GROWTH AND NUTRITION OF CARP IN HEATED EFFLUENTS.

KIM JAUNCEY, B.Sc.

A thesis submitted to the University of Aston in Birmingham for the degree of Doctor of Philosophy.

April, 1979.

SUMMARY

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The growth responses and food utilization of mirror carp were investigated under varied dietary and environmental conditions.
Studies showed that growth rate was least at 35°C, intermediate at 20°C and greatest at 25 & 30°C.
Food conversion ratio increased with increasing level of feeding at each temperature. Temperature and feeding rate influenced the proximate body composition of carp.
Feeding trials showed that the protein level, of diets containing 18% lipid, could be reduced from 45 to 30% with no diminution of weight gain and with improved protein utilization.
Varying the dietary lipid and protein levels influenced the proximate body composition of carp and the apparent digestibilities of protein and energy.
Further feeding trials showed that a methanophilic bacterium and a petroprotein yeast have potential a fishmeal replacements in carp diets. These protein sources were well digested and assimilated when used as the sole protein sources in 30% protein rations. Soyabean and algal protein, although well digested, were poorly assimilated in similar feeds.
When one third of the protein, in a 30% fishmeal protein diet, was replaced by soyabean protein concentrate there was a significant decrease in growth rate and food utilization.
Temperature did not have a profound effect on the protein requirement of carp although it did influence growth, body composition and food utilization.
Growth of carp in a thermal effluent showed no depression compared to that in laboratory recycling systems and the optimum protein content of carp diets under these conditions was 35%.
It was concluded that carp is a suitable species, both nutritionally and in terms of its temperature requirements, for intensive culture in heated effluents.

KEY WORDS: Carp - Nutrition - Temperature - Heated Effluents.

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CONTENTS.

CHAPTER 1.

Chapter 1. INTRODUCTION.

Section 1.1. Background.

The basic concept of aquaculture was first proposed nearly 2,500 years ago when Fan Li, a Chinese scholar, wrote a treatise on the possibility of raising fish in confinement (Olszewski, 1977). Since that time fish culture has been widely, and successfully practised on a small subsistence scale. Farmers and land owners traditionally stocked their ponds with local species and realised that they could not only produce a regular supply of fish for their own consumption, but also a surplus that could be sold. In Europe carp were 'domesticated' a thousand years ago and were extensively raised in the 'stew-ponds' of monasteries and other land owners. A detailed account of the early domestication of the carp is given by Balon (1974).

Until recent times a large proportion of fish farming was performed on a small subsistence scale requiring no advanced technology and very little labour. Such fish farming consisted merely of releasing breeding fish into an enclosed body of water and harvesting the product at a later date. However, space limitations and the high commercial value of the product, due to increased demand, have encouraged the intensification of aquaculture operations. Modern aquaculture is typified by intensive, high technology systems which attempt to control all of the factors affecting fish growth in order to maximise production.

By the year 2,000 it is estimated that the world population will have doubled with a concomitant rise in demand for fishery products from 11.8 kg per capita per annum to 16.2 kg (Descamps, 1977). Aquaculture could significantly contribute towards this as, if production increased tenfold by the year 2,000, 60×10^6

tonnes per annum could be produced. Evidence would suggest that the world's marine fish stocks are currently being harvested close to, or beyond, the maximum sustainable yield. Therefore, it is possible that the future supply of fishery products will rely more heavily on aquaculture.

The world-wide harvest of fish for human consumption and for fishmeal increased steadily until 1970/71 and then levelled off despite the increasing amount of money and labour employed (F.A.O., 1973). This situation has given considerable impetus to the further development of marine and freshwater aquaculture in order to increase the yield of food from the aquatic environment.

The culture of warm water fish species, especially carp, is currently going through a period of development and intensification equivalent to that experienced by the salmonid farming industry during the last fifty years. As food for humans, carp is becoming increasingly important throughout Central Europe and Asia. In 1968 Europe, the U.S.S.R. and Israel produced 109,400 tonnes of carp - in order of production the figures were; U.S.S.R. (31,500), Romania (12,000), Hungary (11,900), Poland (11,000), Czechslovakia (10,600), Israel (10,500), France (10,000), Yugoslavia (8,000) and E.Germany (3,900) (E.I.F.A.C., 1968). Most of this production was achieved by extensive farming methods involving low stocking densities of 0.5 tonnes per hectare. However, in Japan as early as 1929 carp were being cultured in high flow rate ponds at densities up to 200 kg/m^3 (Tanaka, 1929 reported by Kawamoto, 1975) the equivalent of 2,000 tonnes per hectare.

At traditional, low, culture densities the fish food consists essentially of natural production supplemented with the by-products of other forms of agriculture, or industry,

such as slaughter house wastes, grain wastes, silk-worm pupae etc.. However, in intensive culture operations natural production can be discounted and the fish depend upon the supply of a nutritionally complete diet.

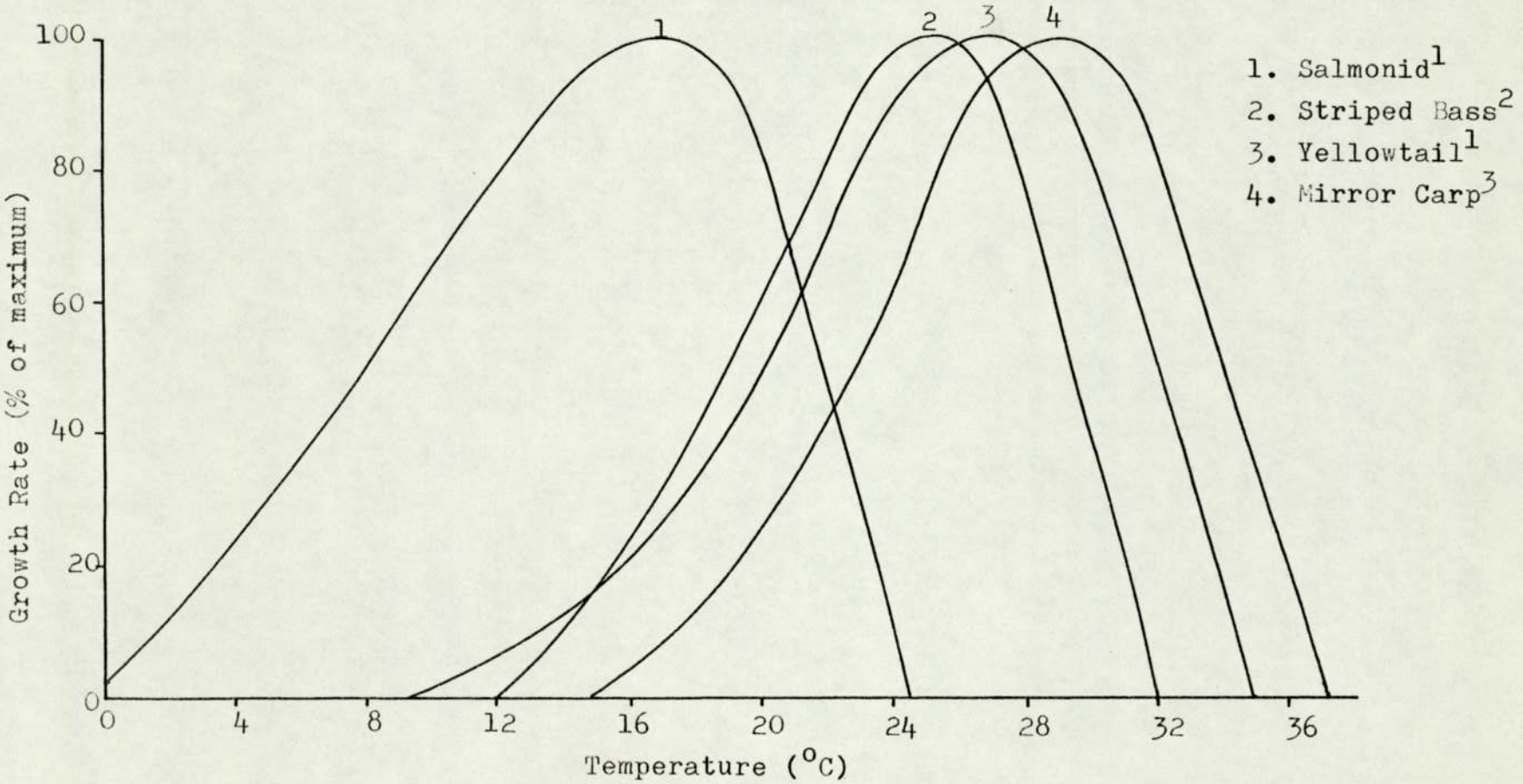
Salmonid production has been vastly increased by balanced feeding, improved culture methods and a better understanding of the economic factors involved. Carp husbandry is on the verge of a similar breakthrough which will be accomplished by paying greater attention to their nutritional requirements and the manufacture of complete pelleted feeds. Bearing this in mind, together with the relative recency of a demand for complete carp rations, it was considered that a broad-based study of carp nutrition would be of great value.

Section 1.2. The Beneficial Effects of Using Waste Heat for Aquaculture

The parameters affecting fish growth include temperature, water quality and diet suitability. Of these, in the context of warm water fish production, temperature is possibly the most important limiting factor in Northern Europe.

The metabolic activity of fish, and hence growth rate, is directly related to temperature (Brett, 1970) with Vant Hoffs' Law predicting, in theory, a doubling or tripling of growth rate with each 10^{o}C rise in temperature up to the optimum. Figure 1 shows the relationship between growth rate and temperature for several species of fish. These serve to illustrate a general trend of an optimum temperature range for growth preceded and succeeded by ranges of declining growth rate. It is also apparent, from Figure 1, that the temperature optima for growth vary widely with species.

Temperature affects the growth rate of fish in several ways



1. Adapted from Guthrie et al.(1975)
2. Adapted from Olzewski (1977)
3. Adapted from Table 12.

Figure 1. The relationship between temperature and growth rate for several fish species.

which combine to produce the resultant growth. Varying the temperature affects the rate of passage of food through the gastrointestinal tract of fish (Shrable et al., 1969; Brett & Higgs, 1970; Shcherbina & Kazlauskene, 1971), the activity of the digestive enzymes (Shcherbina & Kazlauskene, 1971), the rate of absorption of the products of digestion (Shcherbina & Kazlauskene, 1971) and the overall metabolic rate of the fish (Winberg, 1960; Brett, 1970). The effects of temperature on these parameters are discussed in greater detail in the experimental chapters of this dissertation and, therefore, at this stage it suffices to say that the optima observed in Figure 1 are the temperatures at which the greatest proportion of the ingested energy is available for growth.

The temperatures most sought by fish culturists are the optimum temperature for growth and/or the optimum temperature for food conversion. These temperatures vary with species (Figure 1), stage of development, feeding rate and feed quality. The optimum temperature for growth may be the same as, or differ from, the optimum temperature for food conversion. In a study on channel catfish (Ictalurus punctatus) both these optima occurred at 30°C (Andrews & Stickney, 1972). For several salmonids the optimum temperature for food conversion has been found to be lower than that for growth (Elliot, 1975a,b; Elliot, 1976; Brett et al., 1969). The difference in optima occurs because at the higher temperature more of the ingested energy is used for processes such as metabolism and activity, even though these processes occur much more efficiently at higher temperatures, leaving a smaller proportion available for growth.

Thus, in the case of differing optima, the cost of food would decide at which temperarure fish culture operations were more economic. In practice most fish farms capable of some degree of temperature control, together with the majority of

researchers, choose a temperature between the two optima.

In addition to the benefits in terms of growth and food utilization the culture of warm water species at their temperature optima may have certain other advantages. In particular the utilization of lipids in the diets of warm water species is improved with increasing temperature up to the optimum (Andrews et al., 1978; Stickney & Andrews, 1972; Shcherbina & Kazlauskene, 1971). This aspect of fish nutrition is discussed in detail in Chapter 4.

At the present time warm water fish production in the greater part of Europe, Russia, N.America, Asia and Japan is severely limited by the low ambient temperatures prevailing for much of the year. The growth of mirror carp (Cyprinus carpio), for example, is virtually non-existent below 15°C (Ghittino, 1970) thus restricting growth in Europe to the months of June, July and August.

Utilization of a supply of waste heat would enable maintenance of the optimum temperatures for growth of such species for much of the year, extending the growing season and substantially reducing the time taken to produce a product of marketable size. The reduction in the time taken to produce a product of marketable size is in itself important as it would enable more efficient and productive use to be made of the high capital cost facilities required for such intensive culture.

Maintenance of the optimum temperature all the year round has a further advantage, it would enable continuous production to be established. Fish could be cropped throughout the year thus allowing the setting up of an improved system of marketing and preventing seasonal fluctuations in product price and availability.

Section 1.3. The Use of Thermoelectric Generating Station Reject Heat in Aquaculture

From the preceding discussion, and a review of the biological considerations in the use of waste for finfish aquaculture (Sylvester, 1975), it is apparent that a relatively cheap source of heated water would have a significant impact on the fish farming industry. The reject heat from electricity generating stations would seem to be an excellent source of such heat.

Fossil fuelled thermoelectric generating stations convert approximately 30% of the total energy input into electricity with 50% of the energy wasted in the form of heated water (Godfriaux et al., 1975). By comparison nuclear powered stations produce more waste heat per unit of electricity generated than fossil fuelled ones (Olszewski, 1977). Any portion of the waste heat that could be used for aquaculture would represent a net saving in terms of energy utilization.

Reject heat from a thermoelectric generating station is available in the form of condenser cooling water. Figure 2 shows a diagrammatic representation of a power station generating circuit. There are two separate channels of water circulation, namely the steam circuit and the cooling water circuit, which do not come into direct contact. Two basic forms of cooling are used for generating stations, these are 'direct' cooling (a once through system) and closed circuit cooling (employing water recirculation). These differences, although fairly self-explanatory, are diagrammatically illustrated in Figure 3.

The quantity of heated effluent available is directly dependant upon the type of cooling employed. Direct cooled stations are sited where large quantities of cooling water are available; in the U.K. they are sited on the coast or on

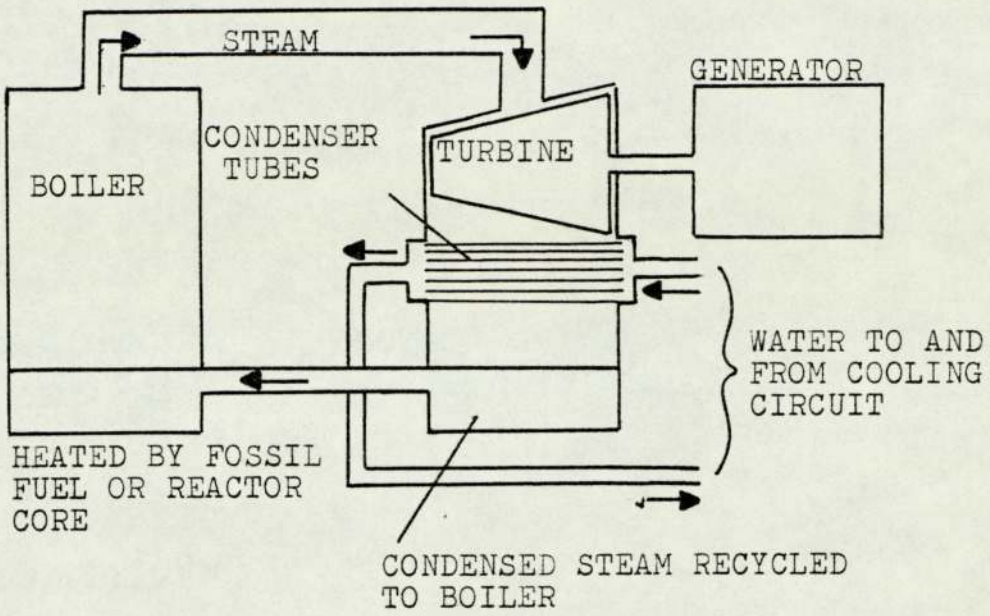


Figure 2. A diagrammatic representation of a steam electric generating circuit.

estuaries. Bradwell nuclear power station (250 MW), which uses direct cooling, discharges approximately $20m^3$/sec. of cooling water whilst Ratcliffe-on-Soar power station (2,000 MW), which employs closed circuit cooling, discharges only $1.7m^3$/sec. even though it has eight times the generating capacity of Bradwell (Aston & Brown, 1978). The only discharge from a closed circuit cooled generating station is the 'purge' water which prevents excessive concentrations of minerals in the evapourative cooling circuit.

The volume of heated effluent available is also dependant upon the generating capacity of the power station, particularly in the case of direct cooling. A 1,000 MW direct cooled nuclear generating station discharges approximately $50m^3$/sec. of heated water (Olszewski, 1977); a 600 MW station produces, by comparison, $25m^3$/sec. (Guthrie et al., 1975).

In most countries, particularly the U.S.A., there is a trend towards the construction of closed circuit cooled generating

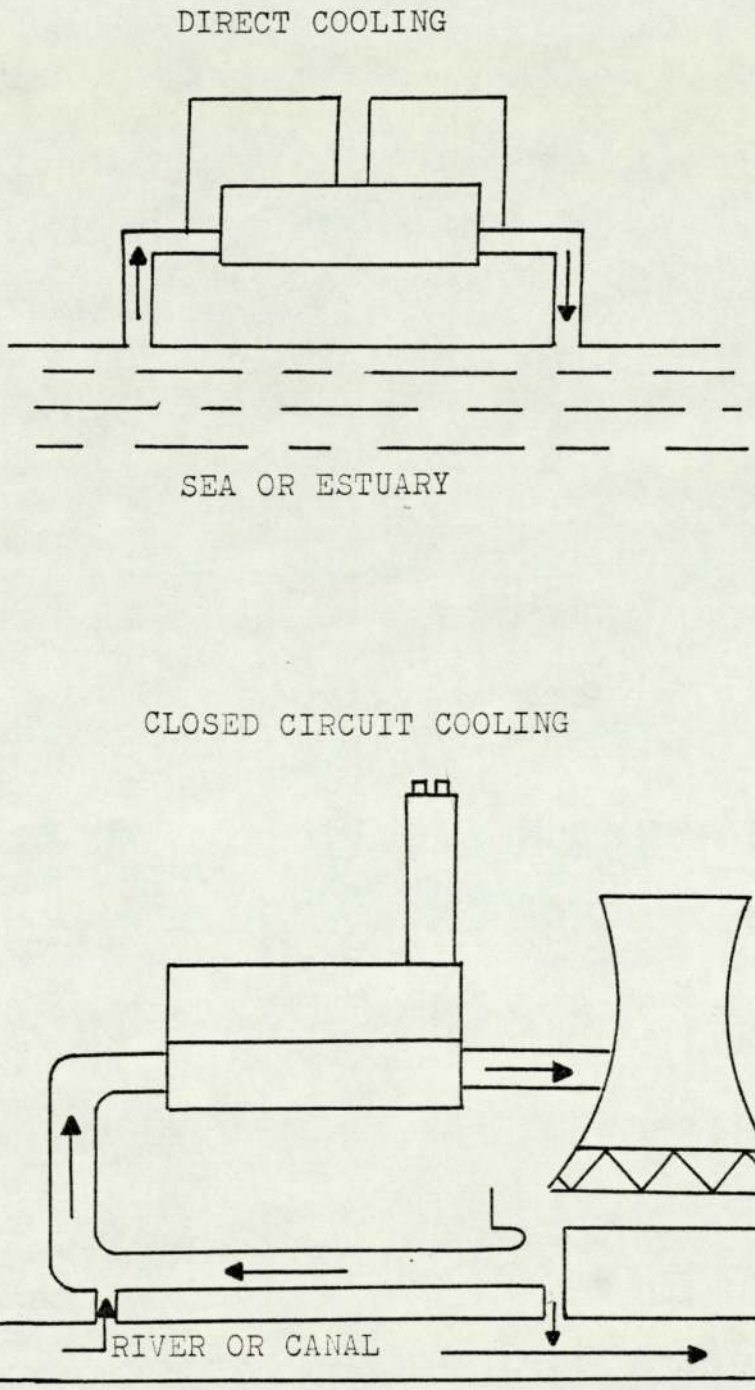


Figure 3. A diagrammatic representation of direct and closed circuit (tower) cooling systems.

stations as they discharge far smaller quantities of thermal effluent and are, therefore, environmentally preferable. The closed circuit can consist of cooling ponds and canals rather than towers, which would be far better suited to the needs of aquacultural enterprises.

The factors affecting the suitability of a particular generating station for aquaculture have been reviewed by Aston & Brown (1978). One of the principal factors involved is the quality of the intake water to the generating station. In many cases this water is of extremely poor quality and, by itself, would limit the variety of species that could be successfully cultured. During the course of power station operation a number of chemicals are added to the cooling water. A comprehensive review of these substances and their toxicities to aquatic organisms is published by the U.S.A. Atomic Energy Commission (Becker & Thatcher, 1973).

In the U.K. chlorine is the principal additive to cooling water being used to eliminate slime growths from condenser tubes and, in addition at coastal power stations, to remove mussels from the cooling water culverts. If ammonia or ammonium compounds are present in the cooling water, chlorine combines with them to form chloramines which may be more or less toxic, according to species, than chlorine itself. A review of the toxicity of chlorine to several species of freshwater and marine fish, and the methodology of chlorination is given by Aston & Brown (1978).

Chlorine concentrations are inevitably higher and more sustained in closed circuits than in the water of direct cooled stations. There are several ways to overcome the problems associated with the dosing of cooling water with chlorine. The simplest approach has been adopted at the direct cooled Mercer power station, New Jersey (Eble et al., 1975; Godfriaux et al.,

1975) where the water supply to the fish farm is automatically cut off for the brief period during which the chlorinators are in operation. Metered addition of sodium thiosulphate to neutralise chlorine doses in a direct cooled station was proposed by Guthrie et al. (1975) as an alternative.

Neither of these systems, however, would be very suitable for use in closed circuit cooled stations. If it was desired to culture chlorine sensitive species, such as salmonids, at a closed circuit cooled station it would be necessary to either replace chlorine dosing with a mechanical method of condenser tube cleaning or to use heat exchangers thus avoiding direct contact between the cultured organism and the chlorinated water (Aston et al., 1976).

A further important factor determining the suitability of a particular power station for aquaculture is for what period of the year the desired culture temperature would be reliably available. This is dependant upon the ambient temperature in the locality of the station, the number of generating sets it possesses and whether it is a 'base load' or 'load following' station. Those power stations that are the most efficient to run, producing the cheapest electricity, are termed 'base load' and are run as continuously as possible. 'Load following' stations are run, as the name suggests, according to demand.

To allow for periods of reduced load the quantity of warm water diverted to a fish farm should only represent a fraction of the total volume discharged. This is because even a base load station is unlikely to run all of its generating machines continuously. Ideally the flow of cooling water to a fish farm should be no more than the rate of discharge of water from one machine at a power station which runs three or four machines, or

from two machines at a power station that operates five or six machines.

There are also differences in the condenser outlet temperatures of generating stations using direct and closed circuit cooling. These may be illustrated using five year temperature records (1972-1976) for the power stations quoted previously, namely Ratcliffe-on-Soar employing closed circuit cooling, and Bradwell, which has direct cooling. At Ratcliffe-on-Soar the temperatures are generally higher with annual means between 28 and 31°C (range 10-43°C) and temperature fluctuations larger (weekly variation 5-14°C) than at Bradwell where weekly fluctuations rarely exceed 4°C and the annual means lie between 17 and 20°C (range 10.5-34.5°C) (Aston & Brown, 1978). Thus, in terms of maintaining a high (28°C) optimum temperature throughout the year closed circuit stations would seem to be most suitable.

It can also be seen, from the figures given above, that the condenser water would require cooling at some times of the year to prevent temperatures, not only in excess of the optimum, but also in excess of the upper lethal temperature for many species. Cooling would also enable the smoothing of the rapid temperature variations which occur, particularly at closed circuit cooled stations. It has been shown that mirror carp (Cyprinus carpio) growth is better in condenser water cooled to 28°C than in condenser water with no temperature control (Aston & Brown,1978).

From the preceding discussion it would seem that thermoelectric generating stations may be a suitable source of waste heat for aquaculture, provided that the constraints mentioned above are taken into consideration. For power stations in the design stage it would appear to be an excellent idea to consider

modifications in construction which would enable the use of the heated effluent produced for aquaculture. In this respect the construction of power stations employing canals and ponds as the closed circuit cooling stage would be the most promising.

For existing power stations the most immediate application for aquaculture would be to divert the condenser outfall of a direct cooled station to a suitable facility. Stations using closed circuit tower cooling, as at the majority of freshwater sites in the U.K., would require modification to make them suitable for aquaculture and the quantity of water available would be far less. Any expenses incurred by such modifications could be recouped by charging for the waste heat and/or leasing the site used. Even if no economic benefit was to be derived by the electricity generating company, from an aquacultural enterprise, the favourable publicity created by the use of power plant reject heat to produce food would be of great value in our environment and energy conscious age.

Section 1.4. The Current Status and Potential of Fish Culture in Fresh Water Heated Effluents.

During the past 15 to 20 years there has been a rapidly growing amount of interest and investment in the use of heated effluents for aquaculture. Experiments on growing carp in cages placed in heated effluents began in the U.S.S.R. in the early 1960s and by 1969 a pilot scale operation producing 35 tonnes per year was in operation (Backiel, 1975).

Reports have since been published of facilities on either a commercial or pilot scale at more than 30 power stations world wide of which about 20 are inland (Aston et al., 1976). The

species cultured at various inland power stations throughout the world are listed in Table 1. The nine species cultured include four species of carp, channel catfish, trout, eels, tilapia and the freshwater prawn Macrobrachium. Most of the stations listed use direct cooling, as described in the preceding section, and are situated on large lakes or rivers. However, three of the U.K. power stations listed, Ratcliffe-on-Soar, Ironbridge and Drax, use closed circuit tower cooling.

Several of the generating stations listed employ a double-cropping system in order to maximise the use of the temperature regime of the heated effluents. This is because the condenser water of a direct cooled station is generally only 8-10°C above the temperature of the intake water and thus high temperatures are not available all the year round. Because of the temperature variation a warm water species is grown during the summer and a cold water species during the winter.

Such a system is employed at the Flevo power station in Holland where carp are grown during the summer and rainbow trout during the winter (Huisman, 1974). A similar double-cropping system is employed at the Mercer power station, New Jersey, where trout are produced during the winter and freshwater prawns during the summer (Eble et al., 1975).

The fish holding facilities differ widely at the various power stations ranging from ponds to concrete raceways. Ponds are used at the Konin power station in Poland (Backiel, 1975) and at the Szazlombatta power station in Hungary (Anon., 1974a). At both of these sites the heated effluent from direct cooled power stations is gravity fed to earth ponds and there is a heavy reliance on natural production for feeding, consequently production figures are not very high, reaching only 2.2 tonnes

Table 1. Species Cultured in the Effluent of Inland Generating Stations World Wide.*

Country	Power Station	Species Cultured	Reference
U.S.A.	Gallatin	Catfish	Goss (1973)
	Colorado City	Catfish	Tilton & Kelley(197
	Hanford	Catfish	Furlong & King(1974
	Fremont	Catfish & Tilapia	" "
	Lake Trinidad	Catfish	" "
	Hutchinson	Catfish	" "
	Mercer	Trout & Macrobrachium	Eble et al., (1975)
U.K.	Ratcliffe-on-Soar	Carp & Eels	Aston & Brown(1975)
	Trawsfynydd	Trout	Bulleid (1974)
	Ironbridge	Carp	Aston et al.,(1976)
	Drax	Eels	Dr.Aston (pers. comm.,1978)
France	Cadarache	Eels	Decamps (1977)
Netherlands	Flevo	Carp, Grass Carp & Trout	Huisman (1974)
E. Germany	Finkenheerd	Carp	Steffens (1969)
	Rheinberg	Carp, Eels & Trout	Mitzinger (1974)
Poland	Konin	Carp, Grass Carp, Silver Carp & Bighead Carp	Thorslund (1971)
Hungary	Szazamlombatta	Carp, Grass Carp, Silver Carp & Bighead Carp	Anon (1974a)
U.S.S.R.	Klasson	Grass Carp & Carp	Verygin (1963)
	Krworozh	Grass Carp & Carp	"
	Shaktinsk	Tilapia	Krayev (1966)
	Kirishi	Carp & Trout	Anon (1974b)

*Adapted from Aston et al., 1976

per hectare.

A complete pond 'ecosystem' utilizing the effluent from a direct cooled, 1,000 MW nuclear power station has been subjected to a theoretical assessment in the U.S.A. (Olszewski, 1977). A system of ponds acting as a complete food chain and producing algae, zooplankton, fish, crayfish and clams was proposed. Theoretically the system, supplied with 50m^3/sec. of heated effluent, was capable of producing 410 tonnes of fish, 1,500 tonnes of clam meat and 15 tonnes of live crayfish per year at an estimated pre tax profit of 1.05 million dollars in ponds covering 133 hectares.

Cages have been widely adopted for holding fish in heated effluents due to the relatively low cost of incorporating them into existing power station facilities. Experiments at the Klasson power station in the U.S.S.R. have achieved stocking densities, with carp, up to 160 kg/m^3 (Korneyev, 1969) in cages.

Cylindrical cages were employed for the culture of channel catfish at the Colorado City power station in the U.S.A. and in preliminary trials stocking densities reached 100 kg/m^3 at harvest (Tilton & Kelley, 1970). In more controlled experiments, carried out at the Finkenheerd generating station in E. Germany, using cages moored to a barge in the effluent canal, the stocking density of carp at harvest reached 120 kg/m^3 (Steffens et al., 1969). These latter experiments achieved food conversion ratios (g. dry food fed per g. live weight gain) of 1.45 when feeding a 34% protein diet at 2% of the body weight per day. More recent reports indicate that with improved management much higher stocking densities might be achieved. Professor Huisman (1978) reported that stocking densities in excess of 250 kg/m^3 had been achieved with carp held in cages placed in the outfall

of the Flevo generating station in Holland.

Raceways are also used for the culture of fish in heated effluents, however, they are probably the most expensive type of holding facility to construct. Raceways are employed at the Colorado City station in the U.S.A. for the production of channel catfish fingerlings which are then 'planted out' into cages for ongrowing to market size (Anon., 1974c). Concrete raceways are also being used at the Drax power station, in the U.K., for the culture of eels at a farm which is currently being scaled up for commercial production (Dr.R.J.Aston, pers. comm., 1978).

A project study of rainbow trout culture in the effluent of a 600 MW direct cooled nuclear powered generating station at Pickering, Ontario proposes the production of 340 tonnes per year in raceways (Guthrie et al., 1975). This production could, theoretically, be achieved by using one fortieth of the total flow ($0.6m^3$/sec.) from the direct cooled station. These authors estimate that if the total flow of the station was used ($25m^3$/sec.) it could produce 12,000 tonnes of trout per year. Such a large facility would, however, present effluent disposal problems and they estimate that if one tenth of the total flow was utilized the dilution of the effluent would be sufficient to meet existing water quality standards.

In addition to the use of power station thermal effluent several industries produce waste heat as a by-product. With current rising energy costs such industries are becoming increasingly interested in the use of reject heat for aquaculture. In E. Germany two such effluents are being used by the State Fishery Enterprise in Wermsdorf (Backiel, 1975). The effluent from a paper mill is being used to supply the heat for a carp

fry raising facility and a 'briquette' plant is suppling heated water to concrete raceways for raising carp fingerlings to table size.

In the U.K. a full scale commercial eel farm, aiming to produce 100 tonnes per year, is being established in the heated effluent from the Tomatin whisky distillery in Scotland (Anon., 1978).

Before heated effluent aquaculture can take place on a wider scale there are several major points which require further investigation and clarification;

a) The engineering, biological implication and economics of management of the water temperature so as to achieve continuous production and optimum economic growth.

b) The manipulation of photoperiod and feeding schedules to optimise growth rates and feed utilization.

c) The design and management of disease control systems suited to high temperatures and the intensive culture conditions of cages and raceways.

d) The engineering of reliable and economic systems for the culture of fish in heated effluents.

e) The study of potential market acceptance of products cultured in heated effluents, especially those cultured at nuclear power stations and novel species.

f) An improved understanding of the nutrition of fish in relation to temperature.

g) The quality of the heated effluent and its' effects on the cultured species.

h) The treatment and disposal of effluents from an intensive aquaculture project.

It would seem that, at the present time, all of the points mentioned above are soluble provided that sufficient financial backing is made available. The setting up of more pilot-scale operations to test the theoretical studies and projections is the next logical step in the development of heated effluent aquaculture.

The ultimate goal should be the conversion of foodstuffs unfit for human consumption into a high quality protein product with a concomitant improvement in the energy utilization of industrial processes, particularly electricity generation.

Section 1.5. The Aims of the Research

In the preceding sections of this chapter the background to, and status of, fish farming in heated effluents has been given. In view of the potential for the culture of warm water fish species in the heated effluent of a thermoelectric generating station the present study was conceived in conjunction with the Central Electricity Generating Board. Mirror carp (*Cyprinus carpio*) was chosen as the experimental animal because of its tolerance of a wide range of environmental conditions, its rising economic importance as a table fish in Europe and its predicted temperature requirements being in the range attainable at an inland closed circuit generating station in the U.K..

It was decided to investigate as many of the basic nutritional parameters as possible in order to construct an overall picture of the suitability of mirror carp, both nutritionally and in terms of its' temperature requirements, as a species for

culture in heated effluents. Consequently the following experimental work was designed and undertaken.

In Experiment 1 (Chapter 3) an endeavour was made to determine the effects of temperature on growth, food utilization and carcass composition of carp held at 20, 25, 30 & 35°C and fed 3, 6 & 9% of their body weight per day.

High temperatures have been shown to increase the metabolic rates of fish (Brett, 1970) with a concomitant rise in demand for dietary metabolisable energy. Hence, it was considered that investigation of the efficacy of dietary lipid as an energy source would be of great value.

Experiment 2 (Chapter 4) was undertaken to examine the effects of varying lipid levels.

Following the examination of dietary energy levels it was decided to examine the possibility of replacing, or at least reducing, the amount of fish meal in carp diets. fish meal is an expensive source of dietary protein whose future price and availability is questionable (Anon., 1973). Experiment 3 (Chapter 5) was conducted to determine the utilization, by mirror carp, of yeast, bacterial, algal, soyabean, casein and herring meal proteins as the sole protein sources in semipurified rations.

From the results of Experiment 3 it was decided to investigate the effects of partial substitution of fish meal by soyabean protein as soyabean is the dominant oilseed protein on a world wide basis (Anon., 1973) and therefore freely available at relatively low cost. This study was undertaken in Experiment 4 (Chapter 6).

As it is unlikely that the optimum temperature would be

continuously available in thermal effluents Experiment 5 (Chapter 7) was conducted to determine the effects of a range of temperatures, 20 to 35°C, on the growth of carp fed three levels of dietary protein, 20, 30 & 40%. It was hoped that such a study would serve to demonstrate the effects of varying temperatures on the protein requirements of carp.

Experiments 1 to 5 were conducted in laboratory recycling systems and it was not possible to determine the accuracy with which the results could be projected to forecast growth in an actual thermal effluent. For this reason Experiment 6 (Chapter 8) was carried out in water from the cooling circuit of the C.E.G.B.s' coal fired thermoelectric generating station at Ratcliffe-on-Soar, Nottingham. In order to facilitate comparison with growth under laboratory conditions diets, having varying protein levels, were used with formulations similar to those used in Experiment 5 .

CHAPTER 2.

Chapter 2. GENERAL MATERIALS AND METHODS

Section 2.1. The Experimental Facilities

Section 2.1.1. 'System 1' The Principal Recirculation System

From the outset it was realised that the nature of the experimental work envisaged would entail the design, construction and operation of a system that could maintain a large volume of water at an accurately controlled temperature with adequate water quality. In order to fulfill these criteria it was decided that water recirculation systems would be required. These would enable minimisation of the quantity of water needed, an important consideration when mains tap water was the sole supply, and the amount of heating - provided that there was adequate insulation.

Holding fish in a restricted volume of water causes deleterious changes in water quality that must be rectified. These changes include increases in the concentrations of dissolved ammonia, dissolved organic material, solid faecal matter and dissolved carbon dioxide as well as a decrease in the dissolved oxygen content of the water.

The first limiting change is the depletion of oxygen due to the metabolic requirements of the fish. This situation is easily corrected by mechanical agitation and aeration of the water. Huisman (1969) found that, for mirror carp (Cyprinus carpio),no depression of growth or toxic effects occurred provided that dissolved oxygen concentrations in excess of 3 mg/l were maintained.

The second limiting change is the production of ammonia, by the fish, which is their primary method of nitrogen elimination. Unless steps are taken, potentially harmfull levels are quickly reached. The 'safe' level for mirror carp is

reported, by Huisman (1969), to be 2 mg/l at an undefined temperature, dissolved oxygen concentration or pH. Smith (1972) reports a value of 1 mg/l as 'safe' for rainbow trout (Salmo gairdneri) with oxygen levels in excess of 7 mg/l. As the dissolved oxygen level of the water falls, so the threshold of ammonia toxicity falls (Larmoyeaux & Piper, 1973).

It must, however, be borne in mind that the toxicity of the ammonium ion (NH_4^+) is low, but the toxic action of ammonia (NH_3), with which it is in equilibrium, is much greater. This dissociation is pH dependent; the higher the pH the greater the proportion of ammonia (Lloyd, 1961). There is also a slight dependance of the equilibrium on temperature; increasing the temperature increases the proportion of ammonia (Huisman, 1969).

On the basis of the above considerations it was considered that if the total ammonia remained below 0.5 mg/l it would have no toxic or growth inhibitory effects on mirror carp between temperatures of 20 & 35°C at an approximate pH of 7 and a level of dissolved oxygen in excess of 5 mg/l.

A great deal of information on water purification, by various methods of recirculation, for fish culture has been published (Spotte, 1970; Hirayama, 1966; Hirayama, 1974; Parker & Simco, 1973; Meske, 1971; Meske, 1976; Parker & Broussard, 1977). These methods vary widely in principle, degree of complexity and capital cost. Previous researchers in the University of Aston Fish Culture Unit had successfully used, for rainbow trout, recirculation systems based on a downflow submerged gravel filter (Roberts, 1976; Atack & Matty, 1978). This system had the proven advantages of relatively low capital cost, reliability and simplicity of construction and

operation. Such a method of biological filtration involves the passage of ammonia laden water down through a gravel substrate which provides a large surface area on which nitrifying bacteria may grow.

Bacteria growing on a filter bed are chemosynthetic autotrophs which oxidise simple organic compounds to more complex carbohydrates, lipids and proteins, in this case by using ammonia as their energy source. The oxidation of ammonia produces nitrate and nitrite ions, thus a base is required to combine with these ions. Birmingham tap water is extremely soft and, for this reason, crushed cockle shells were mixed with the gravel filter medium to provide the required base. Absence of sufficient base would cause a fall in the pH due to the accumulation of nitrous and nitric acids. The calcium carbonate from the crushed cockle shells reacts with the nitrate ions thus;-

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\text{NITROSOMONAS}} 2H^+ + H_2O + NO_2^-$$

$$4NH_3 + 7O_2 \longrightarrow 4NO_2^- + 6H_2O$$

$$2NO_2^- + O_2 \xrightarrow{\text{NITROBACTER}} 2NO_3^-$$

$$2CaCO_3 + 4NO_3^- \longrightarrow 2Ca(NO_3)_2 + 2CO_2 + O_2$$

The toxicity of the nitrate, produced by the biological oxidation of ammonia, to fish is fairly low. Huisman (1969) reports no depression of growth in mirror carp with total NO_2^-/NO_3^- concentrations up to 300 mg/l. In view of this it was decided to regulate nitrate levels in the recycling systems by the simple expedient of adjusting the quantity of make-up water flowing through the system rather than attempting to

incorporate a denitrifying stage.

In order to decide upon the materials and temperature control system to be used in the construction of 'System 1' some initial studies had to be undertaken. The ambient temperature of the building which was to house the proposed recycling systems was subject to large fluctuations both diurnally and annually. In view of this it was realised that effective insulation would be required to enable the envisaged operating temperatures, up to 35°C, to be accurately maintained without excessive heating costs.

To minimise heat losses the header, filter and experimental tanks were of double-skinned fibre glass with a 20 mm cavity filled with polyurethane foam (see Appendix 1). The header and filter tanks were also supplied with double-skinned insulated lids and the experimental tanks with translucent single thickness lids. The solids trap was a cone of heavy duty polythene and was supplied with a 3cm thick polystyrene cover.

Temperature control studies commenced with the construction of a single unit of three experimental tanks (Figure 4). The first arrangement examined consisted of a 3kW titanium immersion heater (Appendix 1), in the header tank, connected via a heavy duty industrial relay to an aquarium thermostat placed in the solids trap. A multiple probe temperature recorder (Cambridge L 454) was used to monitor the performance of this set up.

The aquarium thermostat was found to be totally inadequate as a temperature control device as the difference between switching on and switching off temperatures was 6°C. This series of trials, however, served to demonstrate the good thermal properties of the system as the rate of cooling was only 3°C/h when the system temperature was 35°C and the ambient temperature

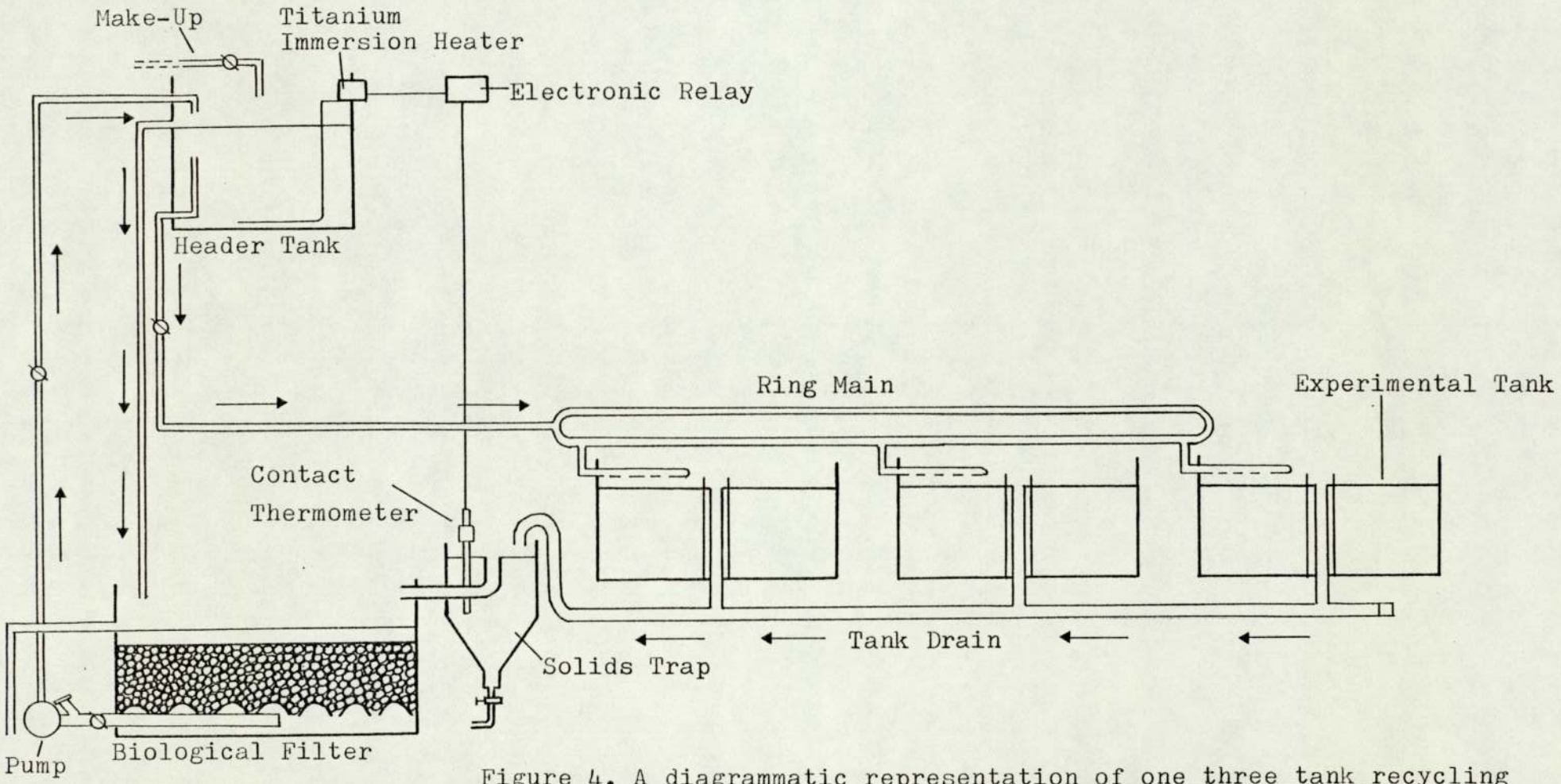


Figure 4. A diagrammatic representation of one three tank recycling unit of 'System 1'.

16°C. This slow rate of cooling may, in part, be attributed to the heat storage properties of the filter gravel.

As a result of these preliminary studies it was decided that a far more sensitive temperature control device was required. The combination finally selected consisted of a contact thermometer connected to a three kilowatt immersion heater via a solid state electronic relay (Appendix 1). Temperature records with this arrangement showed that fluctuations in the temperatures of the experimental tanks were less than $\pm$0.5°C of the preset temperature.

It was decided to equip a further three such recycling units with 4kW heaters to ensure adequate capacity if a temperature of 35°C was to be maintained in winter (at very low ambient temperatures) with high stocking and feeding rates necessitating increased make-up water flow. The complete system finally developed consisted of four similar three tank recycling systems (as depicted in Figure 4). The manufacturers and specifications of the materials used are listed in Appendix 1.

A 300 l header tank, containing a titanium immersion heater, was supported on a platform 2.5m above the floor. Heated water flowed down a standpipe (to prevent the header tank draining in the case of a pump failure) into which was built an expanded chamber containing an airstone that was supplied with 1.5l of air per minute. The aerated water passed, via a pressure equalising ring main, to the three 250l experimental tanks.

Water was tangentially jetted into these tanks both to increase aeration and to induce a circular flow. The circular experimental tanks (Figure 5) with sloping bottoms, together

with the circular flow, were effectively self-cleaning. Overflow water from the three tanks drained into a common pipe which carried the water to a 68l conical solids trap (Figure 6). Water was introduced into the solids trap at an angle to induce a circular flow which spun solids to the outside edge where they then sank to the bottom. Solid material collecting in this trap was siphoned out at weekly intervals.

Water passed from an abstraction point in the centre of the solids trap onto the surface of the biological filter (Figure 7). This consisted of a 600l oblong tank containing 400l of 1 to 1.5cm broken gravel supported on a corrugated, perforated plate which was, in turn, supported on house bricks. Water was drawn down through the filter gravel into the cavity below the filter plate by a pump. This pump drew water through an in-line filter (to prevent gravel and detritus obstructing the pump) and returned it to the header tank where it was jetted in to increase aeration. The pumping rate was double that of the flow to the experimental tanks, the remainder being returned to the surface of the filter via the header tank overflow.

Make-up water was continuously added to the header tank both to compensate for losses through splashing and evaporation and to keep nitrate levels within acceptable limits. Excess water overflowed from the filter to a drain.

Two thirds of the total depth of gravel in the filter was dug over once a month and the displaced detritus siphoned off. This was performed as the suction of the pump tended to pack the gravel down very tightly and, in addition, prolific growth of micro-organisms on the filter surface began to restrict the flow.

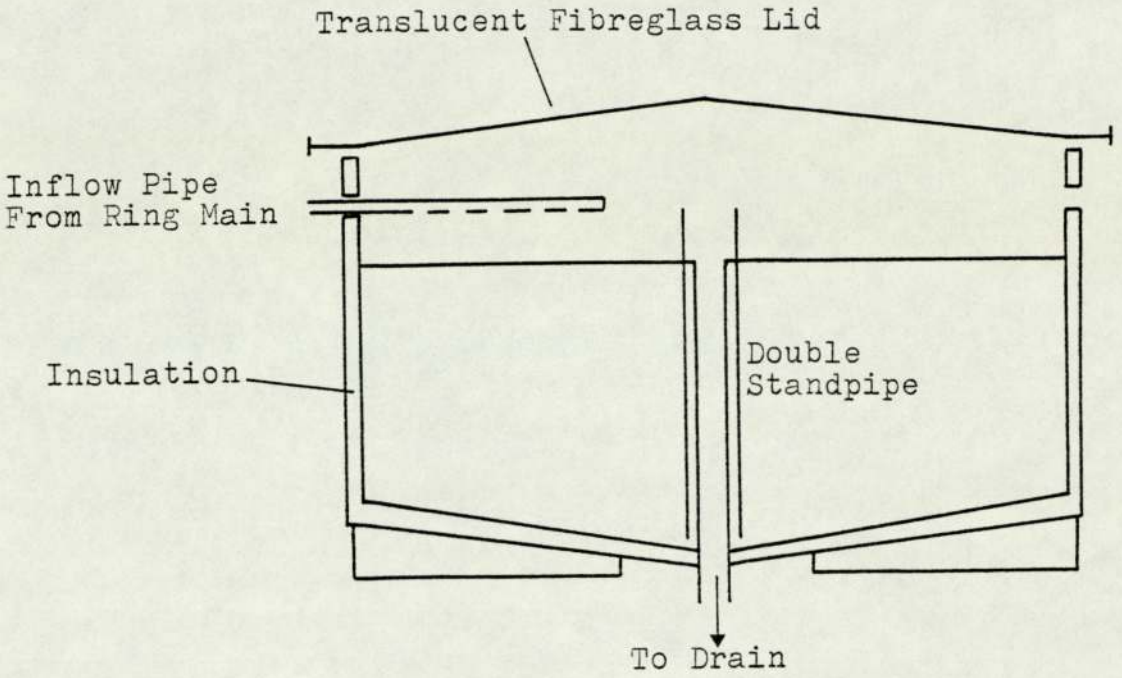


Figure 5. A transverse section of an experimental tank of 'System 1'.

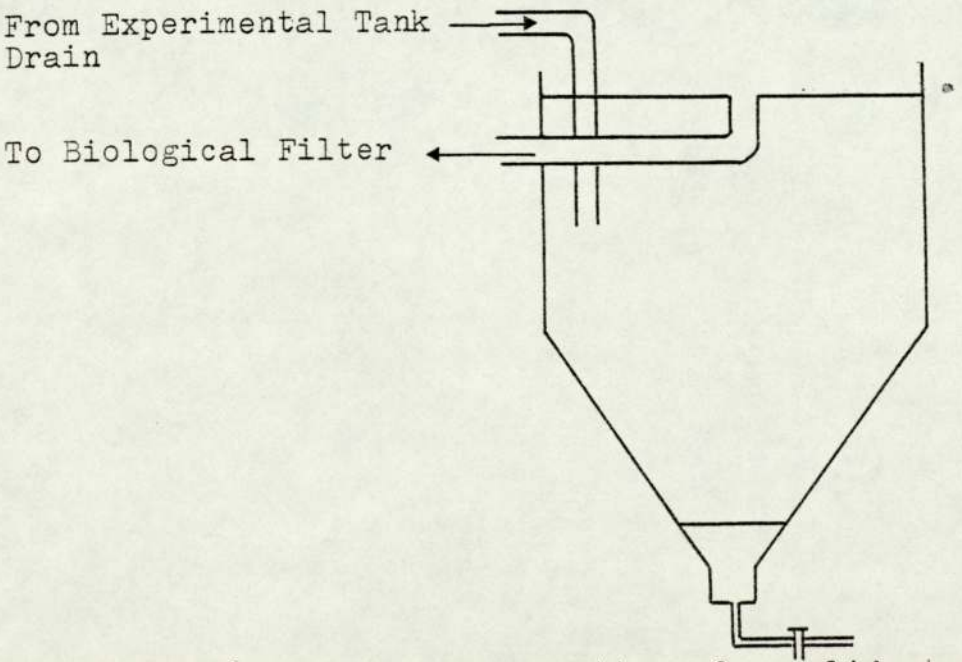


Figure 6. A transverse section of a solids trap of 'System 1'.

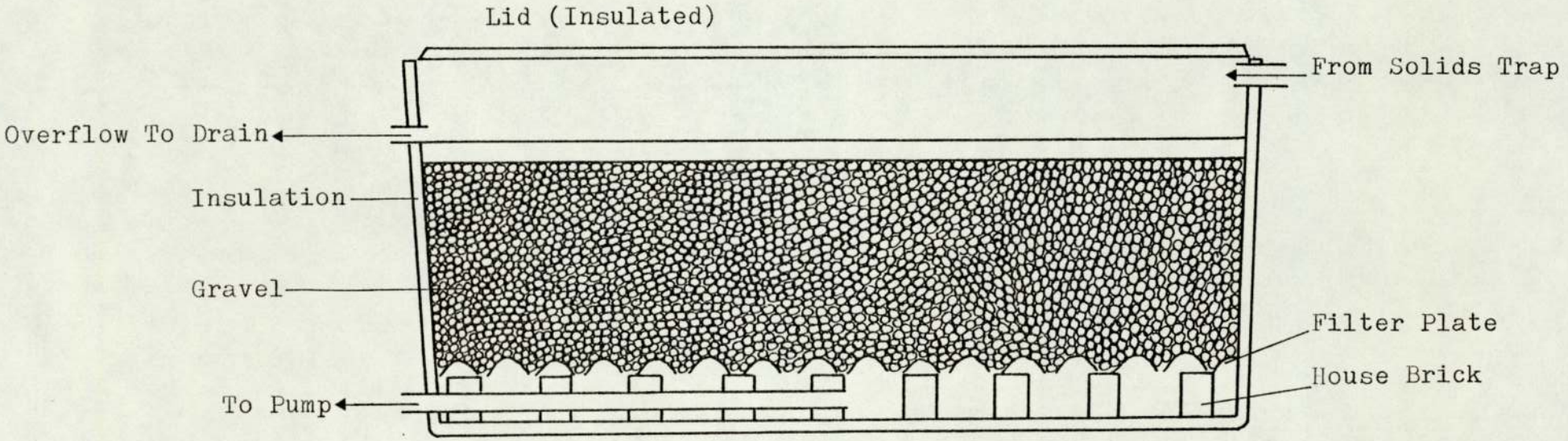


Figure 7. A transverse section of a biological filter of 'System 1'.

The flow-rates employed in 'System 1' are shown in Table 2.

Table 2. Flow-Rates Employed in 'System 1'.

Make-Up Water	0.6 l/min.
Header to Ring Main	15.0 l/min.
Header to Filter	15.0 l/min.
Inlow to each Experimental Tank	5.0 l/min.
Filter to Header (via pump)	30.0 l/min.

Several water quality parameters were measured twice weekly throughout all of the experimental periods and the values recorded are shown in Table 3.

Table 3. Water Quality Criteria in 'System 1'.

Temperature	As preset $\pm$ 0.5°C
Dissolved Oxygen	>5 mg/l
Total Ammonia	<0.1 mg/l
Total Nitrate/Nitrite	<50,0 mg/l
pH	6.8 - 7.2

Section 2.1.2. Secondary Recycling Systems.

In addition to 'System 1' it was decided to construct, from available materials, two smaller recirculating units. These would allow experimentation with smaller numbers of fish and greater control over feeding. Systems 2 & 3 were constructed in a small room and thus ambient temperature fluctuations were greatly reduced.

Section 2.1.2.1. 'System 2'.

The arrangement of this system is shown in Figure 8 together with flow rates and tank volumes. The principles employed in this recyling system are the same as those described for System 1 (section 2.1.1.). However, the limited space available dictated

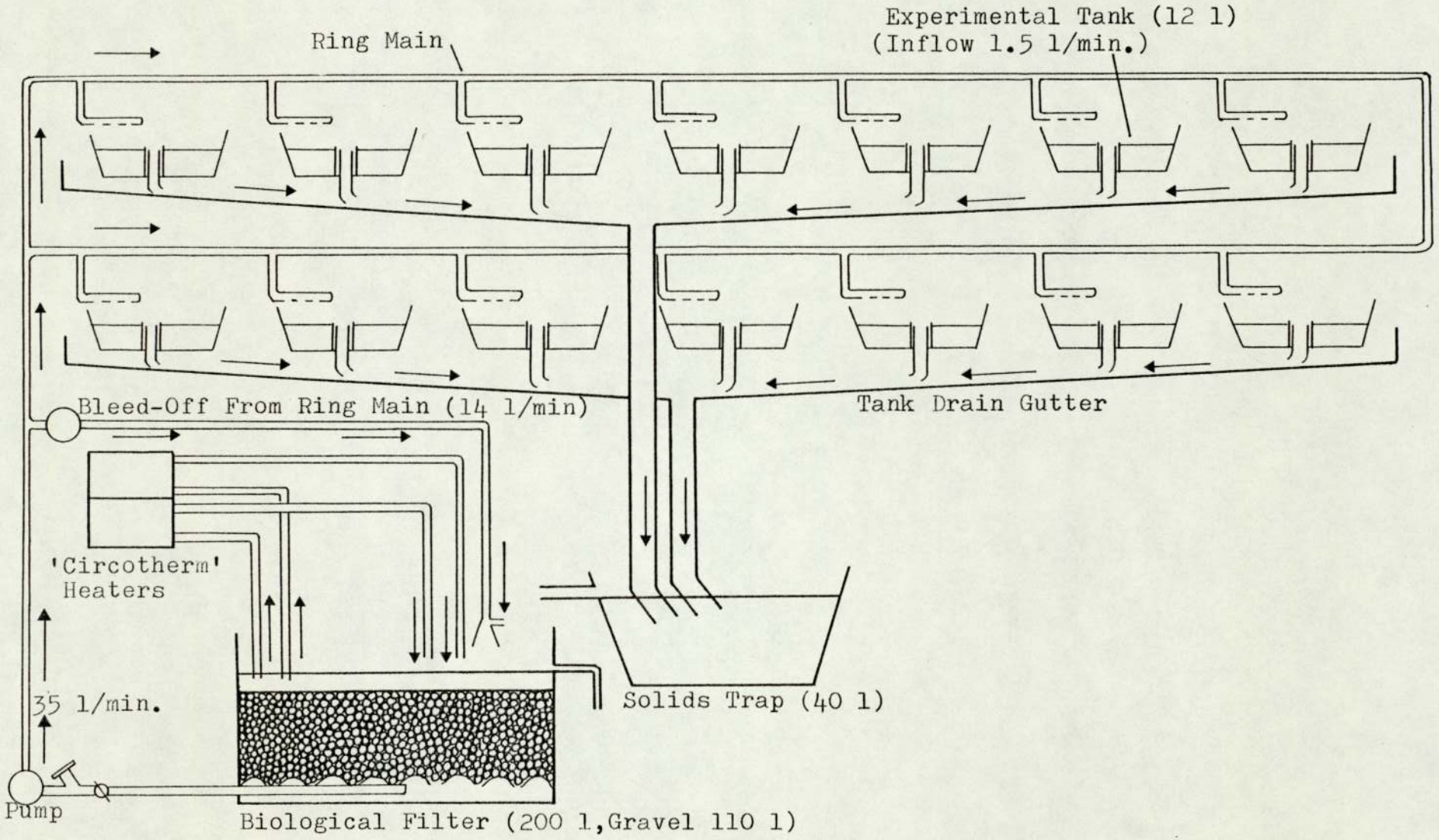


Figure 8. A diagrammatic representation of 'System 2'.

that this system could not have a header tank. Water was pumped directly from the bottom of the filter tank into the ring main supplying the experimental tanks.

Flow rate was adjusted by regulating the amount of water 'bled-off' and returned to the surface of the filter via an aerating venturi. As there was no header tank in which re-aeration of the water, deoxygenated by passage through the biological filter, could take place it was found necessary to place an airstone, supplied with 1.5 l of air per minute, in each of the experimental tanks.

Temperature control was achieved by two 'circotherm' heaters (FH15, Grant Instruments, Cambridge) which abstracted water, heated it to a thermostatically regulated temperature, and returned it to the surface of the filter.

This system was used in Experiment 3 (Chapter 5) during which water quality was measured twice weekly; the values obtained are presented in Table 4.

Table 4. Water Quality Criteria in 'System 2'.

Temperature	$25^{o}C \pm 0.5^{o}C$
Dissolved Oxygen	>5 mg/l
Total Ammonia	<0.1 mg/l
Total Nitrate/Nitrite	<50 mg/l
pH	7-7.3

Section 2.1.2.2. 'System 3'

The arrangement of this system is shown in Figure 9 together with the flow rates and tank volumes. The principles employed in this recycling system are the same as those described for 'System 1' (section 2.1.1.).

Additional aeration was not required as this system was

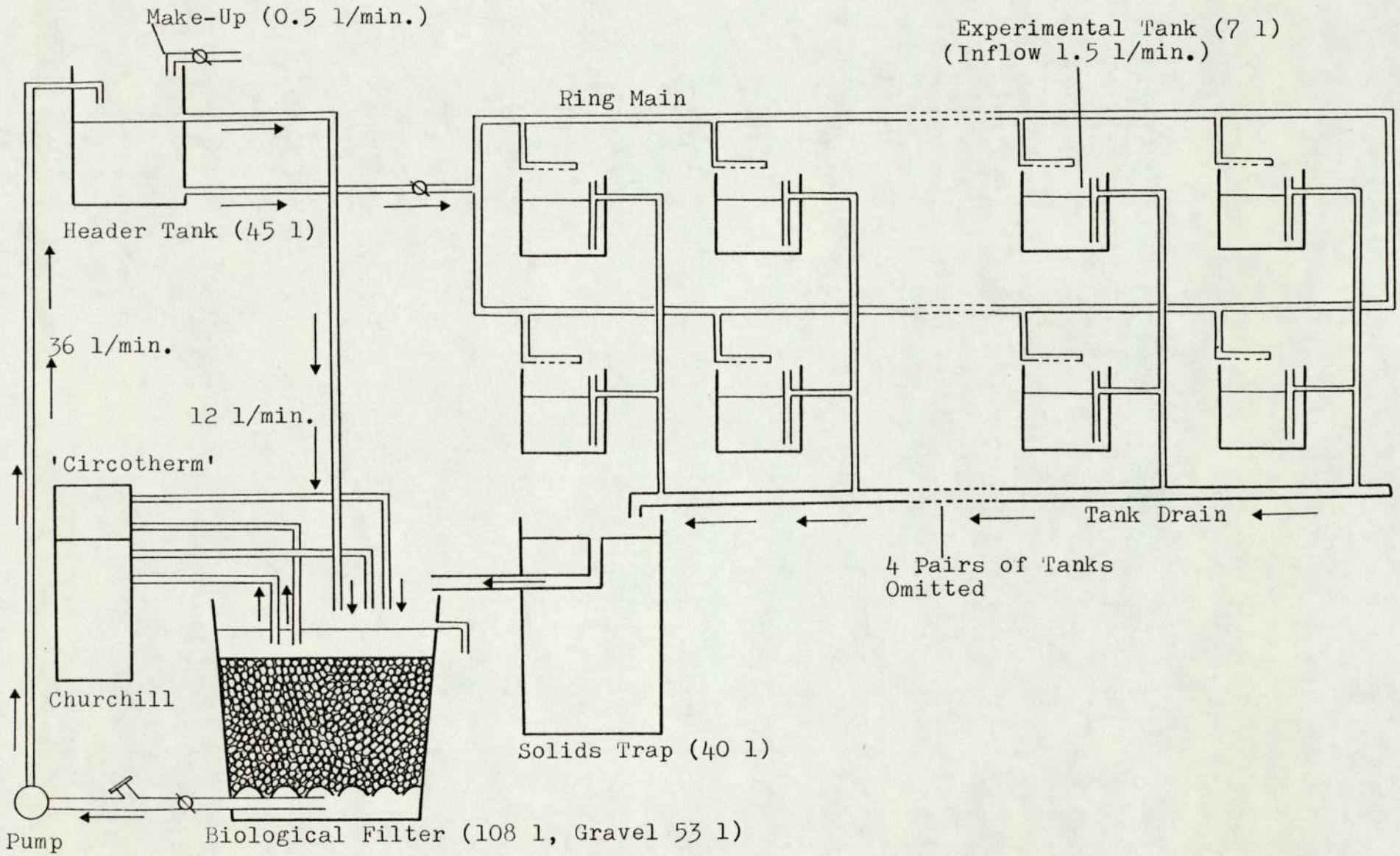


Figure 9. A schematic representation of 'System 3'.

supplied with a small header tank in which sufficient reaeration took place to maintain the dissolved oxygen levels in the experimental tanks.

Temperature control was achieved by a 'circotherm' heater (FH15, Grant Instruments, Cambridge) and a 'heater-cooler' (Thermocirculator, Churchill Instruments, Middlesex) which abstracted water, heated it to a thermostatically regulated temperature, and returned it to the surface of the filter.

This system was used in Experiment 4 (Chapter 6) during which water quality was measured twice weekly and the values obtained are presented in Table 5.

Table 5. Water Quality Criteria in 'System 3'.

Temperature	$28^{\circ}C \pm 0.5^{\circ}C$
Dissolved Oxygen	>7 mg/l
Total Ammonia	<0.2 mg/l
Total Nitrate/Nitrite	<100 mg/l
pH	6.9 - 7.1

Section 2.1.3. 'System 4'.

This system was contructed in a small fish culture building on-site at the Central Electricity Board's coal fired generating station at Ratcliffe-on-Soar, Nottingham. Due to the remoteness of this facility it was designed to require minimum supervision and, to this end, it was equipped with automatic feeders that required filling only once per week.

The arrangement of this system is shown in Figure 10. Condenser and pond water were abstracted from the power station cooling circuit, as shown in Figure 11, and mixed in a header tank, using thermostatically controlled valves, to obtain a temperature of approximately $28^{\circ}C$.

Water from this mixing/header tank flowed to a constant head

trough which supplied 5 l/min. to each of six 30 litre oblong experimental tanks. Six automatic feeders were used to dispense food into the tanks at a point away from the outflow. The feeders used were an early model supplied to the C.E.G.B., by Shearwater Fish Farming Ltd., Penrith, which dispensed food every fifteen minutes. The feeders used a screw auger which was turned by a motor that ran for an adjustable period of time thus regulating the quantity of food dispensed.

A 24 hour timer was used to control the feeders so that they operated for 2 hours in the morning (09.30 - 11.30) and 2 hours in the afternoon (15.30 - 17.30) thus supplying a total of sixteen feeds per day. The amount of food dispensed 'per feed' was adjusted after each weekly weighing so that the calculated weeks ration would be dispensed in six days.

This system was used in Experiment 6 (Chapter 8) which was conducted in October/November. Photoperiod was not controlled with lighting relying upon natural photoperiod and being approximately 10 hours light and 14 hours darkness throughout the experiment. Temperature varied from 23 to 28°C, the lower temperature resulting from a fall in the condenser water temperature due to reduced load on the station and low ambient temperatures. Levels of dissolved oxygen always approached 100% saturation (9 mg/l) due to the vigorous aeration occurring in the cooling towers. The level of suspended solids in the cooling circuit, and thus the experimental tanks, was very high due to the poor water quality of the river Soar (a tributary of the river Trent) and the large quantities of coal dust contaminating the water.

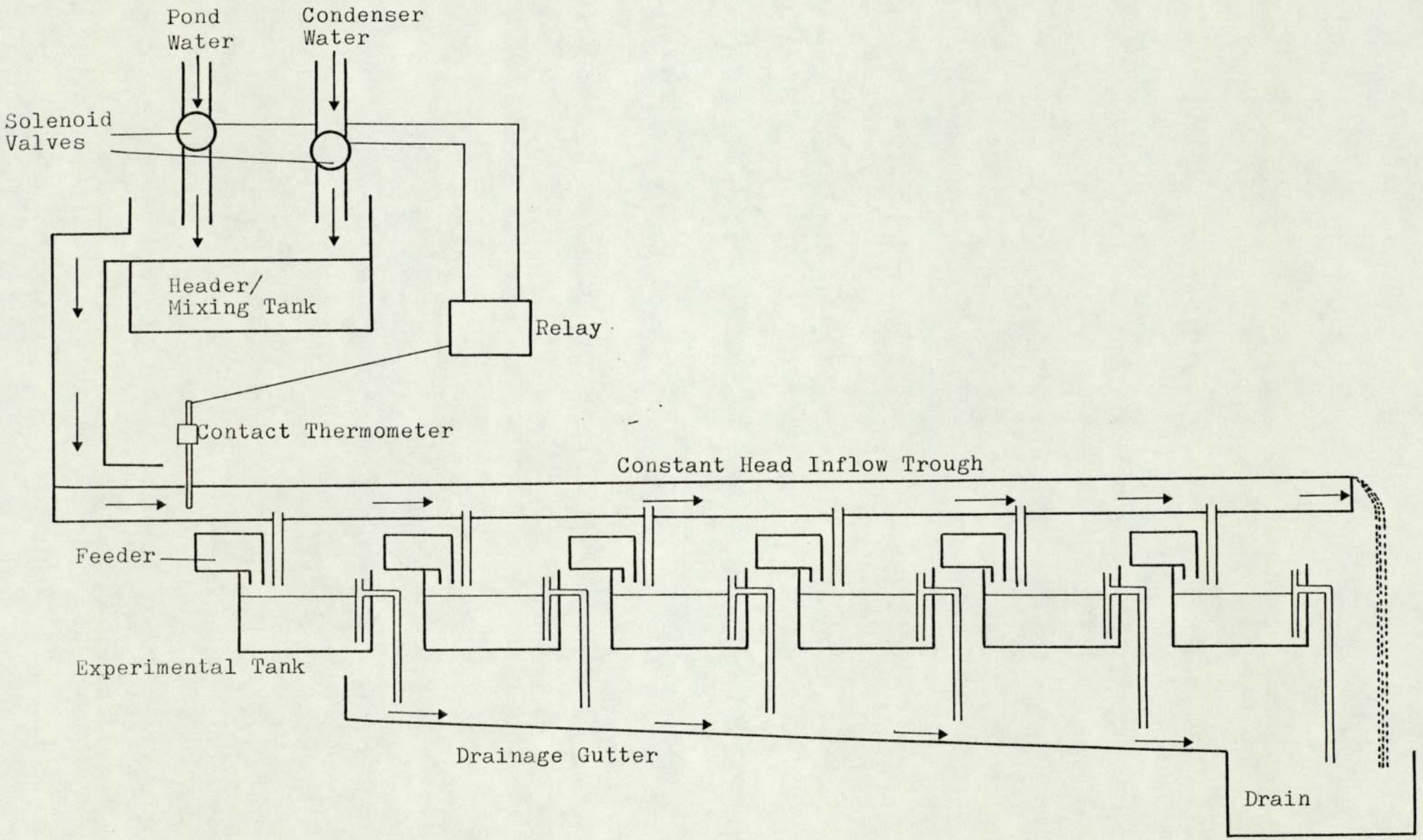


Figure 10. A schematic representation of 'System 4'.

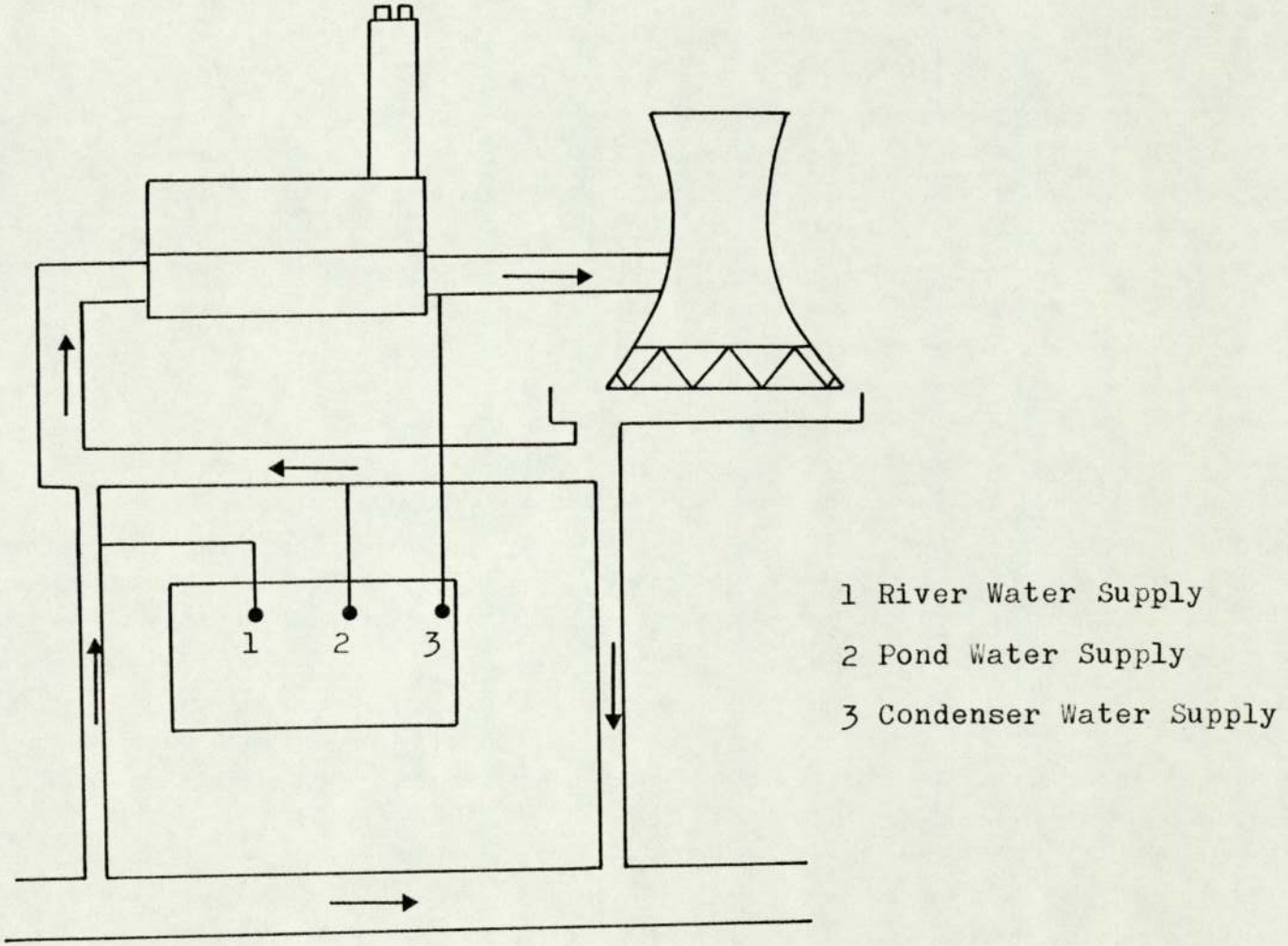


Figure 11. A diagrammatic representation of the power station cooling circuit showing the points of water abstraction for supplying 'System 4'.

Section 2.2. Diet Formulation.

For Experiments 2 - 6 semipurified rations were prepared in the laboratory. With the exception of Experiment 4 these diets contained a single complex protein source to which were added purified ingredients to form a nutritionally complete ration. In Experiment 4 (Chapter 6) two protein sources were used but the basic principles of formulation remained the same.

The method of formulation adopted would, it was hoped, minimise the effects of dietary ingredient variation and thus allow preparation of isonitrogenous and isoenergetic diets. The protein source to be used was first analysed for moisture, crude lipid, crude protein, ash and nitrogen free extractives (NFE) as detailed in section 2.6. The amount of protein source required (X g), per 100 g of diet, in order to give the desired level of protein was then calculated.

The quantity of crude lipid in X g of the protein source was then calculated and the amount of herring oil to be added, to 100 g of diet, to achieve the desired final lipid level, found. Similarly the quantity of ash in X g of the protein source was balanced to the desired level with a mineral mixture (Table 6.). NFE in the protein source was assumed to be a mixture of carbohydrates with an average digestibility equal to that of starch with which the NFE was balanced to the desired level.

The amount of moisture in each dietary ingredient was determined analytically and the quantity in the diet subtracted from the running total so that formulation was on a dry weight basis for all ingredients. To all diets were added 1% vitamin and trace element supplement (Table 7), 1% binder (carboxy-methylcellulose, sodium salt, high viscosity) and 0.5% chromium III oxide (as an inert indicator). The remainder of the diet

Table 6. Mineral Supplement Composition.

From test diet H440 Western Fish Nutrition Laboratory (NAC, 1973).

A combination of; -

Premix No. 5 (Mineral) in grams

Aluminium chloride	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganese sulphate	0.080
Cobalt chloride	0.100
Zinc sulphate	0.300

and Salt mixture No. 2 in grams

Calcium biphosphate	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate	23.98
Sodium biphosphate	8.70
Sodium chloride	4.35
Total	100.00

Table 7. Composition of Vitamin Premix.

This premix was supplied as a commercial fish diet additive initially by Coopers Nutrition Products and subsequently by B.P. Nutrition.

	mg/kg of premix
Vitamin A	0.012 m.i.u.
Vitamin D_3	0.0015 m.i.u.
Vitamin E	60
Vitamin K	15
Thiamine	10
Riboflavin	25
Pyridoxine	15
Biotin	60
Vitamin B_{12}	2
Nicotinic acid	150
Pantothenic acid	50
Folic acid	4
Choline chloride	1130
Vitamin C	60
BHT (antioxidant)	1000
Iron	20
Cobalt	200
Manganese	30
Copper	200
Zinc	50
Iodine	4500

was filled with a mixture of glucose, dextrin, starch and α-cellulose to achieve the desired energy level.

Section 2.3. Diet Preparation.

The amount of diet required for each experiment was estimated from the starting weight of the fish and the expected maximum growth rate assuming a food conversion ratio (g dry food fed per g live weight gain) of 1. Ten percent was added to this figure to allow for losses during pelleting, drying and sieving of the dry diet to the required size, as well as the taking of samples for analysis.

All dietary ingredients were sieved to a particle size of less than 1 mm prior to weighing and pelletising to ensure that a homogeneous mixture was obtained. The dry ingredients were then weighed out, according to the formulation, placed in the bowl of a Hobart A200 (Hobart Ltd., London) food mixer and thoroughly blended for 3 minutes. To this mixture was added the weighed quantity of herring oil and blending continued for a further 3 minutes.

Warm water (25°C) was then added to the diet, with mixing, until a stiff dough was obtained. If the diets were particularly light in colour, due to the nature or level of the protein source used, then a food colouring (Edicol Blue 12070, D.F.Anstead Ltd., Essex) was added to the water prior to its' addition to the diet. Colouring the diet had two advantages; firstly in the light background experimental tanks it was easier to determine whether or not the fish had consumed all of their ration and, secondly, it was noted that the fish had difficulty locating light coloured pellets against the light background of the experimental tanks.

After addition of the water the stiff dough was extruded through the mincer attachment of the food mixer, using a 3 mm die, into long sphagetti - like strands. These were dried on porous trays by having warm ($< 40^{\circ}C$) air blown over them from an electric fan convector heater.

When almost dry to the touch these strands were broken into approximately 5mm lengths by rubbing between the hands and then further dried. The dry diets were sieved to remove 'fines', a sample was taken for proximate analysis (section 2.6.) and the remainder stored in sealed polythene bags at $-20^{\circ}C$ until required for feeding.

Section 2.4. Quarantine Procedures.

Upon arrival each batch of fish was placed in a prepared, sterilized tank situated in a building seperate from the main fish culture facilities. Access to this area was via a caustic footbath and all the equipment used was sterilized and maintained solely for use in the quarantine area.

For the first ten days after arrival the fish were fed a specially prepared antibiotic diet, to eliminate bacterial pathogens, and for the remainder of the time in quarantine were fed a commercial fish feed.

On the 5th and 6th days after arrival the fish were bathed in 150 ppm formalin for one hour. After a further five days the fish were bathed on three successive days in 2 ppm malachite green for one hour. These treatments with formalin and malachite green were to eliminate external parasites and were accompanied by vigorous aeration.

Throughout the quarantine period fish were carefully observed for any abnormalities and samples were examined

microscopically for pathogens. Fish were held in quarantine for a total of 21 days before being transferred to the experimental facility.

Section 2.5. Tagging and Weighing Procedures.

In Experiments 1, 2 and 5 the fish were individually tagged. The tags were made out of 5 mm wide 'Dymo' embossing tape trimmed to an oval shape with a 1.5 mm diameter hole punched in the left hand end. Various combinations of tape colours and symbols (letters and numbers) enabled identification of each individual fish.

The tags were attached by a loop of 0.5 mm diameter silver wire inserted just in front of, and below, the dorsal fin. Fish were anaesthetised in MS222 (Sandoz, Basle) at a concentration of 50 mg/l. They were then removed from the anaesthetic and a 1 mm diameter hypodermic needle inserted through the fish at the desired tag attachment position. One end of a 5 cm length of silver wire was inserted into the needle and drawn through the fish by withdrawal of the needle.

A tag was then threaded onto the silver wire and the wire bent over the back of the fish, allowing a generous loop for growth, the free ends were then securely twisted together and the excess trimmed off.

Individual weighing of fish permitted the distribution of growth rates in different populations to be statistically compared. In addition tagging enabled instant identification of any fish which succeeded in escaping from an experimental tank, thus allowing, in the case of mortalities, adjustment of daily feeding rate and calculation of growth parameters.

Individual weighing of fish was undertaken in Experiments 1, 2 and 5. This was achieved by removing

the fish from their tank and anaesthetising them, a few at a time, in MS222 at a concentration of 50 mg/l. They were then removed from the anaesthetic, lightly blotted on a paper towel, and placed on a tared small rectangular pad of absorbent material situated on the pan of a Sartorius 3719MP dual range balance. The fish and pad were weighed to an accuracy of $\pm$0.01g and the fish returned to their tank where complete recovery occurred within three minutes. The absorbent pad was then weighed and the difference between this weight and the weight of the pad and fish was taken as the live weight of the fish.

In Experiments 3, 4 and 6 the fish were batch weighed a tank at a time. Again the fish were anaesthetised, a whole tank at a time, they were then allowed to drain for 15 seconds in a net, with gentle shaking, before being transferred to a tared bucket of water on a balance. In Experiments 3 and 4 the balance was a Sauter 10 kg side pan balance weighing to 1 g and in Experiment 6 a Sartorius 4 kg top pan balance accurate to 0.1 g was used. The fish, when returned to their tanks, recovered completely from the anaesthetic within three minutes.

In all experiments the fish were starved for twelve hours prior to weighing.

Section 2.6. Methods of Proximate Analysis.

Proximate analysis of diets, dietary ingredients, fish and faeces were carried out by the following procedures.

Moisture

Moisture content was determined by air drying the samples in an oven at 105°C for 48 hours.

Crude Lipid

Crude lipid content was determined by extracting dried samples for four hours, using a Soxhlet apparatus (A. Gallenkamp & Co. Ltd.), with 40 to 60°C boiling range petroleum ether and measuring, by weight difference, the amount of ether soluble material extracted.

Crude Protein

Crude protein content was determined by the microkjeldahl method for determining nitrogen (A.O.A.C. Methods, 1970) and applying the empirical factor of 6.25 to the results to convert total nitrogen to total crude protein.

Ash

Ash content was determined by heating samples in a muffle furnace (FR 610A, A. Gallenkamp & Co. Ltd.) for twelve hours at a temperature of 500 - 550°C.

Nitrogen Free Extractives (NFE)

NFE was determined by calculation.

NFE = 100 - (%moisture + %crude lipid + %crude protein + %ash)

Energy

Energy contents were determined using a ballistic bomb calorimeter (A. Gallenkamp & Co. Ltd.).

Section 2.7. Analysis of Experimental Data.

Section 2.7.1. Specific Growth Rate.

In the early stages of life the growth of a fish under controlled conditions follows an exponential curve the equation of which is ;

$$w_2 = w_1 \cdot e^{g(T-t)}$$

Where w_2 is the final weight (at time T) and w_1 the initial size (at time t); T and t are expressed in units of time (usually days); w_2 and w_1 in units of weight (usually grams); e is the base of natural logarithms; g is a constant for a particular curve known as the specific, instantaneous, geometrical or multiplicative growth rate.

Rearrangement of the above equation, to obtain g, and multiplying by 100 gives the rate of change in weight of the fish, expressed as percent per day; this is commonly termed the Specific Growth Rate (SGR).

$$\text{SGR (\%/day)} = \frac{\log_e w_2 - \log_e w_1}{T - t} \times 100$$

(Brown, 1957)

Section 2.7.2. Food Conversion Ratio.

The food conversion ratio (FCR) is defined as the amount of dry food fed per unit live weight gain of fish.

$$\text{FCR} = \frac{\text{Food Fed, g dry food}}{\text{Live Weight Gain, g wet fish}}$$

In calculation of FCRs for all experiments the true weight of dry food fed was used with correction being made for the analysed moisture content of each diet.

Section 2.7.3. Protein Utilization.

The efficiency with which fish were able to utilize dietary protein was determined by calculation of values for Protein Efficiency Ratio (PER), defined as the gain in wet weight of

fish per gram of crude protein consumed (Osborne et al., 1919).

$$PER = \frac{\text{Weight Gain, g wet fish}}{\text{g Crude Protein Fed}}$$

Although PER values give a somewhat better indication of the nutritional status of the fish than FCRs, they do not take into account the proportion of the ingested protein used for maintainance and are based on the assumption that the growth of the fish consists of tissues with identical composition in all groups. An improved assessment of the nutritional status of the fish, with respect to dietary protein utilization, is the apparent efficiency of deposition of dietary protein as body tissue, the Net Protein Utilization (NPU).

In all of the present trials NPU was determined by the carcass analysis method of Bender & Miller (1953) and Miller & Bender (1955). Since no correction was made for endogenous nitrogen losses during experiments 1, 2, 4, 5, and 6 the results are expressed as Apparent NPU.

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

Where Nb is the body nitrogen at the end of the test, Na the body nitrogen at the start of the test and Ni the amount of nitrogen ingested.

In Experiment 3 (Chapter 5) a low protein diet was fed in order to enable estimation of endogenous nitrogen losses and thus calculation of True NPU. The feeding of a low protein diet, in order to overcome the problems of feeding a non-protein diet, was first proposed by Cowey et al. (1974). Hence;

$$\text{True NPU (\%)} = \frac{B - (Bk - Ik)}{I}$$

Where B is the total body nitrogen of the fish fed the test diet and Bk the total body nitrogen of the fish fed the low protein diet, with nitrogen intakes of I and Ik respectively.

Obtaining a value for True NPU in this way, together with the determination of true protein digestibility (section 2.7.4), permits calculation of the Biological Value (BV) of the dietary protein.

$$BV = \frac{\text{True NPU}}{\text{True Digestibility}}$$ (Bender & Miller, 1953)

Section 2.7.4. Digestibility Determination.

The use of inert indicators, which pass unaffected by digestion through the alimentary tract, has provided a convenient method of measuring digestibility in a number of animals without the need to collect the faeces quantitatively. This method has been successfully applied to fish digestion studies using Chromium III oxide as the indicator (Furukawa & Tsukahara, 1966; Nose, 1967).

For the purpose of determining digestibility the diets used in Experiments 2 - 5 contained 0.5% chromic oxide. After the terminal weighing of each of these experiments feeding was continued at the same rate for six days, with faeces being stripped from the fish once per day, and the six day samples for each group pooled.

Faecal stripping was accomplished by lightly anaesthetising the fish and gently applying pressure on the abdomen from the pelvic fins to the anus. Faeces obtained in this way were

collected on filter paper, dried at 105°C for 12 hours and transferred to sealed dry containers.

Chromic oxide determinations by the spectrophotometric method of Furukawa & Tsukahara (1966) were carried out on the diet and faeces.

Apparent digestibility was derived from the following equation (Maynard & Loosli, 1969);

$$\text{Apparent Digestibility (\%) (Digestion Coefficient)} = 100 - \left(100 \times \frac{\text{indicator in feed \%}}{\text{indicator in faeces \%}} \times \frac{\text{nutrient in faeces \%}}{\text{nutrient in feed \%}}\right)$$

In Experiment 4 a low protein diet was fed which enable approximation of endogenous nitrogen losses and thus calculation of true digestibility assuming the low protein diet to be 100 % digestible.

Section 2.7.5. Statistical Methods.

Statistical comparisons between means were made by multiple analysis of variance using Duncan's Multiple Range F -Test (Duncan, 1955). Standard Errors ($\pm$ SE) were calculated from the residual mean square in the analysis of variance and are presented, where relevant, to indicate the range of the means tested.

CHAPTER 3.

Chapter 3.

3. Experiment 1. The Effects of Different Environmental Temperatures and Feeding Levels on the Growth Performance, Food Conversion, Body Composition and Protein Utilization of Fingerling Mirror Carp (Cyprinus carpio).

Section 3.1. Introduction.

In recent years there has been a great deal of interest in the utilization of heated effluents for the culture of finfish, as described in sections 1.3 and 1.4. The most important consideration in this respect is determination of the optimum temperature for growth and food conversion of the proposed species.

The literature contains some references to optimum temperatures for mirror carp (Pitt et al., 1956; Shpet & Khabitonaova, 1963; Gribanov et al., 1966; Reynolds & Casterlin, 1977; Adelman, 1977; Adelman, 1978; Aston & Brown, 1978). However, these studies have not, in general, been conducted under defined conditions of temperature, water quality and feeding level.

The present 8 week growth study was undertaken to determine the combined effects of temperature and feeding rate on the growth of carp and, thus, at what combination of temperature and feeding rate to conduct future experiments.

Groups of fish were maintained at temperatures of 20, 25, 30 & 35°C and feeding rates, at each temperature, of 3, 6 & 9% (dry food) of the wet body weight per day were fed.

Section 3.2. Materials and Methods.

Section 3.2.1. The Experimental System and Animals.

The experimental facility used in the present study was 'System 1' as described in detail in section 2.1.1.

400 fingerling mirror carp (5 - 8 cm) were obtained from the Cotswold Carp Farm, Bourton-on-the-Water, Gloucestershire. The fish were subjected to quarantine and prophylaxis, as described in section 2.4, and then transferred to three of the 230 l tanks of System 1 at the prevailing ambient temperature of 13°C.

The temperature was then raised at approximately 3°C per day to 25°C; during this period the fish fed actively on a commercial trout ration and showed no signs of stress. 30 fish at a time were then allocated to each of the twelve experimental tanks. 10 fish were removed for proximate carcass analysis (section 2.6.) and the remainder discarded.

The fish in each tank were then individually tagged (section 2.5.) and the temperatures of the recycling systems adjusted at approximately 3°C per day to the required experimental temperatures. One, three tank recycling system was adjusted to each of 20, 25, 30 & 35°C.

No losses were experienced during tagging or temperature acclimation and the fish fed actively over this period. Photoperiod was controlled at 14 hours light and 10 hours darkness throughout the experiment.

Section 3.2.2. The Experimental Diet.

The diet used in this experiment was a commercially available fingerling trout food supplied by 'Trouw', Witham, Essex. This diet was selected as it had been used in a previous study by the Central Electricity Generating Board (Aston & Brown, 1975). Proximate analysis was performed on this diet and the results are presented in Table 8.

Table 8. Proximate Analysis of 'Trouw' Fingerling Feed.

Moisture	9.5 %
On a moisture free basis;	
Crude Lipid	8.2 %
Crude Protein	50.5 %
Ash	11.6 %
NFE*	20.7 %

* Nitrogen Free Extractives

Section 3.2.3. Feeding Rates.

During this experiment it was only practicable to feed the fish four times per day between 08.30 h and 18.30 h . In addition to this, feeding a stomachless fish, such as carp, to satiation presents difficulties as they are, in nature, continuous feeders. In view of this it was decided that in the present, and all subsequent, experiments a restricted ration would be fed.

Each of the four daily feeds was distributed over a period of 15 to 20 minutes to ensure, as far as possible, consumption of the whole ration. The quantity of food fed was corrected for its' analysed moisture content so that a true percentage (dry food) of the body weight was dispensed.

In the present study one of each of the three tanks at each temperature was fed 3, 6 or 9% of the total body weight of fish per day. The quantity of food fed was adjusted after each weekly weighing and fed for the subsequent six days.

Section 3.2.4. Weighing and Sampling.

Details of the weighing procedure employed are presented in section 2.5. Fish were individually weighed ($\pm$ 0.01 g), under anaesthesia, every seven days after 12 hours of starvation.

An intermediate sample of fish (3 per group) was removed at the end of the fourth week and subjected to proximate analysis (section 2.6.). At the end of the eighth (and final) week a further sample (3 per group) was removed for proximate carcass analysis. The results of carcass analyses of initial, intermediate and final fish samples are presented in Tables 9 & 10.

Section 3.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

Section 3.3. Results.

At 20, 25 & 30°C all groups of fish became accustomed to both the diet and conditions, feeding actively throughout the experiment. It was noticed, however, that fish held at 35°C appeared 'stressed', being darker than those in the other experimental groups and, although appearing to consume all of their daily ration, they fed nervously and erratically. No mortalities occurred during the experiment.

Section 3.3.1. Growth Performance.

The growth responses of the fish under the different regimes are shown graphically in Figures 12 to 18. Figures 12 to 15 show the effect, on growth, of varying the feeding level at each temperature and Figures 16 to 18 the effect of temp-

Table 9. The Results of Proximate Carcass Analysis of Initial and Intermediate Samples from Experiment 1.

Sample		Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Initial		79.7	5.0	10.0	2.4	97.1
Intermediate;						
Temp./Ration						
20°C	3%	79.9^{ab}	3.3	11.9	2.8	97.9
"	6%	79.7^{ab}	3.9	13.0	2.5	99.1
"	9%	78.1^{ab}	5.1	12.2	2.5	97.9
25°C	3%	80.2^{a}	3.7	12.6	2.6	99.1
"	6%	78.8^{ab}	4.4	11.9	2.6	97.7
"	9%	78.1^{ab}	4.9	13.0	2.5	98.5
30°C	3%	79.9^{ab}	3.0	12.2	3.1	98.2
"	6%	78.7^{ab}	4.7	12.3	3.7	99.4
"	9%	78.7^{ab}	4.2	12.1	2.4	97.4
35°C	3%	80.2^{a}	3.5	11.7	3.2	98.6
"	6%	79.2^{ab}	3.0	12.8	3.2	98.2
"	9%	77.8^{b}	4.8	13.1	3.0	98.7
S.E.±		0.666				

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).
Standard Errors (S.E.±) indicate the range of the means tested.

Table 10. The Results of Proximate Carcass Analysis of the Final Sample from Experiment 1.

Sample		Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Final; Temp./Ration						
20°C	3%	78.1^{a}	2.6^{c}	14.0^{ab}	3.3^{b}	98.0
	6%	77.2^{a}	3.7^{bc}	13.7^{ab}	2.8^{bc}	97.4
	9%	76.2^{a}	4.6^{b}	12.1^{b}	2.4^{c}	95.3
25°C	3%	78.9^{a}	2.5^{cd}	13.4^{ab}	3.0^{bc}	97.8
"	6%	77.4^{a}	3.8^{bc}	14.3^{a}	2.6^{c}	98.1
"	9%	74.9^{a}	5.5^{ab}	14.3^{a}	2.6^{c}	97.3
30°C	3%	79.3^{a}	2.1^{d}	13.8^{ab}	3.4^{ab}	98.6
"	6%	76.4^{a}	4.0^{b}	13.4^{ab}	2.8^{bc}	96.6
"	9%	75.6^{a}	5.6^{ab}	13.5^{ab}	2.6^{c}	97.3
35°C	3%	78.7^{a}	2.6^{c}	11.9^{b}	3.9^{a}	97.1
"	6%	76.9^{a}	4.8^{ab}	12.1^{b}	3.4^{ab}	97.2
"	9%	74.2^{a}	6.1^{a}	11.7^{b}	3.2^{b}	95.2
S.E.±		1.298	0.507	0.643	0.168	

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).
Standard Errors (S.E.±) indicate the range of the means tested.

erature on growth at each feeding level.

Figures 12 to 15 show that increasing the feeding rate at each temperature increased the growth response. Figures 16 to 18 show that, at each feeding rate, increasing the temperature from 20 to 30°C increased growth and that further raising it to 35°C gave depressed growth.

Statistical analysis of initial fish weights (Table 11) shows significant differences between certain groups ($p<0.05$). There were, however, no significant differences between groups at the same temperature. The differences in initial weights were not reflected in the final weights obtained for each group nor in the growth curves.

No significant differences ($p>0.05$) in Specific Growth Rates (SGRs, section 2.7.1.) or average final fish weights (Table 11) were observed at the 3% feeding level at temperatures of 20, 25 & 30°C, although at 35°C the lowest SGR and final average fish weight obtained in any group were recorded. At the 6% feeding level SGRs and final average fish weights at 25 & 30°C were not significantly different ($p>0.05$) although growth at 20°C was significantly lower ($p<0.05$) and growth at 35°C significantly the lowest. Similarly at the 9% feeding rate SGRs and final average fish weights were not significantly different ($p>0.05$) at 25 & 30°C, growth at 20°C was significantly lower ($p<0.05$) and growth at 35°C significantly the lowest.

Section 3.3.2. Food Conversion.

Poor Food Conversion Ratios (FCRs, section 2.7.2.) were obtained for all groups (Table 11) possibly reflecting the poor water stability of the commercial diet used. Disinteg-

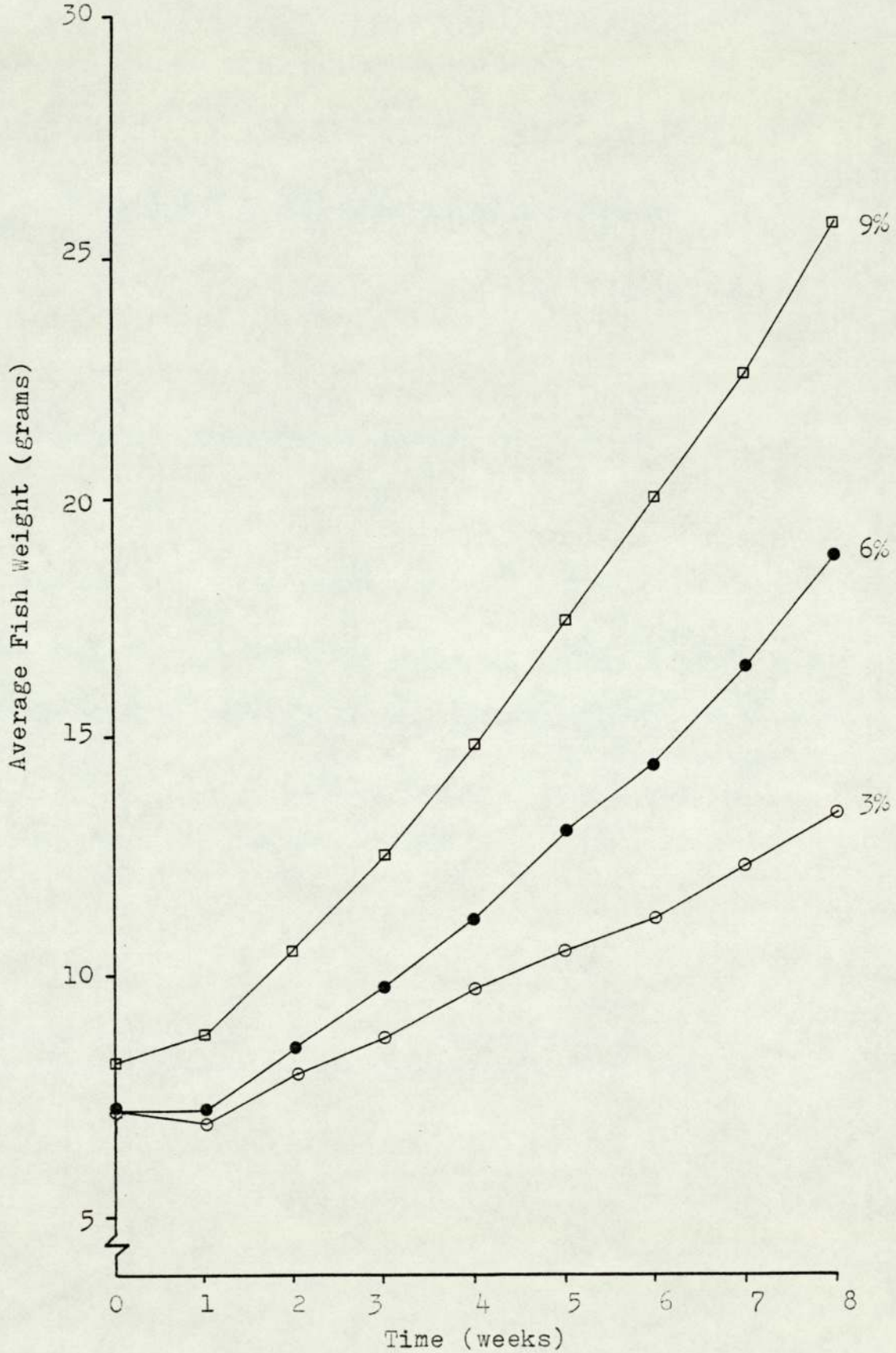


Figure 12. Growth responses of carp at 20°C fed three different rations.

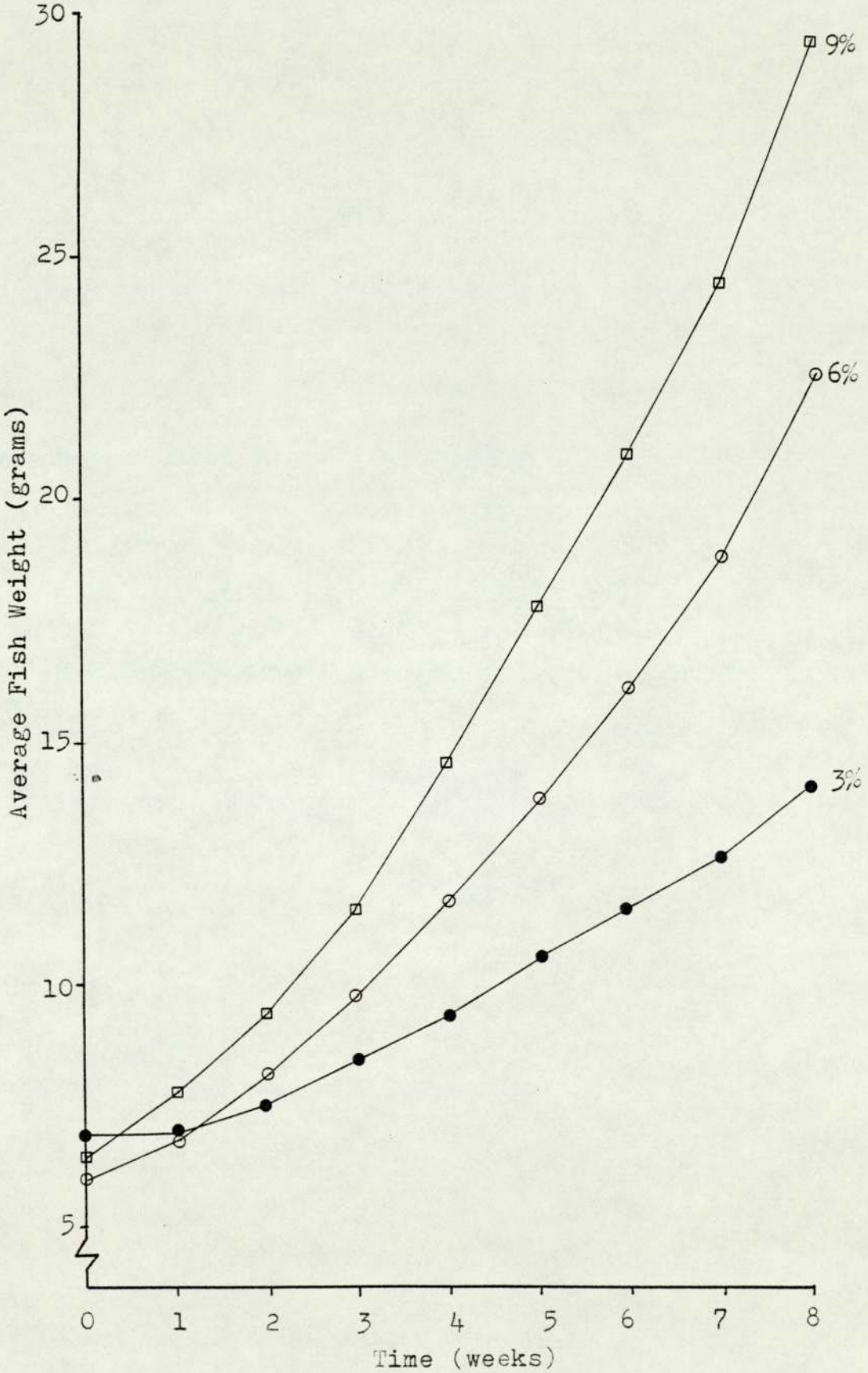


Figure 13. Growth responses of carp at 25°C fed three different rations.

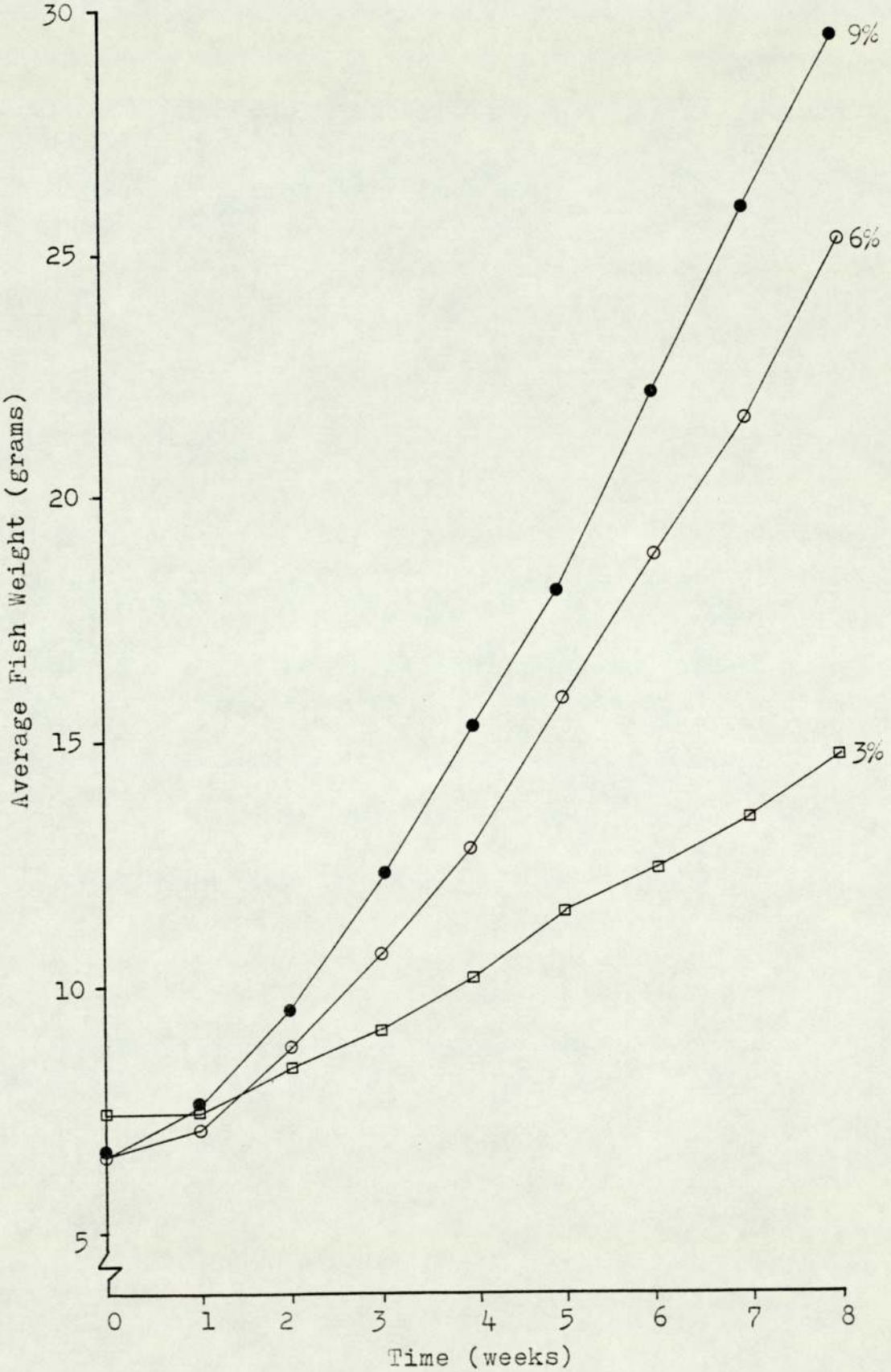


Figure 14. Growth responses of carp at 30°C fed three different rations.

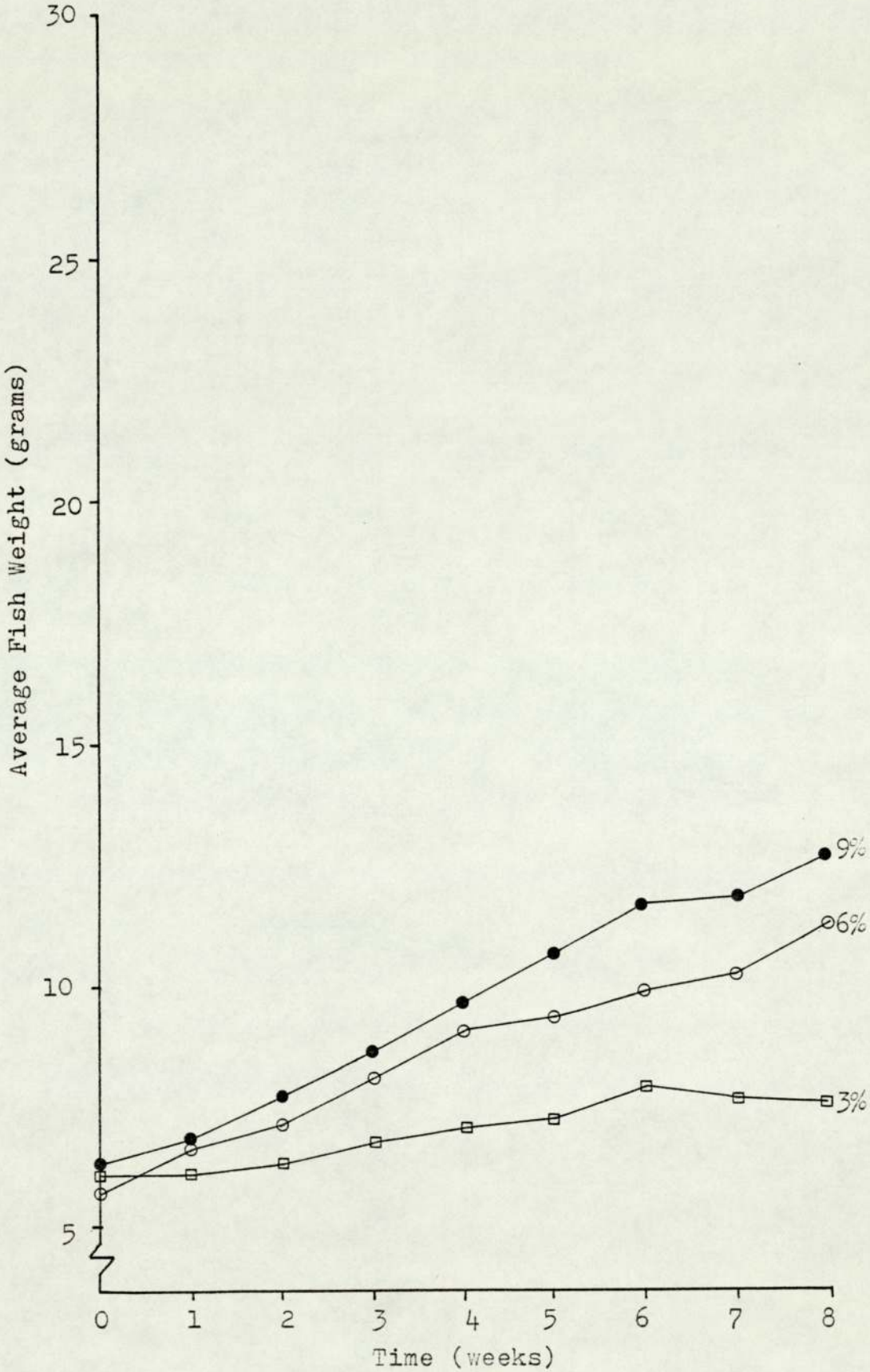


Figure 15. Growth responses of carp at 35°C fed three different rations.

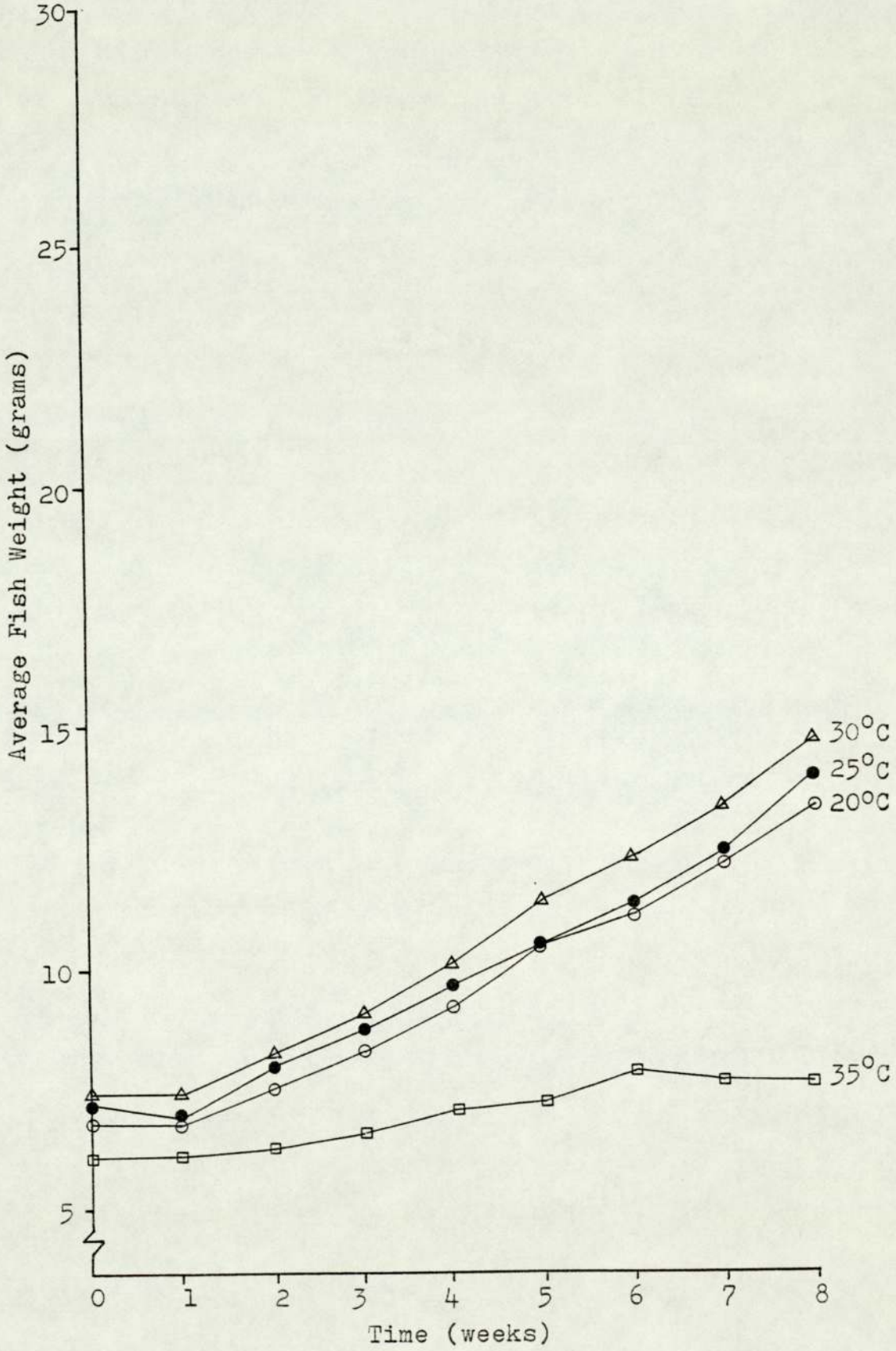


Figure 16. Growth responses of carp fed 3% of the body weight per day at four different temperatures.

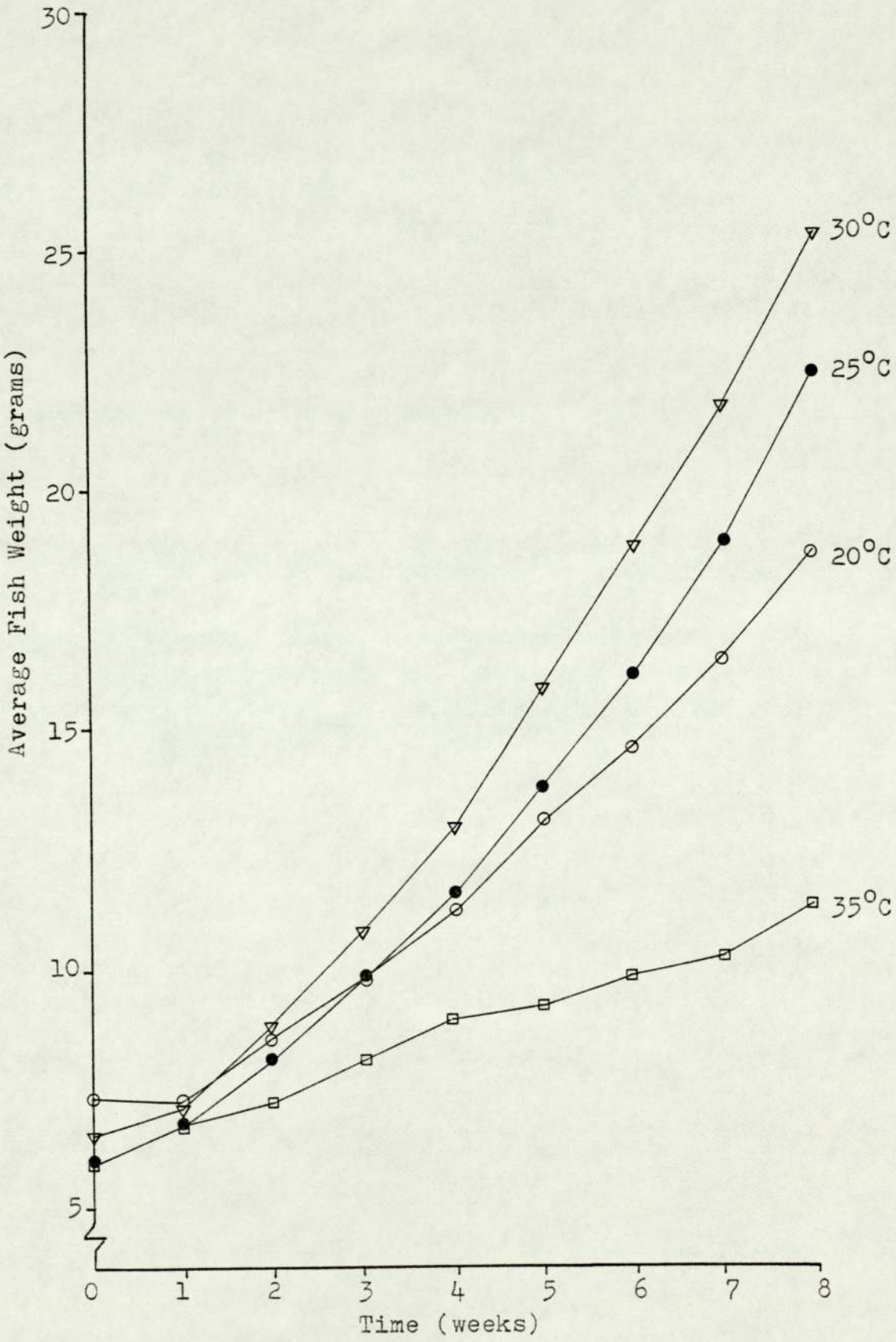


Figure 17. Growth responses of carp fed 6% of the body weight per day at four different temperatures.

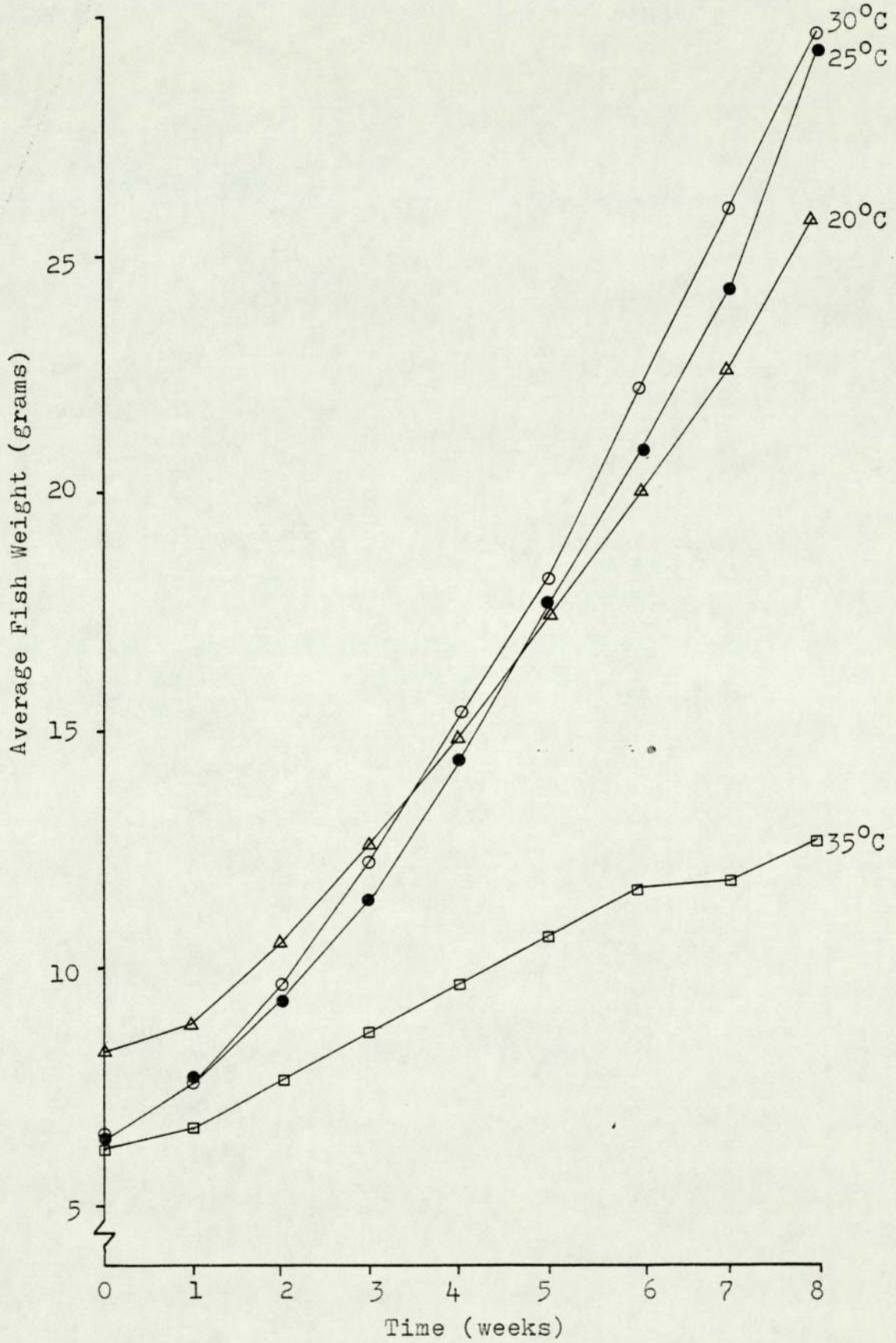


Figure 18. Growth responses of carp fed 9% of the body weight per day at four different temperatures.

Table 11. Growth and Food Utilization Data from Experiment 1.

Group		Average Initial Weight(g)	Average Final Weight(g)	S.G.R. (%/day)	FCR.	PER.	Apparent NPU(%)
Temp./Ration							
20°C	3%	7.24^{bcd}	13.40^{bc}	1.15^{b}	2.03	1.05	16.58
"	6%	7.35^{bcd}	18.81^{d}	1.65^{c}	2.54	0.87	11.14
"	9%	8.25^{d}	25.76^{e}	1.91^{d}	3.40	0.60	7.54
25°C	3%	6.88^{abc}	14.08^{bc}	1.27^{b}	1.78	1.14	17.46
"	6%	5.90^{a}	22.50^{e}	2.39^{ef}	2.02	1.01	15.83
"	9%	6.51^{abc}	29.50^{f}	2.64^{g}	2.64	0.74	11.21
30°C	3%	7.40^{cd}	14.86^{c}	1.19^{b}	1.82	1.11	17.48
"	6%	6.66^{abc}	25.31^{e}	2.32^{e}	1.99	1.04	13.77
"	9%	6.99^{abc}	30.65^{f}	2.56^{fg}	2.91	0.78	10.61
35°C	3%	6.17^{ab}	7.71^{a}	0.42^{a}	3.65	0.61	3.73
"	6%	5.84^{a}	10.67^{ab}	1.08^{b}	5.05	0.45	6.23
"	9%	6.26^{abc}	12.76^{bc}	1.24^{b}	5.73	0.37	4.30
	S.E.±	0.380	1.302	0.071			

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

ration of the diet soon after feeding was particularly noticeable at the higher temperatures employed in this study. The pellet ingredients also appeared to be fairly coarse contributing to its' friability and, possibly, poor digestibility.

On a general basis increasing the feeding rate at each temperature led to increased FCRs. FCRs were found, at each feeding level, to be lower at 25 & 30°C than those observed at 20°C which were, in turn, lower than those at 35°C.

Section 3.3.3. Carcass Composition.

Neither temperature nor feeding rate appeared to have a profound effect on either the intermediate (Table 9) or final (Table 10) proximate carcass composition of the fish.

A possible explanation of this is that too small a sample of fish was taken from each group and thus individual variation resulted in comparatively large standard errors. Statistical analysis on the intermediate samples was only performed on moisture content as the dried fish had to be pooled in order to obtain sufficient material for subsequent analyses. On a general basis moisture content decreased, and lipid content increased, with increasing feeding rate at each temperature. Temperature appeared to have no effect on these two parameters in the intermediate sample.

Proximate analysis of the final sample was performed on individual fish thus allowing statistical analysis of all four parameters (Table 10). Although moisture contents show no statistically significant differences ($p > 0.05$) they do, on a general basis, decrease with increasing feeding rate at each temperature although there appear to be no differences between temperatures. Carcass lipid contents increased with

increasing feeding rate at each temperature, in some cases significantly ($p < 0.05$), although temperature appeared to have little effect.

Protein and ash contents of the final sample (Table 10) showed some significant variations ($p<0.05$) although these appeared to have little correlation with the experimental conditions.

Section 3.3.4. Protein Utilization.

The efficiency with which fish were able to utilize dietary protein was determined by the calculation of Protein Efficiency Ratios (PERs) as described in section 2.7.3. The PER values (Table 11) were low for all groups, reflecting the poor FCRs, and decreased with increasing feeding rate at each temperature. PERs at 25 & 30°C were higher, at all three feeding levels, than those observed at 20 and 35°C.

Values for apparent Net Protein Utilization (NPU) were determined (section 2.7.3.) and they, like FCR and PER, were low for all groups (Table 11). Apparent NPU decreased with increasing feeding rate at temperatures of 20, 25 & 30°C being greatest, at all feeding levels, at 25 & 30°C indicating improved protein utilization in these groups. Apparent NPU at 35°C increased when the ration was increased from 3 to 6% and decreased when the ration was further increased to 9%. A possible explanation for this is that the 3% feeding rate only provided sufficient energy for the high metabolic demands at this temperature. Increasing the ration to 6% provided sufficient energy to spare a small amount of the dietary protein for growth thus increasing apparent NPU. At the 9% feeding rate, however, some of the ration may have been unconsumed causing a fall in

apparent NPU.

Section 3.4. Discussion and Conclusions.

From the results of the present study it may be inferred that the optimum temperature for growth and food conversion of mirror carp fingerlings lies between 25 and 30°C. This finding is in agreement with that of other authors whose results are summarized in Table 12.

Table 12. The Effects of Temperature on Mirror Carp.

Temperature(s)	Notes	Author(s)
23 - 30°C	Optimum for growth in cages	Gribanov et al, 1966
33 - 34°C	Growth greatly depressed	Korneev, 1969
22°C	Growth sub-optimal	
23 - 29°C	Optimum for growth	Shpet & Kharitonaova, 1963
32°C	Final preferendum in a graduated temperature apparatus	Pitt et al., 1956
29°C	Preferred temperature in a thermoregulatory shuttlebox	Reynolds & Casterlin, 1977
28 - 30°C	Optimum for growth	Adelman, 1977
29.6°C	Optimum for growth	Adelman, 1978
28°C	Optimum for growth	Aston & Brown, 1978

The effects of temperature and feeding rate on body composition were not as profound in this study as reported by other authors. At low feeding rates Brett et al. (1969), working on sockeye salmon (Oncorhynchus nerka), found that as temperature increased carcass moisture content increased and carcass lipid content decreased. This is to be expected as more of the stored lipid should be utilized to meet the higher

metabolic energy demands of the fish as the temperature increases.

In the present study, at the 3% feeding level, final carcass lipid contents (Table 10) decreased, although not significantly ($p>0.05$), with increasing temperature from 20 to 30°C in agreement with the results of Brett et al. (1969). However, at 35°C the carcass lipid content of the fish fed the 3% level was higher than at 25 & 30°C.

At higher feeding levels (6%) Andrews & Stickney (1972) found an almost linear increase in the carcass lipid content of channel catfish (Ictalurus punctatus) with increasing temperature. Similar results have been reported at high feeding levels for rainbow trout, Salmo gairdneri, (Papoutsoglou & Papoutsoglou, 1978) and mirror carp (Aston & Brown, 1978).

The present experiment demonstrated a similar effect. At the 6 & 9% feeding levels carcass lipid contents increased with increasing temperature, however these differences were not significant ($p> 0.05$). The reason for the increase of carcass lipid content, at high feeding levels, with increasing temperature is not known.

The effect of varying the feeding rate, at any one temperature, on carcass composition was the same in this study as reported by others (Brett et al., 1969; Nijkamp et al., 1974; Murray et al., 1977). Moisture content decreased and lipid content increased with increasing feeding rate at each temperature implying that dietary energy, excess to requirements, was stored as carcass lipid (Brett et al., 1969).

The effects of temperature and feeding rate on carcass composition in this experiment may have been less pronounced than reported by others due to the generally poor

water stability, digestibility and assimilibility of the diet as reflected in the poor values for food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization presented in Table 11. This assumption is supported by the fact that final carcass lipid levels in the present study were lower than those previously reported for mirror carp (Nijkamp et al., 1974; Sin 1973a,b; Takeuchi et al., 1978a; Meske & Pfeffer, 1978).

The combined effects of temperature and feeding rate on the growth and food utilization of fish have been reported by several authors (Brett et al., 1969; Andrews & Stickney, 1972; Huisman, 1969; Huisman, 1976). In the present study the efficiency of food utilization decreased with increasing feeding rate at each temperature (Table 11) in agreement with the results of others.

Brett et al. (1969) conclude, from the study of sockeye salmon, that as the feeding rate is lowered the optimum temperature for growth is also lowered. This, they say, is because the decrease in maintainance metabolism at lower temperatures should permit better use of a restricted ration for growth if the temperature dependent activities of digestive enzymes and growth processes were unaffected. Examination of Figure 19, however, shows that in the present study the optimum temperature for growth was approximately the same at all three levels of feeding. This may be due to a lack of data at lower feeding levels or because the temperature dependant activities of carp were more greatly affected than those of sockeye salmon.

The specific growth rate (SGR) of fish in this experiment increased with increasing feeding rate at each temperature and did not appear to have reached a maximum at the 9% feeding

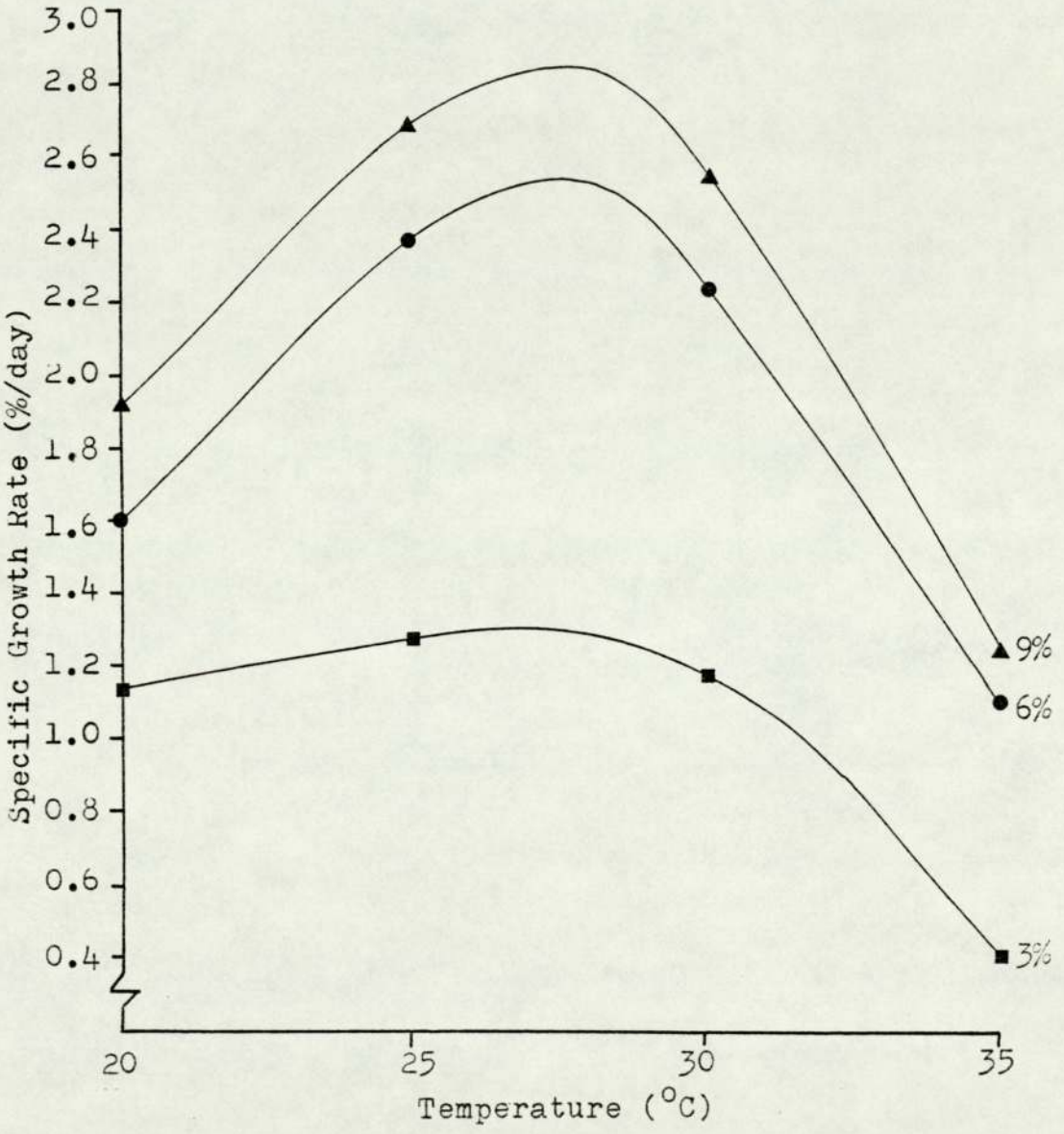


Figure 19. Growth responses of carp at three feeding levels and four temperatures.

level. However, Figure 19 shows that increasing the feeding level resulted in proportionately smaller increases in SGR as the feeding level increased suggesting that SGR was approaching a maximum.

Brett et al. (1969) found, with sockeye salmon, that increasing the feeding rate at any one temperature increased the growth rate up to an asymptote beyond which growth remained constant.

It should be emphasised at this stage, however, that contrary to the results of the present study, and those of Brett et al. (1969), Huisman (1969), with mirror carp, found that increasing the ration above 3% at 17°C and above 8% at 23°C caused a fall in SGR. Such a fall in SGR at high levels of feeding was also reported for rainbow trout (Roberts, 1976) and it was postulated that a high feeding rate combined with a relatively high temperature resulted in poorer growth rates due to the high induced metabolic rate and consequent reduction in the amount of energy available for growth.

That the metabolic rate of fish increases 4 - 5 times with rations increasing from maintainance to satiation was shown by Paloheimo et al. (1966a) who postulated that this may have been due to the energy required to metabolise excess nutrients, principally in the deamination of excess amino acids. However, this conclusion of the theoretical study of Paloheimo et al. (1966a) is not borne out by the recent experimental work of Smith et al. (1978) who found, by direct measurement, little heat increment in rainbow trout with increasing food intake.

In conclusion from this study it was decided that future experimentation would be conducted at 28°C as this should result in optimal, or near optimal, growth and food conversion.

It was also decided that in future trials a fixed feeding

rate of 5% of the body weight per day would be adopted. This ration was chosen as experience had shown that it would readily be consumed when fed, by hand, in four equal feeds per day. It would also permit the attainment of low FCRs and should result in high growth rates.

CHAPTER 4.

Chapter 4.

4. Experiment 2. The Interaction of Varying Dietary Lipid and Protein Levels and their Effects on Growth Performance, Food Conversion, Body Composition and Protein Utilization of Fingerling Mirror Carp (Cyprinus carpio).

Section 4.1. Introduction.

Fish are efficient converters of food to flesh with food conversion ratios (g dry food fed per g wet weight gain) frequently in the range 1 to 1.5 as compared to values of 3 or more obtained for conventional livestock such as pigs and poultry. This improvement in food conversion is outweighed, in economic terms, by the fact that fish feeds contain three times as much protein as conventional livestock feeds. A large proportion of the protein in fish diets must, therefore, be catabolised for energy rather than being deposited as growth.

In order to satisfy the energy requirements of fish it is wasteful to use dietary protein as, per kilocalorie, protein is an expensive energy source. It has been estimated that between 50 and 70% of the available energy in commercial trout feeds is supplied by protein.(Austreng, 1976; Phillips, 1969).

Therefore, investigation of the possibility of reducing the proportion of dietary protein used for energy, and thus increasing the utilization of protein for growth, by the use of high energy diets would seem to be necessary.
Such studies are of even greater importance when considered

with respect to the higher metabolic energy requirement of a warm water species, such as carp reared at 28°C, as compared with that of a cold water species, such as rainbow trout reared at 12 - 15°C (Winberg, 1956).

The total energy contents of protein, lipid and carbohydrate have been estimated as 5.56, 9.5 and 4.1 kcal/g respectively (Brody, 1945). Thus, in terms of energy supplied per gram, dietary lipid should have the greatest protein sparing effect.

Numerous studies have been made of the effects of increasing the dietary energy intake, by increasing levels of lipid, on food conversion, protein utilization and growth of various fish species (Tiemeier et al., 1965; Stickney & Andrews, 1972; Lee & Putnam, 1973; Sin, 1973a,b; Adron et al., 1976; Higuera et al., 1977; Viola & Rappaport, 1978; Reinitz et al., 1978; Takeuchi et al., 1978a,b,c).

The present study is particularly relevant as other authors have reported that lipid metabolism (digestion, absorption and utilization) is improved with increasing temperature (Atherton & Aitken, 1970; Shcherbina & Kazlauskene, 1971; Stickney & Andrews, 1972; Andrews et al., 1978).

Thus an experiment was designed to investigate the protein sparing capacity of lipid in carp diets. Rations were formulated to contain 21, 29, 37 & 45% protein with lipid levels, at each protein level, of 6, 12 & 18%. These diets were fed to mirror carp fingerlings at 28°C for a period of seven weeks and their effects on growth performance, carcass composition and food utilization determined.

Section 4.2. Materials and Methods.

Section 4.2.1. The Experimental System and Animals.

The experimental facility used in the present study was 'System 1' as described in detail in section 2.1.1.

250 fingerling mirror carp (6 - 10 cm) were obtained from Humberside Fisheries, Skerne, Driffield. The fish were subjected to quarantine and prophylaxis, as described in section 2.4., and were then transferred to three of the 230 l tanks of System 1 at the prevailing ambient temperature of 15°C. The temperature was then raised at approximately 3°C/day to 28°C. 20 fish at a time were then allocated to each of the twelve experimental tanks and the remainder discarded.

The fish in each tank were then individually tagged (section 2.5.) and fed on a commercial trout ration for a further week. During the period of temperature acclimation and tagging there were no mortalities, fish feeding actively throughout. 12 fish, one from each group, were then removed for initial proximate carcass analysis (section 2.6.).

Photoperiod was controlled at 14 hours light and 10 hours darkness throughout the experiment.

Section 4.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general procedure described in section 2.2 and the ingredients used are listed in Table 13. Each group of three diets with a common protein level was isonitrogenous and increased levels of dietary lipid were attained by the replacement of ∝-cellulose with herring oil. The diets were prepared by wet extrusion as detailed in section 2.3. Proximate analysis (section 2.6.) was carried out on samples of the diets and the results are

Table 13. Ingredient Composition of the Diets used in Experiment 2.

Ingredient (dry weight)	DIET 1	2	3	4	5	6	7	8	9	10	11	12
Herring Meal	29.55	29.55	29.55	40.81	40.81	40.81	52.06	52.06	52.06	63.32	63.32	63.32
Herring Oil	3.83	9.83	15.83	3.00	9.00	15.00	2.17	8.17	14.17	1.34	7.34	13.34
Mineral Mix[1]	8.71	8.71	8.71	6.32	6.32	6.32	3.93	3.93	3.93	1.53	1.53	1.53
Starch	4.91	4.91	4.91	4.88	4.88	4.88	4.84	4.84	4.84	4.81	4.81	4.81
Binder[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
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
Glucose	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
∝-Cellulose	36.50	30.50	24.50	28.49	22.49	16.49	20.50	14.50	8.50	12.50	6.50	0.50
Vitamins[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated;-												
Crude Protein (%)	21.0	21.0	21.0	29.0	29.0	29.0	37.0	37.0	37.0	45.0	45.0	45.0
Crude Lipid (%)	6.0	12.0	18.0	6.0	12.0	18.0	6.0	12.0	18.0	6.0	12.0	18.0

1 Composition given in Table 6 section 2.2.

2 Carboxymethylcellulose, Sodium Salt, High Viscosity.

3 Composition given in Table 7 section 2.2.

presented in Table 14 with estimated components of the diets in Table 15.

Section 4.2.3. Feeding Rates.

The fish were fed four times per day between 08.30 h and 18.30 h with each feed being distributed over a period of 15-20 minutes. All groups were fed 5% of their body weight per day with allowance made for the moisture content of the diets. The quantity of food delivered per day was adjusted after each weekly weighing and fed for the subsequent six days.

Section 4.2.4. Weighing and Sampling.

Details of the weighing procedure are presented in section 2.5. Fish were individually weighed ($\pm$ 0.01 g), under anaesthesia, after 12 hours starvation, every seven days. At the end of the seventh (and final) week 4 fish were removed from each group for proximate carcass analysis (section 2.6.) the results of which are presented in Table 18. Faeces were obtained from the remaining fish, as described in section 2.7.4, and analysed for crude protein, energy (section 2.6.) and chromic oxide (section 2.7.4.).

Section 4.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

Section 4.3. Results.

Fish in all groups fed aggressively throughout the experiment appearing to consume all of their daily ration. No mortalities occurred during the experiment.

Table 14. Proximate Analyses of the Diets used in Experiment 2.

Component	DIET 1	2	3	4	5	6	7	8	9	10	11	12
Moisture (%)	8.00	6.83	7.46	8.60	8.62	7.79	8.66	8.81	8.26	8.13	8.86	7.17
On a moisture free basis;-												
Crude Lipid (%)	6.33	12.53	18.42	5.83	12.68	18.59	6.44	12.74	19.13	6.41	12.78	19.01
Crude Protein (%)	21.11	20.76	20.94	29.20	29.08	28.67	36.95	36.75	36.85	45.45	45.49	45.37
Ash (%)	13.66	13.94	13.96	14.77	15.11	14.42	15.14	15.95	15.87	16.59	17.07	16.57
NFE[1] (%)	58.90	52.77	46.67	50.20	43.13	38.31	41.47	34.57	28.14	31.57	24.65	19.05
Chromic Oxide (%)	0.53	0.40	0.43	.0.49	0.48	0.48	0.44	0.50	0.48	0.44	0.49	0.44
Energy (kcal/g)	4.28	4.64	4.96	4.39	4.72	5.05	4.47	4.83	5.13	4.59	4.89	5.17

1 NFE = Nitrogen Free Extractives.

Table 15. Estimated Components of the Diets used in Experiment 2.

Component	DIET 1	2	3	4	5	6	7	8	9	10	11	12
Carbohydrate (%) (including NFE from fishmeal)	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Fibre (%) (∝-cellulose)	36.50	30.50	24.50	28.49	22.49	16.49	20.50	14.50	8.50	12.50	6.50	0.50
Total Energy (kcal/g)	4.07	4.39	4.70	4.15	4.54	4.83	4.32	4.65	5.02	4.47	4.82	5.16
Metabolisable Energy (ME) (kcal/g)	2.22	2.74	3.25	2.55	3.12	3.61	2.95	3.47	4.02	3.33	3.87	4.40
P:E Ratio (mg protein per kcal of ME)	95.0	75.8	64.4	114.5	93.2	79.4	125.3	105.9	91.7	136.5	117.5	103.11
% of the ME supplied by Protein	42.72	34.15	29.03	51.63	41.92	35.76	56.45	47.63	41.23	61.50	52.89	46.46

Section 4.3.1. Growth Performance.

Growth responses of the various groups fed the different diets are represented graphically in figures 20 - 26. Figures 20 - 23 show that increasing the dietary lipid level increased growth at each protein level.

Figures 24 - 26 show the effect of increasing the dietary protein level at each level of lipid. Figure 24 shows that, at a dietary lipid level of 6%, increasing the protein level from 21 to 45% increased the growth response. Figures 25 & 26 show that at dietary lipid levels of 12 & 18% increasing the dietary protein level from 21 to 37% increased the growth response but that further increasing the dietary protein level to 45% resulted in decreased growth and a final weight similar to that at the 29% protein level.

Statistical analysis of the initial fish weights (Table 16) shows no significant differences ($p > 0.05$) between any of the groups. Significant differences ($p < 0.05$) in final average fish weights showed a general trend of increasing final weight with increasing dietary lipid level at each protein level. Statistical analysis of final average fish weights ranks the diets in the following order 6, 8, 9 & 12 > 5, 7, 10 & 11 > 2, 3, & 4 > 1.

The differences in final average fish weights were reflected by differences in Specific Growth Rates (SGRs, section 2.7.1.) which are presented in Table 16. SGRs of fish fed dietary lipid levels of 12 & 18% were significantly greater ($p < 0.05$) than growth on diets containing 6% lipid at each protein level with the exception of 45% protein where no significant difference occurred at any lipid level. Statistical analysis of SGRs ranks the diets in the following order 6, 8, 9 & 12 > 5, 10 & 11 > 3, 4, & 7 > 2 > 1.

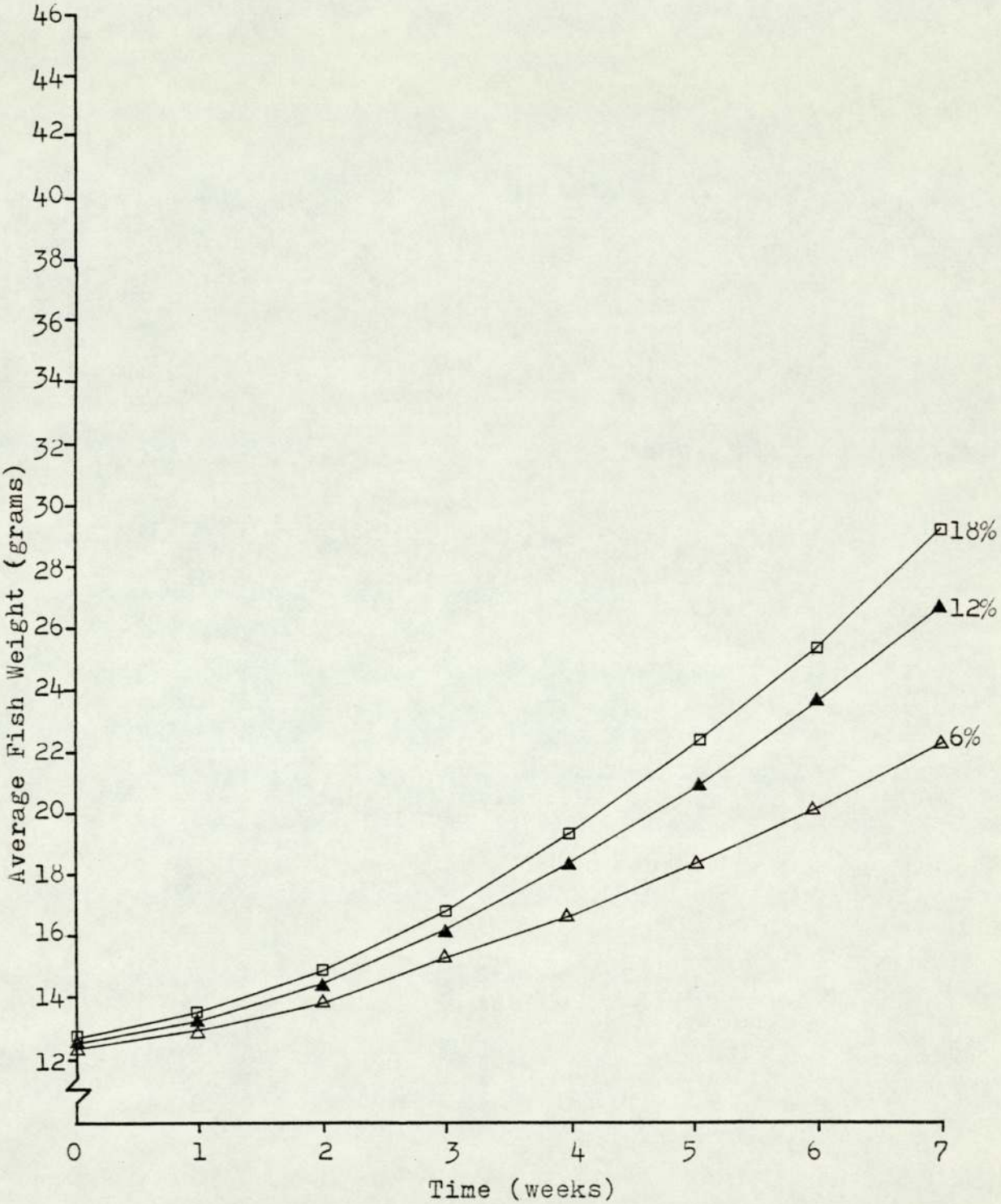


Figure 20. Growth responses of carp fed 21% protein and three levels of dietary lipid.

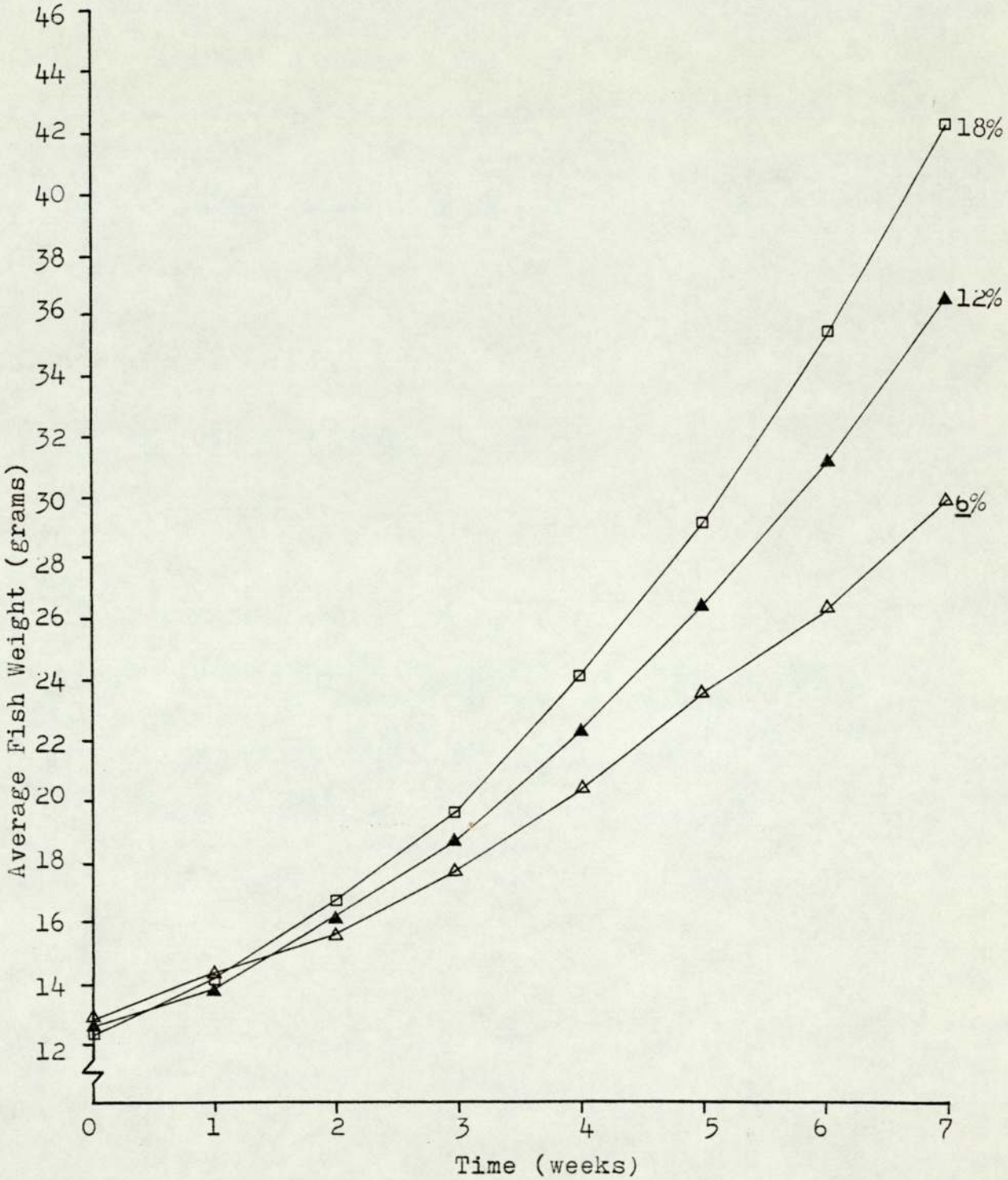


Figure 21. Growth responses of carp fed 29% protein and three levels of dietary lipid.

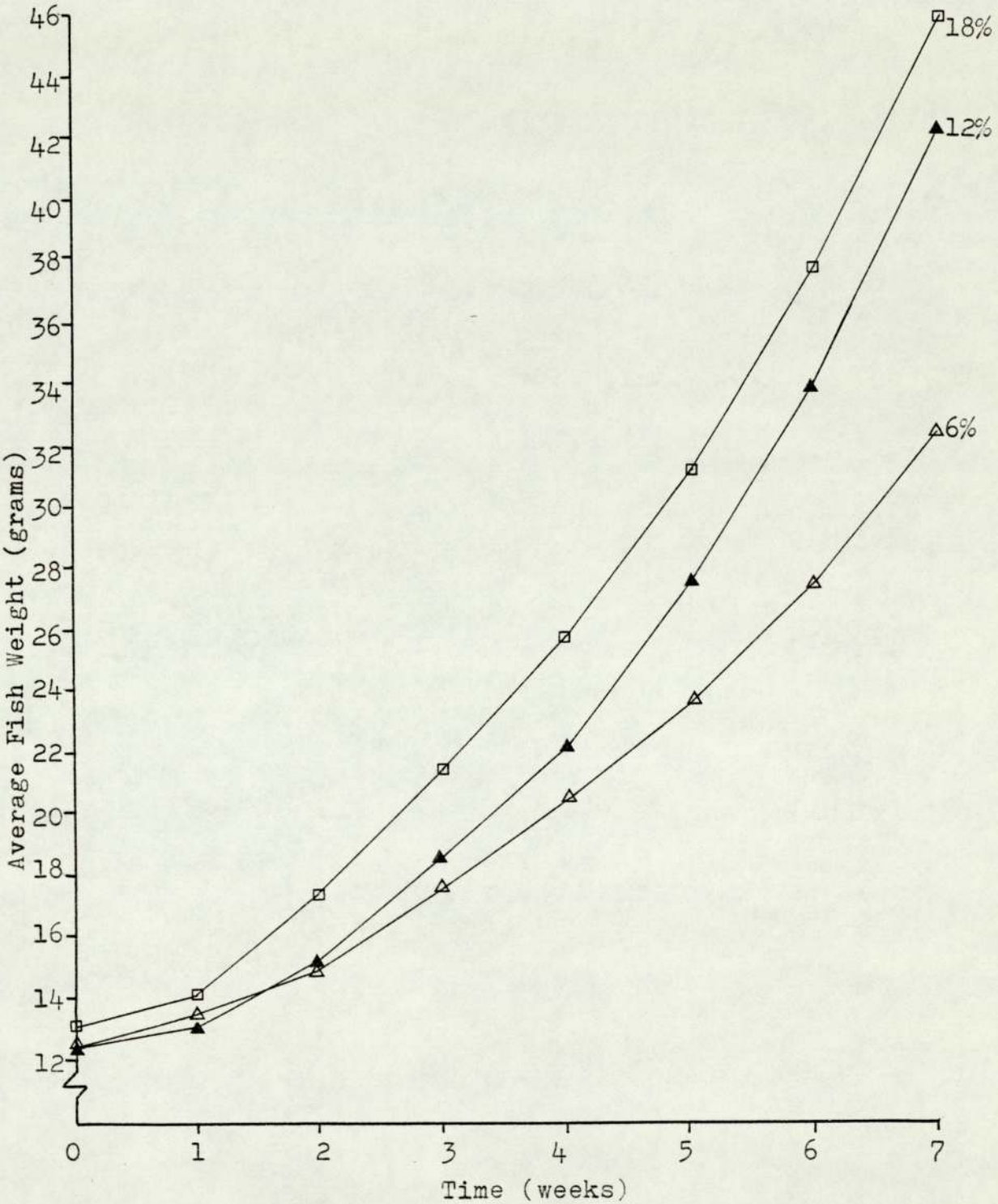


Figure 22. Growth responses of carp fed 37% protein and three levels of dietary lipid.

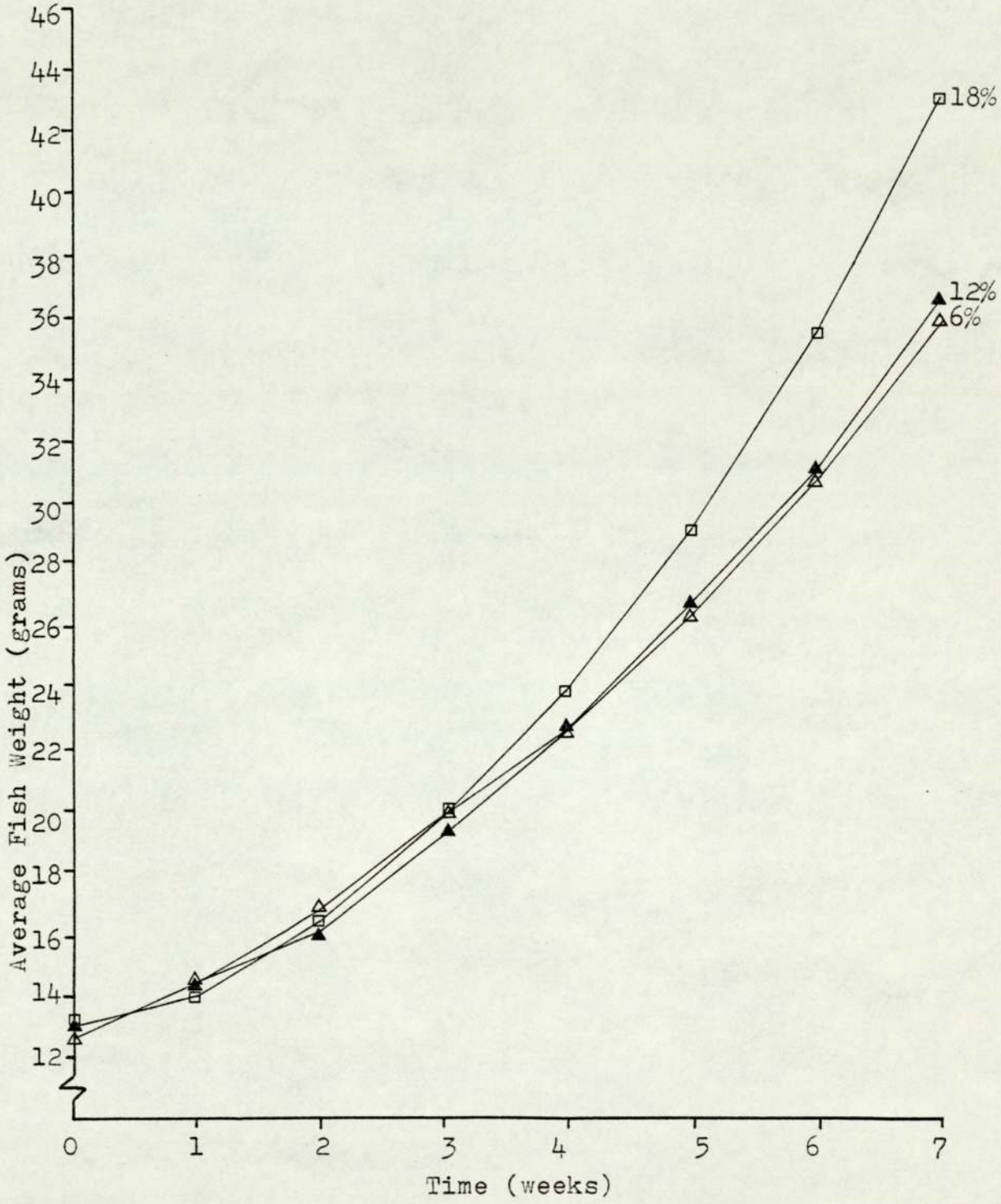


Figure 23. Growth responses of carp fed 45% protein and three levels of dietary lipid.

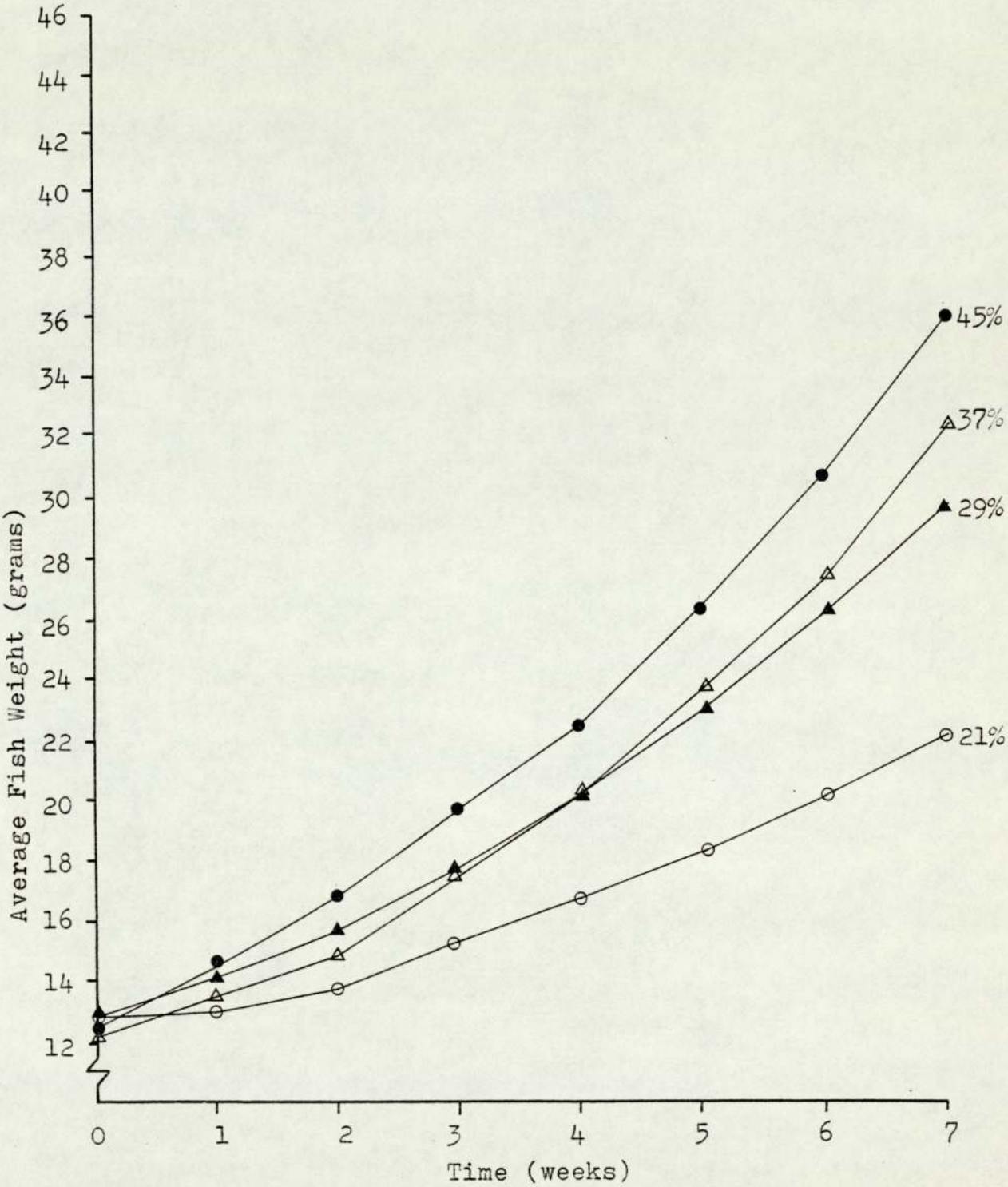


Figure 24. Growth responses of carp fed 6% lipid and four levels of dietary protein.

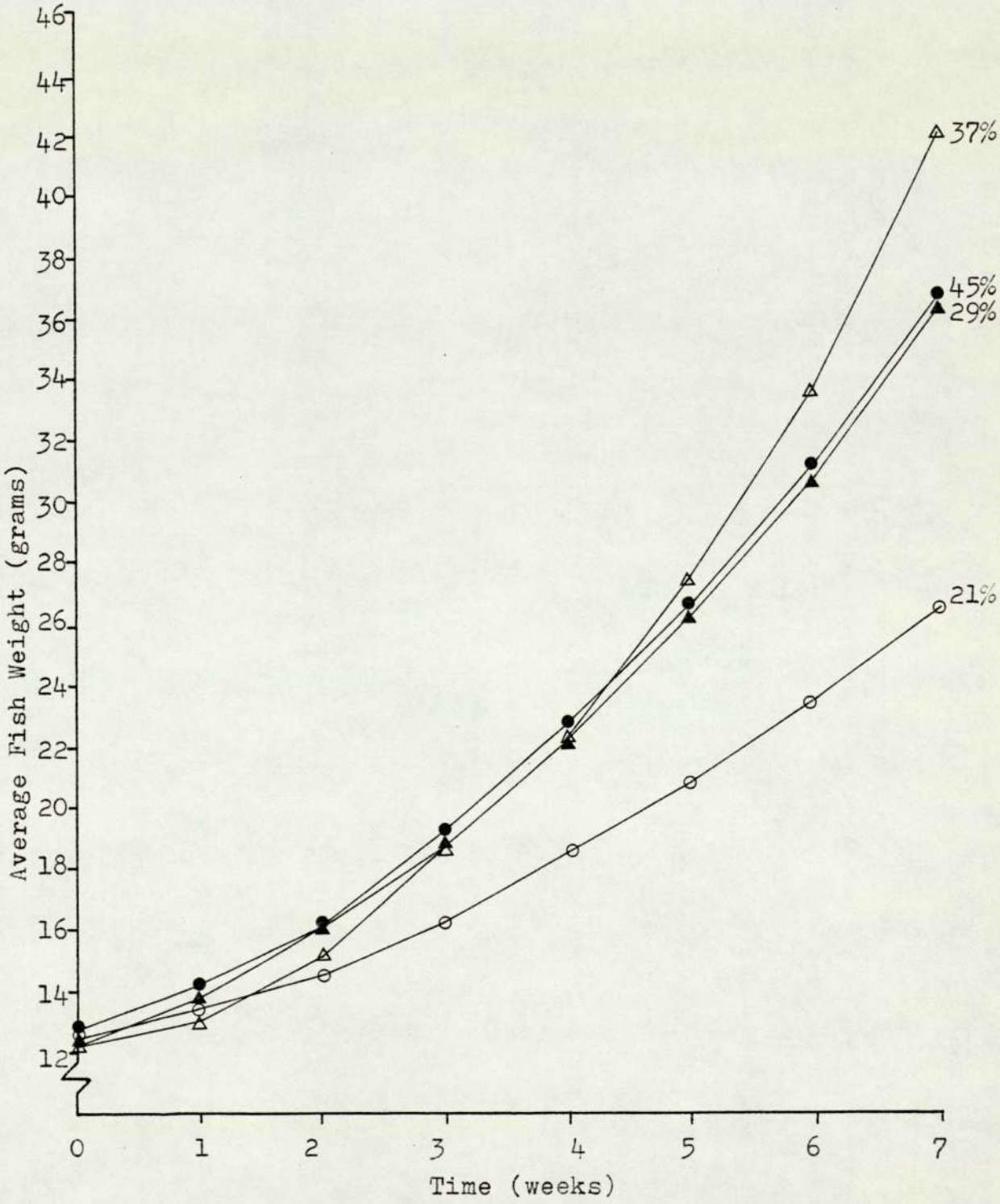


Figure 25. Growth responses of carp fed 12% lipid and four levels of dietary protein.

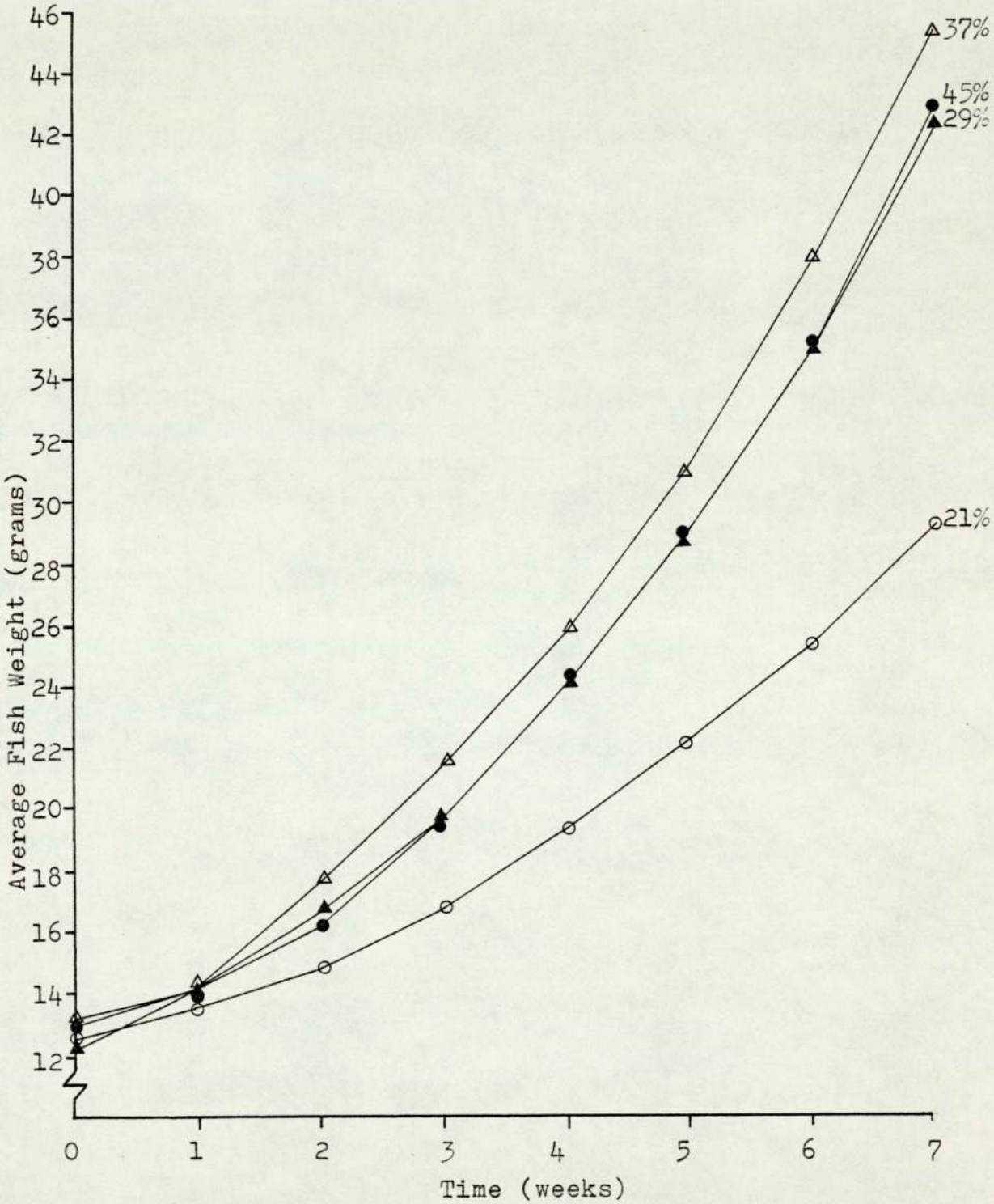


Figure 26. Growth responses of carp fed 18% lipid and four levels of dietary protein.

Table 16. Growth and Food Utilization Data from Experiment 2.

	DIET											
	1	2	3	4	5	6	7	8	9	10	11	12
Protein(%)	21	21	21	29	29	29	37	37	37	45	45	45
Lipid(%)	6	12	18	6	12	18	6	12	18	6	12	18
Initial Av.Wt.(g)	12.38^{a}	12.44^{a}	12.53^{a}	12.75^{a}	12.34^{a}	12.27^{a}	12.28^{a}	12.30^{a}	13.12^{a}	12.56^{a}	12.74^{a}	12.84^{a}
Final Av.Wt.(g)	22.20^{d}	26.58^{c}	29.22^{c}	29.82^{c}	36.59^{b}	42.65^{a}	32.44^{bc}	42.28^{a}	45.92^{a}	36.02^{b}	36.74^{b}	43.00^{a}
SGR[1](%/day)	1.28^{e}	1.56^{d}	1.73^{cd}	1.71^{cd}	2.27^{b}	2.47^{ab}	1.96^{c}	2.58^{a}	2.56^{a}	2.15^{bc}	2.21^{bc}	2.40^{ab}
FCR[2]	4.03	3.09	2.77	2.28	1.74	1.49	1.93	1.47	1.43	1.76	1.79	1.51
PER[3]	1.41	1.91	2.13	1.50	1.98	2.34	1.40	1.85	1.90	1.15	1.23	1.46
Apparent NPU[4](%)	28.39	29.77	30.49	23.28	27.43	31.63	19.12	23.37	27.62	17.90	16.20	17.90

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)
1 Specific Growth Rate
2 Food Conversion Ratio
3 Protein Efficiency Ratio
4 Net Protein Utilization

Section 4.3.2. Food Conversion.

Food Conversion Ratios (FCRs, section 2.7.2.) were obtained for each group and are presented in Table 16. FCR decreased with increasing dietary protein level, at each level of dietary lipid, with the exception of 45% protein. Diets containing 12 & 18% lipid at the 45% protein level had poorer FCRs than those at 37 or 29% protein.

Increasing the level of dietary lipid at each protein level resulted in decreased FCR, again with the exception of the 45% protein level where increasing the dietary lipid level from 6 to 12% increased FCR.

Section 4.3.3. Carcass Composition.

The initial and final proximate carcass analyses (section 2.6.) are presented in Table 18. As reported by other authors, and discussed in Experiment 1 (Chapter 3), the final carcass levels of moisture and lipid appear to be inversely related. Increasing the level of dietary lipid at each protein level increased the carcass lipid content as did increasing the protein content at each level of dietary lipid; in several instances these differences were significant ($p < 0.05$).

Section 4.3.4. Protein Utilization.

Protein Efficiency Ratios (PERs, section 2.7.3.) were calculated for each group and are presented in Table 16. PER increased with increasing dietary lipid level at all protein levels. Little difference existed between PERs at 21 & 29% protein but above 29% PER decreased with increasing dietary protein level at each level of dietary lipid.

Values for apparent Net Protein Utilization (NPU, section 2.7.3

Table 17. Digestibility Data from Experiment 2.

	Diet											
	1	2	3	4	5	6	7	8	9	10	11	12
Protein(%)	21	21	21	29	29	29	37	37	37	45	45	45
Lipid(%)	6	12	18	6	12	18	6	12	18	6	12	18
Apparent Protein Digestibility (%)	82.43	83.52	85.89	84.63	86.86	87.54	85.29	88.08	90.08	82.95	67.95	82.29
Apparent Energy Digestibility(%)	60.08	64.55	69.77	70.46	73.43	71.35	73.02	74.54	69.91	74.73	48.19	71.43
Digestible Energy (kcal/g)	2.57	2.99	3.46	3.09	3.47	3.60	3.27	3.60	3.59	3.43	2.35	3.69
P:E Ratio (mg protein/kcal of digestible energy)	82.1	69.4	60.5	94.5	83.8	79.6	113.0	102.1	102.6	132.5	193.6	123.0

Table 18. The Results of Proximate Carcass Analysis of Initial and Final Samples from Experiment 2.

Sample		Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Initial		82.3	2.0	11.1	2.8	98.2
Final;-						
Diet(Protein/Lipid)						
1	(21/6)	76.2^{a}	5.2^{d}	13.6^{a}	3.4^{a}	98.4
2	(21/12)	76.0^{a}	6.0^{d}	13.1^{ab}	3.3^{a}	98.4
3	(21/18)	75.1^{ab}	6.9^{cd}	12.9^{ab}	3.2^{ab}	98.1
4	(29/6)	75.6^{ab}	5.7^{d}	13.6^{a}	3.2^{a}	98.1
5	(29/12)	75.5^{ab}	6.6^{cd}	12.9^{ab}	3.0^{b}	98.0
6	(29/18)	73.9^{b}	8.3^{b}	12.8^{ab}	2.9^{bc}	97.9
7	(37/6)	76.3^{a}	6.4^{cd}	12.7^{ab}	2.7^{c}	98.1
8	(37/12)	75.4^{ab}	7.3^{c}	12.2^{b}	2.5^{c}	97.4
9	(37/18)	73.5^{b}	8.6^{b}	13.5^{a}	2.4^{cd}	98.0
10	(45/6)	75.3^{ab}	6.9^{cd}	13.3^{ab}	2.4^{cd}	97.9
11	(45/12)	75.0^{ab}	8.0^{bc}	12.4^{ab}	2.4^{cd}	97.8
12	(45/18)	74.2^{b}	9.5^{a}	11.9^{b}	2.2^{d}	97.8
	S.E.±	0.462	0.287	0.368	0.084	

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

were determined for each group and are presented in Table 16. Apparent NPU increased with increasing lipid content at dietary protein levels of 21, 29 & 37%. At the 45% protein level apparent NPUs for all dietary lipid levels were extremely low with that at the 12% lipid level being the lowest.

Section 4.3.5. Protein Digestibility.

Apparent protein digestibilities were determined as described in section 2.7.4 and are presented in Table 17. Protein digestibility tended to increase with increasing lipid at each dietary protein level, except 45% where digestibility at 6 & 18% lipid was equal and digestibility at 12% lipid exceptionally low.

Apparent protein digestibility at the 37% protein level was higher, at all three levels of dietary lipid, than at the other protein levels.

Section 4.3.6. Dietary, Metabolisable and Measured Digestible Energy Values.

The total energy contents of the diets were estimated by assuming total energy values for protein, lipid and carbohydrate of 5.65, 9.45 and 4.1 kcal/g respectively (Brody, 1945). These calculated values are presented in Table 15 and are consistently lower than the total energy contents of the diets as measured by bomb calorimetry (section 2.5.) and presented in Table 14. The reasons for this are not clear, the calculated value being based on analysed protein and lipid together with estimated carbohydrate and fibre. It may have been that some of the dietary ingredients not included in the calculation, vitamins, minerals, binder etc., may have produced energy when

burnt in the bomb calorimeter.

The calculated metabolisable energy (ME) contents of the diets are presented in Table 15 and are based on literature values for the ingredients. Protein was assigned a value of 4.5 kcal/g (Smith, 1971), a value of 8.51 kcal/g for crude lipid was based on an assumed digestibility of 90% (Austreng, 1978). The value used for glucose was 4.0 kcal/g assuming a digestibility of 99% (Phillips, 1972) and that for combined nitrogen free extractives (from the fishmeal) and starch was 3.49 kcal/g based on a digestibility of 85% (Chiou & Ogino, 1975).

The measured digestible energies (section 2.7.4.) presented in Table 17 are higher than the calculated metabolisable energies (Table 15) where the calculated ME is less than 3.6 kcal/g. Where the calculated ME is 3.6 kcal/g or greater the measured digestible energy remained at approximately 3.6 kcal/g.

At dietary protein levels of 21 & 29% increasing the level of dietary lipid increased the energy digestibility. This is to be expected as highly digestible lipid replaced poorly digestible α-cellulose in the diets.

Section 4.4. Discussion and Conclusions.

The protein sparing action of dietary lipid is evident, in this study, from the increasing values for Specific Growth Rate (SGR), Protein Efficiency Ratio (PER) and apparent Net Protein Utilization (NPU) at each protein level with increasing levels of dietary lipid. In this experiment it proved possible to reduce the protein content of diets containing 18% lipid from 45 to 29% with no diminution in weight gain and with improved utilization of dietary protein. This finding is in

general agreement with that of others. Sin (1973a,b) showed that the optimum protein content of diets for mirror carp (Cyprinus carpio) was reduced from 38 to 33% by increasing the dietary energy from 2.8 to 3.1 kcal/g by the addition of 5% soybean oil to the diets.

Fish oil, used as the sole lipid source, was found to spare dietary protein for growth in rainbow trout (Phillips, 1969). More recently Takeuchi (1978b) found that protein levels could be reduced from 48 to 35%, in trout diets, with no loss in weight gain given dietary lipid levels of 15 to 20%.

Results obtained with turbot (Scopthalamus maximus) present a similar picture (Adron et al., 1976). Protein levels were reduced from 50 to 35% with no loss of weight gain and with improved protein utilization provided that the diets had a gross energy content of 3 kcal/g.

Garling and Wilson (1976), working on the channel catfish (Ictalurus punctatus), found that at a total dietary energy level of 2.75 kcal/g fish fed diets containing 24% protein had a significantly higher protein deposition (which is the same as apparent NPU) than fish fed higher levels of protein at a protein to energy (P:E) ratio of 88 (mg of protein per kilocalorie). In the present study the highest value for apparent NPU occurred at a P:E ratio of 79.4 mg of protein per kcal (of calculated metabolisable energy, ME) or 79.6 mg of protein per kcal (of measured digestible energy). This result was obtained for diet 6 which contained 29% protein and 18% lipid.

Buhler & Halver (1961) found, conversely to the above, that increasing the lipid content of diets for chinook salmon (Onchorhynchus nerka) from 7 to 14%, in diets containing 36% protein, did not increase the growth rate. They concluded that the additional calories, supplied by corn oil, were not available to this species.

Murray et al. (1977) found, with channel catfish, that increasing the dietary lipid from 5 to 12% resulted in increased weight gain on a diet containing 35% protein but not on a diet containing 25% protein. Conversely, in the present study increasing the dietary lipid level resulted in increased weight gain even at the lowest level of protein (21%). At low protein levels it would be expected that increasing the level of dietary non-protein energy would result in increased growth as a proportion of the protein would be spared for growth rather than being metabolised for energy.

The maximum amount of dietary lipid which could be tolerated, without growth depression, by carp did not appear to have been reached, even at the 18% level, in this experiment. Results for channel catfish, however, showed depression of growth when dietary lipid levels were raised from 12 to 16% of the dry diet (Dupree, 1969). Other authors report no growth depressing or pathological effects of very high levels of dietary lipid. Higashi et al. (1964) fed diets containing 25% marine oil to rainbow trout with no ill-effects and Kitamikado et al. (1964) fed diets containing up to 30% lipid. More recently Takeuchi (1978d) fed diets containing 54% protein and 20% lipid to rainbow trout with no observed pathological disturbances. Higuera et al. (1977) fed a diet containing 18% lipid to rainbow trout for six months with no pathological disturbances and, in fact, found functional adaptation to the 'high fat' diet as the excretion of bile salts increased by approximately 27%.

The digestibility data obtained in this study (Table 17) are not in complete agreement with that obtained by others. The results presented here show a trend of increasing apparent

protein digestibility with increasing dietary lipid at protein levels of 21, 29 & 37%. In addition, protein digestibility increases with increasing protein level from 21 to 37% at each level of dietary lipid.

Higuera et al. (1977), with rainbow trout, reported no change in apparent protein digestibility with changes in either dietary protein or lipid. Increasing apparent protein digestibility with increasing dietary protein level has, however, been reported for rainbow trout (Nose, 1967; Austreng, 1976), channel catfish (Smith & Lovell, 1973) and carp (Ogino & Chen, 1973). This may be because apparent protein digestibility contains no correction for endogenous nitrogen excretion (ENE). In fish fed low protein diets the ENE makes up a large percentage of the faecal nitrogen thus lowering apparent protein digestibility. On high protein diets, however, ENE is a small fraction of the faecal nitrogen and apparent digestibility approaches the true digestibility.

Page & Andrews (1973) reported, with channel catfish, that decreasing the P:E ratio increased the apparent protein digestibility - a similar result to that found here. The observed increase in apparent protein digestibility with increasing level of dietary lipid might be explained by the results of Shcherbina et al. (1976) who reported that the rate of absorption of protein from the gut of carp, as measured by the activity of proteases, increased with increasing levels of dietary lipid.

A further possible explanation is that as the percentage of dietary lipid increased in the diets the level of α-cellulose decreased. Both high fat and low fibre levels are known to increase the gut retention time in higher animals (Bender,

1967) so increasing the time available for enzymic breakdown and absorption of the protein.

Takeuchi (1978c) found no change in protein or lipid digestibility, with varying dietary lipid levels of 5 to 25% at a fixed protein level of 35%, in rainbow trout. This author also reports a constant energy retention (equivalent to apparent energy digestibility) of 60% regardless of the dietary lipid level. In the present study apparent energy digestibility (Table 17) increased with increasing dietary lipid level at the lowest protein level (21%), but at higher levels of dietary protein varied between 70 & 75% independant of the dietary lipid level.

Apparent protein digestibilities (Table 17) obtained in this experiment were generally lower than those previously reported for fishmeal. Ogino & Chen (1973) reported a digestibility of 95% for white fishmeal in carp at 23°C and a feeding rate of approximately 3% of the body weight per day. The lower values obtained in the present study may have been due to the decrease of protein digestibility with increasing temperature, the present study being conducted at 28°C, or with increasing feeding rate, 5% of the body weight per day in this trial.

That temperature affects the rate of passage of food through the gastrointestinal tract of fish has been shown by several authors (Shrable et al., 1969; Brett & Higgs, 1970). Shcherbina & Kazlauskene (1971) reported a fall in the digestibility of sunflower protein in carp diets with increasing temperature from 16 to 27°C which they attribute to the increased rate of passage of food through the gut. Ogino et al. (1973) found that the endogenous nitrogen excretion (ENE) of carp increased from 7.2 mgN/100 g of fish/day at 20°C to 8.6 at 27°C. This

increase in ENE with increasing temperature would also cause a fall in apparent protein digestibility with increasing temperature as no correction is made for its prescence in the faeces.

A decrease in digestibility with increasing food intake has been reported by other authors (Brown, 1946a,b; Kinne, 1960) and has been attributed to an influence of meal size on either the rate of passage of food through the intestine or on absorptive processes.

Table 18 presents the results of proximate carcass analysis of the fish at the termination of the experiment. The levels of moisture and lipid appear to be inversely related as reported by other authors (Andrews & Stickney, 1972; Brett et al., 1969; Papoutsoglou & Papoutsoglou, 1978; Takeuchi, 1978a). Carcass lipid contents rose with increasing dietary lipid level at each protein level, and with increasing protein level at each level of dietary lipid. Increased carcass lipid contents with increasing levels of dietary lipid have been reported for channel catfish (Page & Andrews, 1973; Murray et al., 1977; Garling & Wilson, 1976), chinook salmon (Buhler & Halver, 1961), turbot (Adron et al., 1976), rainbow trout (Lee & Putnam, 1973; Austreng, 1976; Takeuchi, 1978d; Reinitz et al., 1978) and mirror carp (Sin, 1973a,b).

The observed increase in carcass lipid content with increasing dietary protein level may be due to the 'spared' excess dietary protein being metabolised to lipid for energy storage. That carp are able to effectively synthesise lipids from excess dietary amino acids has been shown by Nagai & Ikeda (1973). Increased carcass lipid with increasing dietary protein level has not, in general, been reported by other

authors. Meske & Pffefer (1978) found a decrease in carcass lipid level from 15 to 10% with increasing dietary protein levels from 20 to 40% in mirror carp. Cho et al. (1976) found, with rainbow trout, that increasing the dietary protein level from 40 to 60% had no effect on carcass lipid.

During the present trial the maximum level of carcass lipid obtained was 9.5%; this is considered to be perfectly acceptable to the consumer as carp is generally supposed to be a fatty fish with carcass lipid levels of 20% reported for pond reared fish (Meske & Pffefer, 1978). In intensive culture systems using high culture temperatures carcass lipid levels of 10% are more generally obtained (Sin, 1973a,b; Takeuchi, 1978a).

From the results of this experiment it is apparent that the protein sparing effect of dietary lipid is pronounced at protein levels below 45%. Even at a dietary lipid level of 18% it does not appear that the maximum protein sparing effect has been obtained. Thus, theoretically, levels of lipid in excess of 18% might be expected to spare even more protein for growth. However, in the present study it was found that when the calculated metabolisable energy (Table 15) exceeded 3.6 kcal/g there was no increase in observed digestible energy (Table 17) indicating that a maximum amount of utilizable energy may have been reached under these experimental conditions.

In addition, reducing the P:E ratio further would probably result in a reduced protein intake if carp do, in fact, feed to a set level of dietary energy. Lee & Putnam (1973) reported that rainbow trout adjust their total food intake to a set energy level and thus diets with very low P:E ratios resulted in reduced growth due to reduced protein intake. Rozin &

Mayer (1961) reported that increasing the caloric density of diets reduced the voluntary food intake of goldfish (Carassius auratus).

Further difficulties in the formulation of feeds containing levels of dietary lipid in excess of 18% are problems with the mechanics of the pelletising process and the difficulty in storing such diets so as to prevent oxidative rancidity of the lipid. These obstacles might be reduced by the use of saturated fats in carp feeds.

Andrews et al. (1978) reported excellent utilization of solid animal fat by channel catfish which they attributed to the high culture temperatures employed (28°C). These authors theorise that highly saturated fats are poorly utilized by salmonids due to their solidification in the gastrointestinal tract at the low culture temperatures employed.

However, Yu et al. (1977) reported that growth of rainbow trout was not depressed when 50% of the fish oil, in a diet containing 22% lipid, was replaced by swine fat (lard). For mirror carp Takeuchi (1978a) found that if more than 50% of the pollack oil, in a diet containing 10% lipid, was replaced with saturated fats then growth was depressed.

It has been theorised that the requirement of fish for polyunsaturated fatty acids (PUFA) is lower at higher temperatures due to a change in the composition of fish phospolipids (Cowey & Sargent, 1977; Castell, 1978). Thus the ability of warm water fish to grow adequately on diets containing saturated fats may be due to a reduction in their requirement for PUFA.

Carp may also be able to utilize acidulated soap-stocks (acid treated crude lipids) which contain high levels of free

fatty acids (Viola & Rappaport, 1978). In experiments on the nutrition of carp in cages they grew as well on a diet containing acidulated soyabean soapstocks as they did on neutral lipids, including fish oils. The utilization of 'soapstocks' in carp diets would be of economic significance as they are a cheap source of dietary lipid and are already extensively used in animal feedstuffs.

Some general conclusions may be drawn from the results and discussion presented here. It is possible to reduce the protein content of diets for mirror carp from 45 to 29%, at a dietary lipid level of 18%, with no reduction in growth performance, with improved protein utilization and with no unacceptable accumulation of carcass lipid. This corresponds to a reduction in the P:E ratio from 123 to 80.

The diet which resulted in the highest NPU and PER contained 29% protein and 18% lipid with only 36% of the calculated metabolisable energy of the diet being supplied by protein.

These results demonstrate the beneficial effects, both economic and nutritional, of increasing the level of dietary lipid as compared to conventional 'low fat' fish feeds.

CHAPTER 5.

Chapter 5.

5. Experiment 3. The Evaluation of Yeast, Bacterial, Soyabean, Algal, Casein and Fishmeal Proteins in Semipurified Diets for Fingerling Mirror Carp (Cyprinus carpio).

NOTE: This Experiment was conducted jointly with Mr.T.H.Atack and will also be submitted by him as a part of his dissertation for the degree of Ph.D. of the University of Aston in Birmingham.

Section 5.1. Introduction.

In recent years there has been much interest in the possibility of using various unconventional protein sources as a replacement for fishmeal in compounded fish feeds. Most of this has centered on feeds for salmonids as this family includes the most commonly reared species raised on artificial diets.

Many of the results obtained so far have proved encouraging. At least partial replacement of fishmeal with yeast, bacterial and soyabean proteins seems possible (Hoshai, 1972; Andruetto et al., 1973; Cho et al., 1974; Bergstrom, 1978; Spinelli et al., 1978). However, there has been little information published on the utilization of such materials as the sole protein sources in fish rations and thus little is known of their basic characteristics. Atack & Matty (1978) and Tiews et al. (1978) have recently reported on the utilization of petroyeast, bacterial and soyabean proteins as the sole protein sources in diets for rainbow trout (Salmo gairdneri).

Replacement of fishmeal in diets of warm water fish species has received less attention since complete compounded rations are only recently being employed in the commercial culture of such species. Some results have been reported on the utilization

of soyabean in channel catfish feeds, Ictalurus punctatus, (Dupree & Sneed, 1966; Krishnandhi & Shell, 1967; Andrews & Page, 1974) and for various yeasts in carp diets (Hoshai, 1972; Ogino & Chen, 1973a; Omae et al., 1978). There have also been attempts to use algal material in diets for mirror carp (Terao, 1960; Meske & Pruss, 1977; Hepher et al., 1978).

Since there is now a trend towards the intensive culture of warm water fish species, such as carp, in heated effluents there is an increasing need for specialised compounded feeds. In view of their digestive system and natural feeding habits it seems possible that species such as carp may be able to use protein sources of lower quality than those normally included in salmonid diets.

Hence the following 40 day growth trial was undertaken to determine the value of petroyeast, bacterial, soyabean and algal proteins, as compared to casein and fishmeal, as the sole protein sources in mirror carp diets.

Section 5.2. Materials and Methods.

Section 5.2.1. The Experimental System and Animals.

The experimental facility used in the present study was 'System 2' as descibed in detail in section 2.1.2.

Fingerling mirror carp (5 - 10 cm) were obtained from the Cotswold Carp Farm, Bourton-on-the-Water, Gloucestershire. The fish were quarantined, as detailed in section 2.4, and then randomly allocated, 10 fish per tank, to the fourteen 12 litre experimental tanks at the prevailing ambient temperature of 17°C. The temperature of the system was then raised at approximately 3°C/day to the experimental temperature of 25°C (this lower temperature was employed as the heating

system could not reliably maintain a temperature higher than 25°C).

During temperature acclimation, and for the subsequent 7 days, the fish were fed a commercial trout ration. They were then starved for 12 hours prior to batch weighing (section 2.5) and adjustment of tank weights, by redistribution of fish, to ensure a uniform starting weight.

Photoperiod was controlled at 12 hours light and 12 hours darkness throughout the experiment.

Section 5.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general method described in section 2.2. The diets (Table 19) were formulated to be identical to those used by Atack & Matty (1978), being isonitrogenous (30% protein) and, as far as possible, isoenergetic on a digestible energy basis. Although 30% is below the 'optimum' level of 38% recommended by Sin (1973a) and Ogino & Saito (1970) it proved optimum in Experiment 2 (Chapter 4) and would minimise the use of dietary protein for energy.

The protein sources used were a petroyeast ('Toprina', BP Proteins Ltd.) a methanophilic bacterium ('Pruteen', ICI Ltd.), an algal protein (Spirulina maxima, Sosa Texacoco, SA Mexico) and an extracted soyabean protein concentrate ('Newprod', T.Lucas & Co. Bristol). The control diets consisted of commercial grade casein and herring meal. A low protein diet was also included (10% casein) so that biological value (BV, section 2.7.3.) and Net Protein Utilization (NPU, section 2.7.3) could be obtained and also that true digestibilities (section 2.7.4.) could be evaluated.

Table 19. Ingredient Composition of the Diets used in Experiment 3.

Ingredient (dry weight)	Low Casein	Casein (CS)	Petroyeast (PY)	Bacterial (MB)	Algal (SA)	Herring Meal (HM)	Extracted Soyabean (SY)
Protein Meal	11.13	37.78	51.56	38.62	47.84	37.03	44.27
Capelin Oil	20.00	15.00	13.55	13.27	12.27	13.22	14.76
Mineral Mix[1]	10.25	7.75	5.53	7.54	6.27	7.73	9.21
Binder[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamins[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glucose	20.15	5.00	5.00	5.00	5.00	5.00	5.00
Starch	28.10	28.10	13.99	25.20	18.25	26.64	16.39
∝-Cellulose	7.87	7.87	7.87	7.87	7.87	7.87	7.87
Chromic Oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated;-							
Crude Protein(%)	30	30	30	30	30	30	30

1 Composition given in Table 6 section 2.2.

2 Carboxymethylcellulose, Sodium Salt, High Viscosity.

3 Composition given in Table 7 section 2.2.

Diet preparation was by wet extrusion (section 2.3.), the results of proximate analysis (section 2.6.) are presented in Table 20 and estimated dietary components in Table 21. Unfortunately proximate analysis revealed that an error in formulation had led to the fishmeal control having a lower protein content, and the bacterial diet a higher protein content, than intended. The implications of this error are considered in the discussion.

Section 5.2.3. Feeding Rates.

Due to the relatively poor food conversion ratios (section 2.7.2.) obtained in Experiment 2 (Chapter 4) it was decided to feed 4% of the body weight per day in the present trial. This lower feeding rate was also adopted as it was foreseen that there might be palatability problems with some of the protein sources.

The fish were fed four times daily between 08.30 h and 16.30h with each feed distributed over a period of 15 to 20 minutes with each diet being fed to duplicate tanks. With certain feeds the fish were unwilling to consume the whole days' ration, if this occurred they were fed only the amount they would consume within a 30 minute feeding period and uneaten food was weighed and deducted from the daily ration. The quantity of food fed was adjusted after each weekly weighing and fed for the subsequent period.

Section 5.2.4. Weighing and Sampling.

Details of the weighing procedure are presented in section 2.5. Fish were batch weighed ($\pm$ 1 g), under anaesthesia, after 12 hours starvation every ten days for forty days. At the end

Table 20. Proximate Analyses of the Diets used in Experiment 3.

	Low Casein	Casein (CS)	Petroyeast (PY)	Bacterial (MB)	Algal (SA)	Herring Meal (HM)	Extracted Soyabean (SY)
Moisture (%)	10.13	9.08	6.53	9.49	8.97	6.74	8.82
On a moisture free basis;-							
Crude Lipid(%)	18.22	15.77	14.23	16.17	14.24	13.69	16.01
Crude Protein(%)	10.30	30.24	30.84	35.19	30.21	25.08	29.93
Ash (%)	7.08	5.84	7.23	9.60	9.46	9.31	8.90
NFE[1](%)	64.40	48.15	47.70	39.04	46.09	51.92	45.16
Chromic Oxide	0.49	0.49	0.47	0.46	0.58	0.50	0.64

1 NFE = Nitrogen Free Extractives

Table 21. Estimated Components of the Diets used in Experiment 3.

	Low Casein	Casein (CS)	Petroyeast (PY)	Bacterial (MB)	Algal (SA)	Herring Meal (HM)	Extracted Soyabean (SY)
Total Carbohydrate(%)	48.25	33.10	33.10	33.10	33.10	33.10	33.10
Fibre (∝-cellulose)(%)	7.87	7.87	7.87	7.87	7.87	7.87	7.87
Total Energy (kcal/g)	4.60	4.88	4.77	5.20	4.74	4.39	4.88
Metabolisable Energy (kcal/g)	4.50	4.06	3.96	4.32	3.93	3.66	4.07
P:E Ratio (mg protein per kcal ME)	22.90	74.50	77.90	81.50	76.90	68.50	73.50

of this period two fish were removed from each group and proximate carcass analysis (section 2.6.) performed, the results of which are presented in Table 22. Faeces were obtained from the remaining fish, as described in section 2.7.4, and analysed for protein, energy (section 2.6.) and chromic oxide (section 2.7.4.).

Section 5.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

Section 5.3. Results.

Groups of fish fed those diets except soyabean and algal fed actively throughout the experiment. Fish fed the soyabean (SY) and algal (SA) diets were reluctant to feed and did not consume all of their allocated 4% ration indicating that these diets were less palatable. No mortalities occurred during this experiment

Section 5.3.1. Growth Performance.

Growth responses of the groups fed the various diets are represented in Figure 19 where the average fish weights, as a mean of the two replicate tanks, are plotted against time.

The methanophilic bacterium (MB) produced the greatest growth response, followed by the casein (CS), herring meal (HM) and petroyeast (PY) diets. The soyabean (SY) and algal (SA) diets produced growth no better than that on the low casein (CS Low) diet.

Statistical analysis of the Specific Growth Rates (SGRs, section 2.7.1.), presented in Table 23, showed that the methanophilic bacterium (MB) gave significantly ($p < 0.05$) the

Table 22. The Results of Proximate Carcass Analysis of Final Samples from Experiment 3.

Sample	Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Low Casein	72.80^{c}	9.46^{a}	12.10^{b}	3.19^{a}	97.55
Casein	75.99^{ab}	5.72^{b}	13.54^{ab}	2.96^{a}	98.21
Petroyeast	75.59^{b}	6.84^{b}	13.20^{ab}	3.02^{a}	98.65
Bacterial	76.83^{ab}	5.90^{b}	13.24^{ab}	3.19^{a}	99.16
Algal	77.32^{a}	6.18^{b}	12.32^{b}	3.04^{a}	98.86
Herring Meal	76.85^{ab}	5.25^{b}	13.84^{a}	2.90^{a}	98.84
Extracted Soyabean	76.82^{ab}	6.34^{b}	13.02^{ab}	3.00^{a}	99.18

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

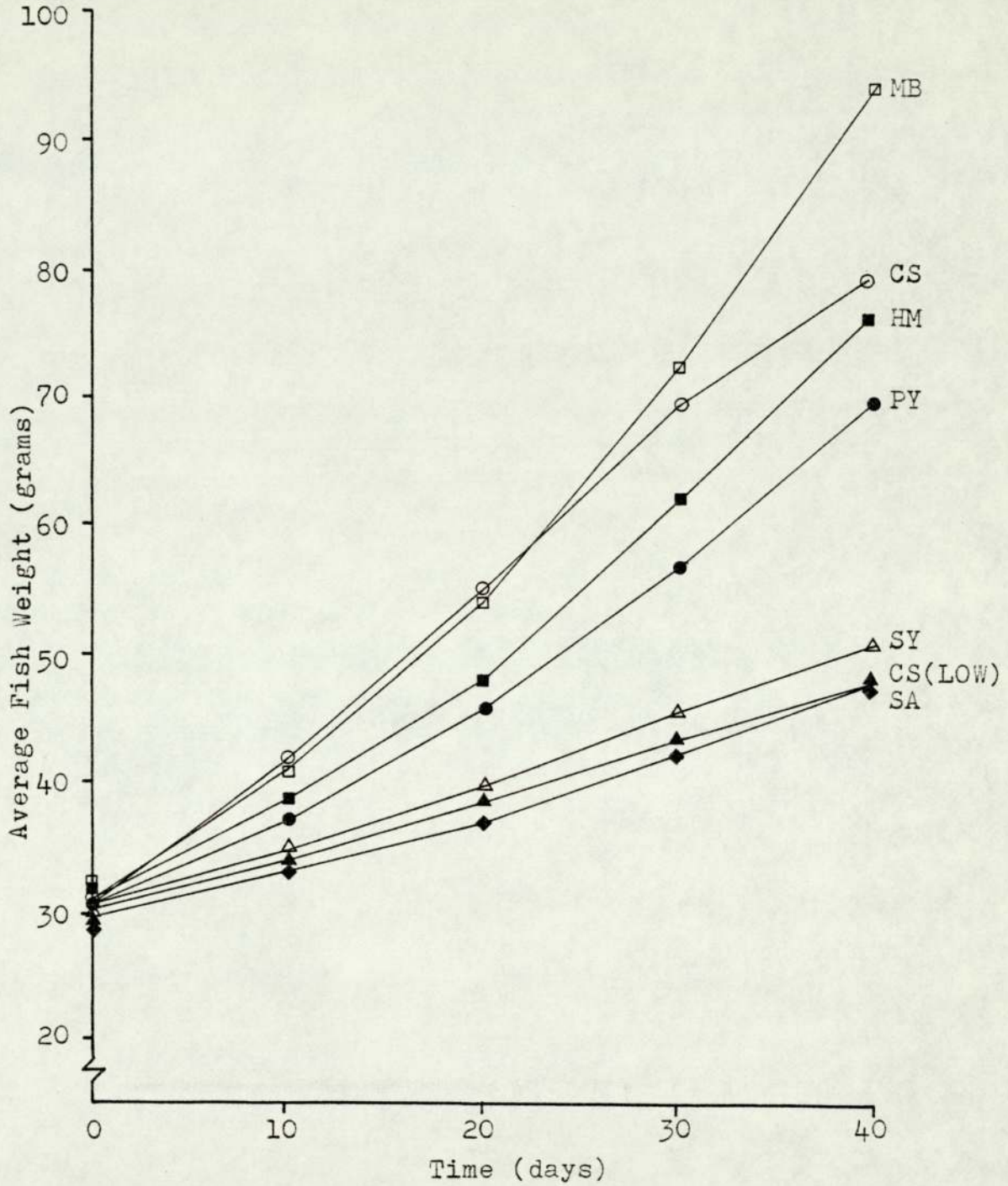


Figure 19. The growth responses of mirror carp fed 7 diets containing various protein sources.

Table 23. Growth and Food Utilization Data from Experiment 3.

	Low Casein	Casein (CS)	Petroyeast (PY)	Bacterial (MB)	Algal (SA)	Herring Meal (HM)	Extracted Soyabean (SY)
Initial Av.Wt.(g)	30.3	30.9	30.5	31.0	29.9	31.2	30.9
Final Av.Wt.(g)	47.8	79.7	70.1	94.2	47.9	77.0	50.8
SGR[1](%/day)	1.13^{a}	2.40^{c}	2.08^{b}	2.78^{d}	1.18^{a}	2.26^{c}	1.24^{a}
FCR[2]	3.04^{a}	1.39^{c}	1.55^{c}	1.14^{d}	2.50^{b}	1.42^{c}	2.86^{a}
PER[3]	3.24^{a}	2.48^{c}	2.08^{d}	2.54^{c}	1.15^{e}	2.82^{b}	1.15^{e}
NPU[4] (%)	-	49.0^{b}	47.0^{b}	49.0^{b}	36.0^{d}	64.0^{a}	42.0^{c}
BV[5]	-	52.0^{b}	49.0^{c}	52.0^{b}	41.0^{d}	79.0^{a}	51.0^{c}

1 Specific Growth Rate

2 Food Conversion Ratio

3 Protein Efficiency Ratio

4 Net Protein Utilization

5 Biological Value

Figures in the same row having the same superscript are not significantly different ($p > 0.05$).

highest growth, followed by the casein (CS) and herring meal (HM) diets. The petroyeast (PY) gave significantly lower growth ($p < 0.05$) than the above but higher than the soyabean (SY) and algal (SA) diets. Fish on these latter two diets grew no faster than those fed the 10% casein diet.

Of interest was the slight decline in growth rate of the casein (CS) control group between day 30 and day 40. The reason for this is not immediately apparent, but the same effect was noted in a previous trout experiment (Atack & Matty, 1978).

Section 5.3.2. Food Conversion.

Mean Food Conversion Ratios (FCRs, section 2.7.2.) were obtained for each group and are presented in Table 23. The bacterial protein gave significantly ($p < 0.05$) the lowest FCR although this could be attributed to its' higher protein content. The yeast, herring meal and casein feeds produced good FCRs that were insignificantly ($p > 0.05$) different. The soyabean and algal diets gave significantly poorer FCRs ($p < 0.05$).

Hoshai (1972) has reported an FCR of 1.11 for a petroyeast based carp diet, slightly better than that found here, whilst the casein value is very similar to that found by Ogino & Saito (1970).

Section 5.3.3. Carcass Composition.

The results of proximate analysis of final carcass composition are presented in Table 22. Gross body composition was little affected by the dietary regime except in the group fed the low protein diet. These fish had a significantly higher ($p < 0.05$) body lipid content and lower moisture content

than fish from other groups due to the higher dietary lipid level.

Section 5.3.4. Protein Utilization.

Protein Efficiency Ratios (PERs, Section 2.7.3.) were calculated for each group and the means of results for duplicate tanks are presented in Table 23. The low protein diet gave significantly ($p < 0.05$) the highest PER, which was to be expected as Ogino & Saito (1970) have shown, in carp, that PER increases as the dietary protein level decreases. However, consideration of PER at very low levels of dietary protein shows that PER must fall as the protein level decreases. PER would be zero when the dietary protein level was equal to the maintenance requirement. At still lower levels of dietary protein the fish would loose weight. Fish fed a zero protein diet would, if anything, exhibit a negative PER approximating to the endogenous nitrogen excretion.

Both of the plant proteins, algal and soyabean, gave poor PERs which were significantly lower than that for petroyeast. The bacterial protein was found to be comparable to the casein control although neither performed as well as the fishmeal control.

True Net Protein Utilization (NPU, section 2.7.3.) values were calculated for each group and the means of results for duplicate tanks are presented in Table 23. Since the body composition of the various groups was fairly stable the results in general reflect the PER values. The algal and soyabean diets exhibited the lowest values; the petroyeast, bacterial, and casein had intermediate values whilst the herring meal showed significantly the highest degree of utilization.

Section 5.3.5. Protein Digestibility.

True protein digestibilities were determined for each group, as described in section 2.7.4., and the results are presented in Table 24.

Table 24. Digestibility Data from Experiment 3.

	Low Casein	Casein (CS)	Petroyeast (PY)	Bacterial (MB)	Algal (SA)	Herring Meal (HM)	Extracted Soyabean (SY)
Apparent Protein Digestibility (%)	86.91	88.40	91.91	90.99	82.82	75.69	78.96
True Protein Digestibility(%)	-	92.99	96.62	95.54	87.10	80.31	83.74

The protein of the low casein diet was assumed to be 100% digestible which thus allowed calculation of the metabolic faecal nitrogen (MFN). This was found to be 217 mgs N/100 g of diet fed, a value which is higher than that reported by Ogino et al. (1973) of 170 mgs on a non-protein diet and 144 mgs with protein containing diets. This may indicate that the 100% digestibility assigned to the 10% protein diet was incorrect. If, in fact, a value of 93% digestibility, found for the high casein diet, is used then the MFN becomes comparable to that previously reported. However, for the purposes of this experiment such small differences do not affect the results.

Most of the test proteins showed good digestibility, the petroyeast and bacterial proteins being exceptionally well assimilated (96.6 & 95.5% respectively). The plant proteins showed good, but comparatively lower, digestibility and both these proteins were better digested than fishmeal. The value for the algal diet is similar to that reported by Hepher at al. (1978).

Section 5.3.6. Biological Value.

Biological Values (BVs, section 2.7.3.) were determined for each group and the means of results for duplicate tanks are presented in Table 23. Most of the values thus obtained proved similar although statistically significant ($p<0.05$) differences could be detected between certain of the protein sources. The Spirulina alga gave the lowest BV and fishmeal the highest.

Section 5.4. Discussion and Conclusions.

One immediate problem in evaluation of the results obtained lies in the varying protein content of the diets used. For instance, since all the protein levels were probably below the optimum for carp, Cyprinus carpio, (Sin, 1973a,b; Ogino & Saito, 1970; Ogino et al., 1976) the higher growth obtained with the bacterial diet in comparison to that with herring meal could be ascribed to its' higher protein content. Equivalent protein levels would probably have led to the bacterial protein giving growth comparable to that of the casein and petroyeast diets whilst the herring meal would possibly have ranked somewhat higher.

Most of the other evaluation data can be considered in the same way as it has been shown by numerous authors that the level of protein in the diet markedly affects most aspects of its utilization (Ogino & Saito, 1970; Nose 1971; Cowey et al., 1972; Ogino & Chen, 1973a; Ogino et al., 1976; Matty & Smith, 1978). Thus a 30% protein level in all of the diets may have resulted in herring meal protein exhibiting lower and bacterial protein higher Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and Biological Value (BV) values than those found, though the extent to which this would tend to negate or reverse the differences found between these and the other proteins is difficult to judge. However, it seems likely that overall the bacterial protein would be ranked as being of intermediate value between casein and herring meal.

An attempt was made to correlate the BVs obtained in this experiment with the amino acid profiles of the test proteins. The results (Table 25) show that in absolute terms none of the chemical methods accurately predicted the BVs

Table 25. A Comparison of Biological Values with Chemical Prediction of Protein Utilization.

BV[1]	EAAI[2]	Chemical Score[3]	Chemical Score[4]	Chemical Score[5]
HM(79)	HM(74)	HM(58)	HM(110)	HM(93)
MB(52)	SY(71)	MB(47)	MB(87)	MB(75)
CS(52)	PY(68)	CS(46)	SY(82)	SA(67)
SY(51)	SA(68)	SA(42)	PY(80)	SY(63)
PY(49)	MB(66)	SY(40)	CS(74)	PY(63)
SA(41)	CS(61)	PY(40)	SA(70)	CS(53)

1. Biological Value obtained experimentally.
2. Essential Amino Acid Index - reference whole egg protein.
3. Chemical Score - reference protein whole egg.
4. Chemical Score - based on the amino acid requirements of carp (Nose, 1978).
5. Chemical Score - based on the amino acid requirements of chinook salmon (Mertz, 1969).

HM - Herring Meal

MB - Methanophilic Bacterium

CS - Casein

SY - Soyabean Protein

PY - Petroleum Yeast

SA - Spirulina alga

obtained experimentally. The explanation of this probably lies in the fact that the BV of a protein varies with the level of that protein in the diet, the level of feeding, the energy content of the diet and the physiological state of the animal (Cowey, 1978).

Table 25 shows that all of the chemical methods used correctly predicted the herring meal as the best protein source but overall the chemical scores were more consistent with the experimental results than the Essential Amino Acid Indices (EAAI). The chemical score based on whole egg protein incorrectly placed the algal protein whilst that based on the amino acid requirements of carp misplaced the casein and the chinook salmon score wrongly ranked both the casein and algal proteins.

Since the same diet formulations were used in a previous experiment on rainbow trout, Salmo gairdneri, (Atack & Matty, 1978) it is of interest to compare the protein utilization data for the two species, though the effects of different feeding regimes and environmental conditions have to be considered. Similarly dietary ingredients, other than protein, may be used to different extents by the two species and so the diets would not be equivalent on a metabolisable energy basis.

However, the comparison (Table 26) does produce some interesting results. The absolute PER values for fishmeal, bacterial and casein proteins are all higher in carp than their corresponding values for trout whereas the petroyeast and algal proteins exhibited almost identical values. Some carp growth occurred on the soyabean diet resulting in a positive PER whereas trout fed this diet lost weight and, consequently, had a negative PER. In general the ranking of

Table 26. A Comparison of Results for Carp and Trout*.

	PER[1]		NPU[2]		Digestibility		BV[3]	
Diet	Carp	Trout	Carp	Trout	Carp	Trout	Carp	Trout
HM	2.82	1.91	64	38	80.3	91.2	79	41
MB	2.54	1.62	49	37	95.5	93.5	52	40
CS	2.48	1.97	49	40	93.0	98.7	52	41
PY	2.08	2.01	47	42	96.6	91.6	49	46
SY	1.35	-	42	18	83.7	43.6	51	41
SA	1.15	1.33	36	32	87.1	83.1	51	38

*Trout data from Atack & Matty (1978)

1 Protein Efficiency Ratio

2 Net Protein Utilization

3 Biological Value

HM, Herring Meal MB, Methanophilic Bacterium CS, Casein PY, Petroleum Yeast SY, Soyabean Protein SA, Spirulina Alga.

proteins according to PER, for the two species, is fairly similar although with trout the petroyeast and casein ranked the same whereas, in this trial, the bacterial protein and casein ranked similarly.

Again, as for PER, NPUs rank the proteins in roughly the same order for both species, although the absolute values are appreciably higher for carp possibly due to improved utilization of dietary energy. The two plant proteins produced the poorest NPUs in carp and trout.

Biological Values (BVs) for trout were remarkably constant whilst for carp significant differences could be detected between the protein sources which, again, may have been due to the greater available energy of the diets to carp.

One further point that should be considered in the discussion of these results is the effect that non-protein nitrogen in the diets may have on growth. Certain of the novel proteins, notably the bacteria and yeast, can contain non-protein nitrogen in the form of nucleic acids (A. Tacon, Pers. Comm., 1978). As the total nitrogen digestibility of these two proteins was high in the present trial it appears that a portion of these nucleic acids may be absorbed. If it is assumed that the absorbed nucleic acids are not utilized to any great extent then the retention of true dietary protein may have been appreciably greater than the values shown here. However, there is a lack of information on the metabolism of dietary nucleic acids in fish, especially any protein sparing effect they may have.

In conclusion the results of this experiment indicate that carp, like salmonids, require a diet containing high

quality protein for optimal growth in tanks. A major proportion of this protein could be provided by bacterial and yeast proteins but not by soyabean or algal proteins which were poorly utilized in this experiment.

CHAPTER 6.

Chapter 6.

6. Experiment 4. The Progressive Substitution of Fishmeal with Soyabean Protein and its Effects on Growth Performance, Food Conversion, Body Composition and Protein Utilization of Fingerling Mirror Carp (Cyprinus carpio).

Section 6.1. Introduction.

Up until the present time fishmeal has been the principal source of protein in complete commercial fish rations. However, in recent years the supply of fishmeal has become increasingly uncertain and the price has risen rapidly. The most prominent event in this respect was the fishmeal crisis in 1972/1973 when the fishery for the Peruvian anchovy (Engraulis ringens) failed; at this time it was estimated to supply more than 80% of the world wide production of saleable fishmeal (Anon., 1973). For this reason it has become of great importance to either replace fishmeal in commercial fish rations or, at least, to reduce its' use to a minimum.

The use of plant proteins in complete rations for fish is therefore desirable as such proteins are likely to be more constantly available, and cheaper to produce, than fishmeal. Soyabean meal is the dominant oilseed protein on a world wide basis and is readily available at a cost per tonne half that of peruvian or menhaden fishmeal (Anon., 1978a). Assuming a protein content for fishmeal of 70% and 50% for defatted soybean meal the relative cost per unit of protein from soyabean is 70% of that of fishmeal.

Attempts to replace fishmeal with commercially processed soyabean meals have met with variable success (Krishnandhi & Shell, 1967; Cowey et al., 1971 & 1974; Nose, 1971; Lovell et

al., 1974; Rumsey & Ketola, 1975; Viola, 1975; Cho et al., 1976; Koops et al., 1976; Davis & Stickney, 1978). Complete replacement of fishmeal with soyabean meal has generally been unsuccessful as soyabean protein is deficient in amino acids that are essential for fish, principally methionine.

Before an attempt can be made to incorporate soyabean into commercial carp rations it is necessary to evaluate the effect of varying levels on carp growth. In Experiment 4 (Chapter 6) the digestibility of soyabean protein was found to be in excess of 80%, despite this, when fed as the sole protein source, it resulted in poor growth and protein utilization. The present study was designed to determine what proportion of the dietary protein could be supplied by soyabean meal before growth became depressed.

Seven diets were formulated on an isonitrogenous and isoenergetic basis to supply 30% protein and 3.4 kcal/g of metabolisable energy. The proportion of the dietary protein supplied by each protein source was varied with percentages of fishmeal protein and soyabean protein (in the diet) in the following ratios; 30:0, 25:5, 20:10, 15:15, 10:20, 5:25, and 0:30. These diets were fed to duplicate tanks and their effects on growth performance, food utilization and body composition determined in a 5 week growth trial.

Section 6.2. Materials and Methods.

Section 6.2.1. The Experimental System and Animals.

The experimental facility used in the present study was 'System 3' as described in section 2.1.2.

150 fingerling mirror carp (5 - 8 cm) were obtained from the Cotswold Carp Farm, Bourton-on-the-Water, Gloucestershire.

The fish were quarantined, as detailed in section 2.4, and then randomly allocated, 10 fish per tank, to 14 of the 16 seven litre experimental tanks of 'System 3' at the prevailing ambient temperature of 17°C and the remaining 10 fish were placed in one of the spare tanks of this system.

The temperature was then raised at approximately 3°C/day to the experimental temperature of 28°C. During temperature acclimation, and for the subsequent 7 days, the fish were fed a commercial trout ration. They were then starved for 12 hours prior to batch weighing (section 2.5.) and tank weights were adjusted by redistribution of fish to ensure a constant average initial fish weight. The 10 'spare' fish were then removed and subjected to proximate carcass analysis (section 2.6.). Photoperiod was controlled at 12 hours light and 12 hours darkness throughout the experiment.

Section 6.2.2. The Experimental Diets.

Formulation of the diets was carried out by the procedure described in section 2.2. although being complicated by the need to balance the composition of the two protein sources. The ingredients used are presented in Table 28, the soyabean protein concentrate was the same as that used in Experiment 3 (Chapter 5)('Newprod', T.Lucas & Co., Bristol). Diet preparation was by wet extrusion (section 2.3.), the results of proximate analysis (section 2.6.) of the diets are presented in Table 29 and estimated dietary components in Table 30.

Section 6.2.3. Feeding Rates.

Due to the relatively poor food conversion ratios obtained in Experiment 2 (Chapters 4) and based on the experience

Table 28. Ingredient Composition of the Diets used in Experiment 4.

Ingredient (dry weight)	DIET 1	2	3	4	5	6	7
Herring Meal	38.85	32.88	25.89	19.42	12.95	6.47	0.00
Soyabean	0.00	7.60	15.20	22.80	30.80	38.00	45.59
Herring Oil	7.16	7.59	8.03	8.45	8.89	9.32	9.75
Mineral Mix[1]	4.33	4.88	5.43	5.99	6.53	7.08	7.64
Starch	14.67	12.56	10.45	8.34	6.23	4.12	2.02
Vitamins[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Binder[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic Oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
∝-Cellulose	16.49	16.49	16.49	16.49	16.49	16.49	16.49
Glucose	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated;-							
% Protein from Herring Meal	30.00	25.00	20.00	15.00	10.00	5.00	0.00
% Protein from Soyabean	0.00	5.00	10.00	15.00	20.00	25.00	30.00

1 Composition given in Table 6 section 2.2.

2 Composition given in Table 7 section 2.2.

3 Carboxymethylcellulose, Sodium Salt, High Viscosity.

Table 29. Proximate Analysis of the Diets used in Experiment 4.

	DIET						
	1	2	3	4	5	6	7
Moisture (%)	6.47	7.36	8.66	8.36	8.93	8.80	10.01
On a moisture free basis;-							
Crude Lipid (%)	10.04	10.01	10.40	9.90	10.18	10.11	10.21
Crude Protein (%)	30.56	31.07	31.28	30.85	31.43	31.03	31.35
Ash (%)	8.98	9.27	9.55	9.47	9.53	9.36	9.25
NFE[1] (%)	50.42	49.65	48.77	49.78	48.86	49.50	49.19
Chromic Oxide (%)	0.41	0.46	0.41	0.45	0.43	0.47	0.45
Energy (kcal/g)	4.81	4.88	4.79	4.83	5.08	5.05	5.09

1 Nitrogen Free Extractives

Table 30. Estimated Components of the Diets used in Experiment 4.

	DIET						
	1	2	3	4	5	6	7
Carbohydrate (%) (including NFE from proteins)	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Total Energy (kcal/g)	4.58	4.61	4.66	4.58	4.64	4.62	4.64
Metabolisable Energy (kcal/g)	3.35	3.37	3.42	3.35	3.40	3.38	3.40

gained in Experiment 3 (Chapter 5) it was decided to feed 4% of the body weight per day with allowance being made for the moisture content of the diets so that a true feeding rate of 4% dry food was fed. Each of the 7 diets was fed to duplicate tanks in four daily feeds, each distributed over a period of 15 - 20 minutes, between 08.30 h and 18.30 h. The quantity of food fed was adjusted aftereach weekly weighing and fed for the subsequent six days.

Section 6.2.4. Weighing and Sampling.

Details of the weighing procedure are presented in section 2.5. Fish were batch weighed, under anaesthesia, after 12 hours starvation, every seven days for 5 weeks. At the end of the fifth (and final) week 3 fish were removed from each group and proximate carcass analysis (section 2.6.) performed, the results of which are presented in Table 31. Faeces were obtained from the remaining fish, as described in section 2.7.4, and analysed for protein, energy (section 2.6.) and chromic oxide (section 2.7.4.).

Section 6.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

Section 6.3. Results.

Fish in all groups fed actively throughout the experiment and no mortalities occurred. Those fish fed diets 6 & 7 took longer to consume their rations than those fed diets with a lower proportion of soyabean.

Table 31. The Results of Proximate Carcass Analysis of Initial and Final Fish Samples from Experiment 4.

	Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Initial	76.5	7.1	11.5	2.1	97.2
Final;- (diet)					
1	74.7[a]	6.3[a]	14.8[a]	2.8[a]	98.6
2	74.7[a]	6.2[a]	14.8[a]	2.5[a]	98.5
3	75.1[a]	5.8[a]	14.9[a]	2.7[a]	98.3
4	75.1[a]	6.3[a]	14.4[a]	2.5[a]	98.3
5	74.8[a]	6.3[a]	14.5[a]	2.4[a]	98.0
6	75.8[a]	5.8[a]	13.7[b]	2.5[a]	97.8
7	75.5[a]	6.4[a]	13.6[b]	2.8[a]	98.3
S.E.±	0.647	0.607	0.187	0.299	

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

Section 6.3.1. Growth Performance.

Growth responses of the groups fed diets 1 - 7 are represented graphically in Figure 20 where the average fish weight, as a mean of two replicate tanks, is plotted against time. Figure 20 shows that the growth responses fell into three general groups. Diets 1 & 2 (fishmeal to soyabean protein ratios, FM:SBP, of 30:0 & 25:5) gave the best growth response; diets 3, 4 & 5 (FM:SBP of 20:10, 15:15 & 10:20) gave a growth response slightly less than that of diets 1 & 2 and diets 6 & 7 (FM:SBP of 5:25 & 0:30) gave a very much reduced growth response.

Statistical analysis of the average initial fish weights (Table 32) shows significant ($p<0.05$) differences between certain of the groups although these small differences were not reflected in the growth performance or average final fish weights. Analysis of average final fish weights (Table 32) shows that diets 1 & 2 produced significantly ($p<0.05$) heavier fish than diets 3, 4 & 5 which, in turn, produced significantly heavier fish than diets 6 & 7.

Significant differences ($p<0.05$) in Specific Growth Rates (SGRs, section 2.7.1.), presented in Table 32, placed the diets in the following order;-

$1>2>3,>4 \ \& \ 5>6>7.$

Section 6.3.2. Food Conversion.

Mean Food Conversion Ratios (FCRs, section 2.7.2.) were obtained for each diet and are presented in Table 32. Statistical analysis of FCRs showed a significant ($p<0.05$) increase with increasing levels of soyabean (decreasing FM:SBP) in the diets.

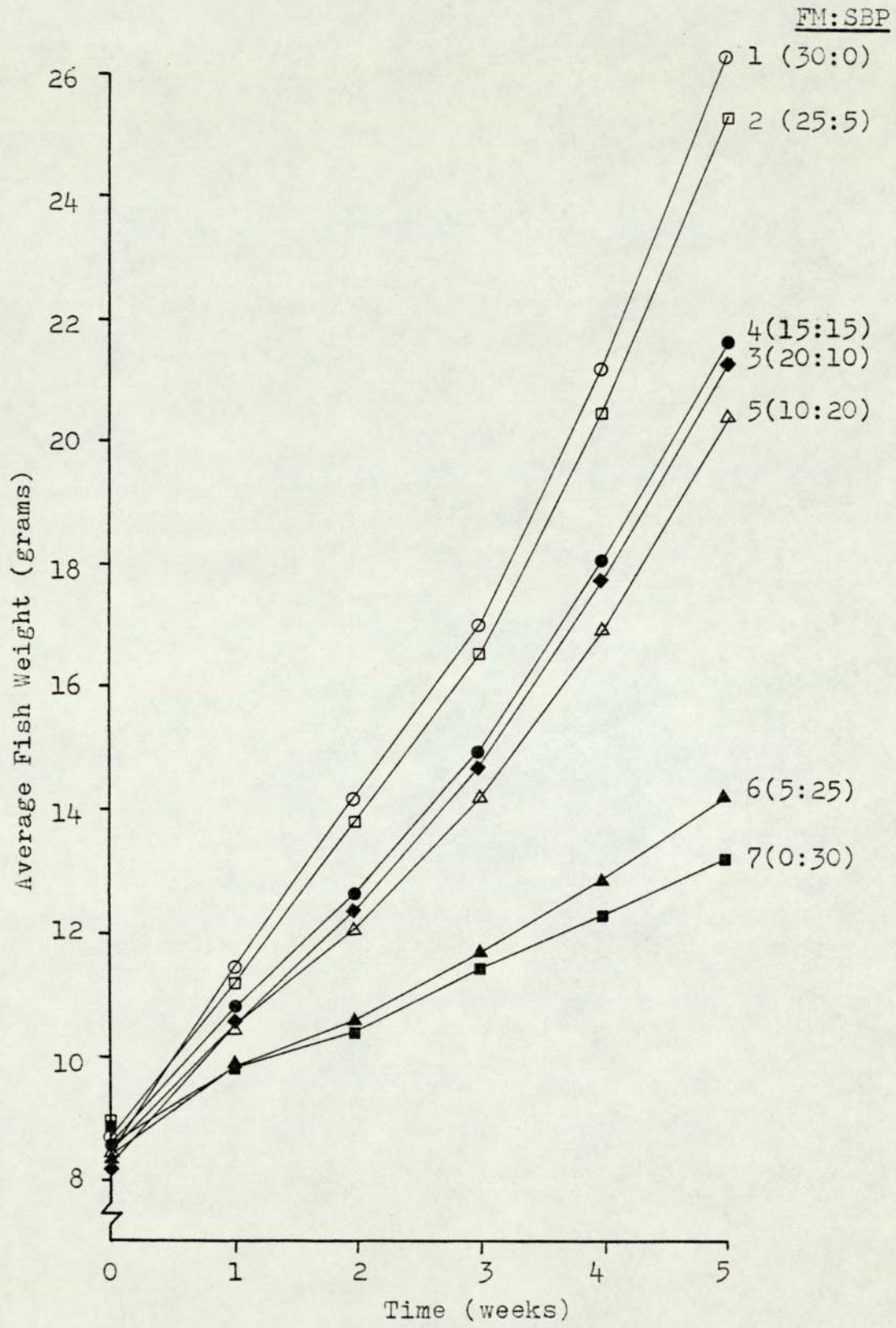


Figure 20. Growth responses of carp fed diets containing varying levels of fishmeal and soyabean proteins.

Table 32. Growth and Food Utilization Data from Experiment 5.

	DIET							
	1	2	3	4	5	6	7	
(FM:SBP)	(30:0)	(25:5)	(20:10)	(15:15)	(10:20)	(5:25)	(0:30)	S.E.±
Initial Av.Wt.(g)	8.50^{bc}	8.70^{ab}	8.30^{c}	8.45^{b}	8.40^{c}	8.80^{a}	8.60^{b}	0.056
Final Av.Wt.(g)	26.25^{a}	25.20^{a}	21.35^{b}	21.50^{b}	20.40^{b}	14.15^{c}	13.20^{c}	1.071
SGR[1](%/day)	3.23^{a}	3.04^{b}	2.70^{c}	2.67^{c}	2.54^{c}	1.49^{d}	1.23^{e}	0.053
FCR[2]	1.33^{a}	1.40^{ab}	1.56^{b}	1.59^{b}	1.69^{b}	2.82^{c}	3.43^{d}	0.052
PER[3]	2.46^{a}	2.31^{b}	2.05^{c}	2.02^{cd}	1.89^{d}	1.15^{e}	0.93^{f}	0.045
Apparent NPU[4](%)	40.49^{a}	38.12^{a}	34.78^{b}	33.30^{bc}	31.52^{c}	19.44^{d}	16.39^{e}	0.722

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

1 Specific Growth Rate.

2 Food Conversion Ratio.

3 Protein Efficiency Ratio.

4 Net Protein Utilization.

Section 6.3.3. Carcass Composition.

The results of proximate analysis of initial and final fish samples are presented in Table 32. The only statistically significant ($p<0.05$) variation in carcass composition was the lower protein content of fish fed diets 6 & 7.

Section 6.3.4. Protein Utilization.

Protein Efficiency Ratios (PERs, section 2.7.3.) were calculated for each group and the means of results for duplicate tanks are presented in Table 32. PER decreased significantly ($p<0.05$) with decreasing FM:SBP ratio.

Apparent Net Protein Utilization (NPU, section 2.7.3.) was also calculated for each group and the means of results for duplicate tanks are presented in Table 32. Apparent NPU decreased significantly ($p<0.05$) with decreasing FM:SBP ratio.

Section 6.3.5. Protein Digestibility.

Apparent protein digestibilities were determined for each group, as described in section 2.7.4, and the means of results for duplicate tanks are presented in Table 33. Protein digestibility decreased with decreasing FM:SBP ratio reflecting the lower digestibility of soyabean protein as compared to fishmeal. In the previous experiment (Experiment 3, Chapter 5) the apparent digestibility of the soyabean protein was 78.96%. The lower value obtained in the present study (72.96%) may be due to the slightly higher temperature employed (28°C as compared to 25°C).

Table 33. Digestibility Data from Experiment 5.

	DIET							
	1	2	3	4	5	6	7	
FM:SBP	30:0	25:5	20:10	15:15	10:20	5:25	0:30	S.E.±
Apparent Protein Digestibility (%)	87.21[a]	85.36[b]	83.60[c]	81.32[d]	80.12[d]	73.41[e]	72.69[e]	0.568
Apparent Energy Digestibility (%)	67.36	66.60	66.60	66.87	64.96	65.74	64.64	
Digestible Energy (DE, kcal/g)	3.24	3.25	3.19	3.23	3.30	3.32	3.29	

Figures in the same row having the same superscript are not significantly different ($p>0.05$).

Standard Error (S.E.±) indicates the range of the means tested.

Section 6.3.6. Dietary, Metabolisable and Measured Digestible Energy Values.

The total energy contents of the diets (Table 30) and the metabolisable energy contents (Table 30) were estimated using the figures and principals detailed in section 4.3.6. The total energy contents of the diets, as measured by bomb calorimetry, (Table 29) were, in all cases, higher than the estimated values probably due to the small calorific values of the vitamins and binder not included in the calculation.

The apparent Digestible Energy (DE, section 2.7.4.) contents of the diets were obtained and are presented in Table 33. The average DE of the diets was 3.26 kcal/g compared with an average calculated metabolisable energy of 3.38 kcal/g. The lower DE of the diets may have been due to overestimation of the metabolisable energy content of the NFE (Nitrogen Free Extractives) portion of the diets by assuming a digestibility equivalent to that of starch.

Section 6.4. Discussion and Conclusions.

The results of the present study show that in a 30 % fishmeal protein diet substitution of only one third of the protein with a soyabean protein concentrate caused a significant decrease in growth rate and food utilization in a five week experiment. The results of previous studies of the isonitrogenous replacement of fishmeal with soyabean protein are in general agreement with the results of this experiment.

Cowey et al. (1971) replaced approximately half of the protein, in a 40% codmeal protein diet, with soyabean meal and found that this depressed the growth and protein utilization of plaice (Pleuronectes flatessa).

Similar results have been reported for channel catfish, Ictalurus punctatus, (Andrews & Page, 1974) where isonitrogenous replacement of dietary menhaden meal with soyabean meal depressed growth and food utilization even when the soyabean meal was supplemented with methionine, cystine and lysine to the levels found in the fishmeal control. These three amino acids are considered to be the most limiting in soyabean protein (NAC, 1973). However, Krishnandhi & Shell (1967) found that channel catfish grew as well on a 30% protein diet comprising a 50:50 mixture of soyabean and casein proteins as they did on a diet containing casein alone.

Koops et al. (1976) evaluated isonitrogenous replacement of fishmeal, at two protein levels (39 & 47%), with either soyabean meal or soyabean protein in diets of rainbow trout (Salmo gairdneri). They found that 25% of the dietary fishmeal could be replaced by soyabean protein but that total replacement resulted in growth depression.

Rumsey & Ketola (1975) found that the growth of rainbow trout fingerlings, fed diets containing 40% protein with soyabean meal as the sole protein source, was significantly improved by supplementation with amino acids in certain combinations. Methionine, leucine, lysine, valine, histidine, tryptophan and tyrosine as well as methionine, leucine, lysine, valine and threonine significantly improved growth when supplemented to levels resembling isolated fish protein. Supplements of methionine, histidine, lysine and leucine alone and in various combinations, however, did not result in improved growth.

Attempts have been made to use soyabean 'grits' (52.4%

protein) to isonitrogenously replace fishmeal in a 45% protein compounded feed for chinook salmon (Oncorhynchus tshawytscha) reducing the percentage of the protein supplied by fishmeal from 22 to 15% (Fowler & Banks, 1976). Addition of soyabean 'grits' inhibited growth of this species and resulted in reduced carcass lipid and protein deposition. The present study also resulted in reduced carcass protein deposition.

A more comprehensive, recent study of the replacement of fishmeal with soyabean meal in tilapia (Tilapia aurea) diets at various protein levels found that at a level of 36% protein tilapia grew as well on an all soyabean protein diet as they did on an all fishmeal protein diet (Davis & Stickney, 1978). All the diets in this study were supplemented with methionine to bring the total in the diet to 1.1%. These authors also reported that if one third of the dietary protein was supplied by fishmeal then the protein content of the diets could be reduced from 36 to 29% with no depression of growth.

Information on the effects of substitution of fishmeal with soyabean meal in carp diets is scarce. Kaneko (1969) reported unpublished Japanese data that one third of the white fishmeal, in a carp diet, could be replaced by soyabean oil meal with no depression of growth. Hepher et al. (1971) reported that, in pond carp feeds, those diets containing fishmeal produced better growth than those containing soyabean meal.

Viola (1975) isonitrogenously reduced the fishmeal content of a 25% protein carp feed from 15 to 5% by replacement with soyabean meal supplemented with amino acids, vitamins and minerals. The group fed the soyabean meal diet did not perform as well as the controls despite the supplementation.

As stated previously methionine is the first limiting amino acid in soyabean protein. The percentage of the dietary protein supplied by methionine has been calculated for the diets used in the current experiment using a value of 1.3% methionine for soyabean protein and a value of 2.9% methionine for fishmeal (Hepher et al., 1978 from NAC, 1973; Cowey et al., 1971). The diets 1 to 7 thus contained, as a percentage of the dietary protein, 2.9, 2.6, 2.4, 2.1, 1.8, 1.6 & 1.3% methionine respectively. It is of interest to compare these levels with the reported methionine requirements of fish.

The methionine requirement of chinook salmon has been found to be 4% of the dietary protein in the abscence of cystine and 1.5% in the presence of adequate cystine (Mertz, 1972). For carp the requirements have been reported as 3.1% in the abscence cystine and 2.1% in the prescence of 2% cystine (Nose, 1978).

Hepher et al. (1978 from NAC, 1973) reported that both soyabean and fishmeal proteins contain 1.5% cystine and thus that figure is assumed for all of the diets in the present study.

The results of this experiment (Table 37) indicate that diets containing 2.9 & 2.6% methionine (diets 1 & 2) performed equally well and slightly better than diets containing 2.4, 2.1 & 1.8% (diets 3, 4 & 5). Growth was, however, greatly depressed in diets containing only 1.6 & 1.3% methionine (diets 6 & 7). These results compare fairly favourably with those of Nose (1978) although differences in the protein content of the diets and feeding rate make absolute comparison impossible.

Amino acid supplementation should, theoretically, improve

the utilization of essential amino acid deficient proteins such as soyabean. However, supplementation of diets for warm water fish species has generally proved unsuccessful (Hepher et al., 1971; Andrews & Page, 1974; Viola, 1975; Hepher, 1978) possibly due to an inability of these fish to utilize free amino acids or peptides (Aoe et al., 1970; Aoe et al., 1974; Page & Andrews, 1974).

In conclusion the present study demonstrated that, in carp diets containing 30% protein, substitution of only one third of the protein with a soyabean concentrate caused a significant decrease in growth rate and protein utilization during a five week growth trial. However, it is possible that this might be offset by the reduction in feed costs achieved by replacing fishmeal with soyabean.

CHAPTER 7.

Chapter 7.

7. Experiment 5. Investigation of the Effects of Varying Temperature and Dietary Protein Level on Growth Performance, Food Conversion, Body Composition and Protein Utilization of Fingerling Mirror Carp (Cyprinus carpio).

Section 7.1. Introduction.

Very little information is presented in the literature as to the effect of varying temperature on the protein requirement of fish. In consideration of the use of heated effluents for the culture of carp it must be borne in mind that the optimum temperature for growth might not be available throughout the year. It would, therefore, be of interest to compare the performance of fish fed different levels of dietary protein at different temperatures.

The only apparent reference in the literature on the effect of temperature on the protein requirement of fish is that chinook salmon (Oncorhynchus tshawytscha) required 40% protein at 8.3°C and 50% at 14.4°C (Delong et al., 1958).

The ways in which temperature affects the protein utilization of fish will be similar to the effects of temperature on digestion and utilization of the whole ration as discussed in Experiment 1 (Chapter 3). These will include;-

1. The motor activity of the digestive tract is affected by to the environmental temperature. The rate of passage of food through the gastrointestinal tract of fish is approximately doubled by a 10°C rise in temperature (Shcherbina & Kazlauskene, 1971). The rate of gastric evacuation of sockeye salmon (Oncorhynchus nerka) was quadrupled by a 10°C rise in temper-

ature (Brett & Higgs, 1970).

2. The activity of the digestive enzymes is increased by increasing temperature and there is evidence that the rate of absorption of nutrients from the fish intestine is also increased by increasing the temperature (Shcherbina & Kazlauskene, 1971).

3.Enzyme substrate affinity may change significantly with temperature and this change may be in the direction to compensate for the lowering of enzyme activity with a fall in temperature.(Cowey & Sargent, 1979).

These opposing effects of temperature combine to determine the degree of digestion and utilization of dietary ingredients but the effect of temperature on growth is further influenced by the metabolic rate of fish being raised by increasing temperature (Brett et al., 1969).

Previous attempts to define the optimum protein content of carp diets have usually been conducted with diets containing insufficient metabolisable energy (Ogino & Saito, 1970; Sin, 1973a,b; Ogino et al., 1976). Thus the diets in the present study were formulated to have a high metabolisable energy content of approximately 3.8 kcal/g.

Diets containing 20, 30 & 40% protein were fed to groups of mirror carp at 20, 25, 30 & 35°C for five weeks and the effects on growth, body composition and food utilization determined.

Section 7.2. Materials and Methods.

Section 7.2.1. The Experimental System and Animals.

The experimental facility used in the present experiment was 'System 1' as described in detail in section 2.1.1.

190 fish (10 - 13 cm) were selected from those remaining from Experiments 2 & 3 that had been maintained on a commercial trout ration at ambient temperatures for approximately

15 weeks. These fish were temperature acclimated, as described for Experiment 1 (Chapter 3), and 10 fish were removed for initial proximate carcass analysis (section 2.5.). 15 fish were allocated to each of the twelve experimental groups. No mortalities occurred during temperature acclimation and photoperiod was controlled at 14 hours light and 10 hours darkness throughout the experiment.

Section 7.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general method described in section 2.2 with increasing protein content achieved by the replacement of dextrin with herring meal. The ingredient composition of the diets is shown in Table 34. Diets were prepared by wet extrusion (section 2.3.), the results of proximate analysis (section 2.6) of the experimental diets are presented in Table 35 and estimated dietary components in Table 36.

Section 7.2.3. Feeding Rates.

All goups were fed 5% of their body weight per day distributed in four equal feeds, each lasting 15 - 20 minutes, between 08.30h and 18.30h. Correction was made for the analysed moisture content of the diets so that a true feeding rate of 5% dry food was fed. The quantity of food dispensed per day was recalculated after each weekly weighing and fed for the subsequent six days.

Section 7.2.4. Weighing and Sampling.

Details of the weighing procedure are presented in section 2.5. Fish were individually weighed, under anaesthesia every

Table 34. Ingredient Composition of the Diets used in Experiment 6.

Ingredient (dry weight)	DIET 1	2	3
Herring Meal	25.76	38.64	51.52
Herring Oil	9.12	7.68	6.24
Mineral Mix[1]	9.26	7.89	6.52
Starch	4.86	4.79	4.73
Vitamins[2]	1.00	1.00	1.00
Binder[3]	2.00	2.00	2.00
Chromic Oxide	0.50	0.50	0.50
Dextrin	27.50	17.50	7.49
Glucose	15.00	15.00	15.00
∝-Cellulose	5.00	5.00	5.00
Total	100.00	100.00	100.00
Calculated;-			
Crude Protein(%)	20.00	30.00	40.00

1 Composition given in Table 6 section 2.2.

2 Composition given in Table 7 section 2.2.

3 Carboxymethylcellulose, Sodium Salt, High Viscosity.

Table 35. Proximate Analysis of the Diets used in Experiment 6.

	DIET		
	1	2	3
Moisture (%)	9.51	8.67	7.62
On a moisture free basis;-			
Crude Lipid (%)	11.97	11.89	12.01
Crude Protein (%)	21.46	31.19	42.12
Ash (%)	10.73	10.16	9.82
NFE[1] (%)	55.84	46.76	36.05
Energy (kcal/g)	4.56	4.73	4.89

1 Nitrogen Free Extractives.

Table 36. Estimated Components of the Diets used in Experiment 6.

	DIET		
	1	2	3
Carbohydrate (%) (including NFE from fishmeal)	47.50	37.50	27.49
Fibre (%) (∝-Cellulose)	5.00	5.00	5.00
Total Energy (kcal/g)	4.49	4.62	4.84
Metabolisable Energy (kcal/g)	3.72	3.80	3.96

seven days for 5 weeks. At the end of the fifth (and final) week 4 fish were removed from each group and subjected to proximate carcass analysis (section 2.6.) the results of which are presented in Table 37. Faeces were collected from the remaining fish, as described in section 2.7.4, and pooled samples were analysed for protein, energy (section 2.6.) and chromic oxide (section 2.7.4.).

Section 7.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

Section 7.3. Results.

At 20, 25 & 30°C all groups of fish became accustomed to both the diet and conditions, feeding actively throughout the experiment. It was noted, however, that fish held at 35°C appeared 'stressed' and were considerably darker in colour than those held at lower temperatures. Fish at 35°C fed nervously and erratically appearing 'hyper - active' although consuming all of their daily ration.

Two fish of group 11 (35°C, 30% protein) jumped out of their tank on day 12 and died due to the lid of this tank being inadvertently left off. Their weights were recorded, feeding rate was adjusted and because all fish were individually marked and weighed their loss did not prevent calculation of growth and food utilization data for that group.

Section 7.3.1. Growth Performance.

The growth responses of the fish under the different regimes are shown graphically in Figures 21 - 27 as average fish weight plotted against time.

Table 37. The Results of Proximate Analysis of Initial and Final Fish Samples from Experiment 6.

Sample		Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Initial		76.5	6.20	13.0	2.80	98.50
Final;-						
Temp./Protein						
20°C	20%	76.2^{bc}	5.6^{d}	13.3^{a}	3.5^{a}	98.6
	30%	75.9^{bc}	6.3^{c}	13.6^{a}	3.5^{a}	99.3
	40%	75.1^{c}	7.4^{b}	13.9^{a}	3.0^{ab}	99.4
25°C	20%	75.4^{c}	6.4^{c}	13.7^{a}	4.0^{a}	99.5
	30%	74.6^{c}	7.4^{b}	13.0^{a}	3.9^{a}	98.8
	40%	74.3^{cd}	7.9^{ab}	14.1^{a}	3.2^{ab}	99.5
30°C	20%	76.1^{bc}	7.6^{b}	13.9^{a}	2.0^{b}	99.6
	30%	75.2^{c}	7.9^{ab}	12.8^{a}	3.6^{a}	99.5
	40%	73.4^{d}	8.3^{a}	13.2^{a}	3.9^{a}	98.8
35°C	20%	78.0^{a}	4.6^{e}	13.3^{a}	3.8^{a}	99.6
	30%	77.4^{ab}	5.3^{d}	13.1^{a}	3.6^{a}	99.3
	40%	76.8^{b}	5.3^{d}	14.1^{a}	2.9^{ab}	99.1
	S.E.±	0.353	0.215	0.399	0.420	

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

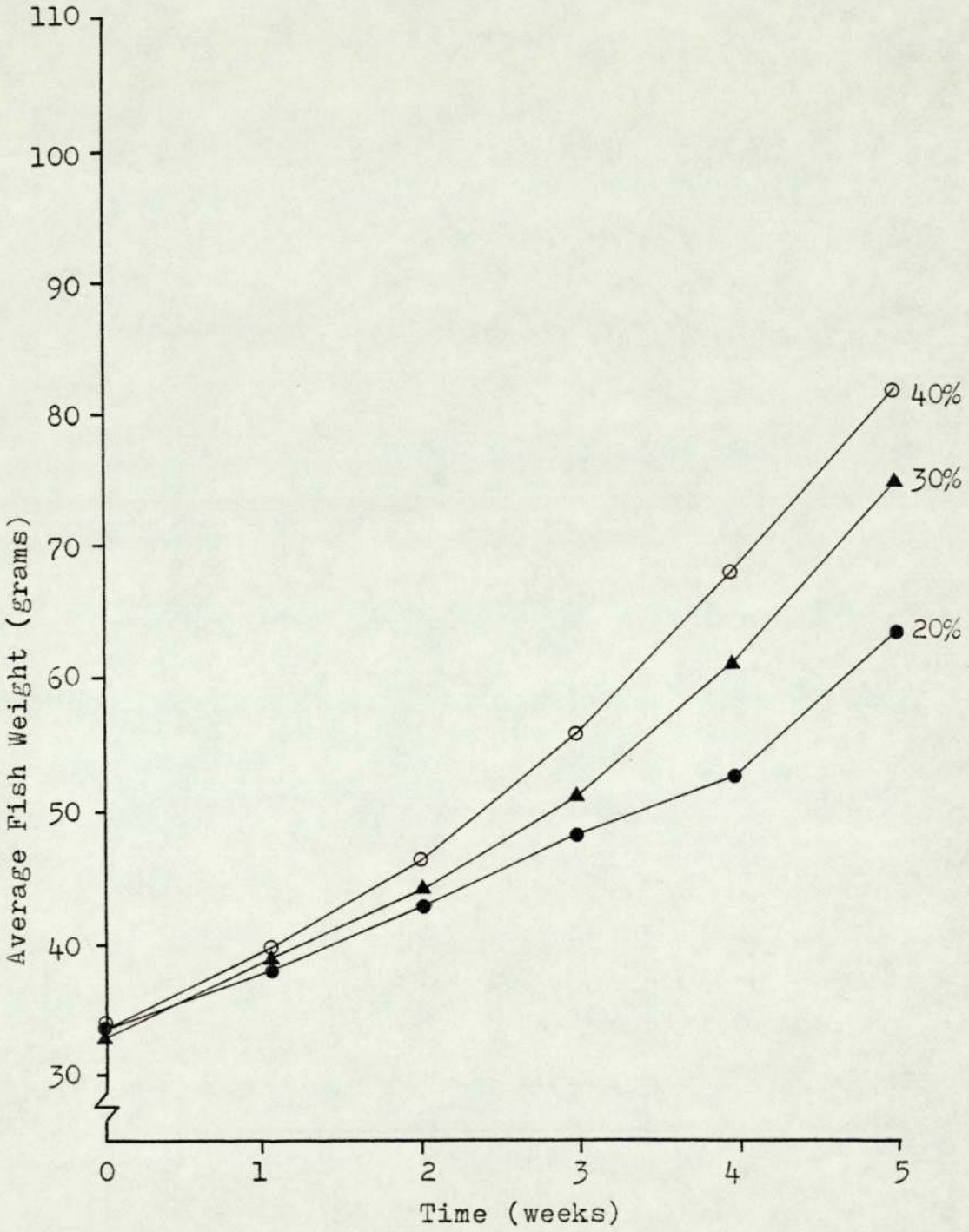


Figure 21. Growth responses of carp at 20°C fed three levels of dietary protein.

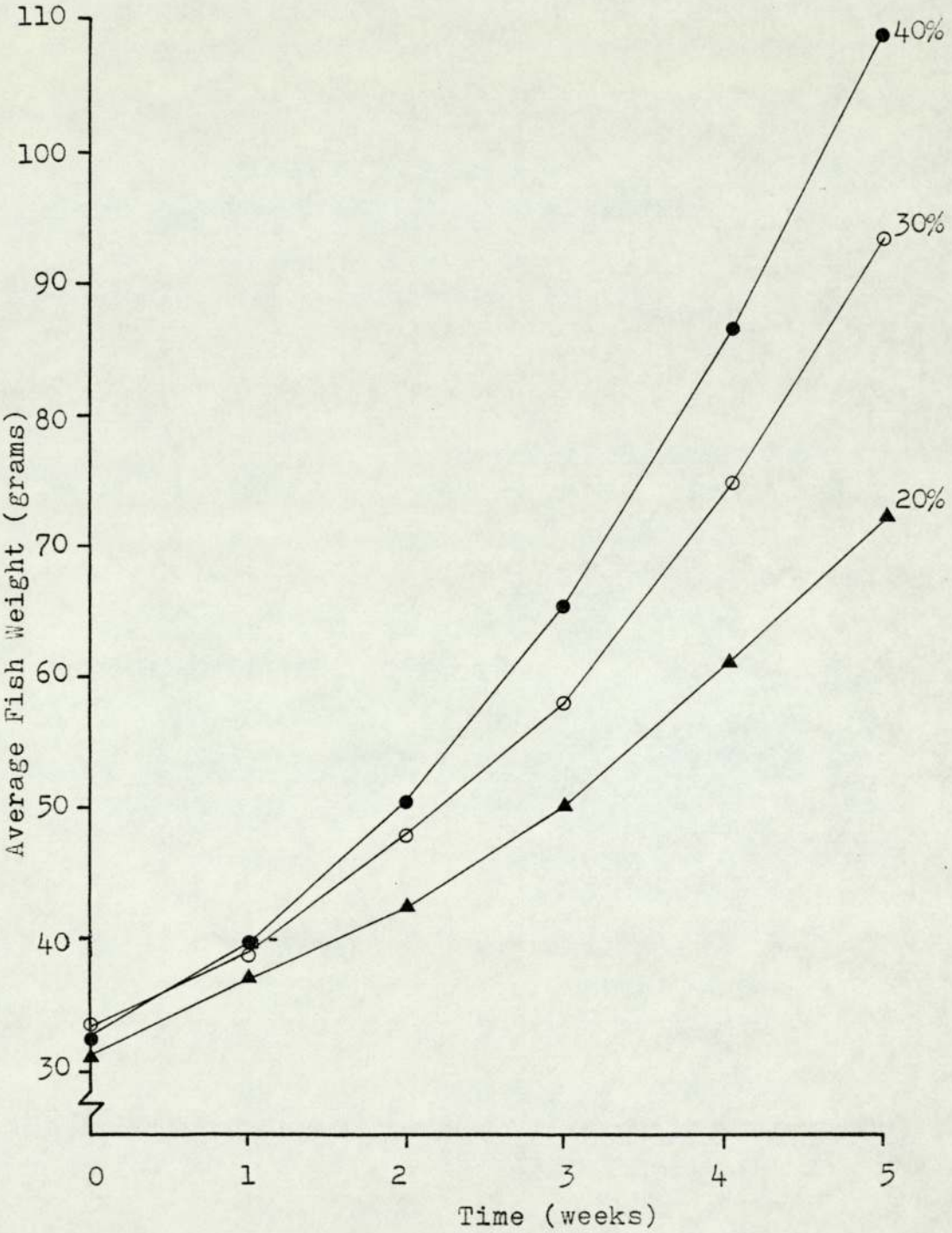


Figure 22. Growth responses of carp at 25°C fed three levels of dietary protein.

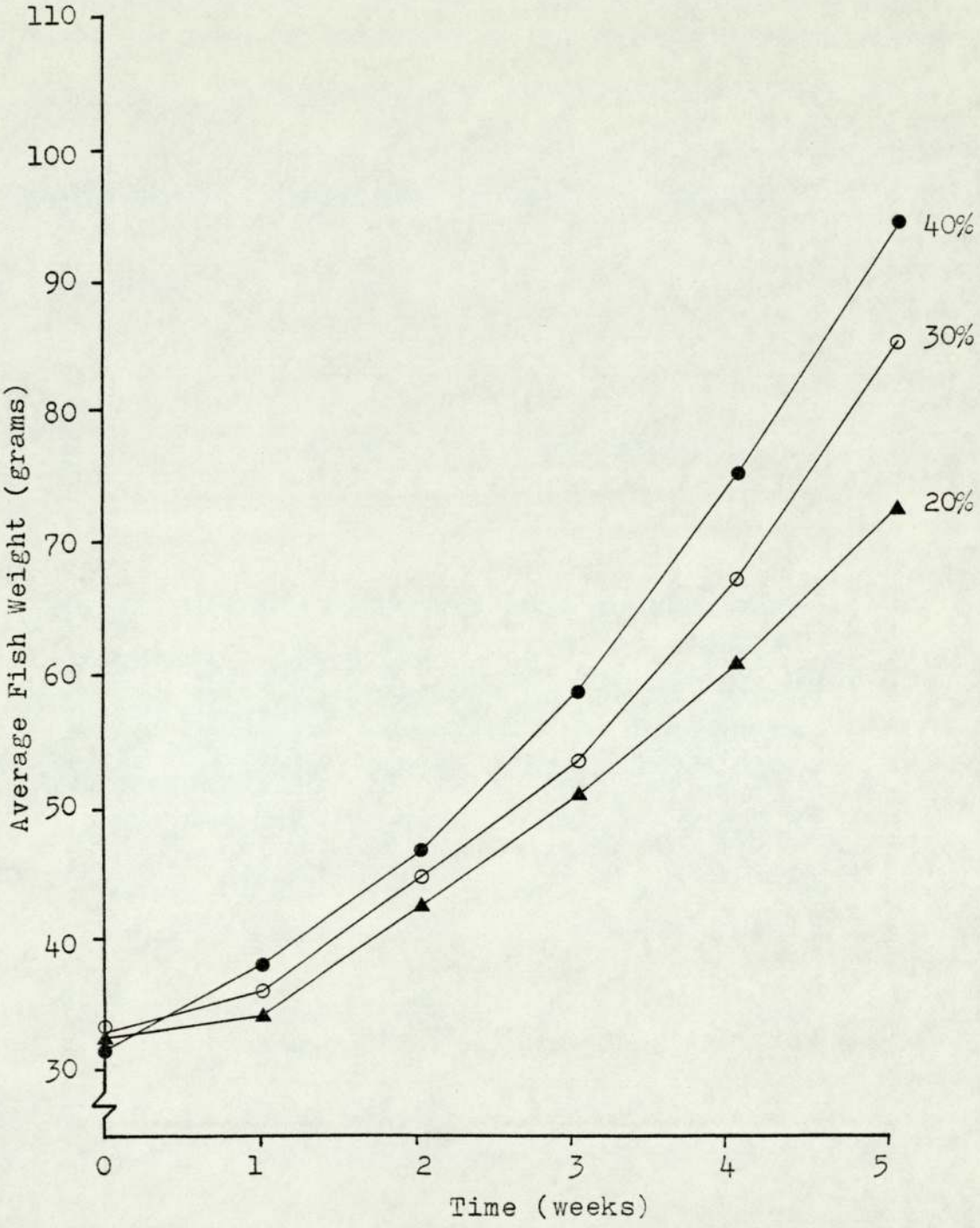


Figure 23. Growth responses of carp at 30°C fed three levels of dietary protein.

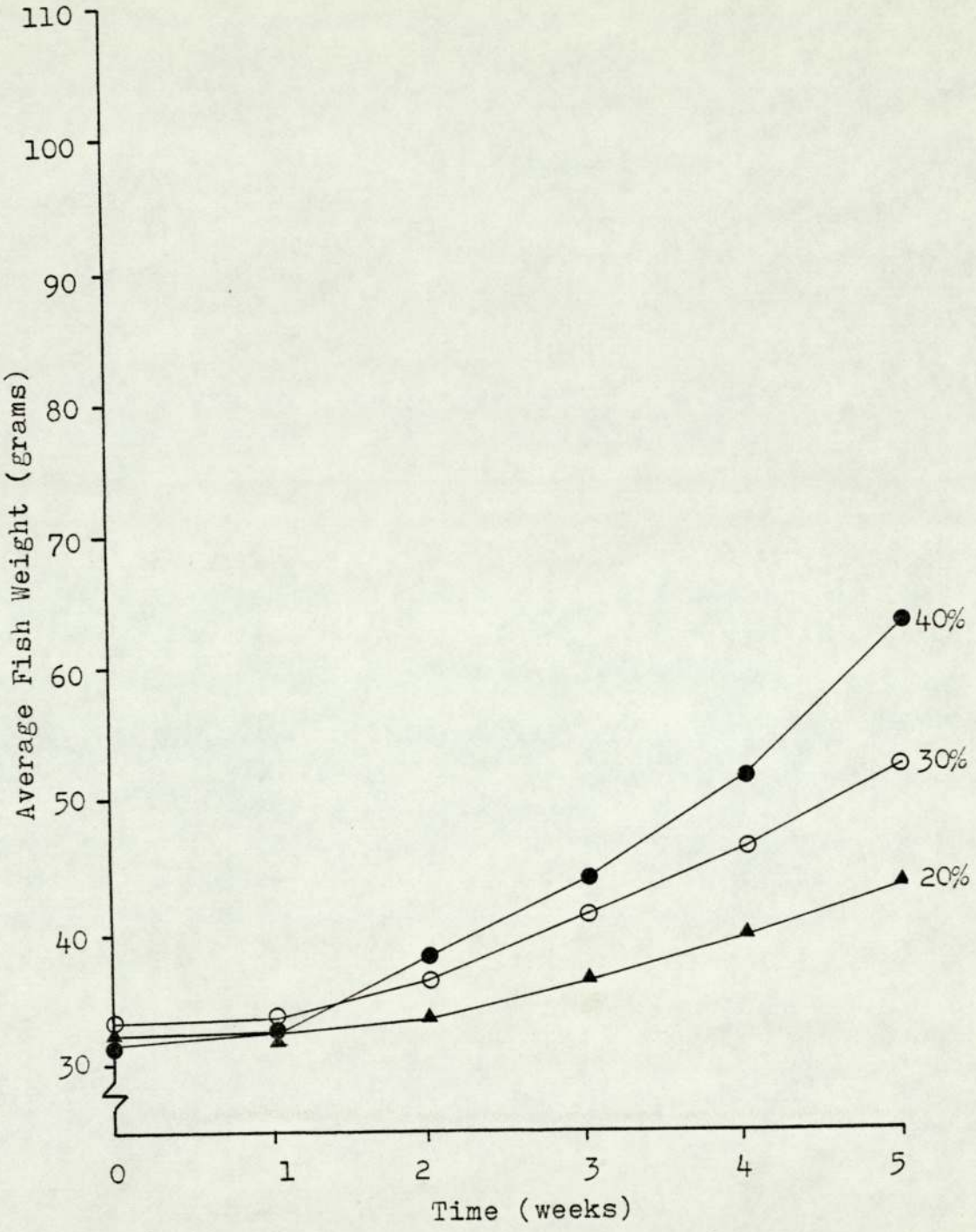


Figure 24. Growth responses of carp at 35°C fed three levels of dietary protein.

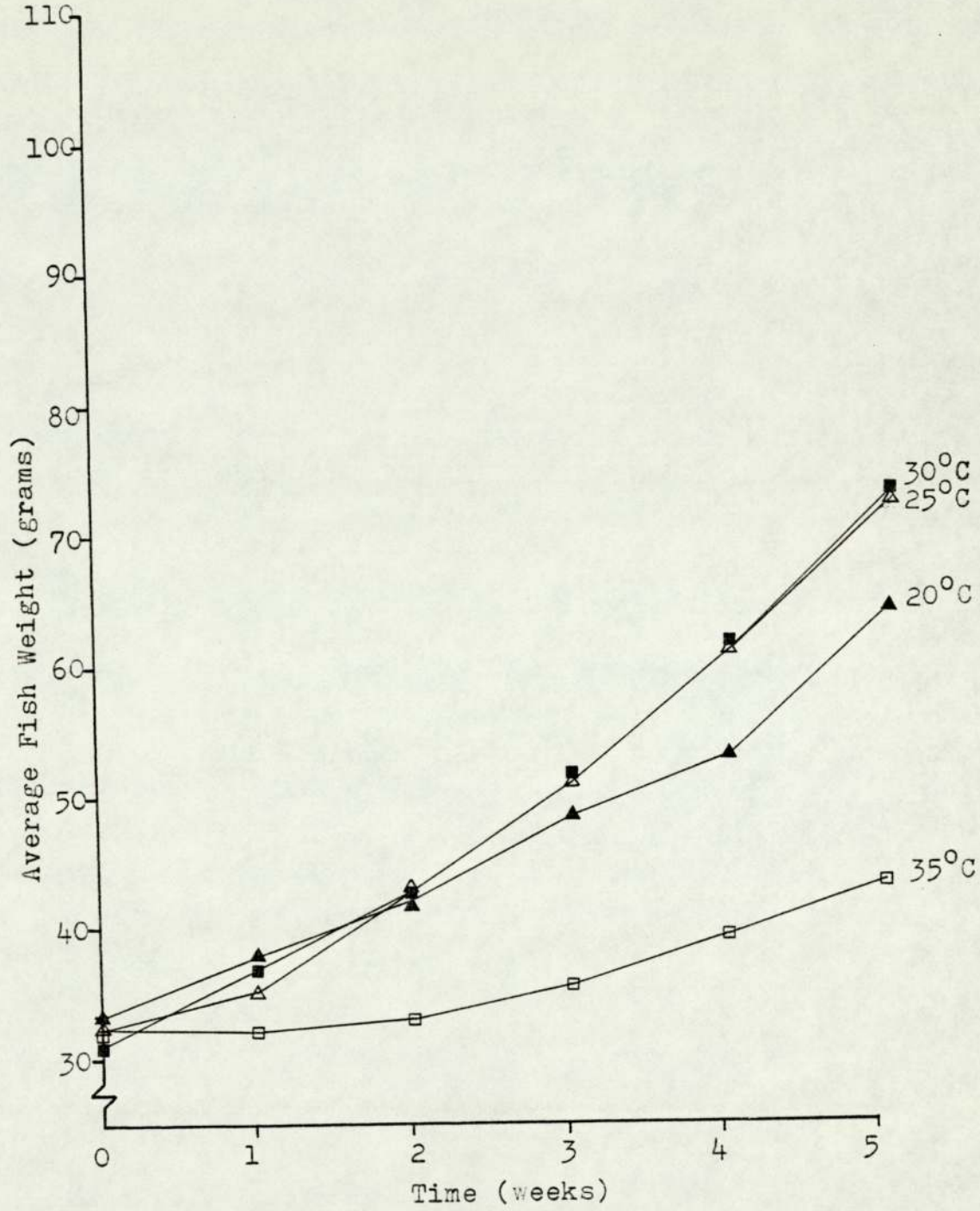


Figure 25. Growth responses of carp fed 20% protein at four different temperatures.

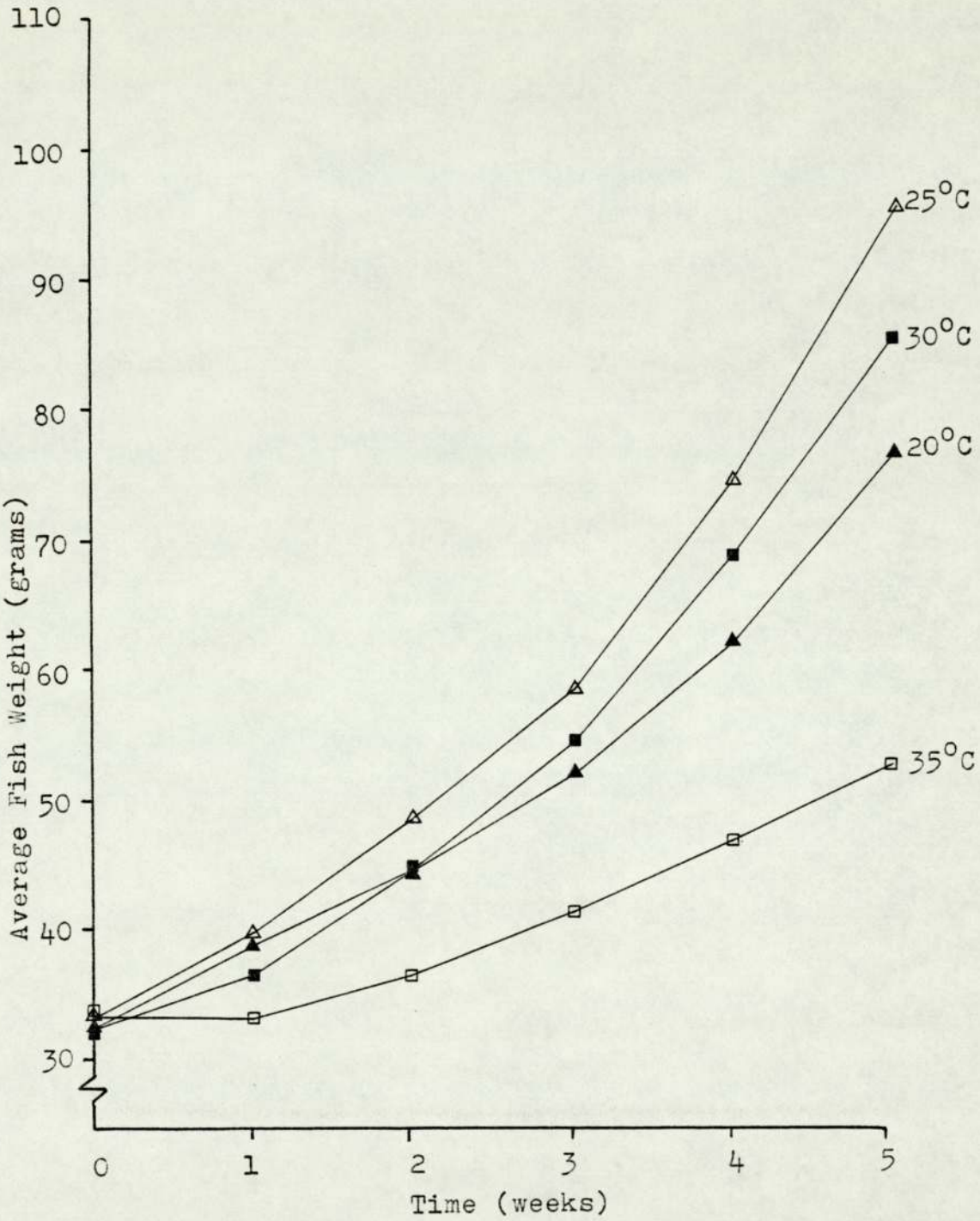


Figure 26. Growth responses of carp fed 30% protein at four different temperatures.

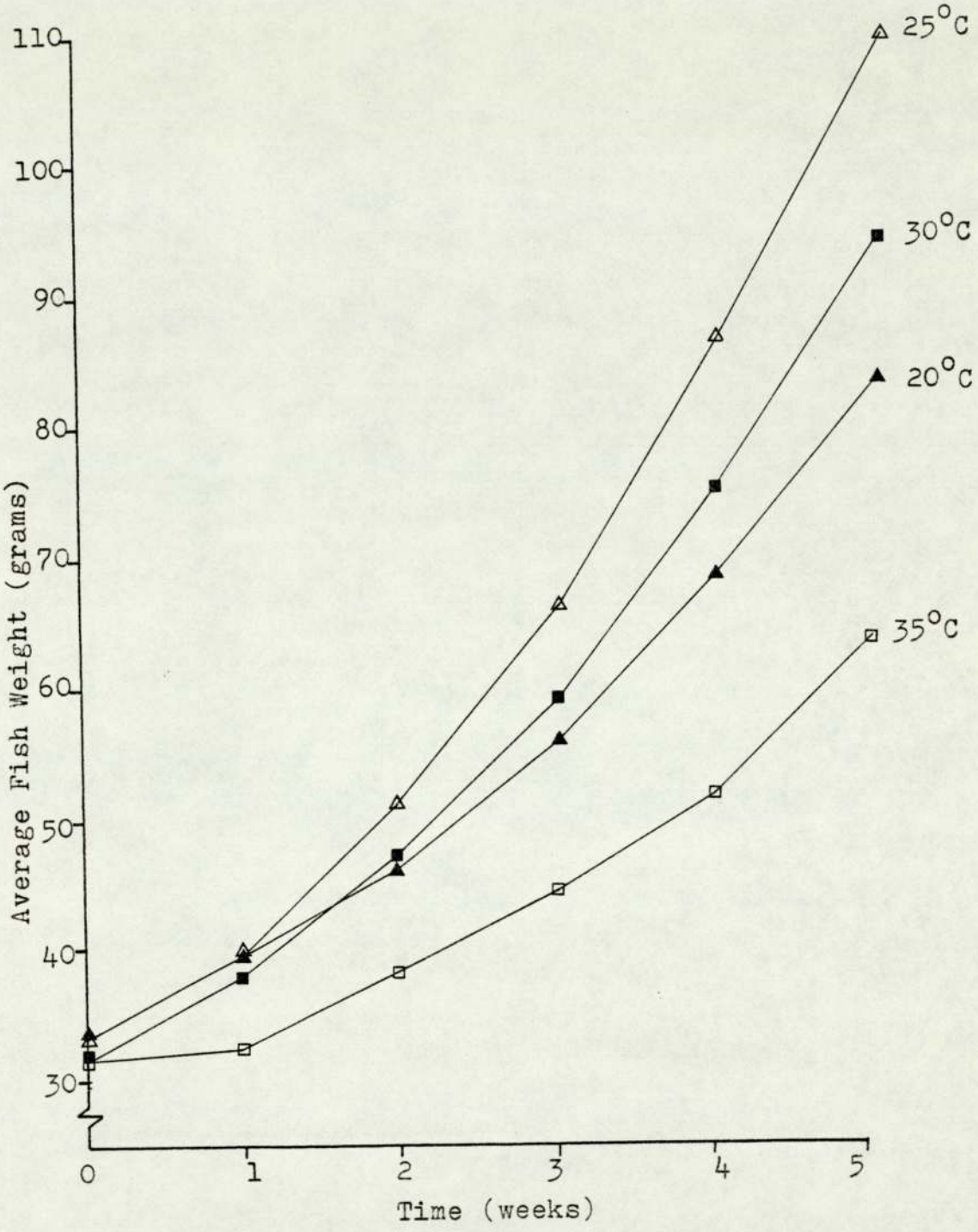


Figure 27. Growth responses of carp fed 40% protein at four different temperatures.

Figures 21 - 24 show that with increasing dietary protein level at each temperature growth increased. Figures 25 - 27 show the effect of increasing the temperature at each level of dietary protein. At the 20% protein level (Figure 25) increasing the temperature from 20 to 25°C increased growth, further increasing the temperature to 30°C had no effect and growth at 35°C was greatly depressed. Figures 26 & 27 show that, at dietary protein levels of 30 & 40%, increasing the temperature from 20 to 25°C increased growth, further increasing the temperature to 30°C resulted in a final weight midway between that at 20 & 25°C and increasing the temperature to 35°C resulted in depressed growth.

Statistical analysis of the average initial fish weights (Table 38) revealed no significant differences ($p > 0.05$) between groups. Statistical analysis of average final fish weights (Table 38) reveals a significant ($p < 0.05$) increase in the average final weight with increasing protein level at each temperature.

Specific Growth Rates (SGRs, section 2.7.1.) were calculated for each group and the results are presented in Table 38. SGRs increased significantly ($p < 0.05$) with increasing dietary protein level at each temperature. At all levels of dietary protein SGRs were significantly lower ($p < 0.05$) at 35°C and next lowest at 20°C. SGRs were significantly higher ($p < 0.05$), at the 30 & 40% protein levels, at 25°C than at 30°C although at the 20% protein level SGRs at these two temperatures were not significantly different ($p > 0.05$).

Section 7.3.2. Food Conversion.

Food Conversion Ratios (FCRs, section 2.7.2.) were

Table 38. Growth and Food Utilization Data from Experiment 6.

Group Temp./Protein		Average Initial Weight(g)	Average Final Weight(g)	S.G.R. (%/day)	FCR.	PER.	Apparent NPU(%)
20°C	20%	33.37[a]	64.31[e]	1.87[e]	2.71	1.72	23.29
"	30%	32.91[a]	75.63[d]	2.40[d]	2.14	1.50	19.69
"	40%	33.37[a]	82.91[c]	2.61[c]	1.97	1.20	17.89
25°C	20%	31.28[a]	72.26[d]	2.40[d]	2.16	2.16	31.48
"	30%	33.27[a]	94.76[b]	3.01[b]	1.37	2.35	29.73
"	40%	33.05[a]	109.26[a]	3.42[a]	1.50	1.58	20.89
30°C	20%	32.65[a]	72.76[d]	2.27[d]	2.20	2.11	30.21
"	30%	32.79[a]	85.15[c]	2.74[c]	1.84	1.75	23.53
"	40%	31.75[a]	93.53[b]	3.09[b]	1.67	1.43	20.89
35°C	20%	32.17[a]	42.43[g]	0.86[g]	5.87	0.79	11.21
"	30%	33.27[a]	52.73[f]	1.33[f]	3.75	0.86	12.36
"	40%	31.77[a]	60.35[e]	1.84[e]	2.72	0.87	13.02
S.E.±		1.132	1.412	0.110			

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).

Standard Errors (S.E.±) indicate the range of the means tested.

calculated for each group and are presented in Table 38. FCR decreased with increasing dietary protein level at each temperature. FCRs were lowest for all three levels of dietary protein at 25°C followed by 30, 20 & 35°C respectively.

Section 7.3.3. Carcass Composition.

The results of proximate analysis (section 2.6.) of initial and final fish samples are presented in Table 37. Moisture content of the final sample decreased with increasing levels of dietary protein at each temperature and was inversely related to crude lipid, in some cases these variations were significant ($p < 0.05$). Moisture content also decreased at each dietary protein level as the temperature was increased from 20 to 30°C and was inversely related to carcass lipid content. Protein contents of the final sample did not vary significantly ($p > 0.05$) and ash content showed little variation except that it was significantly lower ($p < 0.05$) in fish at 30°C fed 20% protein.

Section 7.3.4. Protein Utilization.

The efficiency with which fish utilized dietary protein was determined by calculation of Protein Efficiency Ratios (PERs, section 2.7.3.) which are presented in Table 38. At temperatures of 20 & 30°C PER decreased with increasing dietary protein level. However, at 35°C PER increased slightly with increasing dietary protein level possibly because at a low dietary protein level virtually all of the protein was metabolised for energy whereas at higher protein levels a little was spared for growth. PERs reflected FCRs being highest at 25°C followed by 30, 20 & 35°C respectively.

Section 7.3.5. Protein Digestibility.

Apparent protein digestibilities were determined as described in section 2.7.4 and are presented in Table 39. Apparent protein digestibility increased with increasing level of dietary protein at each temperature. Protein digestibility at each level of dietary protein decreased with increasing temperature with digestibilities at 35°C being greatly depressed.

Section 7.3.6. Dietary, Calculated Metabolisable and Measured Digestible Energy Values.

The total energy contents of the diets and the metabolisable energy contents (Table 36) were estimated using the figures and principles detailed in section 4.3.6. The calculated total energy contents are consistently lower than the total energy values obtained by bomb calorimetry and presented in Table 35. This is probably due to the calorific value of the vitamins and binder not included in the calculation.

The measured digestible energy contents (Table 39) are, in all cases, lower than the calculated metabolisable energy contents possibly due to the effect noted in Experiment 2 (Chapter 4) that metabolisable energies in excess of 3.6 kcal/g did not result in increased digestible energies.

The digestible energy of the diets increased with increasing dietary protein level at each temperature. This is to be expected as the metabolisable energy of protein is higher than that of the dextrin which it replaces in the diets. Digestible energy increased with increasing temperature from 20 to 30°C at each level of dietary protein however, at 35°C digestible energy was low at all protein levels.

Table 39. Digestibility Data from Experiment 5.

Temperature(°C)	20	20	20	25	25	25	30	30	30	35	35	35
Protein Level (%)	20	30	40	20	30	40	20	30	40	20	30	40
Apparent Protein Digestibility (%)	86.83	88.61	90.01	85.59	86.47	88.01	81.81	82.63	83.40	64.41	67.68	72.01
Apparent Energy Digestibility (%)	70.83	69.98	70.76	74.56	73.57	73.62	76.54	74.63	74.03	52.63	49.69	50.10
Digestible Energy (DE, kcal/g)	3.23	3.31	3.46	3.40	3.48	3.60	3.49	3.53	3.62	2.40	2.41	2.45

Section 7.4. Discussion and Conclusions.

This experiment was designed to evaluate the effects of temperature on the protein requirements of mirror carp. It would appear that even at the highest protein level employed (40%) the maximum response to increasing the dietary protein level had not been reached. Conversely in Experiment 2 (Chapter 4) no significant differences ($p > 0.05$) were observed between diets containing 29 & 37% protein. However, if the growth curves in Figures 21 - 23 are compared it can be seen that the lines representing the 30 & 40% protein levels, at temperatures of 20, 25 & 30°C, are parallel for the last two weeks of the experiment indicating a similar growth rate. The slower growth of fish fed the 30% protein diet, at the start of the experiment, is not readily explainable. It may have been that the fish fed the 40% protein diet accumulated body lipid at a greater rate at the start of the experiment, than those fed 30% protein, and the use of a fixed feeding rate amplified the divergence in growth rates as the heavier group was fed more food.

In this experiment growth at 25°C was superior, at dietary protein levels of 30 & 40%, to growth at 30°C but growth at these two temperatures was the same at the 20% protein level. This is the converse result to that of Experiment 1 (Chapter 3) where growth was higher, but not significantly, at 30°C than at 25°C. However, again if the slopes during the last two weeks of the present experiment are compared at 25 & 30°C and 30 & 40% protein (Figures 25 & 26) there is little difference. In fact at the 30% protein level (Figure 25) the growth curves at 25 & 30°C are parallel after the first week.

It would be expected that the level of dietary protein

producing maximum growth would be increased by increasing the experimental temperature as reported for chinook salmon, Oncorhynchus tshawytscha, (Delong et al., 1958). As temperature increases, up to the optimum for growth, growth increases, and thus so must the quantity of dietary protein required for protein synthesis. The metabolic rate also increases with increasing temperature accompanied by an increase in endogenous nitrogen excretion (ENE) and an increase in the protein requirement to cover these losses. The ENE of carp was found to be 7.2 mg N/100 g of fish/ day at 20°C and 8.6 mg N at 27°C (Ogino et al., 1973).

The level of dietary protein producing maximum growth will also depend upon the non-protein metabolisable energy of the diet as reflected by the protein to energy ratio (P:E, mg crude protein per kcal of metabolisable dietary energy) and discussed in Experiment 2 (Chapter 4). The level of dietary protein producing maximum growth in mirror carp has been reported for differing conditions. Ogino & Saito (1970) found that at 23°C and a metabolisable energy (ME) of 3.7 kcal/g (calculated using the values presented in section 8.3.6.) maximum growth occurred on a 38% protein diet. In a subsequent study (Ogino et al., 1976) these authors reported an optimum dietary protein content of 35% at 20°C with an ME of 3.4 kcal/g.

Sin (1973a,b) found that at 24 - 25°C and an ME of 2.7 kcal/g the optimum protein content was 38.4% but that increasing the ME to 3.06 kcal/g reduced the optimum protein content to 33%. In the present study the diets contained a calculated ME of 3.8 - 3.96 kcal/g and growth on a 40% protein diet was significantly greater than that on the 30% protein diet at temperatures of 20, 25 & 30°C although, as previously explained,

this was due to a difference in growth during the first two weeks of the experiment.

At 35°C, however, the 40% protein level resulted in a growth curve (Figure 24) divergent to that at the 30% protein level possibly indicating an increase in protein requirement at this temperature. Growth at 35°C in the present experiment was greatly increased compared with that at 35°C obtained in Experiment 1 (Chapter 3) possibly as a result of the greater water stability of the diets used and the use of purified ingredients leading to higher digestibilities.

In this experiment the dependence of carcass composition on temperature was more marked than in Experiment 1 (Chapter 3). The result that carcass lipid content increased with increasing temperature (from 20 to 30°C) is in agreement with results for channel catfish, Ictalurus punctatus, (Andrews & Stickney, 1972) and rainbow trout, Salmo gairdneri, (Papoutsoglou & Papoutsoglou, 1978). The effects of temperatures close to the upper lethal limit, on carcass composition, do not appear to have been reported for other fish species; in the present experiment carcass lipid content was greatly reduced at 35°C. This to be expected as digestibility decreases with increasing temperature, as discussed below, and metabolic demands are greatly increased resulting in utilization of stored carcass lipid for energy. Increasing the dietary protein level at each temperature also resulted in increased carcass lipid contents as it did in Experiment 2 and as is discussed in section 4.4.

An increase in apparent protein digestibility with increasing dietary protein level was found both in the present experiment and in Experiment 2 (Chapter 4). Such an increase

has been reported by other authors (Nose, 1967; Ogino & Chen, 1973; Smith & Lovell, 1973; Austreng, 1978a) and is attributed to the fact that at low levels of protein intake the ENE is a significant proportion of the total nitrogen excreted whereas at high levels of dietary protein it is not. As no correction is made for ENE in the calculation of apparent protein digestibility it will cause a larger reduction in apparent digestibility at low levels of nitrogen excretion than at high.

The observed decrease in apparent protein digestibility with increasing temperature is probably the result of a combination of two factors. At higher temperatures ENE is increased (Ogino et al., 1976) causing a depression in apparent protein digestibility. In addition, the motor activity of the gastrointestinal tract is doubled by a 10°C rise in temperature (Shcherbina & Kazlauskene, 1971) thus reducing the length of time the food is in the gut and available for digestion and absorption.

Although the protein digestibility decreased with increasing temperature apparent energy digestibility increased, at each level of dietary protein, with increasing temperature from 20 to 30°C and then fell sharply at 35°C. This may be due to the increasing digestibility of dietary lipid with moderate increase in temperature (Atherton & Aitken, 1970; Shcherbina & Kazlauskene, 1971; Stickney & Andrews, 1972; Andrews et al., 1978) but at 35°C the rate of passage of food through the gastrointestinal tract may have become the dominant factor affecting digestibility.

The efficiency of protein utilization (as determined by PER & NPU) decreased with increasing dietary protein level at each temperature and was highest, for each level of protein,

at 25°C followed by 30, 20 & 35°C respectively. These results reflect the growth performance of the different groups. Maximum PER occurred at 30% protein/25°C and maximum NPU at 20% protein/25°C.

In conclusion it may be said that even on diets containing 40% protein maximum growth was not obtained, however, in a practical situation a compromise between growth and protein utilization would have to be reached and, in this respect, diets containing 30% protein and a metabolisable energy content of 3.5 kcal/g would be optimum at temperatures between 25 & 30°C.

CHAPTER 8.

Chapter 8.

8. Experiment 6. The Effects of High Energy Diets with Varying Protein Contents on the Growth Performance, Food Conversion, Body Composition and Protein Utilization of Mirror Carp (Cyprinus carpio) Maintained in an Industrial Thermal Effluent.

Section 8.1. Introduction.

This final experiment was performed in order to enable comparison of the previous laboratory studies with the growth of mirror carp in an actual industrial thermal effluent. The thermal effluent used was at the Central Electricity Generating Boards' coal-fired thermoelectric generating station at Ratcliffe-on-Soar, Nottingham. A small facility existed at this power station where investigations into the growth of carp and eels had already been undertaken (Aston et al., 1975; Aston & Brown, 1976; Aston & Brown, 1978) which had demonstrated that good growth rates were achievable. However, these studies were not directly comparable to those undertaken in the laboratory as commercial trout rations had been fed to excess.

Despite the recent upsurge of interest in the use of heated effluents for fish culture, described in Chapter 1, very few studies of the growth of fish in heated effluents under controlled conditions have been performed. The present study was thus designed to determine the effects of heated effluents on carp growth, as compared to laboratory recycling systems, and to find the optimum protein requirement under these conditions.

Groups of fish, maintained in a thermal effluent at approximately 28°C, were fed diets containing 20, 25, 30, 35, 40 & 45% protein for five weeks and the effects on growth

performance, body composition and food utilization determined.

Section 8.2. Materials and Methods.

Section 8.2.1. The Experimental System and Animals.

The experimental facility used in the present study was 'System 4' as described in section 2.1.3.

54 mirror carp (15 - 20 cm) were selected from the fish remaining from Experiment 3 (Chapter 5) which had meanwhile been maintained at ambient temperatures and fed a commercial trout ration. These fish were transported to Ratcliffe-on-Soar in sealed polythene bags containing a little water and inflated with oxygen. They were then transferred to the 6 experimental tanks (9 per tank) at the prevailing ambient temperature of 10°C and the temperature raised at approximately 3°C/day to the experimental temperature of 28°C.

The automatic feeders were adjusted to dispense approximately 5% of the body weight per day of a commercial trout ration. After one week of acclimation the fish were starved for 12 hours and six fish were removed (one per tank) for proximate carcass analysis (section 2.6.). The remaining fish were batch weighed (section 2.5.) and the tank weights were balanced by redistribution of fish to ensure similar starting weights.

Temperature was monitored throughout the experiment and varied between 23 and 28°C. Photoperiod was not controlled and was dependant upon natural natural daylight. The experiment was conducted during October/November and the natural photoperiod was approximately 10 hours light and 14 hours darkness throughout the experimental period.

Section 8.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general method described in section 2.2 with increasing dietary protein content achieved by replacement of dextrin with herring meal. The ingredient composition of the diets is shown in Table 40. Diet preparation was by wet extrusion (section 2.3.). The results of proximate analysis (section 2.6.) of the diets are presented in Table 41 and estimated dietary components in Table 42. Higher levels of binder (carboxymethylcellulose), 2.5%, were incorporated into these diets to improve pellet water stability and to prevent the diets being crumbled by the screw-auger automatic feeders.

Section 8.2.3. Feeding Rate.

The fish were fed by automatic feeders which dispensed a quantity of diet every 15 minutes for four hours daily (09.30h - 11.30h and 15.30h - 17.30h). The quantity of food dispensed per feed was adjusted after each weekly weighing so that a total of 5% (dry food) of the body weight was dispensed in sixteen such feeds (i.e. in one day). A calculated weeks ration was placed in the feeders after each weekly weighing and any diet remaining at the end of the week subtracted from the total food fed.

Section 8.2.4. Weighing and Sampling.

Details of the weighing procedure are presented in section 2.5. Fish were batch weighed ($\pm$ 0.1 g), under anaesthesia every 7 days for five weeks. At the end of the fifth (and final) week 4 fish were removed from each tank and subjected to proximate carcass analysis (section 2.6.) the results of

Table 40. Ingredient Composition of the Diets used in Experiment 6.

Ingredient (dry weight)	DIET 1	2	3	4	5	6
Herring Meal	27.29	34.11	40.94	47.76	54.59	61.40
Herring Oil	9.78	9.22	8.67	8.11	7.55	7.00
Mineral Mix[1]	7.53	6.42	5.30	4.18	3.07	1.95
Starch	4.40	4.24	4.09	3.94	3.79	3.64
Vitamins[2]	1.00	1.00	1.00	1.00	1.00	1.00
Binder[3]	2.50	2.50	2.50	2.50	2.50	2.50
Dextrin	27.50	22.45	17.50	12.51	7.50	2.51
Glucose	15.00	15.00	15.00	15.00	15.00	15.00
∝-Cellulose	5.00	5.06	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated;-						
Crude Protein (%)	20.00	25.00	30.00	35.00	40.00	45.00

1 Composition given in Table 6 section 2.2.

2 Composition given in Table 7 section 2.2.

3 Carboxymethylcellulose, Sodium Salt, High Viscosity.

Table 41. Proximate Analysis of the Diets used in Experiment 6.

	DIET					
	1	2	3	4	5	6
Moisture (%)	12.18	12.81	13.30	12.99	11.49	12.06
On a moisture free basis;-						
Crude Lipid (%)	11.94	12.05	11.88	12.01	12.14	11.89
Crude Protein (%)	20.66	24.98	30.63	35.42	40.01	45.17
Ash (%)	13.66	14.42	14.81	14.95	15.01	15.40
NFE[1] (%)	53.74	48.55	42.68	37.62	32.84	27.54
Energy (kcal/g)	4.53	4.58	4.69	4.74	4.83	4.90

1 Nitrogen Free Extractives.

1 Nitrogen Free Extractives

Table 42. Estimated Components of the Diets used in Experiment 6.

	DIET					
	1	2	3	4	5	6
Carbohydrate (%) (including NFE from fishmeal)	47.5	42.5	37.5	32.5	27.5	22.5
Fibre (%) (∝-Cellulose)	5.0	5.0	5.0	5.0	5.0	5.0
Total Energy (kcal/g)	4.45	4.50	4.60	4.67	4.74	4.80
Metabolisable Energy (kcal/g)	3.68	3.71	3.78	3.82	3.87	3.90
P:E Ratio (mg Protein per kcal of metabolisable energy)	56.1	67.3	81.0	92.7	103.4	115.8

of which are presented in Table 43.

Section 8.2.5. Statistical Methods and Analysis of Growth Data. These were performed as detailed in section 2.7.

Section 8.3. Results.

Fish in all groups appeared to consume all of their daily rations as no uneaten food was observed in the gutter into which the tanks drained. The automatic feeder on diet 5 (40% protein) failed during the second week of the experiment and dispensed a whole weeks ration in 3 days, thus the data for this group are not comparable. The malfunctioning feeder was replaced at the beginning of week 3. No mortalities occurred during the experimental period.

Section 8.3.1. Growth Performance.

The growth responses of the groups fed the six diets are shown graphically in Figure 28 as plots of average fish weight against time. Increasing the dietary protein level from 20 to 35% increased the growth response, growth on the 40% protein diet was depressed due to a feeder malfunction and growth on the 45% protein diet was slightly less than on the 35% protein diet.

Initial average fish weights (Table 44) varied slightly ($\pm$ 0.6 g) except for that of group two (25% protein) which was 2 grams heavier. Final average fish weights (Table 44) increased with increasing dietary protein level from 20 to 35% and then decreased slightly. This trend was reflected by the Specific Growth Rates (SGRs, section 2.7.1.) which are also presented in Table 44. SGR increased from 2.5 to 3.6 with increasing dietary protein level from 20 to 35% and then decreased slightly to 3.31 for the 45% protein diet.

Table 43. The Results of Proximate Analysis of Initial and Final Fish Samples from Experiment 6.

Sample	Moisture(%)	Crude Lipid(%)	Crude Protein(%)	Ash(%)	Total(%)
Initial	77.4	6.4	13.0	2.2	99.0
Final;-					
1 (20%)	75.9^{a}	6.5^{c}	13.8^{a}	2.9^{a}	99.0
2 (25%)	75.4^{a}	7.0^{c}	13.7^{a}	2.9^{a}	99.0
3 (30%)	74.3^{b}	7.6^{bc}	14.1^{a}	2.8^{a}	98.9
4 (35%)	73.6^{b}	8.1^{b}	14.0^{a}	3.0^{a}	98.7
5 (40%)	73.3^{b}	8.6^{ab}	14.6^{a}	2.6^{a}	99.0
6 (45%)	73.2^{b}	8.8^{a}	13.9^{a}	2.9^{a}	98.8
S.E.$\pm$	0.362	0.410	0.227	0.200	

Figures in the same column having the same superscript are not significantly different ($p > 0.05$).
Standard Errors (S.E.$\pm$) indicate the range of the means tested.

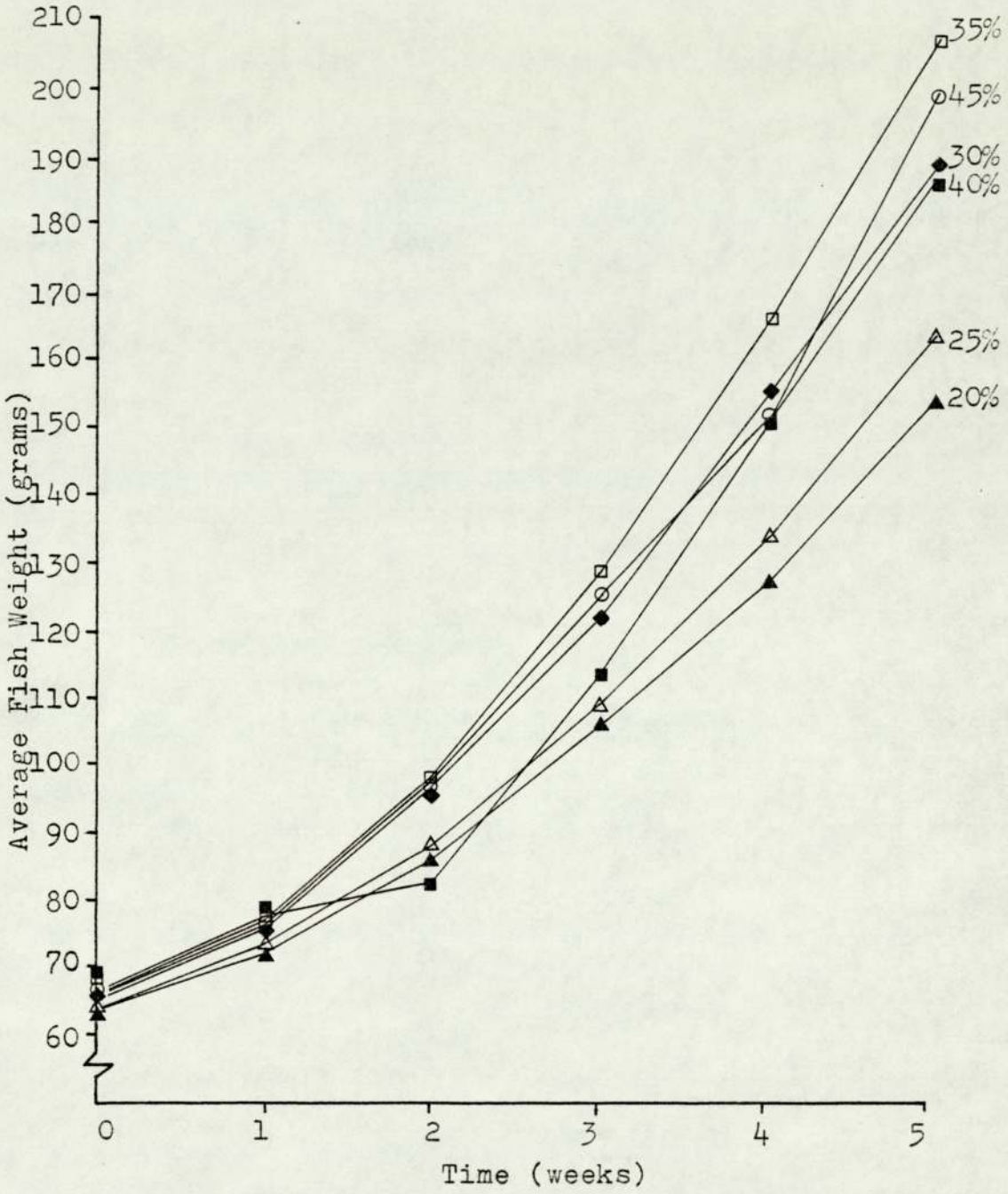


Figure 28. Growth responses of carp fed six different levels of dietary protein.

Table 44. Growth and Food Utilization Data from Experiment 6.

	DIET					
	1	2	3	4	5	6
Protein	20%	25%	30%	35%	40%	45%
Initial Av.Wt.(g)	63.78	63.75	64.70	64.20	66.08	63.53
Final Av.Wt.(g)	152.80	163.33	189.99	207.94	188.30	202.63
SGR[1] (%/day)	2.50	2.69	3.08	3.36	2.99	3.31
FCR[2]	2.04	1.89	1.67	1.53	1.66	1.55
PER[3]	2.38	2.12	1.95	1.85	1.50	1.43
Apparent NPU[4] (%)	30.16	29.71	28.65	26.39	23.35	20.54

1 Specific Growth Rate.

2 Food Conversion Ratio.

3 Protein Efficiency Ratio.

4 Net Protein Utilization.

Section 8.3.2. Food Conversion.

Food Conversion Ratios (FCRs, section 2.7.2.) were calculated for each group and are presented in Table 44. FCR decreased with increasing dietary protein level from 20 to 35% and then increased slightly.

Section 8.3.3. Carcass Composition.

The results of proximate carcass analysis (section 2.7.3.) of initial and final fish are presented in Table 43. Moisture content of the final sample decreased with increasing dietary protein level with the moisture contents of fish fed diets containing 20 & 25% protein being significantly ($p < 0.05$) the highest. Moisture content was inversely related to the carcass lipid content which increased with increasing dietary protein level, in some cases significantly ($p < 0.05$). There was no significant variation ($p > 0.05$) in either protein or ash contents of the final fish sample.

Section 8.3.4. Protein Utilization.

Protein Efficiency Ratios (PERs, section 2.7.3.) were calculated for each group and are presented in Table 45. PER decreased with increasing dietary protein level as did values for apparent Net Protein Utilization (NPU, section 2.7.3.) which are also presented in Table 45.

Section 8.3.5. Dietary and Calculated Metabolisable Energy Values.

Total dietary energy contents were calculated on the same basis as described in section 4.3.6 and are presented in Table 42. As in the previous experiments the calculated total energy contents of the diets were consistently lower

than those obtained by bomb calorimetry and presented in Table 41. Metabolisable energy contents (ME) of the diets were calculated as described in section 4.3.6 and are presented in Table 42.

Section 8.4. Discussion and Conclusions.

This experiment was designed to enable comparison of the growth of mirror carp in a thermal effluent to that obtained under laboratory conditions and to enable determination of the optimum dietary protein content under these conditions.

The results of the present experiment can be directly compared with those of Experiment 5 (Chapter 7). Diets containing 20, 30 & 40% protein in this study are directly comparable with those used in Experiment 5 having a similar formulation, protein level and metabolisable energy content. The temperatures in this experiment (23 - 28°C) should give results comparable to the temperatures of 25 & 30°C employed in Experiment 5.

Growth performance, food conversion and protein utilization were all slightly better in this study than in comparable groups in the previous experiment. This is probably because of the increased frequency of feeding (Huisman, 1969) but serves to demonstrate that the poor water quality of the thermal effluent, as compared to the laboratory recycling system, had no noticeable deleterious effects on growth.

It would appear from the present study that the level of dietary protein producing maximum growth under these conditions is approximately 35%. Figure 29 shows a graph of protein gained per fish over the whole experimental period against dietary protein level. This graph shows that the amount of

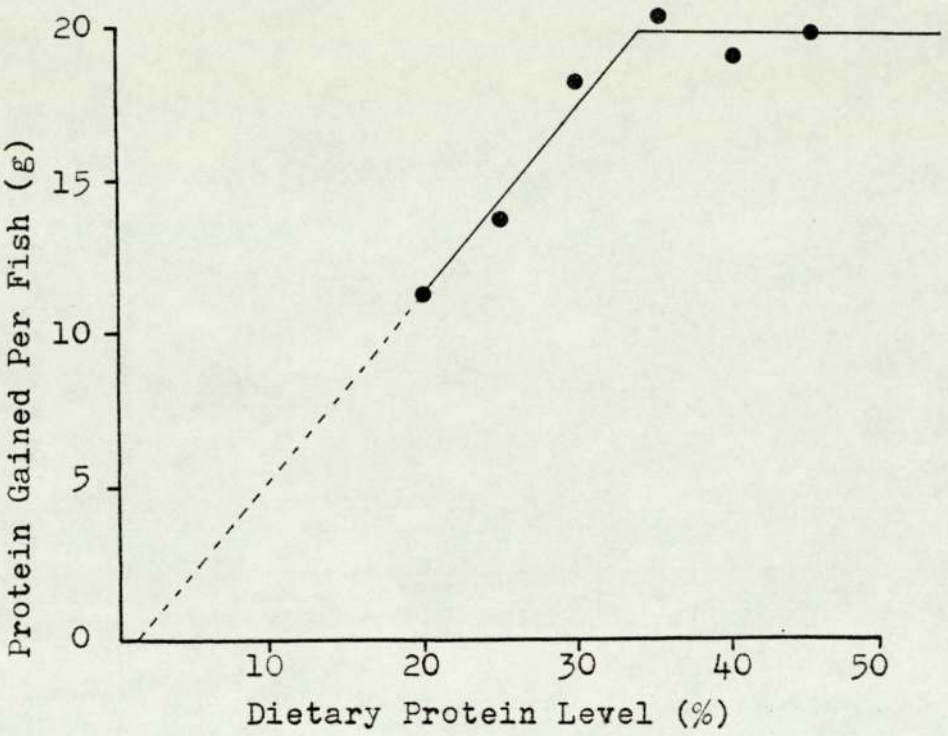


Figure 29. The weight of protein gained per fish versus dietary protein level.

protein gained per fish reached a maximum at the 35% protein level. This result is in agreement with that of Ogino & Saito (1970) who, by plotting a similar graph, found the optimum to be 38% at 23°C using casein as the protein source and with a dietary metabolisable energy (ME) content (using the values presented in section 4.3.6.) of 3.7 kcal/g. In a subsequent study (Ogino et al., 1976) these authors reported an optimum dietary protein content of 35% at 20°C with an ME of 3.4 kcal/g. Sin (1973a,b) also reported, at 24 - 25°C, an optimum dietary protein content of 38.4% with an ME of 2.7 kcal/g and 33% with an ME of 3.06 kcal/g.

Increasing the dietary protein level led to a significant ($p < 0.05$) decrease in carcass moisture content accompanied by

a significant increase in carcass lipid content. This effect was also apparent in Experiments 2 & 5 (Chapters 4 & 7) where it has been fully discussed.

The efficiency of protein utilization, as measured by Protein Efficiency Ratio (PER) and apparent Net Protein Utilization (NPU), was slightly improved in the present study compared to that in Experiment 5. However, both of these values were lower than previously reported for carp. Ogino & Saito (1970) found PERs ranging from 2.7 on a 20% protein diet to just under 2 on a 45% protein diet. NPUs in the same study were also higher decreasing from 46 to 30% over the same range of protein levels. Even higher levels were reported by Ogino et al. (1976) with PERs of 3.9 to 2.9 and NPUs of 60 to 40 over the same range of dietary protein levels (20 to 40%). However, in both of these studies the protein source used was casein which has a digestibility of 98.6% in carp (Ogino & Chen, 1973). As determined in Experiments 2 to 5 the digestibility of the fishmeal used in these studies was approximately 83 to 88% possibly explaining the lower values for PER and NPU.

In conclusion, this study showed no depression of growth of mirror carp in a heated effluent as compared to laboratory studies. The dietary protein level producing maximum growth under these conditions was 35% in a diet containing a calculated metabolisable energy content of 3.82 kcal/g at a protein to energy ratio of 92.7 where 41.6% of the metabolisable energy was supplied by protein.

CHAPTER 9.

Chapter 9 . Final Conclusions.

The main thesis with which this investigation was initiated was whether or not mirror carp were suitable nutritionally, and in terms of their temperature requirements, for culture in heated effluents.

It has been demonstrated that maximum growth of mirror carp occurs at temperatures between 25 & 30°C and that growth at 20 & 35°C is depressed compared to the afore mentioned temperature range. No differences in several growth and food utilization parameters were found between fish held at 25 & 30°C for eight weeks. In the present experiment the effect of feeding level on growth performance and food utilization was also investigated.

Increasing the daily ration from 3 to 6% of the body weight greatly increased growth but further increasing the feeding level to 9% resulted in only a small increase in growth rate and a fall in food utilization.

It was thus inferred that mirror carp is a suitable species for culture in the effluent of an inland, closed circuit thermoelectric generating station as its' optimum temperature range for growth and food conversion is compatible with that obtained in such an effluent (Aston & Brown, 1978).

Mirror carp held in the optimum temperature range achieved specific growth rates (average percentage increase in body weight per day) of 3 at food conversions (g dry food fed per g live weight gain) of 1.5 to 2. Such high rates of growth, as compared to salmonids, would enable maximum utilization of the high capital cost holding facility that would be required for fish culture in heated effluents.

If a specific growth rate of 3 was maintained it would result in fish growing from 1g to 1kg in 33 weeks as compared to approximately 2½ years (128 weeks) in ponds in southern England.

Protein sparing by dietary lipid was also shown. It proved possible to reduce the protein content of mirror carp diets, containing 18% marine fish oil, from 45 to 29% with no reduction in growth performance, with improved protein utilization and with no unacceptable accumulation of carcass lipid. Such a reduction in the protein level of mirror carp feeds is of economic significance as there is, generally, a world shortage of protein particularly for use in animal feeds (Anon., 1978a).

The use of various dietary protein substitutes for fishmeal was also investigated. This study was prompted by the recent rise in the price of fishmeal and its uncertain continuity of supply (Anon., 1978a). It was shown that carp, like salmonids, require a diet containing high quality protein for optimum growth in tanks and that a methanophilic bacterium and a petroprotein yeast could be used to supply a substantial proportion of this protein.

However, two plant proteins tested, algal and soyabean, were poorly utilized despite digestibilities in excess of 80%. As soyabean is the principal oilseed protein on a world wide basis (Anon., 1978a) its potential use in mirror carp diets was further investigated. It was demonstrated that replacement of one third of the fishmeal, in a 30% protein diet, by a soyabean protein concentrate caused a significant decrease in growth rate and food utilization. It is possible that this reduction in performance would be offset by the

lower cost of soyabean (Anon., 1978a).

Finally it was shown that growth and food utilization of mirror carp in water abstracted from a power station cooling circuit was not depressed compared to that in laboratory recycling systems despite temperature fluctuations and poorer water quality.

Thus the present series of experiments has served to demonstrate the suitability of mirror carp for intensive culture in heated effluents. The optimum range for growth and food utilization is compatible with that obtainable at an inland closed circuit thermoelectric generating station. The nutrirional parameters investigated also serve to demonstrate the suitability of mirror carp for intensive culture using complete compounded rations which it may, in practice, be possible to formulate slightly more cheaply than conventional salmonid diets.

Further investigation of the use of alternative, low cost, protein sources, the utilization of saturated fats and dietary carbohydrate may enable reduction of the cost of complete carp rations still further.

APPENDICES.

Appendix 1.

Materials, and their Suppliers, used in the Construction of System 1.

Pumps;-

Beresford PV52, James Beresford & Son Ltd., Birmingham.

Tanks;-

Header, experimental and filter tanks by 'Supaglass', White Lund, Morecambe.

Immersion Heaters;-

Bunting Titanium, Handsworth, Birmingham.

Contact Thermometers;-

0 - 50°C, A. Gallenkamp & Co. Ltd., London.

Electronic Relays;-

EC980, A. Gallenkamp & Co. Ltd., London.

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