graphic solution of time-per cent effects curves. J. Pharmacol. Exp. Ther., 97: 399-408.

Lloyd, R., 1961. Effect of dissolved-oxygen concentrations on the toxicity of several poisons to rainbow trout (Salmo gairdnerii Richardson). J. Exp. Biol., 38: 447-455.

Lloyd. R. and Orr. L.D., 1969. The diuretic response of rainbow trout to sublethal concentrations of ammonia. Water Res., 3: 335-344.

Macintosh, D.J. and DeSilva, S.S., 1984. The influence of stocking density and food ration in fry survival and growth in Oreochromis mossambicus and O. niloticus female x O. aureus male hybrids reared in a closed circulated system. Aquaculture, 41: 345-358.

Mackereth, F.J.H., Heron, J. and Talling, J.F., 1978. Water Analysis: Some Revised Methods for Limnologists. Freshwater Biological Association. Scientific Publication No. 36. 120pp.

Magnuson, J.J., 1962. An analysis of aggressive behaviour, growth, and competition for food and space in Medaka (Oryzias latipes (Pisces, Cyprinodontidae). Canadian J. Zool., 40: 313-363.

Mannan, A., 1977. Nutritional aspects of marine fishes and fisheries products. In: A.L. Bhuiyan, M.J. Islam and A.K. Chowdhury (Editors), Proceeding of National Marine Fisheries Seminar, University of Chittagong, Bangladesh, pp. 15-38.

Mathias, J.A. and Li, S., 1982. Feeding habits of walleye larvae and juveniles: comparative laboratory and field studies. Trans. Am. Fish. Soc., 111: 722-735.

Maynard, L.A. and Looshi, J.K. (Editors)., 1969. Animal Nutrition. 6th Edition. McGraw Hill Book Company.

Meade, T.L. and Perrone, S.J., 1980. Effect of chloride ion concentration and pH on the transport of nitrite across the gill epithelia of coho salmon. Prog. Fish. Cult., 42: 71-72.

Meske, C., 1985. Fish Aquaculture. Pergamon Press, Oxford. 237pp.

Meske, C. and Pfeffer, E., 1978. Growth experiments with carps and grass carp. Arch. Hydrobiol. Beih., 11 : 98-107.

Miller, D.S. and Bender, A.E., 1955. The determination of the net utilisation of proteins by a shortened method. Br. J. Nutr., 9: 382-388.

Mills, E.L., Confer. J.L. and Ready, R.C., 1984. Prey selection by young yellow perch: the influence of capture success, visual acuity, and prey choice. Trans. Am. fish Soc. 113: 579-587.

Ministry of Housing and Local Government, 1969: Fish Toxicity Tests. Her Majesty's Stationery Office, London 15pp.

Mires, D., 1976. Primary nursing of carp fingerlings in tanks. Bamidgeh, 28: 18-24.

Mironova, N.V., 1975. Changes in the energy balance of Tilapia mossambica in relation to temperature and ration size. J. Ichthyol., 16: 120-129.

Mitchell, S.J. and Check, J.J. Jr., 1983. Ammonia-caused gill damage in channel catfish (Ictalurus punctatus): confounding effects of residual chlorine. Can. J. Fish. Aquat. Sci., 40: 242-247.

Montgomery, R.D., 1980. Cyanogens. In: I.L. Liener (Editor), Toxic Constituents in Plant Foodstuffs. Academic Press, New York, pp. 143-160.

Moore, J.W. and Moore, I.A., 1974. Food and growth of anadromous arctic char, Salvelinus alpinus (L), in the Cumberland Sound area of Baffin Island. J. Fish. Biol., 6: 79-92.

Moore, J.W. and Moore, I.A., 1976. The basis of food selection in some estuarine fishes. eels, Anguilla anguilla(L.), whiting, Merlangius merlangius (L), sprat, Sprattus sprattus (L.) and stickleback, Gasterosteus aculeatus. L.J. Fish Biol., 9: 375-390.

Mount, D.I., 1977. An assessment of application factors in aquatic toxicology. In:R.A. Tubb, (Editor). Recent Advances in Fish Toxicology. Proceedings of a symposium sponsored by Corvallis Environmental Research Laboratory, Corvallis, Oregon. 97330, USA, pp. 183-190.

Murray, M.W., Andrew, J.W. and DeLoach H.L. 1977. Effects of dietary lipids, dietary protein and environmental temperatures on growth, feed conversion and body composition of channel catfish. J. Nutr. 107: 272-280.

McCauley, R.W. and Binkowski, F.P., 1982. Thermal tolerance of the alewife. Trans Am. Fish. Soc., 111: 389-391.

McCauley, R.W. and Casselman, J.M., 1980. The final preferendum as an index of the temperature for optimum growth in fish. In: K. Tiews. (Editor), Proc. World Symp. on Aquaculture in Heated Effluents and Recirculation Systems, pp. 81-93.

McCormick, J.H., Jones, B.R., and Syrett, R.F., 1971. Temperature requirements for growth and survival of larval ciscos (Coregonus artedii). J. Fish. Res. Board Can., 28: 924-927.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D., 1981. Animal Nutrition. Longman. London and New York. 479pp.

Nakamura, N. and Kasahara, S., 1955. A study on the phenomenon of the tobi-koi or shoot carp-1. On the earliest stage at which the shoot carp appears. Bull. Jap. Soc. Sci. Fish., 21: 73-76.

Nakamura, N. and Kasahara, S., 1961. A study on the phenomenon of the tobi-koi or shoot carp-IV. Effects of adding a small number of larger individuals to the experimental batches of carp fry and culture density upon the occurrence of shoot carp. Bull. Jap. Soc. Sci. Fish., 27: 958-962.

National Academy of Sciences, 1977. Leucaena, a Promising Forage and Tree Crop for the Tropics. Washington. D.C., 115pp.

National Research Council, 1983. Nutrient Requirement of Warmwater Fishes and Shellfishes. National Academy of Sciences, Washington, D.C., 102pp.

Nemcsok, J., Gyone, K., Olah, J. and Boross, L., 1984. Effect of NH_3 on blood glucose and c a techolamine level, GOT, GPT, LDH enzyme activity and respiration of fishes. Symposia Biologica Hungarica 23: 209-217.

Niimi, A.J. and Beamish, F.W.H., 1974. Bioenergetics and growth of largemouth bass (Micropterus salmoides) in relation to body weight and temperature. Can. J. Zool., 52: 447-456.

Nilsson, N.A., 1957. On the feeding habits of trout in a stream in northern Sweden. Rep. Inst. FW. Res. Drottringholm, 38: 154-166.

Nose, T., 1979. Summary report on the requirements of essential amino acids for carp. In: J.E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Haenemann GmbH & Co. Berlin, pp. 145-156.

Northcote, T., 1954. Observations on the Comparative ecology of two species of fish, Cottus asper and C. rhotheus, in British Columbia. Copeia, 1: 25-28.

Nyambi, T.A.J.S., 1982. The effects of water temperature, pH and dissolved oxygen concentration on the survival and growth of first-feeding stage Oreochromis (=Sarotherodon) mossambicus fry. MSc. Thesis, University of Stirling.

Ogino, C. and Saito, K., 1970. Protein nutrition in fish. I. The utilization of dietary protein by young carp. Bull. Jap. Soc. Sci. Fish., 36: 250-254.

Ogino, C., Cowey, C.B. and Chiou, J.-Y., 1978. Leaf protein concentrate as a protein source in diets for carp and rainbow trout. Bull. Jap. Soc. Sci. Fish., 44: 49-52.

Ohame, H., Suzuki, R. and Shimma, Y. 1979. Influence of single cell protein feeds on the growth and reproductivity of carp with reference to fatty acid composition. In: J.E. Halver and K. Tiews (Editors), Finfish Nutrition and fishfeed Technology, Vol. II, H. Heenemann GmbH & Co., Berlin, pp. 63-73.

Oliver, J.H., 1957. The chemical composition of the sea-water in the aquarium. Proceedings of the Zoological Society of London, 129: 137-145.

Oliver, S.L., McDonald, B.E. and Opuszynska, T., 1971. Weight gain, protein utilization, and liver histology of rats fed low and high thioglucoside content rapeseed meals. Can. J. Physiol. Pharmacol, 49: 448-456.

Olsen, K.R. and Fromm, P.O., 1971. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp. Biochem. Physiol. A, 40: 999-1007.

Olukunle, O.A., 1982. Partial plant substitution in diets of first feeding stage fry of tilapia: Oreochromis (=Sarotherodon) mossambicus. MSc. Thesis, University of Stirling, 55pp.

Opuszynski, K., 1967. Comparison of temperature and oxygen tolerance in grass carp (C tenopharyngodon idella Val.), silver carp (Hypophthalmichthys molitrix Val.), and mirror carp (Cyprinus carpio L.). Ekol. Pol. A. 15: 385-400.

Osborne, T.B., Mendel, L.B. and Ferry, E.L., 1919. A method for expressing numerically the growth promoting value of proteins. J. Biochem., 37: 223-229.

Ostroumova, I.N., Knyazeva, L.M. and Tunetskij, V.I., 1979. On the feeding of carp larvae reared in thermal water. Rybn. Khoz, 3: 17-20.

Otto, R.G., Kitchell, M.A. and O'Hara Rice, J., 1976. Lethal and preferred temperatures of the alewife (Alosa pseudolarengus) in Lake Michigan. Trans. Am. Fish. Soc., 105: 96-106.

Palachek, R.M. and Tomasso, J.R., 1984a. Toxicity of nitrite to channel catfish (Ictalurus punctatus), tilapia (Tilapia aurea) and largemouth bass (Micropterus salmoides): evidence for a nitrite exclusion mechanism. Can. J. Fish. Aqueat. Sci., 41: 1739-1744.

Palachek, R.M. and Tomasso, J.R., 1984b. Nitrite toxicity to fathead minnows: effect of fish weight. Bull. Environ. Contam. Toxicol. 32: 238-242

Paloheimo, J.E. and Dickie, L.M., 1965. Food and growth of fishes. I. A growth curve derived from experimental data. J. Fish. Res. Board. Canada., 22: 521-542.

Paloheimo, J.E. and Dickie, L.M., 1966a. Food and growth of fishes. II. Effects of food and temperature on the relation between metabolism and body weight. J. Fish. Res. Board Can., 12: 869-908.

Paloheimo, J.E. and Dickie, L.M., 1966b. Food and growth of fishes. III. Relations among food, body size, and growth efficiency. J. Fish. Res. Board Canada. 23: 1209-1248.

Pantastico, J.B. and Baldia, J.P., 1979. Supplemental feeding of Tilapia mossambica. In: J.E. Halver and K. Tiews, (Editor), Finfish Nutrition and Fishfeed Technology, Vol. 1. H. Heenemann GmbH & Co. Berlin. pp. 587-593.

Papoutsoglou, S.E. and Papoutsoglou, E.G., 1978. Effect of water temperature on growth rate and body composition of rainbow trout fry (Salmo gairdneri) fed on maximmum rations in a closed system. Thalassographica, 2: 83-97.

Parker, N.C. and Davis, K.B., 1981. Requirement of warmwater fish. Bio-Engineering symposium for fish culture (FCS Publ. 1). pp. 21-28.

Parker, R.E. 1979. Introductory Statistics for Biology. Second Edition. Edward Arnold, London. 122pp.

Pecor, C.H., 1979. Experimental intensive culture of tiger muskellunge in a water reuse system. Prog. Fish-cult. 41: 103-108.

Perrone, S.J. and Meade, T.L., 1977. Protective effect of chloride on nitrite toxicity to coho salmon (Oncorhynchus kisutch). J. Fish. Res. Board Can., 34: 486-492.

Pitt, T.K., Garside, E.T. and Hepburn, R.L., 1956. Temperature selection of carp (Cyprinus carpio L.). Can. J. Zool., 34: 555-557.

Plumb, J.A., 1984. Relationship of water quality and infections in cultured channel catfish. Symposia Biologica Hungarica, 23: 189-199.

Rahman, A.K.A., 1974. A checklist of the freshwater bony fishes of Bangladesh. Freshwater Fisheries Research Station Chandpur Bull., 1: 1-18.

Rao, T.S., Rao, M.S. and Prasad, S.B.S.K., 1975. Median tolerance limits of some chemicals to the freshwater fish "Cyprinus carpio". Indian Journal of Environmental Health, 17: 140-146.

Redner, B.D. and Stickney, R.R., 1979. Acclimation to ammonia by Tilapia aurea. Trans. Am. Fish. Soc., 108: 383-388.

Refstie, T. and Kittelsen, A., 1976. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture, 8: 319-326.

Reynolds, W.W. and Casterlin, M.E., 1977. Temperature preferences of four fish species in an electronic thermoregulatory shuttlebox Prog. Fish.-Cult., 39: 123-124.

Rice, S.D. and Stokes, R.M., 1975. Acute toxicity of ammonia to several developmental stages of rainbow trout. Salmo gairdneri. Fishery Bulletin. 73: 207-211.

Richards, A., 1983. The potential of sunflower and sesame seed meals as alternative sources of protein in diets for Oreochromis niloticus. MSc. Thesis. Inst. of Aquaculture, University of Stirling. 82pp.

Robinette, H.R., 1976. Effects of selected sublethal levels of ammonia on the growth of channel catfish (Ictalurus punctatus). Prog. Fish-Cult., 38: 26-29.

Rothbard, S., 1982. Induced reproduction in cultivated cyprinids- the common carp and the group of Chinese carps: II. The rearing of larvae and the primary nursing of fry. Bamidgeh 34: 20-32.

Rubin, A.J., and Elmaraghy, G.A., 1977. Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. Water Res., 11: 927-935.

Russo, R.C., 1980, Recent advances in the study of nitrite toxicity to fishes. In: W.R. Swain and R.V.R. Shannon (Editors), Proc. Third USA - USSR Symposium on the Effects of Pollutants upon Aquatic Ecosystems; Theoretical Aspects of Aquatic Toxicology, EPA Ecol. Res. Ser. EPA- 600/9.80.034, U.S. Environmental Protection Agency, Duluth, MN. pp. 226-240.

Russo, R.C., Smith, C.E. and Thurston, R.V., 1974. Acute toxicity to nitrite to rainbow trout (Salmo gairdneri). J. Fish Res. Board Can., 31: 1653-1655.

Russo, R.C. and Thurston, R.V., 1977. The acute toxicity of nitrite to fishes. In: R.A. Tubb (Editor). Recent Advances in Fish Toxicology, EPA Ecol. Res. Ser. EPA-600/3-77-085, U.S. Environmental Protection Agency, Corvallis, OR, pp. 118-131.

Russo, R.C., Thurston, R.V. and Emerson, K., 1981. Acute toxicity of nitrite to rainbow trout (Salmo gairdneri): effects of pH nitrite species and anion species. Can. J. Fish. & Aqua.Sci., 38: 387-393.

Saclauso, C.A., 1985. Interaction of growth with social behaviour in Tilapia zilli raised in three different temperatures. J. Fish. Biol., 26: 331-337.

Sadler, K., 1981. The toxicity of ammonia to the European eel (Anguilla anguilla L.) Aquaculture, 26: 173-181.

Sampson, D.R.T. and Macintosh, D.J., 1986. Carp transportation in sealed polythene bags. Aquaculture, 53 (in press).

Sandbank, E. and Hepher, B., 1978. The utilization of microalgae as a feed for fish. Ergeb. Limnol., 11: 108-120.

Sarig, S., 1966. Synopsis of biological data on the Common carp, Cyprinus carpio (Linnaeus 1758) - Near East and Europe. FAO Fisheries Synopis 31.2.42pp.

Sarig, S. and Marek, M., 1974. Results of intensive and semi-intensive fish breeding technique in Israel in 1971-73. Bamidgeh, 26: 28-48.

Saroglia, M.G., Scarano, G. and Tibaldi, E., 1981. Acute toxicity of nitrite to sea bass (Dicentrarchus labrax) and European eel (Anguilla anguilla). J. World Maricul. Soc., 12: 121-126.

Sen, P.R., Rao, N.G.S., Ghosh, S.R. and Rout, M., 1978. Observations on the protein and carbohydrate requirements of carps. Aquaculture, 13: 245-256.

Sharma, K.P. and Kulshreshtha, S.D., 1976. Effect of certain fertilisers, feeds and cobalt chloride on the production and survival of young ones of the common carp Cyprinus carpio L. Bull. Basrah Nat. Hist. Mus. Univ. Basrah. 2: 27-35.

Shaw, J., 1960. The absorption of sodium ions by the crayfish Actacus pallipes Leraboullet. III. The effects of other cation in the external solution. J. Exp. Biol., 37: 548-556.

Shcherbina, M.A. & Kazlauskene, O.P., 1971. Water temperature and the digestibility of nutrient substances by carp. Hydrobiol. J., 7: 40-44.

Shelbourn, J.E., Brett, J.R. and Shirahata, S., 1973. Effect of temperature and feeding regime on the specific growth rate of sockeye salmon fry (Oncorhynchus nerka), with a consideration of size effect. J. Fish. Res. Board Can. 30: 1191-1194.

Shirota, A., 1970. Studies on the mouth size of fish larvae. Bull. Jap. Soc. Sci. Fish., 36: 353-368.

Shrable, J.B., Tiemeier, O.W. & Deyoe, C.W., 1969. Effect of temperature on the rate of digestion by Channel Catfish. Prog. Fish-Cult., 31: 131-133.

Sibbing, F.A., 1986. Specializations and limitations in the utilization of food sources by the carp (Cyprinus carpio), A study of oral food processing. Aquaculture, (in Press).

Smart, G., 1976. The effect of ammonia exposure on gill structure of the rainbow trout (Salmo gairdneri). J. Fish. Biol., 8: 471-475.

Smith, C.E. & Russo, R.C., 1975. Nitrite induced methemoglobinemia in rainbow trout. Prog. Fish-Cult., 37: 150-152.

Smith, R.R., 1971. A method for measuring digestibility and metabolizable energy of fish feeds. Prog. Fish - Cult., 33: 132-134.

Smith, R.R., 1977. Recent research involving full-fat soyabean meal in the salmonid diet. Salmonid, 1: 18.

Smith, C.E. and Williams, W.G., 1974. Experimental nitrite toxicity in rainbow trout and chinook salmon. Trans. Am. Fish Soc., 103: 389-90.

Sokal, R.R. and Rohlf, F.J., 1981. Biometry - The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Company, San Francisco, 776pp.

Soofiani, N.M. and Hawkins, A.D., 1982. Energetic costs at different levels of feeding in juvenile cod, Gadus morhua L. J. Fish. Biol., 21: 577-592.

Sousa, R.J. and Meade, T.L., 1977. The influence of ammonia on the oxygen delivery system of coho salmon hemoglobin. Comp. Biochem. Physiol. 58A: 23-28.

Sousa, R.J., Meade, T.L. and Wolke, R.E., 1974. Reduction of ammonia toxicity by salinity and pH manipulation. In: J.W. Avault (Editor). Proc. 5th Annu. Meat World Mariculture Soc., Charleston, Sc. Publ. Louisiana State Univ. Div. Continuing Education. pp. 343-354.

Speece, R.E., 1973. Trout metabolism characteristics and the rational design of nitrification facilities for water reuse in hatcheries. Trans. Am. Fish. Soc., 102: 323-334.

Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research, 3: 793-821.

Sprague, J.B., 1971. Measurement of pollutant toxicity to fish. 3. Sub-lethal effects and 'safe' concentrations. Water Res. 5: 245-266.

Sprague, J.B. 1973. The ABC's of pollutant bioassay using fish. Spec. Tech. Publ. Am. Soc. Test. Mater. 528: 6-30.

Steel, R.G.D. and Torrie, J.H., 1960. Principles and Products of Statistics. McGraw-Hill Book Company Inc. N.Y. Toronto, London. 480pp.

Stickney, R.R., Murai, T. and Gibbons, G.O., 1972. Rearing channel catfish fingerlings under intensive culture conditions. Prog. Fish. Cult., 34: 100-102.

Stirling, H.P. (Editor), 1983. Water Analysis for Aquaculturists. Institute of Aquaculture, University of Stirling. Stirling, Scotland. 139pp.

Sylvester, J.R., Nash, C.E. and Emberson, C.E. 1974. Preliminary study of temperature tolerance in juvenile Hawaiian mullet (Mugil cephalus) Prog. Fish-Cult., 36: 90-100.

Symons, P.E.K., Metacalfe, J.L. and Harding, G.D., 1976. Upper lethal and preferred temperatures of the slimy sculpin, Cottus cognatus. J. Fish. Res. Board Can., 33: 180-183.

Tabata, K., 1962. Toxicity of ammonia to aquatic animals with reference to the effects of pH and carbon dioxide. Bull. Tokai Reg. Fish Res. Lab., 34: 67-74.

Tamas, G. and Horvath, L., 1979. Fry and fingerlings production under pond farm management conditions (carp and other cyprinids) EIFAC T35. Suppl. 1: 53-58.

Tarzwell, C.M., 1966. Water quality requirements for aquatic life. In proc. Nat. Symp. on Water Quality, Ann. Arbor, Michigan, U.S.A. University of Michigan, School of Public Health, Continued Education Series No. 161: 185-197.

Thorpe, J.E. and Wankowski, J.W.J., 1979. Feed presentation and food particle size for juvenile Atlantic salmon, Salmo salar L. In: J.E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, H. Heenermann GmbH & Co. Berlin, pp. 501-513.

Thurston, R.V. and Russo, R.C., 1983. Acute toxicity of ammonia to rainbow trout. Trans. Am. Fish Soc., 112: 696-704.

Thurston, R.V., Russo, R.C. and Smith, C.E., 1978. Acute toxicity of ammonia and nitrite to cut-throat trout fry. Trans. Am. Fish. Soc., 107: 361-368.

Thurston, R.V., Russo, R.C. and Vinogradov, G.A., 1981a. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Env. Sci. Tech., 15: 837-840.

Thurston, R.V., Phillips, G.R., Russo, R.C. and Hinkins, S.M., 1981b Increased toxicity of ammonia to rainbow trout (Salmo gairdneri) resulting from reduced concentrations of dissolved oxygen. Can. J. Fish. Aquat. Sci., 38: 983-988.

Thurston, R.V., Russo, R.C., and Phillips, G.R., 1983. Acute toxicity of ammonia to fathead minnows. Trans. Am. Fish. Soc., 112: 705-711.

Thurston, R.V., Russo, R.C., Leudtke, R.J., Smith, C.E., Meyn, E.L., Chakoumakos, C., Wang, K.C. and Brown, C.J.D., 1984. Chronic toxicity of ammonia to rainbow trout. Trans. Am. Fish. Soc., 113: 56-73.

Tookey, H.L., Van Etten, C.H. and Daxenbichler, M.E., 1980. Glucosinolates. In: I.E. Liener, (Editor), Toxic constituents of Plant Foodstuffs. Academic Press, New York pp. 103-142.

Trussel, R.P., 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board Can., 29: 1505-1507.

Tomasso, J.R., Simco, B.A. and Davis, K.B., 1979. Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (Ictalurus punctatus). J. Fish. Res. Board Can., 36: 1141-1144.

Tomasso, J.R., Goudie, C.A., Simco, B.A. and Davis, K.B., 1980a. Effects of environmental pH and calcium on ammonia toxicity in channel catfish (Ictalurus punctatus). Trans. Am. Fish. Soc., 109: 229-234.

Tomasso, J.R., Wright, M.I., Simco, B.A. and Davis, K.B., 1980b. Inhibition of nitrite-induced toxicity in channel catfish by calcium chloride and sodium chloride. Prog. Fish-Cult., 42: 144-146.

Tucker, C.S. and Schwedler, T.E., 1983. Acclimation of channel catfish (Ictalurus punctatus) to nitrite. Bull. Environ. Contam. Toxicol., 30: 516-521.

U.S. National Technical Advisory Committee, 1968. Report of the national Technical Advisory Committee on water quality criteria to the Secretary of the Interior. U.S. Fed. Wat. Pollut. Control Admin., Wash. D.C. 234pp.

Vamos, R., 1963. Ammonia poisoning in carp. Acta biol. Szeged, 9: 291-297.

Tookey, H.L., Van Etten, C.H. and Daxenbichler, M.E., 1980. Glucosinolates. In: I.E. Liener, (Editor), Toxic constituents of Plant Foodstuffs. Academic Press, New York pp. 103-142.

Trussel, R.P., 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board Can., 29: 1505-1507.

Tomasso, J.R., Simco, B.A. and Davis, K.B., 1979. Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (Ictalurus punctatus). J. Fish. Res. Board Can., 36: 1141-1144.

Tomasso, J.R., Goudie, C.A., Simco, B.A. and Davis, K.B., 1980a. Effects of environmental pH and calcium on ammonia toxicity in channel catfish (Ictalurus punctatus). Trans. Am. Fish. Soc., 109: 229-234.

Tomasso, J.R., Wright, M.I., Simco, B.A. and Davis, K.B., 1980b. Inhibition of nitrite-induced toxicity in channel catfish by calcium chloride and sodium chloride. Prog. Fish-Cult., 42: 144-146.

Tucker, C.S. and Schwedler, T.E., 1983. Acclimation of channel catfish (Ictalurus punctatus) to nitrite. Bull. Environ. Contam. Toxicol., 30: 516-521.

U.S. National Technical Advisory Committee, 1968. Report of the national Technical Advisory Committee on water quality criteria to the Secretary of the Interior. U.S. Fed. Wat. Pollut. Control Admin., Wash. D.C. 234pp.

Vamos, R., 1963. Ammonia poisoning in carp. Acta biol. Szeged, 9: 291-297.

Viola, S., 1975. Experiments in nutrition of carp in cages. II Partial substitution of fish meal. Bamidgeh, 27: 40-48.

Viola, S., Mokady, S., Rappaport, U. and Arieli, Y., 1982. Partial and complete replacement of fishmeal by soybean meal in feeds for intensive culture of carp. Aquaculture, 26: 223-236.

Von Lukowicz, M., 1979. Experiments on first-feeding of carp fry with alevon and freeze-dried fish. EIFAC Tech. pap. 35, Suppl. 1.

Wales, J. H., 1970. Hepatoma in rainbow trout. In: S.F. Snieszko (Editor), A Symposium on Diseases of Fishes and Shellfishes. Spt. Pub. No. 5. Am. Fish. Soc. 351-364.

Wallen, I.E., Greer, W.C. and Lasater, R., 1957. Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage Ind. Wastes, 29: 695-711.

Wankowski, J.W.J., 1977. The role of prey size in the feeding behaviour and growth of juvenile Atlantic salmon (Salmo salar L.) PhD. Thesis, University of Stirling, Scotland.

Wankowski, J.W.J., 1979. Morphological limitations, prey size selectivity and growth response of juvenile Atlantic salmon, Salmo salar. J. Fish. Biol., 14: 89-100.

Wankowski, J.W.J. and Thorpe, J.E., 1979. The role of food particle size in the growth of juvenile Atlantic salmon (Salmo salar L.). J. Fish Biol., 14: 351-370.

Warren, C.E. and Davis, G.E., 1967. Laboratory studies on the feeding bioenergetics and growth of fishes. In: S.D. Gerking (Editor), The Biological Basis of Freshwater Fish Production. Blackwell Scientific Publications. Oxford. pp. 175-214.

Webber, H.H. and Huguenin, H.E., 1979. Fish feeding technologies. In: J.E. Halver, & K. Tiews, (Editor), Finfish Nutrition and Fishfeed Technology. Vol.1 H. Heenemann GmbH & Co. Berlin 42: 297-316.

Wedemeyer, G.A., 1981. The physiological responses of fishes to the stress of intensive aquaculture recirculation systems. In: K. Tiews (Editor), Proc. World Symp. Aquaculture in Heater Effluents and Recirculation Systems. pp. 3-18.

Wedemeyer, G. and Yasutake, W.T., 1978. Prevention and treatment of nitrite toxicity in juvenile steelhead trout (Salmo gairdneri) J. Fish. Res. Board Can., 35: 822-827.

Westers, H., 1982. Controlled fry and fingerling production in hatcheries. EIFAC Tech. Pap., (35) Suppl. 1: 32-52.

Westers, H. and Pratt, K.M., 1977. Rational design of hatcheries for intensive salmonid culture, based on metabolic characteristics. Prog. Fish-Cult., 39: 157-165.

Westin, D.T., 1974. Nitrate and nitrite toxicity to salmonid fishes. Prog. Fish Cult., 36: 86-89.

Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Wickins, J.F., 1980. Water quality requirements for intensive aquaculture. A review. In: K. Tiews (Editor). Proc. World Symp. Aquaculture in Heater Effluents and Recirculation Systems. H. Heeneman GmbH & Co. Berlin, pp. 17-37.

Wickins, J.F., 1982. Opportunities for farming Crustaceans in Western temperate region. In: J.F. Muir, and R.J. Roberts, (Editor), Recent Advances in Aquaculture. Croom Helm Ltd. London: 87-177.

Wohlfarth, G., 1977. shoot-carp. Bamidgeh, 29: 35-40.

Wohlfarth, G. and Moav, R. 1972. The regression of weight gain on initial weight in carp. I. Methods and results. Aquaculture 1 : 7-28.

Wohlfarth, G., Lahman, M. and Moav, R., 1971. Preliminary experiments in growing carp in recirculating water. Bamidgeh, 23: 10 3-116.

Wood, J.D., 1958. Nitrogen excretion in some marine teleosts. Can. J. Biochem. Physiol., 36: 1237-1242.

Wu, J.L. and Jan, L., 1977. Comparison of the nutritive value of dietary proteins in Tilapia aurea. J. Fish. Soc. Taiwan, 5: 55-60.

Wuhrmann, K. and Woker, H., 1948. Contributions to the toxicology of fishes. II. Experimental investigations on ammonia and hydrocyanic acid poisoning. Schwiez. Z. Hydrol., 11: 210-244.

Wuhrmann, K., Zehender, F. and Woker, H., 1947. Biological significance of the ammonium and ammonia content of flowing water in fisheries. Vierteljahrsschrift der Natur forschung Gesellschaft in Zurich 92: 198-204.

Wurtsbaugh, W.A. and Davis, G.E., 1977a. Effects of temperature and ration level on the growth and food conversion efficiency of Salmo gairdneri, Richardson. J. Fish Biol., 11: 87-98.

Wurtsbaugh, W.A. and Davis, G.E., 1977b. Effects of fish size and ration level in the growth and food conversion efficiency of rainbow trout. Salmo gairdneri Richardson. J. Fish Biol., 11: 99-104.

Yamagata, Y. and Niwa, M., 1982. Acute and chronic toxicity of ammonia to eel Anguilla japonica. Bull. Jap. Soc. Sci. Fish., 48: 171-176.

Yearbook of Agricultural Statistics of Bangladesh. 1985. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh.

Zar, J.H., 1974 Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J. 620pp.

Zeitler, M.H., Kirchgersner, M. and Schwarz, F.J., 1984. Effect of different protein and energy supplies in carcass compositon of carp (Cyprinus carpio L.). Aquaculture, 36: 37-48.

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ACUTE TOXICITY OF AMMONIA TO COMMON CARP FRY

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ABSTRACT

Hasan, M.R. and Macintosh, D.J., 1986. Acute toxicity of ammonia to common carp fry. *Aquaculture*, 54: 97—107.

Two recirculation bioassays were conducted for 168 h each to determine the median lethal concentration (LC50) of unionized ammonia (NH_3) to fry of common carp (*Cyprinus carpio* L.) for different exposure times. In the first trial duplicate groups of carp fry (mean weight 299 mg, SE ± 14.2) were exposed to seven concentrations of ammonia. The 48-, 96- and 168-h LC50's for exposure to unionized ammonia were 1.76 (95% CL 1.67—1.85), 1.74 (95% CL 1.65—1.84) and 1.64 (95% CL 1.53—1.76) mg l^{-1} NH_3-N respectively. Similarly, in the second trial duplicate groups of fry (mean weight 206 mg, SE ± 8.4) were exposed to seven concentrations of ammonia. The 48-, 96- and 168-h LC50's of unionized ammonia were 1.87 (95% CL 1.82—1.93), 1.84 (95% CL 1.78—1.91) and 1.78 (95% CL 1.71—1.86) mg l^{-1} NH_3-N respectively. Acute toxicity ceased after 24 and 48 h, respectively, in the first and second trials, and the two 96-h LC50 values obtained were not significantly different.

INTRODUCTION

Ammonia occurs in natural water in unionized (NH_3) and ionized (NH_4^+) forms. It is a product of biological metabolism and is the principal nitrogenous excretory product of freshwater teleosts (Brockway, 1950; Burrows, 1964; Forster and Goldstein, 1969). It can also enter natural waters from sewage effluents, industrial wastes and agricultural materials.

The toxicity of ammonia to fish has been extensively investigated (Ball, 1967; Flis, 1968a,b; Colt and Tchobanoglous, 1976, 1978; Redner and Stickney, 1979; Thurston et al., 1978, 1984). It was demonstrated that the toxicity of ammonia depends principally upon the presence of NH_3; the toxicity of NH_3 was considered to be relatively independent of pH while NH_4^+ was regarded as having little or no toxicity (Wuhrmann and Woker, 1948; Downing and Merkens, 1955; Lloyd, 1961). The toxicity of NH_3 has been ascribed to the fact that this unionized form of ammonia can readily diffuse across gill membranes due to its lipid solubility and lack of charge, whereas the ionized form occurs as a larger hydrated form

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with charged entities which cannot readily pass through the hydrophobic micropores in the gill membrane (Fromm and Gillette, 1968; Hampson, 1976). However, it has been shown that NH_4^+ may also have considerable toxicity under low pH conditions (Tabata, 1962; Armstrong et al., 1978; Thurston et al., 1981a; Yamagata and Niwa, 1982). The toxicity of NH_3 varies among species. Reported acute toxicity values in tests of 24- and 96-h duration on salmonids range from 0.06 to 0.66 mg l^{-1} NH_3-N, and values for comparable tests on non-salmonids range between 0.29 and 4.64 mg l^{-1} NH_3-N (Ball, 1967; Rice and Stokes, 1975; Thurston et al., 1978; Yamagata and Niwa, 1982).

The common carp, *Cyprinus carpio* L., is, on a world-wide basis, one of the most extensively cultivated fish species (Bardach et al., 1972; Jhingran, 1977). Although there is some published information on the toxicity of ammonia to carp fingerlings and adults (Vamos, 1963; Danecker, 1964, cited by Alabaster and Lloyd, 1980; Flis, 1968a,b; Rao et al., 1975; Table 1), data on the toxicity of ammonia to common carp fry (<1.0 g) are not available.

In view of the growing numbers of carp hatcheries (Woynarovich, 1973) and the lack of information on ammonia toxicity to carp, the present investigation was conducted to determine the acute toxicity of unionized ammonia to common carp fry.

TABLE 1

A comparison of the toxicity of NH_3 to common carp[a]

Size (weight/length)	pH	Temp (°C)	NH_3 Conc. (mg l^{-1} NH_3-N)	Effect	Author
6—8 g	8.2	22—25	0.43—0.55	Toxic; sink to the bottom in 60—75 min	Vamos (1963)
—	—	16	1.23	Lethal in less than 2 days	Danecker (1964, cited by Alabaster and Lloyd 1980)
125—260 g	8.3—8.7	7—15	0.71—0.98[b]	10-day LC16—LC18	Flis (1968a)
4—5 cm (1—2 g approx.)	7.2—7.8	27.6	0.96[b]	96 h TLm[c]	Rao et al. (1975)

[a]All tests were static except that of Danecker (1964), information of which is not available.
[b]Calculated from data given for total ammonia or ammonium chloride concentration, pH and temperature.
[c]Median tolerance limit (= LC50).

MATERIALS AND METHODS

Experimental animals and acclimation

Fry of the mirror variety of common carp (Dinkelsbühler strain from West Germany) were obtained from the hatchery of Newhay Fisheries, Bolton, England. Before the test, the fry were maintained for about 15—30 days at 28°C in 150-l tanks in a recirculating system and fed ground commercial trout pellet (Edward Baker's Omega No. 3; protein content 49%). Two trials were conducted to estimate the median lethal concentration of NH_3 to carp fry. Carp fry weighing 299 mg (SE ± 14.2) and 206 mg (SE ± 8.4) were used for the first and second trials respectively.

Experimental system

Both trials were conducted in a recirculating system. The system consisted of eight independent tank units. Each unit was comprised of six 2-l perspex tanks supplied by common inflow and outflow pipes (Fig. 1) and was connected to a 10-l reservoir tank. Water from the reservoir tank was pumped by an Eheim-brand electric pump through an inflow pipe fitted with valves to supply controllable rates of water to each perspex tank. On the side of the upper region of each tank, a window was cut out and a fine

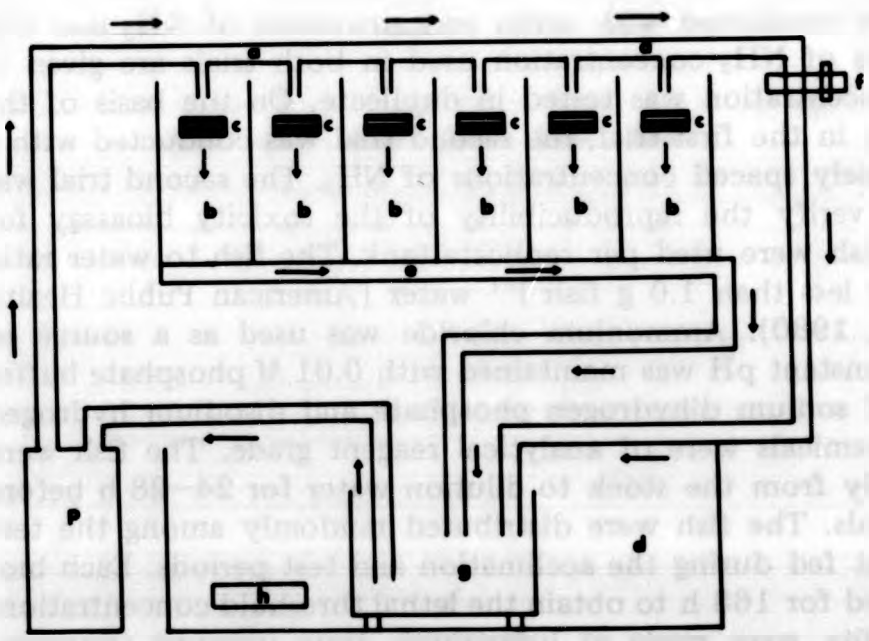

Fig. 1. Layout of one unit of the recirculation system for the ammonia toxicity bioassay. (a) Inflow pipe. (b) Experimental tanks (2-l perspex tank receiving an inflow of water at a rate of 100 ml/min). (c) Outflow. (d) Water bath (150 l). (e) Outflow trough. (f) Clamp for inflow regulation. (g) Reservoir tank (10 l). (h) Aquarium heaters (5 × 200 W). (p) Eheim brand electric pump. → Direction of waterflow.

mesh netting was fixed to this cut portion; this served as the water outflow. All eight reservoir tanks were maintained in a 150-l glass water bath. Thermostatically controlled immersion heaters in the water bath maintained the required temperature. A stone aerator connected to a compressed air supply was used to maintain an adequate level of dissolved oxygen in each reservoir tank. A photoperiod of 12 h light : 12 h dark was maintained by an electronic timer throughout the experimental period.

Test water

To maintain uniform water quality in both tests, synthetic dilution water was prepared with a total hardness of 50 mg l^{-1} as $CaCO_3$ and a pH of about 7.5—8.0 as recommended by Sprague (1973) and Alabaster and Lloyd (1980). The dilution water was prepared following the procedure outlined by the Ministry of Housing and Local Government (1969). Analysed mean values of the chemical characteristics of the dilution water were: total oxidised nitrogen 0.35 mg l^{-1}; total alkalinity 42.5 mg l^{-1} as $CaCO_3$; total hardness 58.5 mg l^{-1} as $CaCO_3$; calcium 7.05 mg l^{-1}; magnesium 3.65 mg l^{-1}; potassium 0.5 mg l^{-1}; sodium 28.3 mg l^{-1}; chloride 31.5 mg l^{-1}; copper 0.01 mg l^{-1} and iron 0.02 mg l^{-1}.

Experimental procedure

Both trials were conducted with seven concentrations of NH_3 and one control. The values of NH_3 concentration used in both trials are given in Table 2. Each concentration was tested in duplicate. On the basis of the observed mortality in the first trial, the second trial was conducted with a series of more closely spaced concentrations of NH_3. The second trial was also intended to verify the reproducibility of the toxicity bioassay for NH_3. Thirty-two fish were used per replicate tank. The fish to water ratio was maintained at less than 1.0 g fish l^{-1} water (American Public Health Association et al., 1980). Ammonium chloride was used as a source of ammonia, and a constant pH was maintained with 0.01 *M* phosphate buffer (a combination of sodium dihydrogen phosphate and disodium hydrogen phosphate). All chemicals were of analytical reagent grade. The fish were acclimated gradually from the stock to dilution water for 24—28 h before adding the chemicals. The fish were distributed randomly among the test tanks and were not fed during the acclimation and test periods. Each bioassay was conducted for 168 h to obtain the lethal threshold concentration. Records of mortality were made at logarithmic time intervals (Sprague, 1973).

Physicochemical analysis

Measurements and analyses of the physicochemical characteristics of the test and control solutions were carried out following procedures recom-

mended by the American Public Health Association et al. (1980). The concentrations of ammonia and nitrite presented in the results are expressed on a nitrogen basis. The temperature, dissolved oxygen and pH were measured every day and ammonia-nitrogen and nitrite-nitrogen were measured once in every two or three days. The unionised ammonia level was calculated using the following formula:

$$NH_3\text{-}N = \frac{\text{Ammonia-N}}{1 + 10^{(pKa-pH)}} \quad \text{(after Emerson et al., 1975)}$$

Where,
Ammonia-N = the measured concentration of total ammonia
pKa = the negative logarithm of the ionization constant. Calculated pKa values for ammonia as a function of temperature were obtained from Emerson et al. (1975)
pH = the measured pH of the solution.

Mean and ranges of the values of NH_3 concentration of the control and each test solution are given in Table 2. Mean and ranges of other physiochemical values of the control and test solution during both trials were dissolved oxygen 7.10 (6.95—7.40) mg l^{-1}; pH 7.72 (7.59—7.79); temperature 28 (28—28)°C; nitrite <0.01 mg l^{-1} NO_2-N. The variation of dissolved oxygen in the single test solutions ranged between ± 0.20 and ± 0.40 mg l^{-1} and that of pH ranged between ± 0.01 and ± 0.10 pH unit.

Analysis of data

Median lethal concentration (LC50) values for different exposure times were calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977). Tests for significant differences were carried out between LC50 values using the method of the American Public Health Association et al. (1980).

RESULTS

The cumulative percentage mortality of carp fry at different concentrations of NH_3 after 168 h exposure are presented in Table 2. In the first trial, very few mortalities were observed at concentrations ranging from 0.19 to 0.69 mg l^{-1} NH_3-N. Mortality higher than 10% occurred only at a concentration of 1.39 mg l^{-1} NH_3-N. In the second trial, however, mortalities were observed only at concentrations greater than 1.0 mg l^{-1} NH_3-N. Two fish died in one of the duplicate control tanks in the first trial at the end of 144 h exposure. This was equivalent to 3.1% mortality of the total control population and was well below the acceptable maximum 10% level for control mortality as recommended by Sprague (1973).

The median lethal concentration of NH_3 for the various exposure times in the first and second trials are presented in Table 3 and shown graphically

TABLE 2

The percentage mortality of common carp fry after 168 h exposure to different concentrations of NH_3

Solution no.	Trial 1		Trial 2	
	NH_3 Conc. (mg l^{-1} NH_3-N) Mean (range)	Mortality (%)	NH_3 Conc. (mg l^{-1} NH_3-N) Mean (range)	Mortality (%)
Control	0.00[1] (0.00[1]—0.00[2])	3.1	0.00[2] (0.00[2]—0.00[3])	0
1	0.19 (0.17—0.21)	0	0.50 (0.49—0.52)	0
2	0.23 (0.22—0.25)	0	0.84 (0.78—0.90)	0
3	0.43 (0.43—0.44)	3.1	1.00 (0.94—1.05)	0
4	0.69 (0.58—0.74)	6.3	1.56 (1.47—1.64)	15.6
5	1.39 (1.27—1.48)	12.5	2.13 (2.12—2.13)	81.3
6	2.54 (2.47—2.62)	100	2.42 (2.35—2.49)	100
7	4.80 (4.62—4.98)	100	2.62 (2.60—2.64)	100

TABLE 3

Median lethal concentrations and 95% confidence limits of NH_3 for various exposure times from Trials 1 and 2

Exposure time (h)	Trial 1		Trial 2	
	LC50 (mg l^{-1} NH_3-N)	95% CL	LC50 (mg l^{-1} NH_3-N)	95% CL
6	2.39[a]*	2.20—2.59	2.61	N.C.
12	1.93[b]	1.81—205	2.55[a]	1.49—2.61
24	1.78[c]	1.70—1.86	2.16[b]	2.10—2.23
48	1.76[c]	1.67—1.85	1.87[c]	1.82—1.93
96	1.74[c]	1.65—1.84	1.84[c]	1.78—1.91
168	1.64[c]	1.53—1.76	1.79[c]	1.71—1.86
Incipient LC50	1.74	—	1.82	—

N.C.: Not calculable.

*Figures in the same column with the same superscripts are not significantly different ($P > 0.05$).

in Fig. 2. In both trials the acute toxicity of NH_3 ceased within 168 h, which is indicated by the toxicity curves becoming asymptotic with the time axis. These asymptotes mark the approximate values of lethal threshold concentration and are given in Table 3. The test of significance between median lethal concentrations of different exposure times showed no significant difference ($P > 0.05$) between LC50's after 24 and 48 h in the first and second trials respectively (Table 3). There was also no significant difference ($P > 0.05$) between the 96-h LC50 values obtained in the first and second trials.

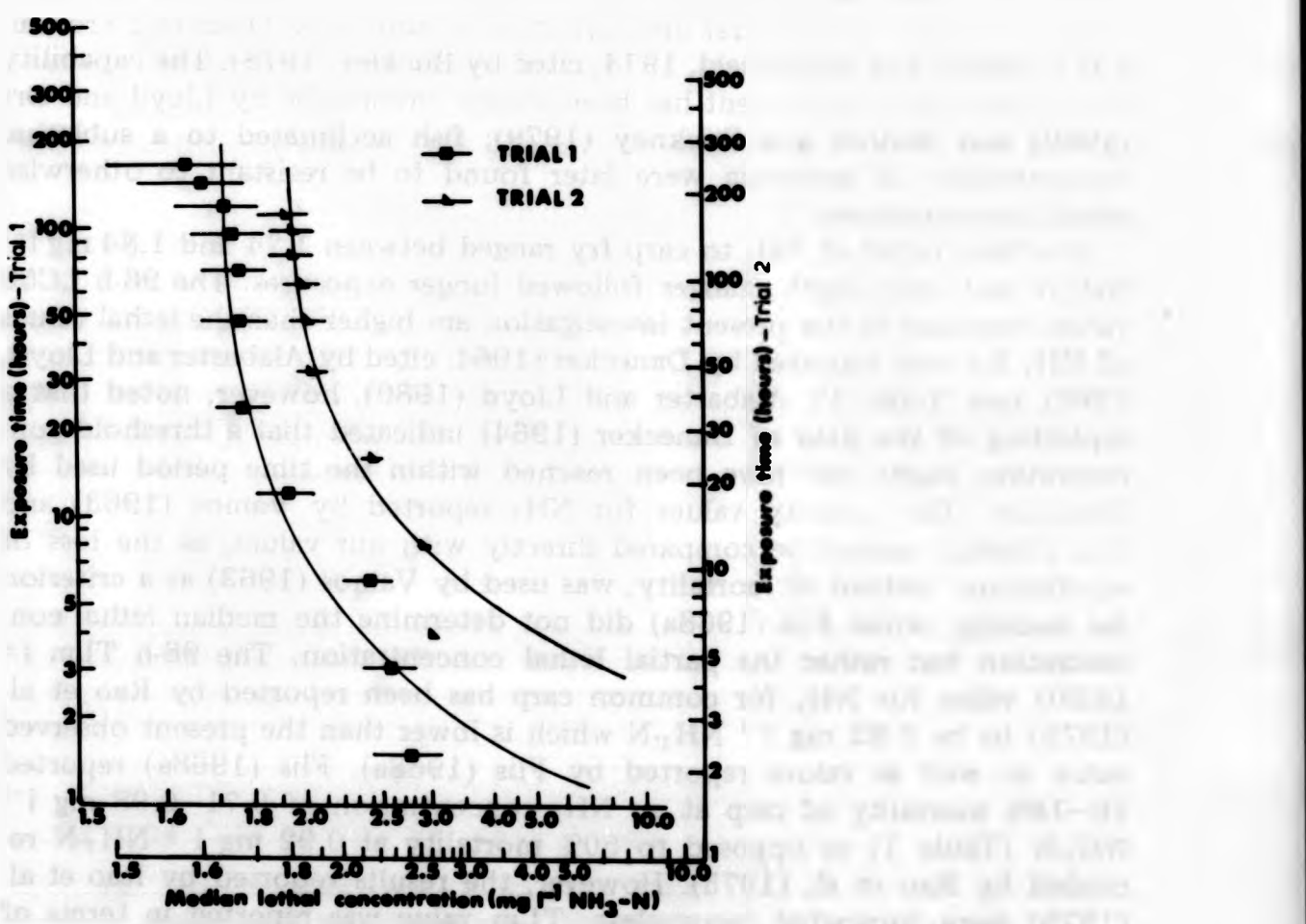

Fig. 2. Toxicity curves of unionized ammonia for common carp fry from Trials 1 and 2. Bars indicate 95% confidence limits.

DISCUSSION

In the present investigation, the acute toxicity of unionized ammonia (NH_3) ceased, and the lethal threshold concentration was reached, within 168 h of exposure. There were no significant differences between the median lethal concentrations for different exposure times after 24 and 48 h, res-

pectively, in the first and second trials. Similar results showing lethal threshold concentration of NH_3 within 96 h of exposure have been reported for rainbow trout *Salmo gairdneri*, bream *Abramis brama* (Ball, 1967), coho salmon *Oncorhynchus kisutch* (Buckley, 1978) and tilapia *Tilapia (Oreochromis) aurea* (Redner and Stickney, 1979). Ammonia, being a non-cumulative poison, its potentially toxic level may be tolerated by fish for longer periods once they have survived a certain exposure. Two mechanisms for the observed increase in resistance to ammonia by fish after an initial exposure have been identified. One involves changes in the permeability of the cell membrane (Vamos, 1963, Lloyd and Orr, 1969) and the other involves excretion or natural detoxification of ammonia (Olsen and Fromm, 1971; Mehrle and Bloomfield, 1974, cited by Buckley, 1978). The capability for physiological adjustment has been shown empirically by Lloyd and Orr (1969) and Redner and Stickney (1979); fish acclimated to a sublethal concentration of ammonia were later found to be resistant to otherwise lethal concentrations.

The 96-h LC50 of NH_3 to carp fry ranged between 1.74 and 1.84 mg l^{-1} NH_3-N and only slight changes followed longer exposures. The 96-h LC50 values obtained in the present investigation are higher than the lethal values of NH_3 for carp reported by Danecker (1964, cited by Alabaster and Lloyd, 1980) (see Table 1). Alabaster and Lloyd (1980), however, noted that a replotting of the data of Danecker (1964) indicated that a threshold concentration might not have been reached within the time period used by Danecker. The toxicity values for NH_3 reported by Vamos (1963) and Flis (1968a) cannot be compared directly with our values, as the loss of equilibrium, instead of mortality, was used by Vamos (1963) as a criterion for toxicity, while Flis (1968a) did not determine the median lethal concentration but rather the partial lethal concentration. The 96-h TLm (= LC50) value for NH_3 for common carp has been reported by Rao et al. (1975) to be 0.92 mg l^{-1} NH_3-N which is lower than the present observed value as well as values reported by Flis (1968a). Flis (1968a) reported 16—18% mortality of carp at an NH_3 concentration of 0.71—0.98 mg l^{-1} NH_3-N (Table 1) as opposed to 50% mortality at 0.92 mg l^{-1} NH_3-N recorded by Rao et al. (1975). However, the results reported by Rao et al. (1975) were somewhat incomplete. TLm value was reported in terms of ammonium chloride concentration and it was never mentioned how the TLm value was estimated from the concentration—mortality data, which were also not given in the results. An approximate estimate of TLm value in terms of NH_3 has been calculated from the TLm value of ammonium chloride, mean temperature and pH, and again pH value had considerable fluctuation (7.2—7.8) (Table 1). However, the lower LC50 value reported by Rao et al. (1975) may be attributable to the variation in the size of fry used. The fry used by Rao et al. (1975) weighed approximately 1—2 g compared to the 0.2 to 0.3 g fry used in this study. It has been demonstrated by several authors (Rice and Stokes, 1975; Holt and Arnold, 1983; Thurston

and Russo, 1983) that the susceptibility of fish to NH_3 varies with age. Thurston and Russo (1983) noted that susceptibility of rainbow trout to NH_3 decreased as the fish developed from the yolk-sac fry to juveniles and increased thereafter. Variation in the lethal concentration of NH_3 may also be attributed to the variation in the dissolved oxygen contents of test waters. A DO content of 7.0 mg l^{-1} or more has been maintained in the present study, compared to the DO content of 5.0 mg l^{-1} or more maintained in the test water used by Rao et al. (1975). A reduction in the level of DO in the water results in an increase of toxicity of NH_3 as demonstrated by Downing and Merkens (1955), Alabaster et al. (1979) and Thurston et al. (1981b). The LC50 values of NH_3 for carp fry obtained in this investigation are considerably higher than those reported for salmonids and some species of coarse fish (Ball, 1967; Rubin and Elmaraghy, 1977; Buckley, 1978; Thurston et al., 1978). However, the above values fall towards the higher range of LC50 values reported for non-salmonids (Colt and Tchobanoglous, 1976, 1978; Redner and Stickney, 1979; Thurston et al., 1983).

The conclusion derived from this study is that carp fry are fairly tolerant of unionized ammonia. In both trials, only concentrations greater than 1.0 mg l^{-1} NH_3-N caused mortalities higher than 10%, therefore, it is unlikely that the concentration of ammonia would increase to such an extent to cause significant mortality in a recirculatory system unless the water pH increased considerably, or the biological filter ceased to function. However, sublethal levels of NH_3 may lead to suppression of fish growth (Colt and Tchobanoglous, 1978; Alderson, 1979; Sadler, 1981; Yamagata and Niwa, 1982) and histopathological changes (Burrows, 1964; Flis, 1968a,b; Smart, 1976; Yamagata and Niwa, 1982; Thurston et al., 1984). Therefore adequate consideration should be given to these aspects when evaluating the toxicity of NH_3 to common carp fry.

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REFERENCES

Alabaster, J.S. and Lloyd, R. (Editors), 1980. Water Quality Criteria for Freshwater Fish. Butterworth, London, 297 pp.

Alabaster, J.S., Shurben, D.G. and Knowles, G., 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo solar* L. J. Fish Biol., 15: 705—712.

Alderson, R., 1979. The effect of ammonia on the growth of juvenile Dover sole, *Solea solea* L. and turbot, *Scophthalmus maximus* L. Aquaculture, 17: 291—309.

American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1980. Standard Methods for the Examination of Water and Waste-water. American Public Health Association, Washington, DC, 1134 pp.

Armstrong, D.A., Chippendale, D., Knight, A.W. and Colt, J.E., 1978. Interaction of ionized and unionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. Biol. Bull., 154: 15—31.

Ball, I.R., 1967. The relative susceptibility of some species of freshwater fish to poisons. I. Ammonia. Water Res., 1: 767—775.

Bardach, J.E., Ryther, J.H. and McLarney, W.C., 1972. Aquaculture. Wiley Inter-Science, New York, NY, 868 pp.

Brockway, D.R., 1950. Metabolic products and their effects. Prog. Fish-Cult., 12: 127—129.

Buckley, J.A., 1978. Acute toxicity of unionized ammonia to fingerling coho salmon. Prog. Fish-Cult., 40: 30—32.

Burrows, R.E., 1964. Effect of accumulated excretory products on hatchery reared salmonids. Research Report 66. United States Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, DC, 12 pp.

Colt, J. and Tchobanoglous, G., 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture, 8: 209—224.

Colt, J. and Tchobanoglous, G., 1978. Chronic exposure of channel catfish, *Ictalurus punctatus*, to ammonia: effects on growth and survival. Aquaculture, 15: 353—372.

Downing, K.M. and Merkens, J.C., 1955. The influence of dissolved oxygen concentration on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). Ann. Appl. Biol., 43: 243—246.

Emerson, K., Russo, R.C., Lund, R. and Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. J. Fish. Res. Board Can., 32: 2379—2383.

Flis, J., 1968a. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part I. Effects of toxic concentrations. Acta Hydrobiol., 10: 205—224.

Flis, J., 1968b. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part II. Effects of subtoxic concentrations. Acta Hydrobiol., 10: 225—238.

Forster, R.P. and Goldstein, L., 1969. Formation of excretory products. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. I. Academic Press, New York, NY, pp. 313—350.

Fromm, P.O. and Gillete, J.R., 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol., 26: 887—896.

Hamilton, M.A., Russo, R.C. and Thurston, R.V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol., 11: 714—719.

Hampson, B.I., 1976. Ammonia concentration in relation to ammonia toxicity during a rainbow trout rearing experiment in a closed freshwater—seawater system. Aquaculture, 9: 61—70.

Holt, G.T. and Arnold, C.R., 1983. Effects of ammonia and nitrite on growth and survival of red drum eggs and larvae. Trans. Am. Fish. Soc., 112: 314—318.

Jhingran, V.G., 1977. Fish and Fisheries of India. Hindustan Publishing Corporation (India), Jawahar Nagar, Delhi, India, 954 pp.

Lloyd, R., 1961. The toxicity of ammonia to rainbow trout (*Salmo gairdneri*). Water Waste Treat. J., 8: 278—279.

Lloyd, R. and Orr, L.D., 1969. The diuretic response of rainbow trout to sublethal concentrations of ammonia. Water Res., 3: 335—344.

Ministry of Housing and Local Government, 1969. Fish Toxicity Tests. Her Majesty's Stationery Office, London, 15 pp.

Olsen, K.R. and Fromm, P.O., 1971. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp. Biochem. Physiol. A, 40: 999—1007.

Rao, T.S., Rao, M.S. and Prasad, S.B.S.K., 1975. Median tolerance limits of some chemicals to the freshwater fish "*Cyprinus carpio*". Indian J. Environ. Health, 17: 140—146.

Redner, B.D. and Stickney, R.R., 1979. Acclimation to ammonia by *Tilapia aurea*. Trans. Am. Fish. Soc., 108: 383—388.

Rice, S.D. and Stokes, R.M., 1975. Acute toxicity of ammonia to several developmental stages of rainbow trout, *Salmo gairdneri*. Fish. Bull., 73: 207—211.

Rubin, A.J. and Elmaraghy, G.A., 1977. Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. Water Res., 11: 927—935.

Sadler, K., 1981. The toxicity of ammonia to the European eel (*Anguilla anguilla* L.). Aquaculture, 26: 173—181.

Smart, G., 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol., 8: 471—475.

Sprague, J.B., 1973. The ABC's of pollutant bioassay using fish. In: J. Cairns and K.L. Dickson (Editors), Biological Methods for the Assessment of Water Quality. ASTM. STP 528, American Society for Testing and Materials, Philadelphia, PA, pp. 6—30.

Tabata, K., 1962. Toxicity of ammonia to aquatic animals with reference to the effects of pH and carbon dioxide. Bull. Tokai Reg. Fish. Res. Lab., 34: 67—74.

Thurston, R.V. and Russo, R.C., 1983. Acute toxicity of ammonia to rainbow trout. Trans. Am. Fish. Soc., 112: 696—704.

Thurston, R.V., Russo, R.C. and Smith, C.E., 1978. Acute toxicity of ammonia and nitrite to cut-throat trout fry. Trans. Am. Fish. Soc., 107: 361—368.

Thurston, R.V., Russo, R.C. and Vinogradov, G.A., 1981a. Ammonia toxicity to fishes. Effect of pH on the toxicity of unionized ammonia species. Environ. Sci. Technol., 15: 837—840.

Thurston, R.V., Phillips, G.R., Russo, R.C. and Hinkins, S.M., 1981b. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentration of dissolved oxygen. Can. J. Fish. Aquat. Sci., 38: 938—988.

Thurston, R.V., Russo R.C. and Phillips, G.R., 1983. Acute toxicity of ammonia to fathead minnows. Trans. Am. Fish. Soc., 112: 705—711.

Thurston, R.V., Russo, R.C., Leudtke, R.J., Smith, C.E., Meyn, E.L., Chakoumakos, C., Wang, K.C. and Brown, C.J.D., 1984. Chronic toxicity of ammonia to rainbow trout. Trans. Am. Fish. Soc., 113: 56—73.

Vamos, R., 1963. Ammonia poisoning in carp. Acta Biol., Szeged, 9: 291—297.

Woynarovich, E., 1973. The role of induced breeding in fish culture development. In: Workshop on Controlled Reproduction of Cultivated Fishes, EIFAC Tech. Pap. No. 25: 18—24.

Wuhrmann, K. and Woker, H., 1948. Beiträge zur Toxikologie der Fische. II. Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. Schwiez. Z. Hydrol., 11: 210—244.

Yamagata, Y., and Niwa, M., 1982. Acute and chronic toxicity of ammonia to eel *Anguilla japonia*. Bull. Jpn. Soc. Sci. Fish., 44: 171—176.

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Effect of chloride concentration on the acute toxicity of nitrite to common carp, *Cyprinus carpio* L., fry

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Abstract. Duplicate static bioassays were conducted for 168 h each to determine the median lethal concentration (LC50) of nitrite (NO_2^-) for common carp, *Cyprinus carpio* L., fry at five different chloride (Cl^-) levels. The acute toxicity of nitrite ceased towards the end of 96 h at all levels of chloride concentration. There was a highly significant positive correlation between the chloride concentration tested and the 96-h LC50. The 96-h LC50 values are 2·55, 5·77, 14·41 27·26 and 48·70 mgl^{-1} NO_2^-–N at chloride concentration of 1·0, 5·0, 10·5, 27·5 and 45·0 mgl^{-1} Cl^- respectively. The linear relationship between chloride concentration and 96-h LC50 is best described by the equation: $y = 1{\cdot}03x + 1{\cdot}49$ ($r = +0{\cdot}996$; d.f. = 3; $P < 0{\cdot}001$), where y = 96 h LC50 of NO_2^-–N and x = concentration of Cl^-. A NO_2^-–N to Cl^- ratio of about 1:1·5–3·0 prevented complete mortality over the 168-h experimental period. A NO_2^-–N to Cl^- ratio of 1:5 is recommended for protection of carp fry against nitrite mortality in fish farms.

Introduction

Generally, in lakes and rivers, nitrite occurs at very low levels (EIFAC 1984). The concentration of nitrite, however, can increase considerably in anoxic regions of stagnant lakes (EIFAC 1984) and streams receiving industrial effluents (Russo, Smith & Thurston 1974). Klingler (1957) reported nitrite concentrations in excess of 30 mgl^{-1} nitrite – nitrogent (NO_2^-–N) for waters receiving effluents from metal, dye and celluloid industries. In newly constructed recirculatory systems where the rate of bacterial nitrification is initially slow, nitrite concentrations can rapidly increase to very high levels (Liao & Mayo 1974; Collins, Gratzek, Shotts, Dawe, Campbell & Senn 1975; Colt & Tchobanoglous 1976).

Nitrite has been shown to be toxic to fish (Gillette, Miller & Redman 1952; Wallen, Greer & Lasater 1957; Smith & Williams 1974), but its toxicity varies greatly between cultured species (Table 7). For example, nitrite concentrations of 0·19 mgl^{-1} NO_2^-–N were shown to be lethal to rainbow trout, *Salmo gairdneri* Richardson, whereas mottled sculpin, *Cottus bairdi* Girard, could tolerate levels in excess of 65·0 mgl^{-1} NO_2^-–N when exposed for 154 h (Russo & Thurston 1977).

The exact mechanism of nitrite toxicity is not clearly understood. It is known that nitrite oxidizes haemoglobin (Hb) in blood to methaemoglobin (met-Hb), which reduces the oxygen-carrying capacity of blood, and thus accumulation of excess met-Hb in the blood of fish may be fatal. Nitrite-induced met-Hb formation has been studied experimentally in salmonids (Brown & McLeay 1975; Smith & Russo 1975) and channel catfish, *Ictalurus punctatus* (Rafinesque) (Huey, Simco & Criswell 1980).

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Recent investigations have shown that different environmental ions e.g. calcium (Crawford & Allen 1977; Wedemeyer & Yasutake 1978) and chloride (Perrone & Meade 1977; Russo & Thurston 1977; Tomasso, Simco & David 1979; Huey *et al.* 1980; Tomasso, Wright, Simco & Davis 1980) have some protective effect against nitrite toxicity. The protective effect of chloride (Cl^-) has been well documented for salmonids (Perrone & Meade 1977; Russo & Thurston 1977; Bath & Eddy 1980; Russo, Thurston & Emerson 1981) and channel catfish (Tomasso *et al.* 1979, 1980; Huey *et al.* 1980; Bowser, Falls, VanZandt, Collier & Phillips 1983). At 9·8 mgl^{-1} NO_2^-–N and undetectable levels of Cl^-, Bath & Eddy (1980) observed 90% mortality of rainbow trout within 24 h; however, when Cl^- levels were increased to 21 mgl^{-1}, only 50% mortality was recorded within 48 h. Perrone & Meade (1977) reported that an ionic ratio of about 17 Cl^- to 1 NO_2^-–N was capable of preventing complete mortality in yearling coho salmon, *Oncorhynchus kisutch* (Walbaum), exposed for 72 h to 9·0–15·0 mgl^{-1} NO_2^-–N.

Common carp, *Cyprinus carpio* L., is, on a world-wide basis, the most extensively cultivated fish species (Jhingran 1977). Although carp are traditionally pond fish in Europe and other parts of the world, indoor rearing of carp fry in artificially heated water is commonly practised (Bryant, Jauncey & Atack 1980). Often, the water in these rearing systems is recirculated to economize on the heating cost. A review of literature indicates only one published report on the toxic effect of nitrite on carp. McCoy (1972), cited by Russo & Thurston (1977), reported no mortality over a 48-h period for carp when exposed to 40 mgl^{-1} NO_2^-–N, though a concentration of 100 mgl^{-1} NO_2^-–N resulted in mortality when exposed for 45 h. Unfortunately, the physicochemical characteristics of the experimental water and the levels of mortality were not reported.

In view of the growing numbers of carp hatcheries (Woynarovich 1973) and the lack of information on nitrite toxicity to carp fry, the present investigation was conducted to study the acute toxicity of nitrite to carp fry and to evaluate the protective effect of chloride ion on nitrite toxicity.

Materials and methods

Experimental animals and acclimation

Fry of the mirror variety of common carp (Dinkelsbühler strain from West Germany) were obtained from the hatchery of Newhay Fisheries, Bolton, England. Prior to test the fry were maintained for about one month at 28°C in 150-l stocking tanks in a recirculatory system at the Institute of Aquaculture, University of Stirling, Scotland and fed with ground commercial trout pellet (Edward Baker's Omega No. 3; protein content 49%).

Experimental system

Five static nitrite bioassays were conducted with different levels of Cl^-. Aquaria containing 8 l of water were used as test tanks. In each tank a 200-W thermostatic heater and stone aerator were used to maintain the required temperature and dissolved oxygen concentration respectively.

Test water

To maintain uniform water quality in all tests, synthetic dilution water was prepared with a total hardness of 50 mgl^{-1} as $CaCo_3$ (Ministry of Housing and Local Government 1969). A complete analysis of the dilution water was carried out with the help of Forth River Purification Board, Stirling (Table 1). Sodium chloride and sodium nitrite were used as sources of Cl^- and NO_2^-–N respectively. All chemicals used were of analytical reagent grade.

Table 1. Chemical characteristics of the dilution water used in the bioassays.*

	Mean	Range
Total oxidized nitrogen	0·3	0·3 –0·4
Conductivity	220·0	194 –245 US/CM
Suspended solids	1·0	1 –2·0
Total ammonia – nitrogen	0·02	0·01–0·02
Orthophosphate (as P)	0·01	<0·01–0·02
Potassium	0·7	0·5 –1·0
Sodium	27·4	26·5 –28·3
Copper	0·01	0·01–0·01
Iron	0·01	<0·01–0·02
Lead	0·005	–
Manganese	<0·005	–
Zinc	<0·006	0·005–0·017

*All values are in mgl^{-1} unless otherwise noted

Experimental procedure

In all the tests, five or more concentrations of NO_2^-–N were tested with one control. A logarithmic range of concentrations (e.g. 0·5, 1·0 2·0 4·0, 8·0 mgl^{-1}) was used. Each concentration was tested in duplicate. Twenty fish were used per replicate concentration as recommended by Jenson (1972). The fish to water ratio was maintained at less than 1·0 g fish l^{-1} water (American Society for Testing and Materials 1970; American Public Health Association [APHA], American Water Works Association & Water Pollution Control Federation 1980). The fish were acclimated gradually from the stock to dilution water for 24–48 h before adding nitrite salt. The fish were randomly distributed among test tanks and were not fed during the acclimation and test periods. Each bioassay was conducted for 168 h to obtain the lethal threshold concentration. Records of mortality were made at logarithmic-time intervals (Sprague 1973). The cessation of opercular movements was used as the criterion for death. Fish which died during the tests and a portion of survivors at the termination of the tests were weighed individually. A sample of the surviving fish was placed in clean water for a further 3–4 days to check for delayed mortality (Wickins 1982). No control or delayed mortality was observed at any time during the tests.

Test water was not changed during the bioassay period as nitrite is fairly stable in solution (Konikoff 1975; Bath & Eddy 1980; personal observation) and endogenous ammonia production by unfed fish is not significant to cause any mortality or stress in a static bioassay (Westin 1974; Colt & Tchnobanoglous 1976).

Physicochemical analysis

Methods used for the physicochemical analyses of the test solutions are listed in Table 2 and the physicochemical characteristics are given in Table 3.

Table 2. Methods used in the analysis of test solutions

Parameter	Frequency	Method
Temperature	1 × day	N_2 filled mercury thermometer
Dissolved oxygen	1 × day	YSI model 57 oxygen meter
pH	1 × day	Extech 651 digital pH meter
Nitrite-N	1 × 2 day	Sulfanilamide based colorimetric reaction (Mackereth, Heron & Talling 1978)
Ammonia-nitrogen	2–3	Nesslerization method (Stirling 1983)
Chloride	Once	Argenometric method (APHA *et al.* 1980)
Total alkalinity	Once	Stirling (1983)
Total hardness	Once	EDTA titrimetric method (APHA *et al.* 1980)
Calcium and magnesium	Once	Atomic absorption spectrophotometer (Golterman, Clymo & Ohnstad 1978)

Table 3. Physicochemical characteristics of test solutions during the period of bioassay*

Test no	Chloride (mgl^{-1}) Mean (range)	pH Mean (range)	Dissolved oxygen (mgl^{-1}) Mean (range)
1	1·0 (1·0–1·0)	7·69 (7·54–7·75)	7·20 (6·7–7·6)
2	5·0 (4·5–5·5)	7·59 (7·52–7·66)	7·30 (7·10–7·50)
3	10·5 (10·0–11·0)	7·78 (7·56–7·94)	7·20 (7·1–7·4)
4	27·5 (26·5–28·5)	7·70 (7·65–7·74)	7·20 (6·85–7·50)
5	45 (44·0–46·5)	7·72 (7·66–7·79)	7·25 (7·00–7·40)

* Other measured variables were: temperature 27·5–28·5°C; total hardness (as $CaCo_3$) 52·0–58·5 mgl^{-1}; total alkalinity (as $CaCo_3$) 40·5–48·5 mgl^{-1}; calcium 7·90–10·38 mgl^{-1}; magnesium 2·23–7·11 mgl^{-1}; total ammonia-nitrogen 0·13–0·50 $^{-1}$

Analysis of data

Median lethal concentration (LC50) values for different exposure times, with their 95% confidence limits, were calculated using the trimmed Spearman-Karber method (Hamilton, Russo & Thurston 1977). Tests for significant differences were carried out between LC50 values using the method of APHA *et al.* (1980). When differences between replicate LC50 values were found to be insignificant ($P > 0{\cdot}05$), LC50s and their 95% confidence limits were calculated after combining the two replicates. Log LC50s for each test were plotted against log exposure time to determine lethal threshold concentration (= incipient LC50). The correlation coefficient and regression equation between chloride concentration and median lethal concentration were calculated according to Sokal & Rohlf (1969).

Results

The medium lethal concentrations of NO_2^-–N for the various exposure times at different levels of Cl^- are presented in Table 4 and shown graphically in Fig. 1. Acute toxicity had ceased towards the end of 96 h, which is indicated by the curve becoming asymptotic with the time axis (Fig. 1). These asymptotes mark the approximate values of lethal threshold concentration and are presented in Table 5. The LC50s between 96 and 168 h were not

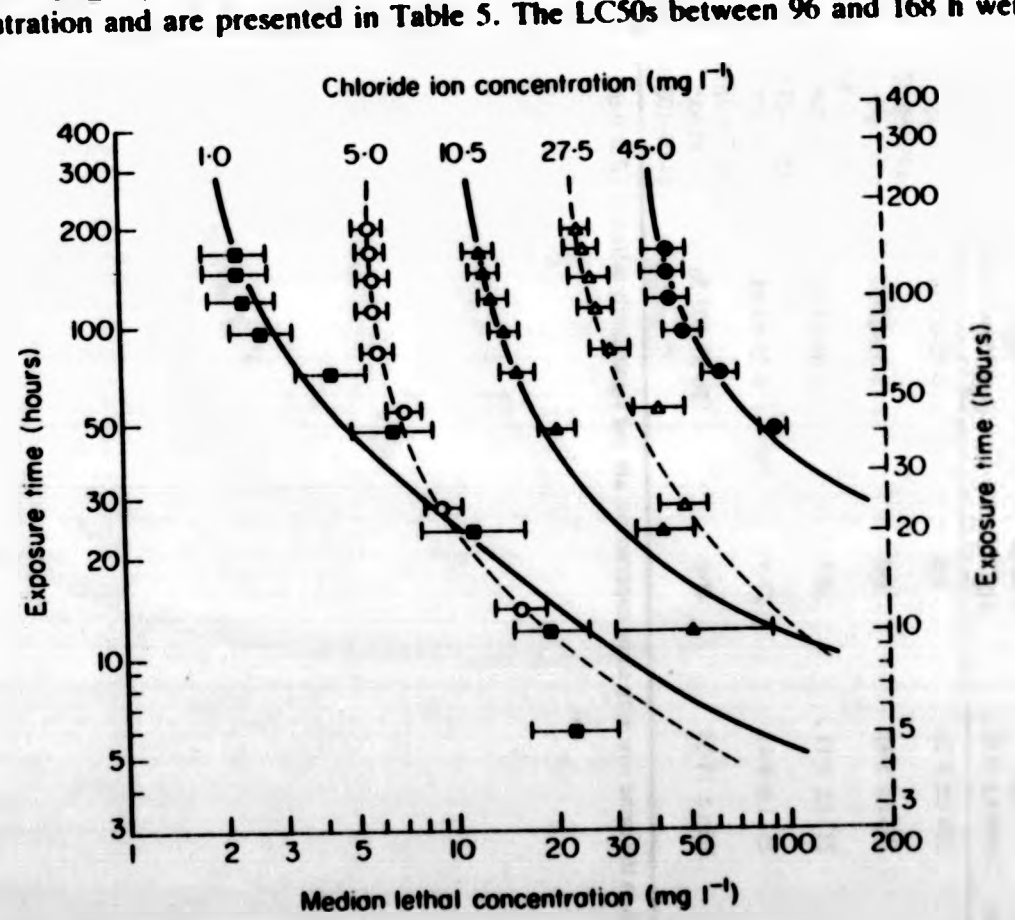

Figure 1. Toxicity curves of nitrite-nitrogen for carp fry at different chloride ion concentration. Bars indicate 95% confidence limits.

Table 4. Median lethal concentration and 95% confidence limits of nitrite-nitrogen for various exposure times at different chloride ion concentrations

Test no	Weight of fry (mg) Mean (± S.E.)	Concentration of chloride (mgl^{-1})	Concentration range tested (mgl^{-1} NO_2^--N)	LC50 (95% C.L.) (mgl^{-1} NO_2^--N) 48 h	72 h	96 h	120 h	168 h
1	216 (± 9·2)	1·0	0·72–25·70	6·46[a*] (4·89–8·53)	4·22[b] (3·28–5·42)	2·55[c] (2·09–3·23)	2·26[c] (1·79–2·86)	2·17[c] (1·72–2·73)
2	226 (± 5·4)	5·0	1·38–23·37	7·16[a] (6·51–7·87)	5·97[b] (5·47–6·51)	5·77[b] (5·30–6·28)	5·77[b] (5·30–6·38)	5·70[b] (5·23–6·20)
3	217 (± 8·1)	10·5	1·69–55·25	20·79[a] (18·37–23·52)	15·87[b] (14·32–17·59)	14·41[bc] (13·10–15·85)	13·36[cd] (12·16–14·67)	12·04[d] (11·01–13·06)
4	251 (± 9·4)	27·5	9·55–53·81	42·37[a] (35·79–50·16)	30·18[b] (26·61–34·24)	27·26[bc] (24·40–30·44)	26·38[bc] (23·64–29·43)	24·54[c] (22·24–27·08)
5	291 (± 11·5)	45·0	26·50–107·38	92·85[a] (84·09–102·54)	64·17[b] (57·10–72·11)	48·70[c] (43·40–54·65)	45·18[c] (39·73–51·37)	43·89[c] (38·37–50·21)

*Figures in the same row with same superscripts are not significantly different ($P > 0·05$)

significantly different ($P > 0{\cdot}05$) for chloride concentrations of 1·0, 27·5 and 45·0 mgl^{-1}. Similarly no significant differences were observed in the LC50s between 72 and 168 and 120 and 168 h for chloride concentrations of 5·0 and 10·5 mgl^{-1} respectively (Table 4).

There was a highly significant positive correlation ($r = +0{\cdot}996$; d.f. = 3; $P < 0{\cdot}001$) between Cl^- and 96-h LC50 of NO_2^--N; as the chloride concentration increased, nitrite toxicity decreased (Fig. 2). This linear relationship is best described by the equation: $Y = 1{\cdot}03x + 1{\cdot}49$ (d.f. = 3; $P < 0{\cdot}001$), where Y = 96-h LC50 and x = concentration of Cl^-.

The highest nitrite concentrations not resulting in mortality at each Cl^- level over the 168-h period are presented in Table 6. The NO_2^--N and Cl^- ratio of about 1:1·5–3·0 prevented complete mortality over the 168-h period.

Table 5. Lethal threshold concentration values obtained from the toxicity curve for nitrite-nitrogen

Test no	Lethal threshold concentration (mgl^{-1} NO_2^--N)
1	2·2
2	5·8
3	13·0
4	25·0
5	45·0

Table 6. Highest levels of nitrite tested that resulted in no mortality of common carp fry over a 168-h exposure period at different chloride ion concentrations

Concentration of chloride (mgl^{-1})	Highest concentrations tested with no mortality (NO_2^--N mgl^{-1})	Ratio of NO_2^--N to Cl^- that caused no mortality
1·0	0·72[1]	–
5·0	3·35	1:1·49
10·5	7·09	1:1·48
27·5	10·07	1:2·73
45·0	26·50[2]	–

[1]Lowest concentration tested with 5% mortality
[2]Lowest concentrations tested with 15% mortality

Discussion

In the present investigation the acute toxicity of nitrite ceased towards the end of the 96 h which is in agreement with observations made by Sprague (1969), Armstrong, Stephenson & Knight (1976) and Thurston, Russo & Smith (1978).

Since the water quality parameters in all trials were similar (Table 1 and 3) the higher LC50s for carp fry were clearly due to the antagonistic effect of Cl^- towards nitrite toxicity. Similar protective effects of Cl^- on nitrite toxicity were reported by Russo & Thurston

(1977) for rainbow trout. The protective effect of Cl^- is probably due to its competition with the NO_2^- for transportation across the gills at the chloride uptake sites (Perrone & Meade 1977; Tomasso *et al.* 1979; Huey *et al.* 1980; Bath & Eddy 1980). Bath & Eddy (1980) suggested that fish gills have the ability to transport nitrite against a concentration gradient by means of a branchial anion (Cl^-/HCO_3^-) exchange mechanism. They proposed that the Cl^- uptake mechanism present in freshwater fish gills has a low affinity for NO_2^- ions and thereby NO_2^- uptake may be competitively inhibited by external Cl^- ions.

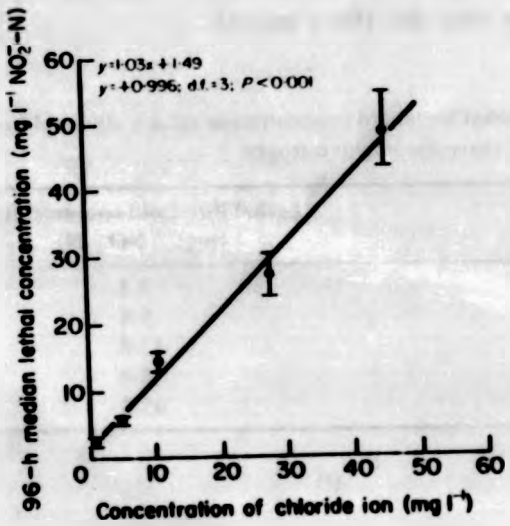

Figure 2. Effect of chloride ion concentration on the acute toxicity of nitrite to carp fry: 96-h LC50 *vs* CL^-. Bars indicate 95% confidence limits.

The 96-h LC50s of nitrite-nitrogen for carp fry are considerably higher than those reported for salmonids (Table 7). At chloride concentrations of 1·0 and 5·0 mgl^{-1} the 96-h LC50s for carp fry of 2·55 and 5·70 mgl^{-1} NO_2^--N (Table 4) are considerably higher than those for rainbow trout (Table 7). At a chloride concentration of 22·0 mgl^{-1}, the 96-h LC50s of channel catfish, tilapia, *Tilapia aurea* (Steindachner), fathead minnow, *Pimephales promelas* Rafinesque, and largemouth bass, *Micropterus salmoides* (Lacépède), were 7·10–12·78, 16·2, 45·3 and 140·2 mgl^{-1} NO_2^--N respectively (Table 7) compared to 24·11 mgl^{-1} NO_2^--N for carp fry (calculated from the regression equation). These comparisons, however, should be viewed with caution since there were variations in the water chemistry and fish size between individual test conditions.

The ratio of nitrite to chloride preventing mortality or suppressing complete methaemoglobin formation has been reported by several authors (Perrone & Meade 1977; Tomasso *et al.* 1979, 1980; Bath and Eddy 1980; Bowser *et al.* 1983). In this investigation the NO_2^--N:Cl^- ratio of about 1:1·5–3·0 prevented complete mortality of carp fry over the 168-h period, which is similar to the ratio (1 NO_2^--N:4 Cl^-) reported by Perrone & Meade (1977) for coho salmon fry. Tomasso *et al.* (1980) observed that a ratio of about 1 NO_2^--N:20 Cl^- produced met-Hb levels similar to those of control channel catfish. Bowser *et al.* (1983)

Table 7. A summary of 96-h LC50 values of nitrite to different fish species

	Weight (g)	Temperature (°C)	pH	Total hardness (mgl^{-1} as $CaCo_3$)	Total alkalinity (mgl^{-1} as $CaCo_3$)	Concentration of chloride (mgl^{-1})	96-h LC50 of NO_2^--N (mgl^{-1})	Author
Rainbow trout. *Salmo gairdneri* Richardson	24·3–188·0	9·8–10·4	7·68–8·10	188–207	171–191	0·35	0·19–0·28	Russo & Thurston (1977)
	69·5	10·4	7·92	188–207	171–191	1·2	0·46	Russo & Thurston (1977)
	69·1	10·4	8·01	188–207	171–191	5·1	2·36	Russo & Thurston (1977)
Cut-throat trout. *Salmo clarkii* Richardson	1–3	11·8–12·4	7·80–7·88	199	176	0·44	0·5–0·6	Thurston, Russo & Smith (1978)
Channel catfish *Ictalurus punctatus* (Rafinesque)	40	21	7·4–7·8	N.R.	60–70	N.R.	7·55	Konikoff (1975)
	3–4[a]	22	8·6–8·8	102	220	22·0[a]	12·78	Colt & Tchobanoglous (1976)
	3	23	7·7–8·2	202·4–254·8	165·6–222·7	22·0	7·1	Palachek & Tomasso (1984a)
Tilapia. *Tilapia aurea* (Steindachner)	3·4	23	7·7–8·2	202·4–254·8	165·6–222·7	22·0	16·2	Palachek & Tomasso (1984a)
	2·8	23	7·7–8·2	202·4–254·8	165·6–222·7	22·0	140·2	Palachek & Tomasso (1984a)
Largemouth bass. *Micropterus salmoides* (Lacepède)	2·3	12·7–13·0	8·05	188–207	171–191	0·35	2·30–2·99	Russo & Thurston (1977)
Fathead minnow. *Pimephales promelas* Rafinesque	0·9–3·3	23	7·7–8·2	191–268	153–250	22·0	45·3[b]	Palachek & Tomasso (1984b)

N.R. Not reported

[a] Reported by Colt & Tchobanoglous (1978)

[b] Obtained from toxicity curve given

reported that a NO_2^-–N to Cl^- ratio of 1:3 prevented met-Hb from reaching 50% levels during 48-h laboratory trial with channel catfish. However, it has been suggested that elevated levels of met-Hb do not necessarily result in mortality (Smith & Williams 1974; Crawford & Allen 1977; Perrone & Meade 1977; Palachek & Tomasso 1984a). The above findings have been substantiated by a recent study of Arillo, Gaino, Margiocco, Mensi & Schenone (1984), which suggests that liver hypoxia, not tissue hypoxia, caused by the nitrite-induced high methaemoglobinaemia, is at the root of the acute toxicity mechanism in rainbow trout. Similarly Bowser *et al.* (1983) recorded that met-Hb levels only in excess of 50% may be lethal for channel catfish and recommended a NO_2^-–N:Cl^- ratio of 1:3 for preventative management in production ponds.

The physiological basis of the NO_2^-:Cl^- ratio which prevents mortality or completely suppresses met-Hb formation has yet to be investigated. This apparent nitrite to chloride ratio in the water might be affected by the presence of other ions as calcium (Crawford & Allen 1977; Wedemeyer & Yasutake 1978), nitrate (Russo *et al.* 1981) and bicarbonate (Bath & Eddy 1980; Huey *et al.* 1980; Bowser *et al.* 1983) have some protective effect against nitrite toxicity. In addition, variation in the nitrite to chloride ratios that did not result in mortalities may also be attributed to variations in the tolerance of met-Hb level among different fish species (Perrone & Meade 1977; Russo 1980), to the presence of an active met-Hb reductase system (Cameron 1971; Huey & Beitinger 1982) or to the variations in the numbers, proliferations and differential selectivity of chloride cells to nitrite or chloride ion (Laurent 1984; Palachek & Tomasso 1984a).

From the present investigation it can be concluded that carp fry are highly susceptible to nitrite toxicity in water containing very low levels of Cl^- (<1·0–5·0 mgl^{-1}). However, the lethal levels of nitrite, though transitory in culture systems, could be rendered harmless if an adequate concentration of Cl^- is present in the water. The fish farmer, may, therefore, avert nitrite mortalities of carp fry by adding Cl^- to the water. Based on the results of the present study, a NO_2^-–N to CL^- ratio of 1:5 (i.e. safely above the level of 1:3 observed in this study) may be recommended for protection of carp fry against nitrite mortality.

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References

American Society for Testing and Materials (1970) *Standard Method of Test for Evaluating Acute Toxicity of Industrial Water to Freshwater Fishes.* ASTM B1345 – 59. American Society for Testing and Materials, Philadelphia.

American Public Health Association, American Water Works Association and Water Pollution Control Federation (1980) *Standard Methods — for the Examination of Water and Wastewater*. American Public Health Association, Washington.

Arillo A., Gaino E., Margiocco C., Mensi P. & Schenone G. (1984) Biochemical and ultrastructural effects of nitrite in rainbow trout: Liver hypoxia as the root of the acute toxicity mechanism. *Environmental Research* **34**, 135–154.

Armstrong D.A., Stephenson M.J. & Knight A.W. (1976) Acute toxicity of nitrite to larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii*. *Aquaculture* **9**, 39–46.

Bath R.N. & Eddy F.B. (1980) Transport of nitrite across fish gills. *Journal of Experimental Zoology* **214**, 119–121.

Bowser P.R., Falls W.W., VanZandt J., Collier N. & Phillips J.D. (1983) Methemoglobinemia in channel catfish: methods of prevention. *Progressive Fish-Culturist* **45**, 154–158.

Brown D.A. & McLeay D.J. (1975) Effect of nitrite on methemoglobin and total haemoglobin of juvenile rainbow trout. *Progressive Fish-Culturist* **37**, 36–38.

Bryant P., Jauncey K. & Atack T. (1980) *Backyard Fish Farming*. Prism Press, Dorchester.

Cameron J.N. (1971) Methemoglobin in erythrocytes of rainbow trout. *Comparative Biochemistry and Physiology* **40**, 743–749.

Collins M.T., Gratzek J.B., Shotts E.B., Dawe D.L. Jr., Campbell L.M. & Senn D.R. (1975) Nitrification in an aquatic recirculating system. *Journal of the Fisheries Research Board of Canada* **32**, 2025–2031.

Colt J. & Tchobanoglous G. (1976) Evaluation of the short term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. *Aquaculture* **8**, 209–224.

Colt J. & Tchobanoglous G. (1978) Chronic exposure of channel catfish, *Ictalurus punctatus*, to ammonia: effects on growth and survival. *Aquaculture* **15**, 353–372.

Crawford R.E. & Allen G.H. (1977) Seawater inhibition of nitrite toxicity to Chinook salmon. *Transactions of the American Fisheries Society* **106**, 105–109.

EIFAC Working Party on Water Quality Criteria for European Fresh Water Fish (1984) *Report on Nitrite: Draft Summary and Conclusions, and Tentative Water Quality Criteria*. EIFAC/XIII/84/16/Sup.1.4.

Gillette L.A., Miller D.L. & Redman H.E. (1952) Appraisal of a chemical waste problem by fish toxicity tests. *Sewage and Industrial Wastes* **24**, 1397–1401.

Golterman H.L., Clymo R.S. & Ohnstad M.A.M. (1978) *Methods for Physical and Chemical Analysis of Fresh Waters*. Blackwell Scientific Publications, Oxford.

Hamilton M.A., Russo R.C. & Thurston R.V. (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology* **11**, 714–719; Correction (1978) **12**, 417.

Huey D.W. & Beitinger T.L. (1982) A methemoglobin reductase system in channel catfish *Ictalurus punctatus*. *Canadian Journal of Zoology* **60**, 1511–1513.

Huey D.W., Simco B.A. & Criswell D.W. (1980) Nitrite-induced methemoglobin formation in channel catfish. *Transactions of the American Fisheries Society* **109**, 558–562.

Jenson A.L. (1972) Standard error of LC_{50} and sample size in fish bioassays. *Water Research* **6**, 85–89.

Jhingran V.G. (1977) *Fish and Fisheries of India*. Hindustan Publishing Corporation (India), Jawahar Nagar, Delhi, India.

Klingler K. (1957) Sodium nitrite, a slow-acting fish poison. *Schweizerische zeitschrift fuer Hydrologie* **19**, 565–578.

Konikoff M. (1975) Toxicity of nitrite to channel catfish. *Progressive Fish-Culturist* **37**, 96–98.

Laurent P. (1984) Gill internal morphology. In: *Fish Physiology Vol. X; Gills Part A* (ed. by W.S. Hoar & D.J. Randall), pp. 73–183. Academic Press, New York.

Liao P.B. & Mayo R.D. (1974) Intensified fish culture combining water reconditioning with pollution abatement. *Aquaculture* **3**, 61–85.

Mackereth F.J.H., Heron J. & Talling J.F. (1978) *Water Analysis: Some Revised Methods for Limnologists*. Freshwater Biological Association Scientific Publication No. 36, 120 pp.

Ministry of Housing and Local Government (1969) *Fish Toxicity Test*. Her Majesty's Stationery Office, London. 15 pp.

Palachek R.M. & Tomasso J.R. (1984a) Toxicity of nitrite to channel catfish (*Ictalurus punctatus*), tilapia (*Tilapia aurea*) and largemouth bass (*Micropterus salmoides*): evidence for nitrite exclusion mechanism. *Canadian Journal of Fisheries & Aquatic Sciences* **41**, 1739–1744.

Palachek R.M. & Tomasso J.R. (1984b) Nitrite toxicity to fathead minnows: effect of fish weight. *Bulletin of Environmental Contamination and Toxicology* **32**, 238–242.

Perrone S.J. & Meade T.L. (1977) Protective effect of chloride on nitrite toxicity to coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada* **34**, 486–492.

Russo R.C. (1980) Recent advances in the study of nitrite toxicity to fishes. In: *Proceedings of the third USA-USSR Symposium on the Effects of Pollutants upon Aquatic Ecosystems; Theoretical Aspects of Aquatic Toxicology* (ed. by W.R. Swain & R.V.R. Shannon), pp. 226–240. EPA Ecol. Res.Ser.EPA-600/9-80. 034. U.S. Environmental Protection Agency, Duluth, MN.

Russo R.C., Smith C.E. & Thurston R.V. (1974) Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 31, 1653–1655.

Russo R.C. & Thurston R.V. (1977) The acute toxicity of nitrite to fishes. In: *Recent Advances in Fish Toxicology* (ed. by R.A. Tubb), pp. 118–131. EPA Ecol. Res.Ser.EPA-600/3-77-085. U.S. Environmental Protection Agency, Corvallis, OR.

Russo R.C., Thurston R.V. & Emerson K. (1981) Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species and anion species. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 387–393.

Smith C.E. & Russo R.C. (1975) Nitrite induced methemoglobinemia in rainbow trout. *Progressive Fish-Culturist* 37, 150–152.

Smith C.E. & Williams W.G. (1974) Experimental nitrite toxicity in rainbow trout and chinook salmon. *Transactions of the American Fisheries Society* 103, 389–390.

Sokal R.R. & Rohlf F.J. (1969) *Biometry*., W.H. Freeman and Company, San Francisco.

Sprague J.B. (1969) Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Research* 3, 793–821.

Sprague J.B. (1973) The ABC's of pollutant bioassay using fish. In: *Biological Methods for the Assessment of Water Quality* (ed. by J. Cairns & K.L. Dickson), pp. 6–30. ASTM. STP 528. American Society for Testing and Materials, Philadelphia.

Stirling H.P. (ed.) (1983) *Water Analysis for Aquaculturists*. Institute of Aquaculture, University of Stirling, Stirling, Scotland, 139 pp.

Thurston R.V., Russo R.C. & Smith C.E. (1978) Acute toxicity of ammonia and nitrite to cut-throat trout fry. *Transactions of the American Fisheries Society* 107, 361–368.

Tomasso J.R., Simco B.A. & Davis K.B. (1979) Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (*Ictalurus punctatus*). *Journal of the Fisheries Research Board of Canada* 36, 1141–1144.

Tomasso J.R., Wright M.I., Simco B.A. & Davis K.B. (1980) Inhibition of nitrite-induced toxicity in channel catfish by calcium chloride and sodium chloride. *Progressive Fish-Culturist* 42, 144–146.

Wallen I.E., Greer W.C. & Lasater R. (1957) Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters. *Sewage and Industrial Wastes* 29, 695–711.

Wedemeyer G.A. & Yasutake W.T. (1978) Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 35, 822–827.

Westin D.T. (1974) Nitrate and nitrite toxicity to salmonid fishes. *Progressive Fish-Culturist* 36, 86–89.

Wickins J.F. (1982) Opportunities for farming crustaceans in western temperate region. In: *Recent Advances in Aquaculture* (ed. by J.F. Muir & R.J. Roberts), pp. 87–177. Croom Helm Ltd., London.

Woynarovich E. (1973) The role of induced breeding in fish culture development. In: *Workshop on controlled reproduction of cultivated fishes. FAO, EIFAC Technical Paper* 25, 18–24.