STUDIES ON
INTENSIVE SNAKEHEAD (CHANNA SPP.) CULTURE
WITH SPECIAL REFERENCE TO THEIR NUTRITION

Thesis submitted to the University of Stirling
for the degree of Doctor of Philosophy

by

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February 1983

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ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. James Muir for his supervision of the research project. Special thanks are also due to Dr. Albert Tacon for his encouragement and valuable suggestions in the nutritional experiments. My gratitude to Professor R.J. Roberts for his interest and critical comments on the manuscript and histopathology.

Thanks are also due to all the academic staff at the Institute of Aquaculture, particularly Dr. Christina Sommerville who helped in the parasite screening procedures and Dr. Kim Jauncey for advice and help in analytical techniques in fish nutrition. Thanks also to all the technical staff for their cooperation.

Special thanks are due to Institute of Marine Biochemistry, Aberdeen, for the use of some of their facilities. My gratitude to Dr. Colin Cowey for his valuable suggestions and critical comments on parts of the thesis and Mr Arthur Youngson who helped in the analysis of fish and experimental diets for their amino acid and fatty acid contents in Experiments 2 and 3.

I would like to express my thanks to everybody who in one way or another contributed to the thesis, especially Mrs. Supranee Chinabut at the National Inland Fisheries Institute (NIFI), Bangkok and Dr. Mohamad Shariff at Universiti Pertanian Malaysia (UPM), Malaysia for their unpublished data on ectoparasites of wild snakeheads, Dr. Peter Edwards at Asian Institute of Technology, Bangkok, Dr. Alex Fedoruk at NIFI and Mr. Chen Foo Yan at Network

of Aquaculture Centres in Asia, Bangkok, for their help in arranging visits to fish farms and providing valuable data during the survey of snakehead culture industry in Thailand., Mr. Wirayot Palurak who kindly allowed access to detailed financial records of his farm for examination., Professor R.H. Johnson at Queensland University, Australia who performed the virology work and Dr. Elisabeth Degener, Hanover who helped identify the myxosporidian parasite described in Chapter 4.

My fellow research students, especially Mr. N.A.M. Iqbal, helped in many ways.

Mrs. Ruth Howden typed this thesis and Mrs. Laura Cummings typed the Tables with care and patience. Special thanks to them.

My most sincere thanks to my parents for their support and encouragement throughout the work and to my wife Eng Peng and my son Jo Hann and daughter Shan Nee for their patience. They are a constant source of inspiration and this thesis is dedicated to them.

ABSTRACT

The aim of this thesis was to conduct preliminary studies into the intensive culture of snakeheads (Channa spp.) with special reference to their nutrition. In order to assess the current culture and management techniques employed, identify problematic areas and to guide research needs a field survey of Channa striata farming in Thailand was carried out. Results showed that, at the time of the survey (1980), the C. striata farming industry in Thailand was experiencing difficulties which threatened to undermine the economic viability of the industry. In particular, problems associated with the use of trash fish as feed, especially its ever increasing cost and diminishing supply, was identified to be the major factor regulating the profits of snakehead farms. Dry pelleted feed was suggested as a replacement for trash fish. Simple economic analysis of the net returns of a C. striata farm showed that potential economic benefits can be gained by utilization of dry pelleted feeds. However, at present, such feeds are not available and the basic nutritional requirement data for snakeheads, needed for diet formulation, is almost non existent.

Therefore, in Chapter 3, a series of experiments were designed to determine the basic nutritional requirements of Channa striata and Channa micropeltes. In Experiment 1, the dietary protein requirements of C. striata and C. micropeltes juveniles were investigated. As expected, for carnivorous species, C. striata and C. micropeltes required high levels of dietary protein, approximately 46% and 52% of the diet respectively. Quantitative

essential amino acid requirements of C. striata and C. micropeltes juveniles were determined using the daily tissue essential amino acid deposition method, in Experiment 2. The essential amino acid requirements of both C. striata and C. micropeltes were comparable to those of other fish species, with the exception of the amino acids methionine and phenylalanine. The effects of dietary essential fatty acid intake on growth and fatty acid composition of the liver and muscle were investigated with C. micropeltes juveniles in Experiment 3. Results showed that C. micropeltes are not very demanding in their essential fatty acid requirements during the on-growing phase. Fish fed either dietary linoleic acid, a combination of linolenic and linoleic acids or cod liver oil all grew equally well. Analysis of the fatty acid composition of liver and muscle indicate that C. micropeltes possess some capacity to chain elongate and desaturate linolenic acid producing long chain (n-3) polyunsaturated fatty acids but seem unable to similarly modify linoleic acid. Dietary lipid, and to a lesser extent dietary carbohydrate, were shown to spare dietary protein for growth in C. striata juveniles in Experiment 4. However, the improvements in growth and feed conversion efficiency with increasing dietary lipid at constant dietary protein level, were achieved at the expense of fattier fish carcass.

Incidental studies on the disease problems associated with the translocation of snakehead juveniles were performed. High mortalities were recorded with newly arrived consignments. It was postulated that the high mortality rate was caused either directly by the traumatic effects of the stress induced by the

translocation process or by the attacks of ectoparasites on fish weakened by the induced stress. Successful treatments for the ectoparasitic infections were developed.

In the final chapter, on the basis of the nutritional studies conducted with C. striata and C. micropeltes, a practical diet for on-growing snakeheads was formulated and its cost of production estimated. Suggestions for future work were also presented.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 INTRODUCTION

Fish culture was believed to have begun in China nearly 2500 years ago, the first fish culture manual on the farming of the Chinese common carp was written by Fan Lee during the 5th Century B.C. (Ling, 1977). Since then, aquaculture has developed to encompass the culture of a wide variety of species and culture (Bardach *et al.* 1972).

The traditional Chinese methods of fish culture, mainly the culture of the species of Chinese carps, were introduced throughout South East Asia by migrant Chinese. These methods were adapted and improved by local populations to include the culture of various indigenous species. Owing to their extremely diverse geography, socio-economic conditions, culture and traditions, each country within the region has its own preferred species for consumption and its own methods of aquaculture. Furthermore, as fish is the cheapest commonly available source of animal protein in this region, a wide variety of fish is consumed and cultured in South East Asia.

In most developing countries, the emphasis is on the production of cheap, good quality protein for human consumption. For this purpose, fish species selected for culture are normally those low in the aquatic food chain, for example, the filter feeding planktivores such as milkfish (*Chanos chanos*), and the herbivores and omnivores such as carps and tilapias which are grown in semi-intensive or wholly extensive farming systems with little or

no fertilization or supplementary feeding. However, the choice of species for culture is most often governed by the marketability of the product and the criterion for success is entirely based on the profitability. For this reason, the intensive culture of highly prized luxury fish, such as grouper (Epinephelus tauvina), sand goby (Oxyeleotris marmoratus), sea perch (Lates calcarifer), walking catfish (Clarias batrachus), and the penaeid shrimps and the giant Malaysian prawn (Macrobrachium rosenbergii) in South East Asia, yellowtail (Seriola quinqueradiata), Japanese eel (Anguilla japonica) and Kuruma shrimp (Penaeus japonicus) in Japan, exist not because of their biological advantages but because they generate a high rate of income for the individual and earn valuable foreign exchange for the country. The majority of these organisms are carnivorous and because of their feeding habits, are normally reared highly intensive in monoculture systems, with high capital and operating costs. The culture of snakeheads (Channa spp.) is included within this category of aquaculture.

Snakeheads, commonly known as murrels or mudfish, belong to the family Channidae, have long been regarded as valuable food fish in the Far East, favoured for their firm, lean, and agreeable flavoured flesh (Aldaba, 1931., Willey, 1910., Yapchiongo and Demonteverde, 1959). Snakeheads are often believed to have special nutritional value containing rejuvenating and strength-generating substances (Ling, 1977).

In the past, demands for snakeheads were met entirely

from capture fisheries in rivers, lakes and swamps. However, in recent years the shortfalls in the supply of wild snakeheads has led to the development of snakehead fish farms. The first experiments on pond culture of snakehead (C. striata) were reported to have begun in 1955 in Thailand (Ling, 1977). These were successful and the popularity soon spread to the neighbouring countries in Indo-China in the 1960s and by the 1970s, Hong Kong and Taiwan were farming snakeheads, albeit of a different species (Channa maculatus). The aim of this thesis will be to increase our knowledge on the culture of snakeheads with special reference to their nutrition.

1.2 CLASSIFICATION OF SNAKEHEADS

Under strict rules of zoological nomenclature, the generic name for snakehead is Channa, though frequently the older name Ophicephalus or Ophiocephalus is still used (in the thesis, the generic name of Ophicephalus and Ophiocephalus will be used if they appeared as such in the original reports).

The body of snakehead is elongate, cylindrical and compressed posteriorly. The mouth is large and protractile. The fins are without spines and the dorsal and anal fins extend along the length of the posterior part of the fish. Ventral fins maybe present or absent. The scales are of medium size, cycloid and striated, except on the upper surface of the head where they are larger and scutiform. A swimbladder is present, which often continues into a prolongation of the abdominal

cavity in the tail. Figure 1.1 shows the typical features of the genus Channa sp..

There are 30 named species of snakeheads described in the literature, of which only five species have been reported to be cultured, namely, Channa striata, Channa punctatus, Channa marulius, Channa micropeltes and Channa maculatus.

1.3 BIOLOGY OF SNAKEHEADS

1.3.1 General Biological Characteristics

Snakeheads are native to the freshwater systems of tropical Asia and Africa though they can also occasionally be found in brackish waters. Temperate species can be found in Northern China, Russia and Eastern Europe.

They are generally ubiquitous and can be found in almost every kind of aquatic habitat ranging from swamps to lakes and rivers, surviving in acidic and alkaline waters. It has been shown that the pH tolerance limits for C. striata range from 4.25 to 9.4 (Varma, 1979).

They are very hardy and if kept moist adult fish can remain alive for a long time out of water. This is very significant in the culture and the marketing of snakeheads as live snakeheads fetch a considerably higher price than dead ones, which are reputed to have a poorer taste. Their ability to be able to survive out of water for long periods is attributable to their possession of a pair of suprabranchial cavities

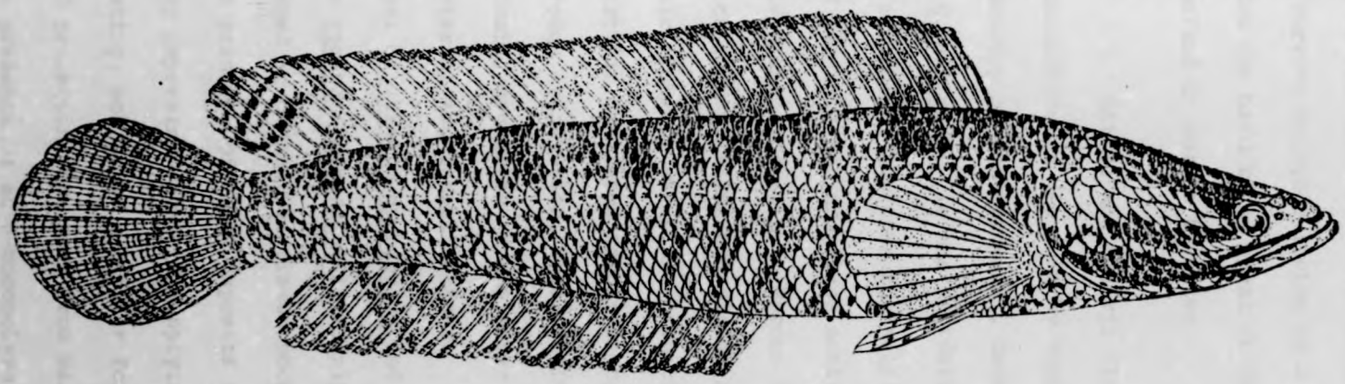

Figure 1.1 Channa striata, exhibiting the typical features of the genus

(the drawing was taken from P.E.P Deraniyagala (1929) The Labyrinthici of Ceylon. Ceylon Journal of Science (B) 15 Plate xxv)

This species can be identified by the following taxonomic features :

Vomer and palatine with a more or less continuous pluriserial band of small teeth, none of them canine. Ocellus may or may not be present at the base of caudal fin; a posterior row of 5 canine teeth on each ramus of lower jaw; lateral line scales 52 to 57, dropping abruptly 2 rows at the 17th to 20th perforated scale; scales in transverse line 4 to 5.5-1.8 to 10; dorsal rays 37 to 45; anal rays 21 to 27 ; black dark green, dark brown, or black; side usually with dark stripes running obliquely upward above, obliquely downward below; underparts white, irregularly blotched with black or brown.

permitting aerial respiration. They have been known to survive for several months without water when buried in moist soil and to survive droughts living in a semi-fluid mud lying torpid below the hard baked crust at the bottom of ponds or wriggling overland in search of water.

Mittal and Banerji (1975) investigated the functional organisation of the skin by studying the structure and cyto-chemistry of the epidermal, dermal and subcuticular components of C. striata. In the epidermis, they found a dense population of mitochondria within the Malpighian cells and strong phosphatase and succinic dehydrogenase activity indicating a high metabolic rate within the skin. Mucous cells were numerous producing a thick coat of slime containing mucopolysaccharides, lipids and basic proteins which are probably important in keeping the skin moist for cutaneous respiration, retarding the rate of water loss through evaporation, facilitating burrowing in the mud and swimming movements in the water and protecting the skin from bacterial and fungal attacks. The dermis consists of an outer layer of stratum spongosium and an inner layer of stratum compactum. The stratum spongosium is mainly composed of well developed scales lodged in connective tissue pockets, which are characterised by the presence of large deposits of lipids. These lipids may play imporatnt roles in supplying energy during periods of fasting, acting as a barrier for water diffusion through skin and protecting the body from mechanical injury during burrowing. The presence of acid-mucopolysaccharides in the substantia

amorphia in the stratum spongosium has been described as an adaptation to prevent dessication.

1.3.2 Air Breathing Characteristics

Das (1927) described the structure and development of the air breathing organ in C. striata, and showed that the air breathing organs are developments of pouches of the pharnyx. The first indication of their development is a thickening of the pharyngeal epithelium, on each side of the mid dorsal line above the first gill arch, the thickening subsequently becoming hollowed to form a pocket. Hughes and Munshi (1973) using electron microscopy were not able to show the presence of pillar cells, which are characteristic of the secondary gill lamellae, in the air breathing organs of snakeheads (C. striata and C. punctatus). This suggests that the air breathing organs of these two species of snakeheads are not modified gills and are thus very different from the accessory organs of other air breathing fish.

Das (1927), in a series of simple experiments, demonstrated the inefficiency of oxygen uptake by the gills of snakeheads and their obligate free air access requirement. He placed his experimental fish in a series of water systems, all without surface access and recorded the length of time they took to succumb when denied access to atmospheric air. When placed in an artificial mixture of mud and water, Ophiocephalus punctatus asphyxiated after 15 minutes. In boiled water (where all the oxygen had

been expelled), O. punctatus survived for eight hours. In fully oxygenated water, this species survived only for 3 to 8 hours. In water saturated with carbon dioxide, the fish died from asphyxiation after 45 minutes. Although these results are somewhat ambiguous they nevertheless clearly demonstrate the necessity of snakeheads for their air breathing organs.

Studies have shown that the period for complete ontogenetic development of the air breathing organ and regular surfacing behaviour in Ophiocephalus striata was about 60 days when the fry grew from 7 mg to 750 mg and reached a 'critical life stage' where breathing from both water and air is obligatory (Vivekanandan, 1977). It is also at this critical period that high mortalities were recorded, particularly when the fry were being transported for stocking, stressing the fry even more and increasing mortalities.

Ojha et al. (1979) studied the oxygen uptake in juvenile and adult C. marulius and found that they had a bimodal gas exchange mechanism extracting oxygen from water through the gills and using the suprabranchial chambers in exchanging respiratory gases with atmospheric air. Juveniles under 'surfacing prevented' conditions did not asphyxiate, whereas adults succumbed. It is presumed from this study that the gills and skin of the juveniles are efficient enough to meet the minimum oxygen requirement for total metabolism of the fish, and so allowing the fish to survive under submerged conditions with continuous flow of normoxic water. In adults, on the other hand, it was presumed

that the oxygen uptake efficiency of gills and skin decreased with increasing size and that therefore they could not cope with the oxygen demand of the fish. These results confirmed the earlier observations of Das (1927) and Vivekanandan (1977). It could thus be concluded that the juveniles are facultative air breathers but adults are obligate air breathers.

In tropical countries where availability of fresh water may be limited and the dissolved oxygen tensions low, air breathing fishes may have significant advantages for aquaculture as they survive and grow in shallow waters deficient in oxygen. However, the advantageous air breathing habits of these fishes and the consequent need to surface more or less at regular intervals imposes a considerable drain of energy which otherwise could have been used for fish production. Pandian and Vivekanandan (1977) investigated the problem of energy loss associated with air breathing.

Ophicephalus striata were reared in tubular aquaria containing different depths of water. Since these fish are obligate air breathers, fish in different depths had to swim varying distances to enable them to exchange atmospheric air. The fish displayed the phenomenon of 'hanging' at the surface, i.e. they stayed on or near the surface for a period of time before returning to the bottom. Results from their experiments with starved and fed fish showed that starved fish surfaced less frequently than fed fish over the different depths of water tested; however, the duration for 'hanging' was longer for

starved fish than fed fish. The duration of 'hanging' increased with increasing intensity of starvation, as the starved fish became exhausted more quickly and more frequently. Feeding rate increased with increasing depth, but food absorption efficiency was not found to vary appreciably between different depth groups. Both conversion rate and conversion efficiency decreased with increasing depths. Consequently, from these results, they deduced that the cultivation of obligatory air-breathing fish in deep waters would result in slow growth rate and poor conversion efficiency despite increased food consumption.

Therefore, although beneficial for survival in oxygen deficient water, the air breathing habits in obligate air breathing fish impose a very significant drain of energy. 'Hanging' appears to be a unique adaptative behavioural strategy of O. striata and, most probably the other Channids, permitting the fish to surface without involving vertical movements and consequent energy expense. 'Hanging' may be regarded as a condition in which the accumulation of oxygen debt and the resulting fatigue reach a maximum threshold, the exhausted fish 'hanging' onto the water surface, repaying its oxygen debt and exchanging respiratory gases without swimming actively.

1.3.3 Life History and Spawning

Snakeheads are generally classified as seasonal breeders. For example, Channa maculatus in Taiwan and Hong Kong breeds from April to September (Chen, 1976); Channa argus warparchowski

spawns in the months of June and July (Frank, 1970) and O. striata during the rainy season (April to September) in North India (Moorkejee et al, 1948).

Snakeheads mature at between 1 and 1.5 years of age (Alikunhi, 1953., Chen, 1976., Frank, 1970). All species of Channa are monogamous and in the wild build a nest for the deposition of eggs, although it has been shown in artificial spawning experiments that snakeheads can still breed in ponds devoid of macrovegetation. The nests are crudely constructed by means of cut portions of aquatic vegetation placed over a slight depression in shallow water near the edge of the water line. The nest is merely a receptacle to receive eggs and a place for courtship. The process of spawning consists of the female lying belly up in the nest and liberating eggs at regular intervals; the male shedding his milt over her at the same time. The fertilized eggs, which are golden yellow in colour, float and are spread like a film flush with the surface in a circular area over the centre of the nest. The developing eggs of the different species are distinguishable by variations in size and colour (Parameswaran and Murugesan, 1976). The bouyancy of the egg is provided by a large single oil globule in the yolk. The hatching time is dependent on the water temperature, for example in O. striata, it varies between 24 - 65.3 hours at incubation temperatures of between 16 - 33^{o}C.

All species of snakeheads exhibit parental care, with both parents participating (Willey, 1910., Alikunhi, 1953). In

general the parents guard the young against predators until the fry begin to separate from the main brood and live independently. There are no reports of any cannibalism by the parents at this stage. On the other hand, no parental care was observed in broods obtained by hypophysation in several species of snakeheads (O. punctatus, O. marulius, O. gachua and O. striata) (Banerji, 1974., Parameswaran and Murugesan, 1976).

Immediately after the mouth is formed, the larvae of O. striata feed on protozoa and algae, while early fry apparently subsisted on planktonic crustacea (Moorkejee et al, 1948., Alikunhi, 1953). With further growth, the fry restrict their diet to purely animal foods such as shrimps, aquatic insects, fish fry and tadpoles. Adults are extremely voracious, carnivorous predators feeding on larger aquatic animals such as frogs, other fish and even small aquatic snakes. Channa punctatus has been reported to feed by chemical attraction as indicated by the well developed olfactory organ, nasal accessory sac and taste buds whose distribution extends into the oesophagus (Panday and Dwivedi, 1974). Channa marulius is reputed to feed by sight, but with a well developed tongue acting as a tactile organ (Singh, 1976). Studies on the morphology and histology of oesophagus and stomach by Singh (1976) on Channa marulius and of Mehrota and Khanna (1969) on O. striata showed that the oesophagus is a short, well developed tubular structure with prominent internal folds running parallel to each other. Taste buds consist largely of gustatory cells and are present in the

intestinal mucosa. The stomach is a large thick walled sac, wider anteriorly and it can be differentiated into cardiac and pyloric regions. The intestine is a long narrow tube with a well developed pyloric valve at the junction of the stomach and intestine. A pair of long and tubular pyloric caeca are present at this junction. The rectum opens out to the exterior through a slit-like anus. Little information is available on feeding regimes but Javaid (1970) found that Channa punctatus fed mainly in daylight, around midday.

At present the supply of seed fish from natural sources appears adequate to meet the demand. However, eventually controlled breeding would seem essential to meet seed requirements and to allow genetic developments to improve growth performances.

Several species of snakeheads have been successfully bred by hypophysation in laboratories (Banerji, 1974., Parameswaran and Murugesan, 1976., Chen, 1976). In general, the method used is similar to that employed for carps; with doses of hormones given in two instalments, an initial low dose followed by a final higher dose, for both females and males. Pituitary glands of other unrelated species have been used successfully. Spawning normally takes place approximately 11 - 24 hours after the second injection of hormones (Parameswaran and Murugesan, 1976).

1.4 CULTURE OF SNAKEHEADS

Although they have long been regarded as valuable food fishes, snakeheads were not cultured on any scientific basis until

very recently. In the past demand for the food market was met entirely by capture fisheries from the wild.

The Indo-Chinese were among the first to culture snakeheads, especially in the Mekong Basin and the Tonle Sap area. Culture of snakeheads in these areas were mainly in cages (Pantulu, 1976). It is considered a lucrative enterprise and thus has progressed very rapidly.

In Kampuchea, Channa micropeltes and Channa striata are normally cultured in cages which are trailed behind boats, those moored near shores are box-shaped, while cages that form part of a fisherman's boat are streamlined to fit into the shape of the boat. Cages vary in size from 40 - 625 m^3. The fry for stocking are obtained from natural sources. They are fed on cooked pumpkin, banana and a combination of cooked, broken and glutinous rice and rice bran. The bigger fish are normally fed on pieces of raw fish, small live fish and kitchen refuse. Generally, monoculture is practised, but occasionally cyprinids such as carps and minnows are stocked with snakeheads. The stocking rate for a large cage measuring 5m x 50m x 2.5m is between 6,000 - 10,000 fry. The stock is harvested nine months later when the fish are between 1.5 - 2.5kg in weight (Pantulu, 1976).

In Vietnam, cage culture of snakeheads is of relatively recent origin. In 1976, snakehead culture formed 18% of the 173 cage farms visited in a survey carried out by Rainboth et al. (as cited by Pantulu, 1976). Generally, fry of C. micropeltes and C. striata are stocked, but if these are not available in the

required numbers, the shortage is made up with more readily available fry of other fish species. The cages used vary in size (averaging 125 m^3) with the fry, fingerlings and early juveniles stocked in cages measuring 1.5m x 2m x 1.5m and grown on to marketable size in larger size cages measuring 5m x 12m x 2.5m. The cages are normally stocked for on-growing with 40 - 65 mm size fry obtained from the wild. The fry are either sold directly to cage owners or are held by wholesalers who raise the fry further to fingerling size for resale. The stocking rate is approximately 80 fry / m^3. They are fed on forage fish, and as supply of forage fish can be expensive and irregular, the culture of snake-heads is practised by rich entrepreneurs only. Supplemental feeds such as cooked or uncooked flesh of snails and mussel are also fed, and the fish are generally harvested after nine months of culture. Production rates averaging 85 kg / m^3/crop were reported (Pantulu, 1976).

In Thailand, O. striata are recommended to be grown in square ponds of area ranging from 800 - 1,600 m^2 with good inlets and outlets for water supply. The depth of the water is generally kept at between 1.5 to 2 metres. Lime is applied at 1 kg / m^2 and left to dry for five to ten days. The recommended stocking rate is 40 - 60 fry / m^2. The feed is forage fish, rice bran and broken rice in a 8:1:1 ratio respectively. The water has to be kept clean and ideally changed three times daily, but in most farms this is not possible. Fish can be expected to grow to marketable size of between 0.5 - 1 kg within 7 - 8 months (Anon, 1979).

In Hong Kong, monoculture of another species of snakehead, C. maculatus is practised (Anon, 1976). They are cultured in ponds that are usually less than 0.5 ha in area and about 1 metre deep. These are normally converted agricultural plots. The water supply is usually rain fed, but may be supplemented by water from wells and drains. Fine mesh wire fencing is required around the pond to prevent the escape of fish. The stocking rate normally varies between 150,000 and 300,000 fry / ha and stocking is usually undertaken in the spring. They are fed with minced trash fish and food conversion ratios (food fed/weight gain) of between 6:1 and 10:1 are reported (Anon, 1976).

In Taiwan, C. maculatus is usually reared in polyculture with Chinese carps where it serves as a 'policeman fish' in the ponds, eliminating small carps and other extraneous or pest fish (Chen, 1976). It is recommended that not more than 500 snakeheads of 10 cm in size be stocked in a one hectare pond, and that the carp should be at least 10 cm in size before stocking the snakehead (Chen, 1976). No supplementary feeding is normally provided. C. maculatus are also cultured with tilapia, which serves as a forage fish. The snakehead stocking rate is 90,000 fingerlings of 10 cm in size per hectare, which is graded two to three times per year and restocked at a lower density to avoid cannibalism. The final stocking density is normally in the order of between 15,000 and 24,000 fish per hectare.

Monoculture of C. maculatus is Taiwan is not common because of high production costs (Chen, 1976). In those monoculture systems which do occur, the feeds consisted of 80% minced trash fish and 20% wheat flour or formulated eel feed. Provided adequate feed levels are maintained, the growth of the fish is rapid; 10 cm fingerlings achieved 600 to 1,000 g weight gain in 9 - 10 months and over 1,000 g in one year. It is reported that a 90% survival rate can be achieved if proper care is taken (Chen, 1976).

In Indonesia, great interest has been shown in the culture of C. micropeltes in cages as evident in Southern Sumatra where in 1976 there was only one operator rearing 500 fish, in 1977 there were seven operators producing 3,455 fish and by 1978 there were 23 operators culturing a total of 39,000 fish (Ondara, 1979). Ondara (1979) in his experiments on the cage culture of C. micropeltes fed trash fish, reported poor conversion rates of between 10.4 and 12.8 : 1. Very high mortalities were also reported particularly during the first two weeks where the mortality rate was between 54 - 61% and the final mortality rate after 14 months was between 66.7 and 76.4%. The fish grew from 6 g to 1,000 g in 14 months.

1.5 AIMS OF RESEARCH

From the literature presented it is clear that there are several areas of research where significant efforts need to be applied to allow for improvements in the culture and

management techniques of snakehead farming.

The aim of this thesis was therefore to conduct preliminary studies into the intensive culture of snakeheads as carried out in Thailand, with subsequent laboratory studies on their nutrition which, it is shown in the study, is the major limiting factor regulating the economic viability of their culture. The study is presented under three major headings:

1) A survey of Channa striata farming in Thailand.

2) Studies on the nutritional requirements of snakeheads.

3) Descriptions of disease problems associated with translocation of juvenile snakeheads.

CHAPTER TWO

A SURVEY OF *CHANNA STRIATA* FARMING IN THAILAND

2.1 INTRODUCTION

The snakeheads (Channa spp.) possess the highly desirable physiological capabilities of air breathing; thus the conventional limits to holding capacity in fish ponds do not apply and remarkable production rates can be obtained in simple pond systems because their stocking numbers are not absolutely dependent on dissolved oxygen levels.

The literature review presented in Chapter One was of necessity limited because there is little or no information available which traces the development of the culture and management techniques currently in use. It is generally believed that the culture of snakeheads in the different Asiatic regions were developed independently through trial and error type experiments by pioneering snakehead farmers. Information is also lacking in the assessment of the significance of variations in the inputs and biological, technical and economic factors on the production and profits of snakehead farming.

It is therefore the primary aim of this study to develop economic, biological and technological information to assess the viability of the C. striata culture industry in Thailand and to provide a more complete concept of different research needs.

2.2 METHODOLOGY

Specific tasks undertaken to achieve the objective of the study include:

a) A review of the pertinent literature on the state of aquaculture and the C. striata culture industry in the study area.

b) A review of the current design and management practices, and to identify problematic aspects through a field survey of C. striata farms and interviews with qualified personnel experienced in aquaculture.

c) An assessment of the profitability of current farming operations with special reference to feeds and to the significant production inputs.

d) Identification of research priorities for the improvement of C. striata culture techniques and management.

2.2.1 Definition of the field of study area

The culture of the species Channa striata in Thailand was selected for study for the following reasons:

a) It is one of the most widely distributed and commonly cultured species in Asia, forming a very important part of the total freshwater culture fish production. In Thailand, C. striata is one of the most popular and commonest staple food fish.

b) The farming techniques employed in the production of C. striata in Thailand are typical of Channa sp. farming in

other regions in Asia, being operated as a monoculture and highly intensive system.

c) Thailand houses several institutions which are actively engaged in the study of the various aspects of aquaculture, who have kindly allowed access to the data gathered and the cooperation of the scientists involved, in particular, the National Inland Fisheries Institute, Bangkok, Asian Institute of Technology, Bangkok and Kasetsart University, Bangkok.

Two provinces in the Central Plains of Thailand were selected as study areas i.e. Suphanburi and Nakhon Nayok provinces (Figure 2.1). The lack of available information prevented the assessment of the study areas in terms of the historical development of C. striata culture. However, it is possible to classify Suphanburi /using available data from the studies on the Clarias sp. catfish farming industry by Muir (1981) and Wattanutcharya and Panayatou (1981)/ as more 'advanced', with a longer Clarias sp. farming history averaging 6.9 years of production, than Nakhon Nayok province (averaging 2.9 years). On the basis of the similarity in the culture practices and the interchangebility between Clarias sp. and C. striata (Muir, 1981; FAO, 1980), similar production histories were expected for C. striata farming in these two provinces.

At the onset of the study, it was intended to visit as many farms as possible to provide a large and random sampling for statistical analysis. As this did not prove possible, the survey

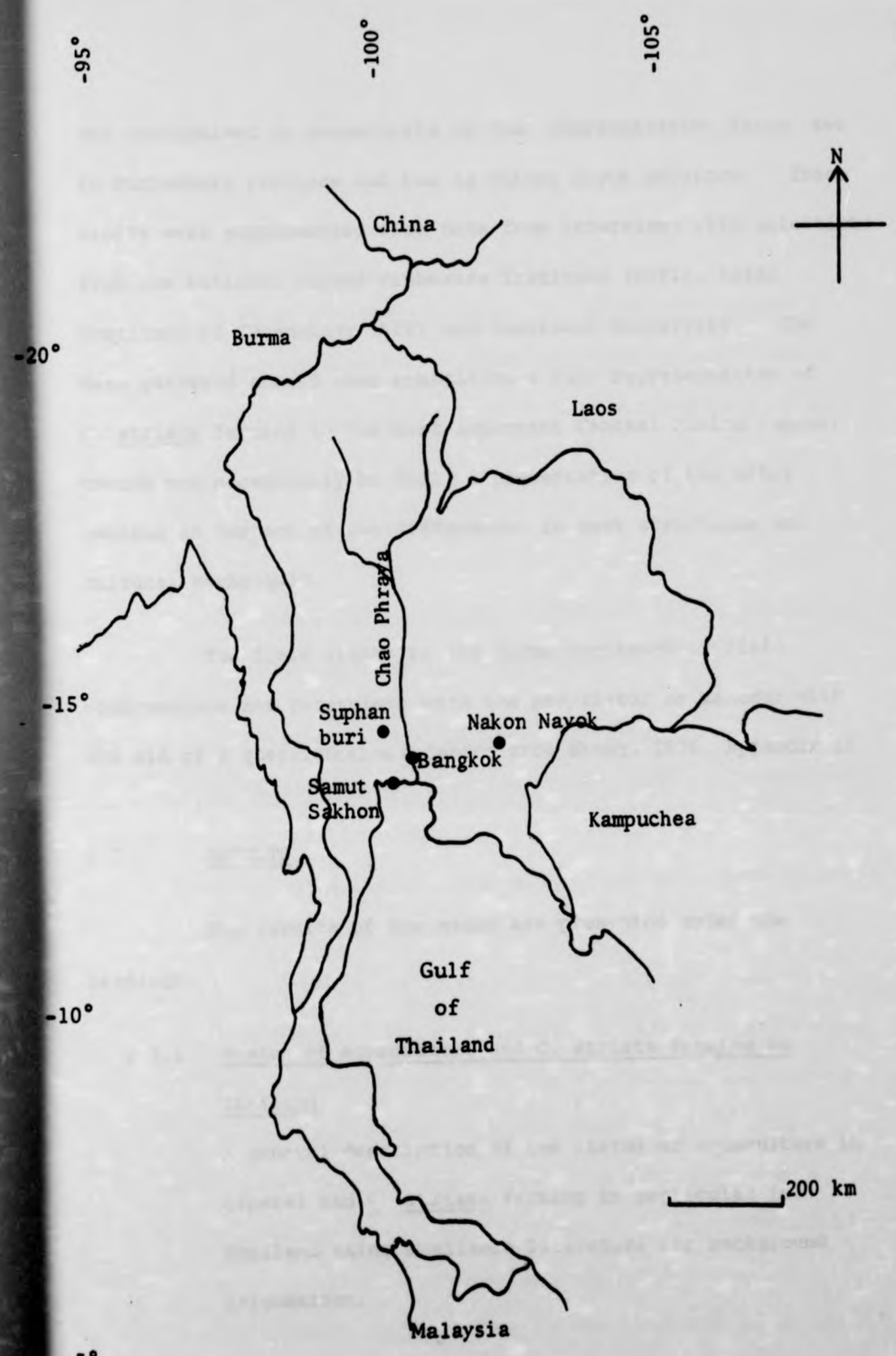

Figure 2.1 Map of Thailand showing the study areas

was reorganised to concentrate on four representative farms, two in Suphanburi province and two in Nakhon Nayok province. These visits were supplemented with data from interviews with scientists from the National Inland Fisheries Institute (NIFI), Asian Institute of Technology (AIT) and Kasetsart University. The data gathered should thus constitute a fair representation of C. striata farming in the most important Central Plains region, though not necessarily be fully representative of the other regions in respect of the differences in cost structures and cultural techniques.

The field visits to the farms consisted of field observations and interviews with the proprietor or manager with the aid of a questionnaire (adapted from Shang, 1976, Appendix 1).

2.3 RESULTS

The results of the study are presented under the headings:

2.3.1 Status of aquaculture and C. striata farming in Thailand

A general description of the status of aquaculture in general and C. striata farming in particular in Thailand using available literature for background information.

2.3.2 Primary data from field survey

Presentation of primary data from field survey,

comparing and contrasting culture and management techniques currently employed in the different farms.

2.3.3 A case study of a C. striata farm

A detailed examination of the design and management practices, problems experienced and economic data leading to an assessment of the profitability of an individual farm.

2.3.1 Status of aquaculture in general and Channa striata farming in particular in Thailand

2.3.1.1 Status of aquaculture in Thailand

It is only in the last two decades that the traditional aquaculture industry in Thailand has undergone a period of advancement and expansion (P Edwards, pers. comm.). The principal reasons appeared to have been:

1) The reduction of the supply of capture fisheries from natural sources, particularly the marine demersal fish stocks, the reduction in the yields from capture fisheries resulted primarily from the uncontrolled fishing when the first modern trawling fleets were introduced in the sixties (these activities decimated most of the fish stocks) and the introduction of a 200 mile exclusive economic zone by countries in the region which effectively reduced Thai fishing grounds by a third (Anon, 1982). This in turn led to the

offshore trawler fleets moving in to inshore fishing grounds to fish an already depleted stock thereby accelerating the extinction of the fish stocks.

2) The early boom in the capture fisheries through the introduction of the modern trawling fleets provided a cheap and plentiful supply of trash fish which led to the development of the culture of carnivorous fish species using trash fish as feed . The species of fish cultured include Clarias batrachus, Clarias macrocephalus, Channa striata, Oxyeleotris mamorata, and Lates calcarifer.

3) The greater public awareness on the viability of aquaculture enterprises, as exemplified by the initial successes of pioneering fish farmers through the mass media and the expert extension services provided by trained personnel (S. Chinabut, pers. comm.).

The efforts of the Royal Thai Government were also important in promoting and aiding aquaculture based national development programmes. The development of an extensive irrigation canal system for agriculture, providing a constant perennial supply of water, has also encouraged the use of this water resource to flood disused or unproductive rice fields for aquacultural purposes (Lawson, 1981).

The rapid growth of the aquaculture industry has severely taxed the ability of the Thai Department of Fisheries (DOF)

to maintain an account of all aquaculture activities in the country. Furthermore, most small scale farmers (especially subsistence farmers) do not generally keep records of their operations. Consequently, exact data on the present status of aquaculture is limited. Estimates on the 1980 freshwater culture production, based on estimates projected from records available for the 1975-76 year, and adjusted by the opinions of the DOF personnel, indicate that more than 20 species of fish are cultured, yielding an annual production of 47,850 metric tonne of which 95% is contributed by 10 species (Suraswadi, 1980). The value of this production, based on farm gate prices, was around US $ 43.5 million per year (1980 prices). There are some 20,000 ponds with a total area of 6000 ha, about 51,000 paddy fish ponds with an area of 35,000 ha and an undetermined number of backyard ponds. There are also some 450 ditch culture and 600 cage culture operations (Suraswadi, 1980).

2.3.1.2 Status of Channa striata farming in Thailand

There are eight known species of Channa in Thailand, all of which are considered as food fishes by the population, but only C. striata, known locally as Pla chon, is cultured (Smith, 1965). The first experiments on the pond culture of C. striata were believed to have started in Thailand in 1955 (Ling, 1977). Early success in this culture coupled with a reduction in the natural production in the wild have led to rapid progress and development of the culture industry. The expansion of the C. striata industry was also believed to have been aided by the availability

of a cheap and plentiful supply of trash fish.

In 1980, production from C. striata culture totalled 7.5% of the annual production from freshwater culture fisheries (Suraswadi, 1980). There were an estimated 500 C. striata ponds averaging 800 m^2 producing 3,600 metric tonne per year. This represented a total net income of about US$ 4 million. C. striata are typically cultured in monoculture, highly intensive systems. They are also cultured in converted paddy fields in a polyculture mix, where the major species cultured is Trichogaster sp.. The snakeheads produced in these paddy fields, which are reputed to have a better coloured flesh and taste better than pond cultured fish and therefore fetch a premium market price, comprise 7% of the total fish production from paddy fields.

One particular feature of fish farming in Thailand is that the farmer may rear more than one species at any one time and the choice of fish selected for culture in a particular year or season is dictated by consumer demands. Normally C. striata farms may also double as Clarias batrachus farms. The similarity in the culture techniques enables the farmer to interchange the production as the market demands without major modifications to the ponds or farm. This practice frequently results in the alternation of the choice of fish for culture from year to year, as a 'good' year for C. striata or C. batrachus inevitably results in overproduction and consequent low profits in the following year as the market becomes saturated with fish produced by other farmers who made the species change. This then encourages

farmers to revert to the former species in which profits have now increased. This practice, in addition to encouraging instability and fluctuation in production also creates difficulty in assessing current or continuous production levels and trends.

There are almost no reports on records on the culture and management techniques of C. striata in Thailand, save that of Suraswadi, (1980) and Ukkatewewat (1980). According to these authors, the rearing ponds average 800 m^2 in size, seed fish are obtained exclusively from the wild and stocked at densities of between 30 to 75 fish per m^2. Survival rates of less than 40% are generally recorded. The feed is trash fish in combination with agricultural by-products, in some cases fortified with a vitamin and mineral premix and antibiotics. A market size of between 0.5 to 1 kg is attained in six to seven months. The yields are usually in the order of 9 kg per m^2/crop.

Suraswadi (1980) and Ukkatewewat (1980), were of the opinion that there was a trend towards selecting C. striata for culture over other species such as Clarias catfish and noted that further expansion was limited by the availability of seed fish. The reason for the preference for C. striata for culture appeared to be the sudden shortfall in the harvest of wild snakeheads. The cost of production in 1980 was reported to be about US$ 1.00 per kg produced and a profit of approximately 49% of the selling price of US$ 2.05 per kg.

2.3.2 Primary data from field survey

The primary data from the field survey and interviews are summarised and presented in this section (Table 2.1). The section is intended to describe and illustrate the range of current culture and management practices and to update existing information beyond that presented in section 2.3.1.2.

2.3.2.1 Farm layouts

C. striata is typically cultured in monoculture systems, primarily in ponds and to a lesser extent in floating cages in large river systems such as the Menam Chao Phyra, the largest river in Thailand (P. Edwards, pers. comm.). As a good quality water supply is a principal prerequisite for efficient and profitable fish farms, it is therefore not uncommon for farms to be situated adjacent to rivers or khlongs (canals). Water from khlongs was used by all the four farms visited (Figure 2.2). Channa striata farms in Suphanburi and Nakhon Nayok province had similar histories in relation to the length of time snakeheads had been cultured, ranging from three to four years of experience. Two of the farms visited were new establishments (one each from Suphanburi and Nakhon Nayok province) and the other two were additions to an existing Clarias or Chinese carp farm (Table 2.1).

All the farms visited had a similar farm layout, all had a pumped water supply from a canal which ran parallel to the farm. The water was pumped, more or less continuously from 10 to 24 hours daily, either directly into the on-growing ponds or into collecting

Table 2.1 Primary data from the field survey of C. striata farms in Thailand

Farm	Aquaculture experience (years)	Size of farm	Stocking rate	Feed	Culture period	Production rate
Palurak Farm	C. striata (4)	Total area of farm = 30 rai *1 Two 1,200 m^2 ponds for C. striata	1,850 'cup' per pond @ 300 fry per 'cup' = 460 fry/m^2	Trash fish and rice bran	7 months	13,536 kg / pond = 112.8 metric tonnes/ha/crop
Pathumthani Farm	Clarias spp. (10) C. striata (3)	Total area of farm = 72 rai. Seven 1,600m^2 Clarias ponds and two 32,000m^2 C. striata ponds	200 kg of fry per pond @ 1,200 fry per kg_2 = 75 fry /m^2	Trash fish and rice bran	10 months	10,000 kg/pond = 31.25 metric tonnes/ha/crop
Khlong Two Farm	Chinese carp (20) C. striata (4)	Total area of farm = 220 rai. Mainly 10 and 20 rai Chinese carp ponds three 1,600m^2 C. striata ponds	220 kg of fry per pond @ 1,200 fry per kg = 165 fry/ m^2	Trash fish	9 months	15,000 kg/pond = 93.75 metric tonnes/ha/crop
Pichit Farm	C. striata (3)	Total area of farm = 23 rai. Seven 1,600 m^2 and three 32,000m^2 ponds	500,000 fry per 1,600m^2 pond = 312.5 fry/m^2	Trash fish and rice bran	8 months	20,000 kg/pond = 125 metric tonnes/ha/crop

*1 Rai is the Thai unit of measurement equivalent to 1,600m^2 .

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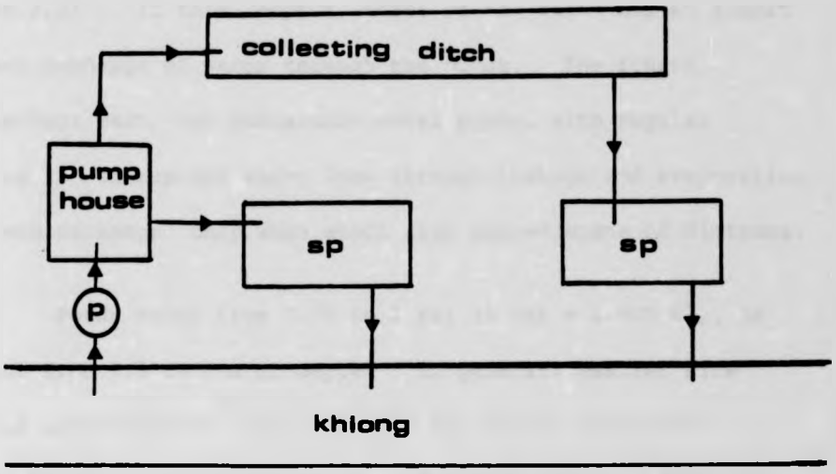
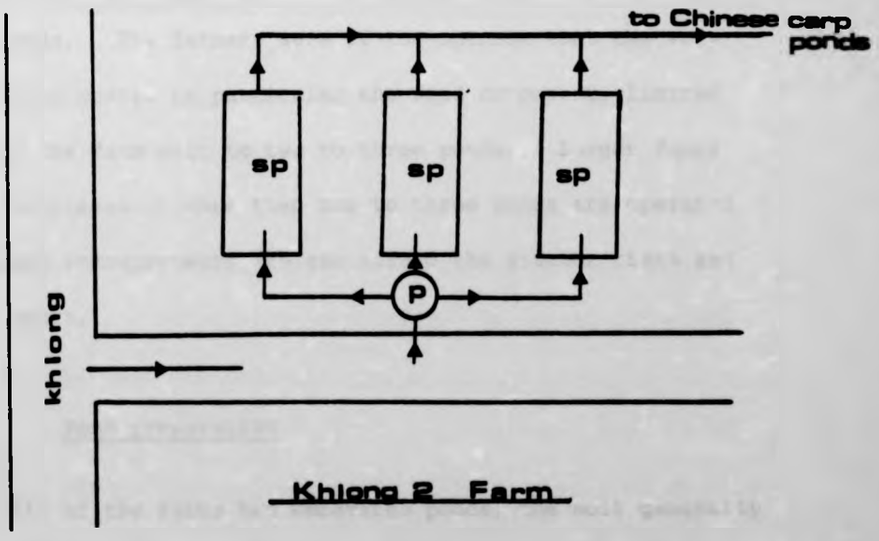


Palurak Farm



Khlong 2 Farm

Figure 2.2 Layout of snakehead farms showing water source and water distribution pattern

(sp : snakehead ponds, P : pump)
→ : direction of water flow

ditches for distribution to the individual ponds by gravity (Figure 2.2). In this respect, three of the farms had an almost constant exchange of water through the ponds. The fourth (Pathumthani Farm) had stationary water ponds, with regular 'topping up' to replace water loss through leakage and evaporation. Water was exchanged only when stock fish showed signs of distress.

Ponds range from 0.75 to 2 rai (1 rai = 1,600 m^2), in size and from 1.5 to 2 m in depth. In general, smaller size ponds of about 1,600 m^2 were favoured for easier management, feeding and harvesting operations. Although suitable land may be available for large size units, the average single C. striata farm unit appears to consist of two to three ponds of 1,600 m^2 in size. Only one of the four farms visited was larger and cultured C. striata in farm complexes of seven 1,600 m^2 ponds and three 3,200 m^2 ponds. The farmers were of the opinion that the very high operating costs, in particular the feed component, limited the size of the farm unit to two to three ponds. Larger farms with pond complexes of more than two to three ponds are operated by the richer entrepreneurs who can afford the greater risks and operating costs.

2.3.2.2 Pond preparation

All of the farms had excavated ponds, the soil generally retained water well and no preparation was required. After each harvest the ponds were repaired and prepared for the next crop. After draining the ponds on the last day of the harvest, the

bottom mud and detritus were scooped out, the base relined with sand and the banks reconstructed and strengthened by packing with bottom mud. The pond was then left to dry for about 15 days after which time clean water was run in and left stationary for three days so that heavy suspended solids can settle and be flushed out of the system. This water was then drained off and the pond refilled with fresh clean water and is ready for restocking.

2.3.2.3 Production Management

The production cycle lasts for 7.5 to 10 months; the principal events relating to the production strategy are illustrated in Figure 2.3. The stocking times are determined in principle by the availability of seed fish which are collected from the wild. The production strategy is however, complicated by the cycle of supply of wild caught C. striata to the market and in order to maximise his profits, the farmer must be able to predict, anticipate and time his harvest to coincide with the expected drop in the supply of wild C. striata, and the subsequent higher market price. This occurs in the months just before and during the wet season (April to September) which is traditionally the period where the market margin between the wholesale price and the retail price at the market is at its highest (Table 2.2 and Figures 2.4 and 2.5). This practice of anticipating and predicting the supply of wild C. striata is extremely difficult as the natural production of C. striata is dependent on a number of factors including the unpredictable onset of the monsoon. It has been postulated that a severe monsoon normally results in a higher production

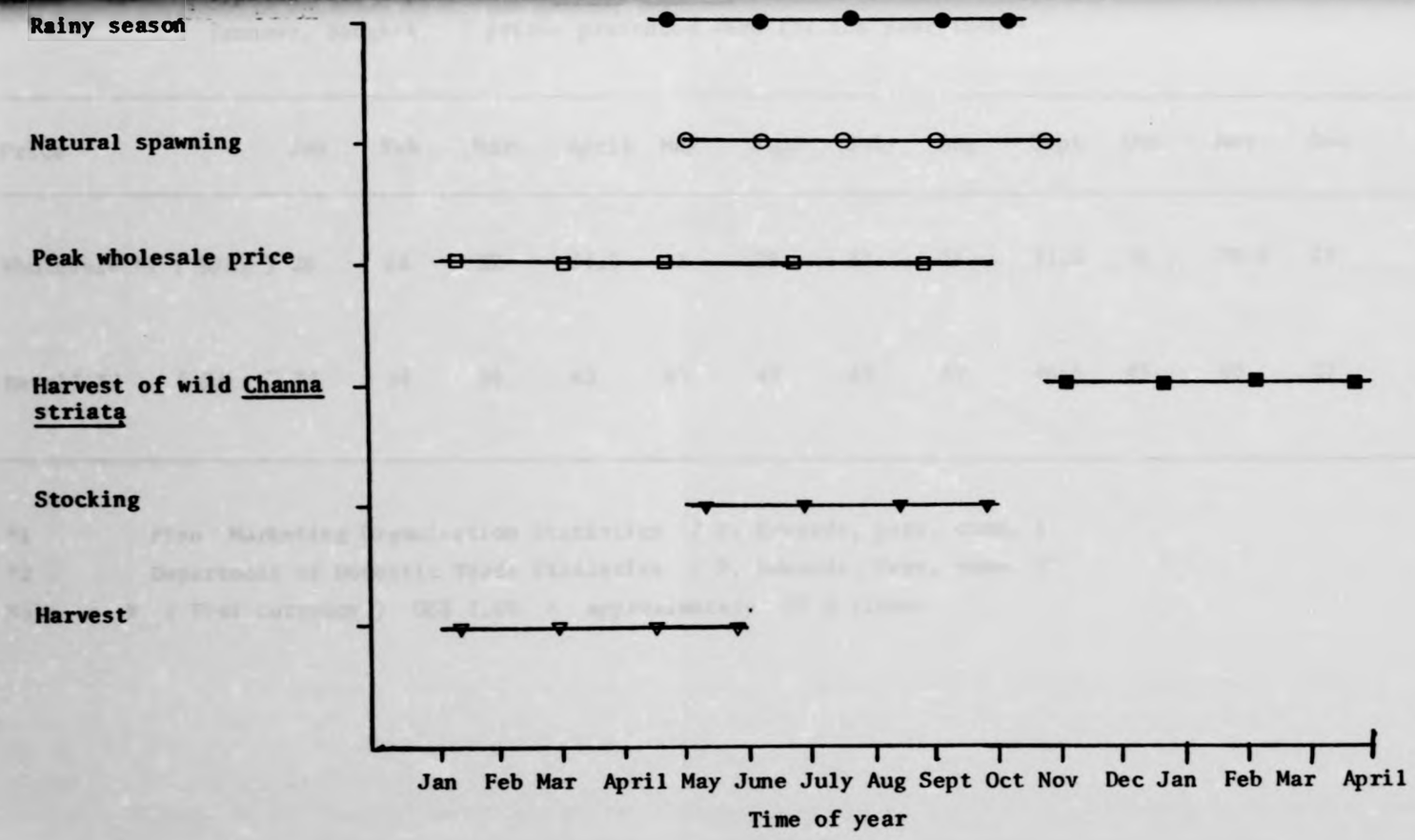


Figure 2.3 Occurrence of principal events relating to Channa striata production strategy in Thailand

Table 2.2 Typical market prices for Channa striata at the Fish Marketing Organisation Central Market, Yannawa, Bangkok. (prices presented were for the year 1980)

Price		Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Wholesale *1	(฿/kg)	26	28	32	34.5	33	35	32	31	31.5	30	29.5	28
Retail *2	(฿/kg)	34	34	36	43	46	47	47	47	46.5	45	40	37

*1 Fish Marketing Organisation Statistics (P. Edwards, pers. comm.)
*2 Department of Domestic Trade Statistics (P. Edwards, Pers. comm.)
Note : ฿ (Thai currency) US$ 1.00 = approximately 20 ฿ (1980)

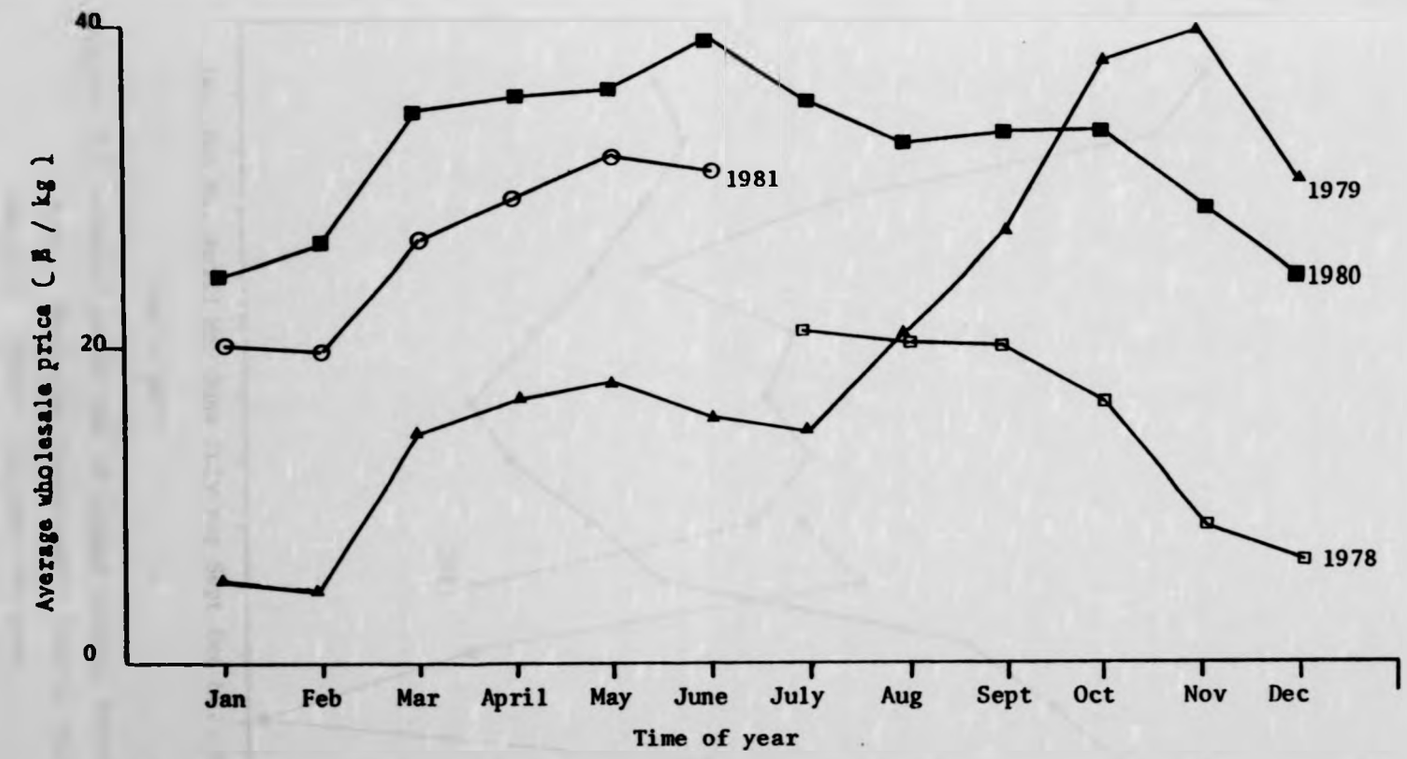


Figure 2.4 Average wholesale price of Channa striata at the Fish Marketing Organisation Central Market, Yannawa, Bangkok . Number indicates the year .

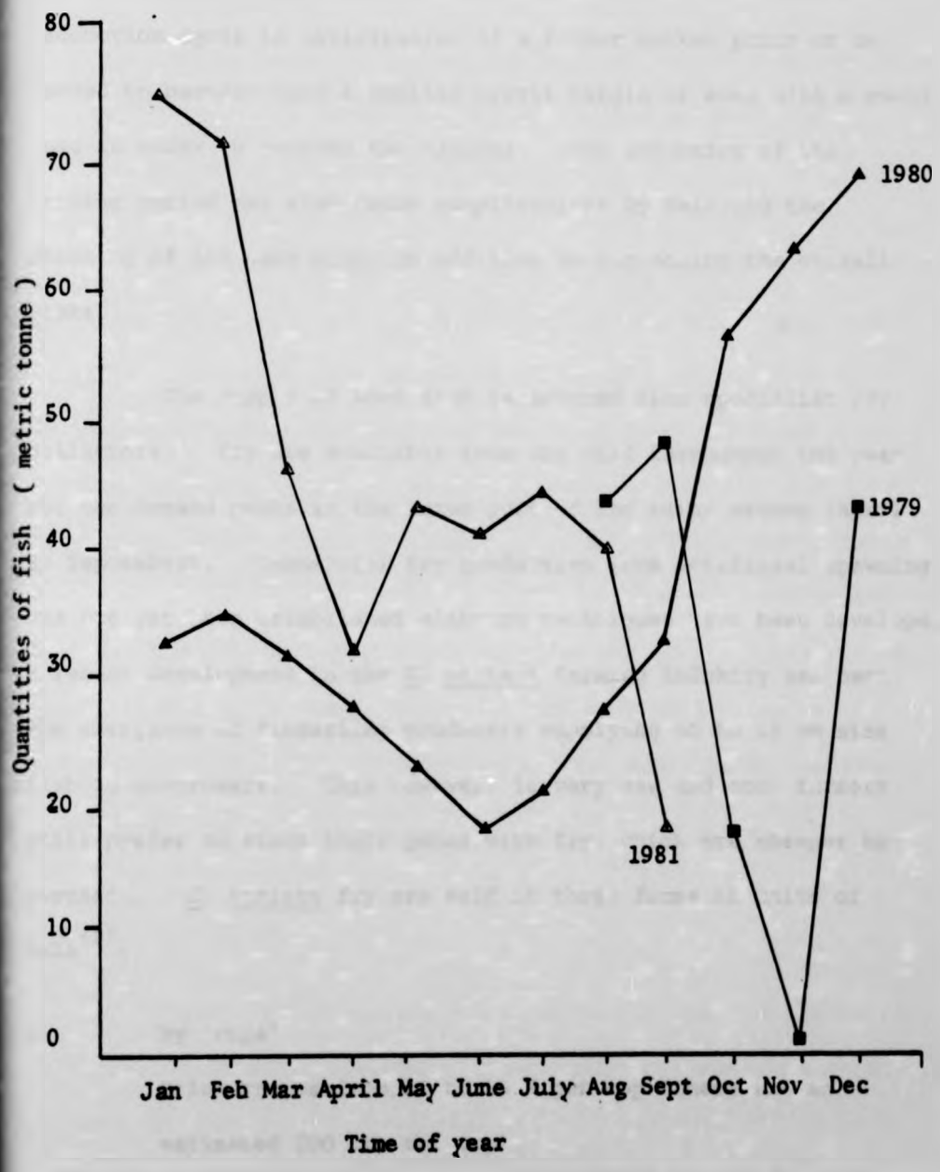


Figure 2.5 Monthly quantities of Channa striata landed at the Fish Marketing Organisation Central Market, Yannawa, Bangkok. Number indicates the year.

(P. Edwards, pers. comm.). Thus, the margin for success and profits is very narrow and in many cases may be beyond the control of the farmers, who can only stock their ponds according to past years' trends. The farmer occasionally may have to extend the production cycle in anticipation of a better market price or be forced to harvest with a smaller profit margin or even with a small loss in order to recover the capital. The extension of the raising period may also cause complications by delaying the stocking of the next crop, in addition to increasing the overall risks.

The supply of seed fish is secured from specialist fry collectors. Fry are available from the wild throughout the year, but the demand peaks in the later part of the rainy season (April to September). Commercial fry production from artificial spawning has not yet been established although techniques have been developed. A recent development in the C. striata farming industry has been the emergence of fingerling producers supplying 10 to 15 cm size fish to on-growers. This however, is very new and most farmers still prefer to stock their ponds with fry, which are cheaper to purchase. C. striata fry are sold in three forms of units of sale[1]:

a) By 'cups'

Price ranges from 10 to 15 ฿ per cup (there are an estimated 300 fry per cup).

1. ฿ = Baht (Thai currency).
US$ 1.00 = approximately 20฿ (1980)

b) By weight

Price ranges from 70 to 80 ฿ per kg of fry (there are an estimated 1,200 fry per kg.)

c) By numbers

Price ranges from 3 to 7 ฿ per 100 fry for 2 cm size fish and 7 to 12 ฿ per 100 fry for 3 to 5 cm size fish.

(all the prices quoted are 1980 prices.)

The stocking rates are not standardised, they are adapted on an arbitrary basis according to local practices. These are variable, ranging from an estimated 75 to 460 fry per m^2 which represent extremely high stocking densities, several times higher than the stocking rates previously described in section 2.3.1.2. Most of the farmers hold the attitude of expecting a high rate of mortality and stocking at corresponding high rates to compensate. Fish fry are introduced directly into the production ponds without going through any nursery facilities for acclimitization or quarantine or prophylactic measures and are grown on in the same ponds until harvested.

2.3.2.4 Feeds

Trash fish (demersal fish species) formed the main feed in all the four farms visited. Trash fish was used alone or in combination with rice by-products, such as rice bran or broken rice (cooked) from the mills. All four farms possessed pickup trucks and the daily rations of fresh trash fish were collected from the ports; however, it is known that other smaller farms in the study areas had their supply of trash fish delivered and were

thus denied the opportunity to select the trash fish for quality.

The trash fish is usually finely chopped to feed the fry, and the juveniles and on-growing fish are fed on a combination of trash fish with rice bran or cooked broken rice. There are no fixed proportions for the ingredients; instead the actual ratio of trash fish to rice bran/broken rice used was dependent on the availability of trash fish. The ratio of trash fish to rice bran was calculated on a weight basis or by volume. Thus Palurak Farm used ratios varying from 8:1 to 13:1 (by weight) and Pathumthani Farm used 4:1 (by volume) (Table 2.1).

Feeds were given once daily, usually in the morning, and as none of the farms possessed freezing or cold storage facilities, the trash fish collected daily was used as soon as possible so as to avoid spoilage. The feeds were prepared by mixing the trash fish and rice bran thoroughly by hand, the mixture being extruded through a 2 cm die plate forming strands (Plate 2.1). The feeds were presented on wooden platforms placed within the ponds, as this enabled the farmer to observe the feeding behaviour and the general condition of the stock fish and also allows the calculation for the next days feed to be made (Plate 2.2). However, some of the farmers merely dumped the basketful of feed into ponds at a fixed position (Pathumthani Farm and Khlong 2 Farm).

As indicated previously, there was no fixed feeding rate and so, fish were fed ad libitum and in some instances, due to the lack of suitable storage facilities, all the feed was given to the fish regardless of appetite. The normal practice was to

Plate 2.1 Feed preparation using trash fish and rice bran in a Channa striata farm

Plate 2.2 Typical Channa striata pond showing the strategically placed wooden feeding platform within the pond

Plate 2.1 Feed preparation using trash fish and rice bran in a Channa striata farm

Plate 2.2 Typical Channa striata pond showing the strategically placed wooden feeding platform within the pond

overfeed, the excess feed being recovered several hours later and the actual amount of feed consumed calculated and the daily feeding rate adjusted accordingly. There were no attempts to sample the stock to estimate the crop weight for the calculation of feeding rate or mortality rate. Quite often, the farmer did not know the exact stock weight until harvest time.

Exact data on the overall feed conversion ratio (FCR, weight of feed (fresh weight)/weight gain), was only available for Palurak Farm, and it ranges between 5.7 to 8.5 : 1 (it can reasonably be assumed that the other three farms visited would obtain similar values, as the feeds used were quite similar to that used at Palurak Farm). These values are comparable to those obtained with Channa maculatus in Hong Kong (6 to 10 : 1, Anon, 1976) but poorer than those obtained with C. batrachus (4.3 to 5.4 : 1, Wattanutcharya, 1982) using similar feeds.

The irregular and unreliable supply of trash fish caused concern to the farmers as they could not store large quantities of trash fish without incurring high capital costs in building and operating adequate freezing facilities. This was stated to be beyond the means of most of the farmers in the study area. The success of the farm therefore, is dependent on a constant and reliable supply of trash fish. The possession of a pickup truck for the collection of trash fish at the ports greatly enhances the rate of success and the profitability of the farm.

2.3.2.5 Water management

There is no information on the effect of poor water quality (resulting from the metabolic wastes and breakdown products from the uneaten feeds) on the growth and production of C. striata in culture conditions. In the short time available for this part of the study, a detailed investigation into this aspect of C. striata farming was not possible. However, results from work carried out with the Clarias catfish, a species which is cultured on very similar lines, have indicated that changes in water quality play a major role in the high mortalities experienced (Muir, 1981).

With the exception of Pathumthani Farm, which had stationary water ponds, the other three farms exchanged the pond water continuously and should thus be exempted to some extent from the problem of deteriorating water quality. This depends, however, on the rate of water exchange and the cleanliness of the water source. Furthermore, as most fish farms in Thailand are aggregated in a few favourable areas, the discharged used water is normally reused by other farms further downstream. Hence, although there were no mass mortalities that could be attributed to poor water quality, its sublethal effects on growth and chronic fish losses are important and deserve further investigation.

2.3.2.6 Disease

Major mortalities, sufficient to jeopardise economic

viability, were reported from all four farms. The cause of fish mortalities are however not often known, as none of the farmers were equipped or trained to perform diagnostic work. It follows therefore that there were no reliable records of any principal disease on cultured C. striata and no corresponding treatment programme such as that already available for the Clarias catfish farming industry.

Ectoparasites such as Trichodina sp., Ichthyobodo sp., Ichthyopthirius multifilis, Argulus sp., and endoparasites such as the acanthocephalid Pallisentis ophicephalli and nematodes Spinitectus sp and Cammallanus sp. were reported in routine parasitological screening exercise on wild C. striata (S. Chinabut, pers. comm.). It is not unreasonable to assume that these parasites also infect C. striata in culture conditions. Bacterial infections are also suspected to have occurred quite commonly, though the causative agent has not been positively identified, it is most probably that the infection arises secondarily from attacks by ectoparasites and wounding from fighting and handling (S. Chinabut, pers. comm.).

Any outbreaks of disease in farms are countered with universal treatment of antibiotics irrespective of whether this is justifiable on clinical grounds. Some farms (Khlong 2, Palurak and Pichit Farm) incorporate antibiotics into feeds as a normal supplement. In some instances, the farmer chose to 'ride out' the mortalities instead of administering the costly antibiotics. Cheap local remedies using herbs have been used though the efficacy

of these treatments have not been evaluated.

2.3.2.7 Production and survival rates

As mentioned previously, sampling of the standing crop is not normally undertaken and most farmers do not know the exact weight or numbers of the fish stock. There is therefore no reliable information on the survival rate.

Production rates ranged from 3.1 to 27.8 kg / m^2 / year which when extrapolated give annual production rates of between 31 to 278 metric tonnes (MT) / ha / year, which is remarkably high by conventional pond production standards. From primary data for the four farms, with differing lengths of culture periods, a typical average annual production rate (arithmetical mean of all the production rate data available from the four farms) was calculated to be approximately 160 metric tonnes / ha / year. This value is comparable to the production rate of *Clarias* farms (100 metric tonnes / ha / year for single crop and 200 metric tonnes / ha / year with double cropping, Muir, 1981) and considerably higher than the average production rate of 10 to 15 metric tonnes / ha / year in a well-managed photosynthesising system, and 30 metric tonnes / ha / year in small experimental ponds in Israel for carps (Sarig, 1978 as cited by Muir, 1981). In comparison, *C. striata* fed chopped *Tilapia* spp. yielded 647 kg / ha / 6 months in Malaysia (the culture conditions were not known, Anon, 1972). Intensive culture of *C. punctatus* in cages in India yielded approximately 46 metric tonnes / ha in

135 days but in intensive pond culture of C. punctatus in the swamps of West Bengal (India) the yield was only 895 kg / ha over 8 months (Anon, 1975).

2.3.2.8 Harvesting and Marketing

A marketable size of between 700 g and 1,000 g is reached within 7 to 10 months. Fish weighing more than one kilogram are unpopular as they then become too expensive as a single purchase for consumers.

The whole crop is harvested at the end of the raising period as there is no multiple cropping. Harvesting consists of seine netting over a 2 to 3 day period and draining the whole pond on the final day. The entire crop is sold to fish wholesalers, who then usually take over the complete operation of harvesting and transportation of fish to the market. This practice releases the farmer from the burden of harvesting and transporting to the markets and locating outlets for his crop. On the other hand, the selling price is inflexible and is normally set by the wholesalers, the farmer often having little or no choice of terms, particularly when, as often happens, the wholesalers act as financiers providing capital for the farmer through credit and loans.

There is only one main fish market in Thailand, where freshwater and marine fish are auctioned for Bangkok city, run by the Fish Marketing Organisation (FMO) in Bangkok. The price of fish is determined both by individual negotiation and trading in

organised auctions. In theory, the fish auction at FMO is based on the Royal Thai Government regulations but in practice, it operates by individual trading i.e. prices are generally determined by demand and supply and the amount of price competition between the wholesalers and retailers.

Channa striata are always sold alive where possible as the price is reduced by 30 to 40% when dead. The fish are transported to market in special containers to keep them alive and presented to the customers on a specially built arena where the fish can be viewed for their 'freshness', normally by assessing their activity. Processed fish, either salted or dried, command only 40 to 50% of the price of the live fish. Fish too stale for processing into salted or dried forms is processed by fermentation and sold for around 20% of the price of the live fish (Nitsmer, 1982). It is important to note that as wet weight is reduced in all these treatments, comparative prices are actually lower than indicated.

There are wide fluctuations in the wholesale price of cultured snakeheads, varying between 16 to 27 ฿ / kg in 1978; 16 to 38 ฿ / kg in 1979; 26 to 40 ฿ / kg in 1980 and 22 to 33 ฿ / kg in 1981 with peak values during the wet season (April to September) of each year. In contrast, the cost of production has increased over this period, mainly due to the increased cost of trash fish.

2.3.2.9 Economics of production

There is no available information on the economics of the C. striata farming industry. The current survey attempted to collect data where possible on the economics of the culture and management techniques, though generally the farmers were reluctant to impart such information because either they did not keep such records or did not wish their figures to be made public. One farm, however, did provide adequate data for an analysis of the inputs and outputs and the cost effectiveness of culture practices, this will be discussed in greater detail in section 2.3.3.

There are apparently three sources of finance available for aquaculture (including snakehead production), namely:

1) Commercial banks: which provide varying sizes of loans at interest rates ranging from 14 to 16% per annum (1982, rates, Thai Farmers Bank, Bangkok. See Appendix 2)

2) FMO registered wholesalers: who provide credit facilities at interest rates of up to 24% per annum (W. Palurak, pers. comm., 1981 rates)

3) Private financiers: these include trash fish suppliers who provide credit facilities at an even higher interest rate of up to 36% per annum (1981 rates).

Fifty per cent of Clarias catfish farmers in Suphanburi and Nakhon Nayok provinces secured loans from commercial banks to

fund their operations (Wattanutcharya and Panayatou, 1981). The sources of finance for the C. striata farms in this survey are not known. Banks were apparently more reluctant to approve loans for C. striata farming enterprise than other fish farming enterprises though their reason for this was not specified (Manager, Thai Farmers Bank, London Branch).

2.3.2.10 Summary of data

The following points can be generalized from the primary data[a]:

1) All the farms appeared to have started C. striata culture at the same time (1977-78), either from existing fish farms or as new establishments.

2) There did not appear to have been any major differences between the practices in the culture and management techniques of the four farms.

3) All farms experienced similar problems which may be summarised as follows:

   a) High and increasing operating costs (in particular the feed item).

   b) Unpredictable and rapid market fluctuations; at the time of the survey low wholesale market price was markedly reducing incomes.

   c) Disease and high mortality; particularly during the early part of the production cycle.

Detailed discussions on these and other problems will be

dealt with specifically in section 2.4.

4) Since all the farmers had similar experience in culturing C. striata (approximately 3.5 years) and used similar culture and management techniques, it would appear that the varying production rates achieved are probably related to the level of management in each individual farm rather than the specific areas or methods used in the two study areas.

In view of the problems facing the C. striata farmers, it would appear that the expansion period in the industry is declining. In fact, all four farms visited are seriously considering moving to raising Clarias catfish because of its shorter production cycle (two crops a year can be achieved) and faster turnover of capital, and indeed two of the farms visited in the survey had indicated their intention to culture Clarias catfish next season and one of the farms has been put up for sale, being bankrupted by the increasing costs and debts incurred in the production.

2.3.3 A Case Study of Palurak snakehead (Channa striata) Farm

There is only one single reference which provides any guidelines to the culture of C. striata in Thailand (Ukkatewewat, 1980, section 2.3.1.2). Pioneering farmers working on a trial and error basis have developed an apparently sound and profitable system. The knowledge gained has been transmitted from farm to

farm by word of mouth, and the culture techniques employed by the farmers have been modified and adapted to suit individual farms.

The primary data for the farm which was the subject for this case study were obtained through an in-depth interview and an extensive analysis of the farm's records, with the aim of describing in fuller detail the management and culture operations, over a production cycle, of a single farm (Palurak Farm). It will serve to compare with the more general information generated by the farm surveys, and with guidelines provided by Government and other agencies.

2.3.3.1 General farm layout

At the time of the visit (February 1981), Palurak Farm had been operating for four years. It was set up in an area where rice and general agriculture predominate, in Nakhon Nayok province. The owner, Mr Wirayot Palurak (age 38), had no training or experience in aquaculture prior to the setting up of the farm.

The plan of the farm is presented in Figure 2.6. The total area averaged 4.8 ha (30 rai). The major species of fish cultured was C. striata with some Chinese carp as a minor activity. The farm also had fruit trees and free ranging chicken and ducks. The economic analysis of the farm's net returns will be based solely on the C. striata production component, though where relevant, inputs or outputs to other sections of the farm are indicated. There were two other C. striata farms of similar size situated 3 km upstream along the canal which supplied the

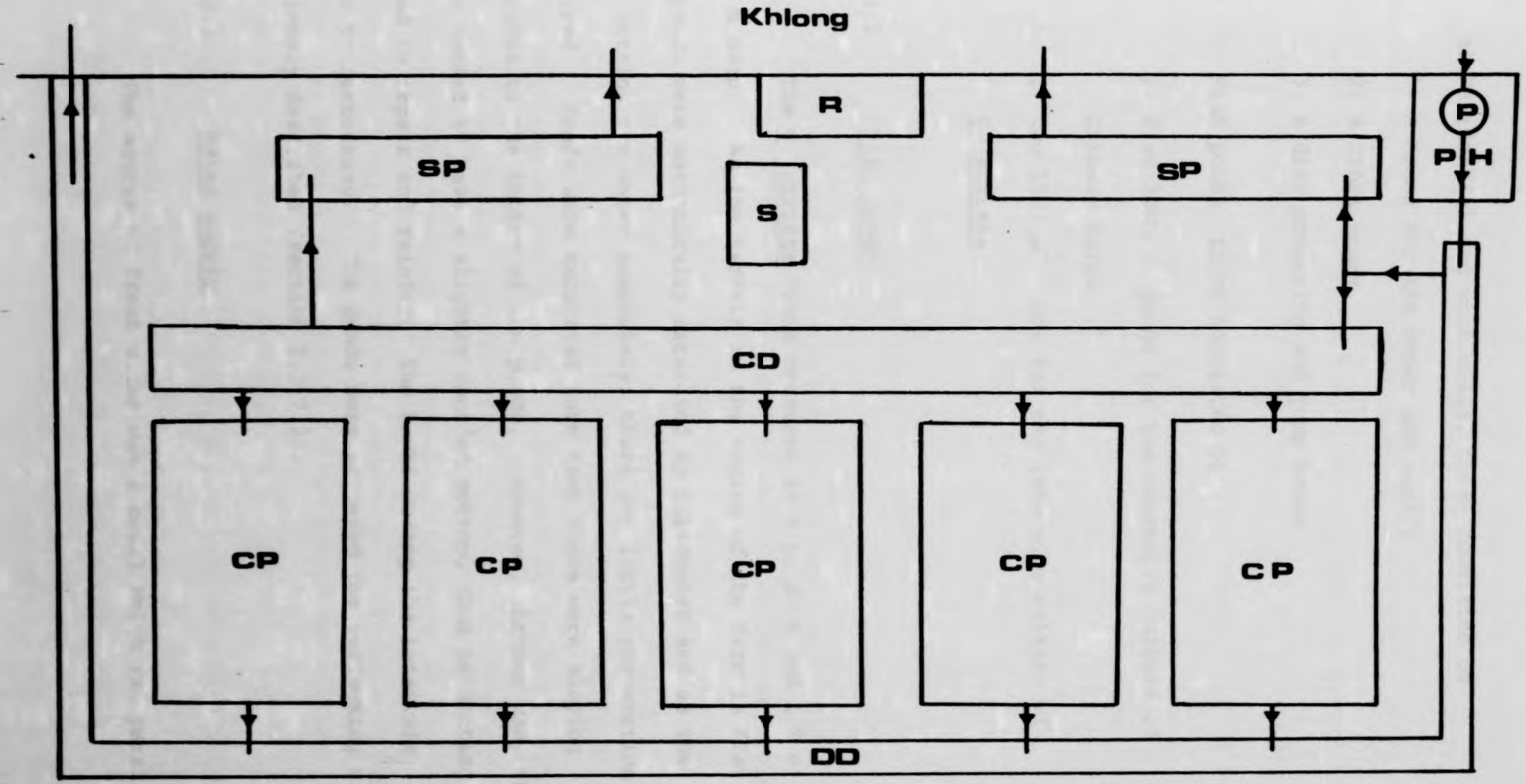


Figure 2.6 Layout of Palurak Farm

→ : direction of water flow; SP: snakehead pond; CP: Chinese carp pond; S: store; PH: pump cum diet preparing house; R:residence; CD: collecting ditch; DD: draining ditch P: pump

farm. The farm was subdivided into:

a) Residential and work areas, these consisted of

1) A house for the owner and family

2) A store house

3) A diet preparing and pump house

b) Fish ponds, these consisted of

1) Five 3060 m^2 ponds for the extensive culture of Chinese carps

2) Two 1200 m^2 ponds for the intensive culture of C. striata.

2.3.3.2 Fish ponds

The C. striata ponds measured 15 m by 80 m and 1.5 m to 2 m deep. As the terrain in the region of the farm is flat, the ponds were mechanically excavated by bulldozers and as the soil retains the water adequately, there was little preparation required. Ponds were excavated such that there were sloping 45° sides to the bottom of the ponds. However, through use, the ponds tended to have a slightly rounded bottom, thus presenting a need to repair and reinforce the banks during the intervals prior to restocking. The ponds were prepared for restocking as previously described (section 2.3.2.2).

2.3.3.3 Water supply

The source of fresh water was a canal which ran parallel

to the farm. Water was pumped continuously to collecting ponds and ditches by a 100 horse power 'long tail' pump, up a height of 2 metres, for 10 to 12 hours daily (from sunrise to sunset). It was then channelled to individual ponds by gravity. There were no attempts to screen the intake water supply for predators. Within the ponds, there was a water head of about 80 cm between the inflow and outflow points. The volume of water discharged at the intake and discharge points was regulated by the use of a single wooden sluice gate. The outflow discharge into the canal downstream from the intake point. The water level at the pond was about 2 metres above the water level of the canal.

2.3.3.4 Production management

A summary of the operations of the farm over the four years of operation is presented in Table 2.3. Fry for stocking were delivered to the farm by specialist collectors. The fry were bought by the 'cup' costing an average of 10 ₦ per cup (1980 price). In the first two years of operation, the farm stocked the ponds in the off season (the peak season for stocking is generally wet season (section 2.3.2.3), when stocked at any other period other than the peak season is referred to as off season) with disastrous economic consequences. In the next two seasons, the ponds were stocked during the wet season for two reasons. Firstly, it was believed that fry produced during this period were in better condition and as such were much hardier and suffered fewer mortalities than those produced later in the year. Secondly, it was a better production strategy where

Table 2.3 Summary of production data for the C. striata production unit , Palurak Farm .

Date of Stocking	Stocking rate (cups per pond @ 300 fry per cup)	Stocking rate (fry /m^2)	Ratio of trash fish to rice bran	Feed conversion rate	culture period (days)	Observed mortality (number of dead fish)	Production rate (kg / pond)	Extrapolated production rate (MT/ha/crop)
2.3.1977	1,000	250	9 : 1	6.5 : 1	255	1523	22,400	187
13.12.1978	1,850	462.5	NA	NA	210	59,400 fry 7400 adults and juveniles	13,536	113
9.8.1979	1,417	354	13.4 : 1	5.7 : 1	225	2031	25,050	209
26.6.1980	1,800	450	14 : 1	8 : 1	380	25497	33,330	278
13.6.1980	1,400	350	13.8 : 1	8.5 : 1	400	12910	31,061	259

NA - records for these data were not available

harvesting would be timed to coincide with the expected high market price as previously described in section 2.3.2.3.

The fry were introduced directly into the production ponds and grown on to marketable size in the same ponds. There were no attempts to determine the actual number and weight of the fish stocked. The stocking rates were adopted from other farmers in the area. In the four seasons, different stocking densities were employed; ranging from 1000 'cups' / pond to 1850 'cups' / pond (i.e. 250 fry / m^2 to 462 fry / m^2, assuming 300 fry per 'cup'). The owner was not aware of the Thai Government Fisheries Department guidelines of stocking between 30 to 75 fry / m^2 (Ukkatewewat, 1980).

2.3.3.5 Feeds

Trash fish formed the main feed item and was collected directly from the port by truck by the farmer who was then able to choose good quality trash fish. Stock fish were fed trash fish and rice bran at an average ratio of 13.5 : 1 (by weight) except between day 1 - day 10 of culture when they were fed on finely chopped trash fish with a minimal amount of rice bran. A typical feeding chart for the 1980 production cycle is presented in Table 2.4. The fish were fed twice a day (early morning and late evening) from day 1 to day 150 of culture and once a day (early morning) from day 150 to harvest. The feeds were prepared as previously described (section 2.3.2.4) and placed on wooden platforms situated in strategic positions in the

Table 2.4 A typical feeding chart for the C. striata production unit , Palurak Farm (1979-80 crop)

Days of culture	Trash fish (kg)	Rice bran (kg)	Trash fish : rice bran
0 - 10	260	2	130 : 1
10 - 20	707	22	32.1 : 1
20 - 30	1,339	77	17.4 : 1
30 - 40	2,426	170	14.3 : 1
40 - 50	4,196	202	20.8 : 1
50 - 60	5,648	428	13.2 : 1
60 - 70	6,971	494	14.1 : 1
70 - 80	7,219	543	13.3 : 1
90 - 100	8,034	640	12.6 : 1
100 - 110	8,655	917	9.4 : 1
110 - 120	6,240	729	8.6 : 1
120 - 130	7,669	553	13.9 : 1
130 - 140	8,063	466	17.3 : 1
140 - 150	9,036	607	14.9 : 1
150 - 160	7,416	510	14.5 : 1
160 - 170	6,641	352	18.9 : 1
170 - 180	6,655	486	13.7 : 1
180 - 190	7,929	584	13.6 : 1
190 - 200	9,026	611	14.8 : 1
200 - 210	5,621	375	15.0 : 1
210 - 220	7,343	593	12.4 : 1
220 - 230	6,769	556	12.2 : 1
Total	133,863	9,917	Average ratio 13.5 : 1

pond (Plate 2.2). There was no fixed feeding rate, the amount fed depended on the appetite of the fish and on the supply of trash fish from the ports. When there was a surplus, the normal practice was to overfeed, recover the excess feed several hours later and to adjust the next days feed accordingly. In times of insufficient supply, the stock fish were underfed and when no trash fish are available, the fish in the farm were not fed.

Feed conversion ratios obtained ranged from 5.7 to 8.5:1 which as was noted in the previous section is poorer than those obtained for Clarias catfish farms but comparable to C. maculatus farms in Hong Kong and Taiwan using trash fish as feeds.

2.3.3.6 Production rate

The rearing periods ranged from 7 to 12 months. The time of harvest was governed by the prevailing market price. This was evident from the record of the sale of the four crops produced (Table 2.3). It was apparent that for the 1980 crop, the rearing period was extended to over 400 days because of a low selling price. It also became apparent that it was not beneficial to extend the production time beyond 10 months; firstly because the fish produced would weigh more than 1 kilogram and secondly the biomass of the standing crop would be approaching its maximum, resulting in reduced growth and poor feed conversion efficiency (as evident by the high feed conversion ratio values as shown in Table 2.3) and also an increase in the incidence of disease. As will be shown later, the consequence of extending

the production time can be very significant to the economics of production.

The production rate ranged from 13,536 kg / pond to 33,330 kg / pond which when extrapolated gives a value of 113 metric tonnes / ha / crop to 278 metric tonnes / ha / crop, which is higher than the average value of 160 metric tonnes / ha / year previously described in section 2.3.2.7.

The entire crop was purchased by a wholesaler at the end of the production cycle who undertook to harvest and transport the fish to market. There was no multiple cropping at any time during the production cycle. Harvesting was normally carried out over three to four days and consisted of seining the ponds and draining them on the last day of harvesting to collect the last of the crop.

2.3.3.7 Water management

Water at various intake and outflow points in the collecting ditch and fish ponds was sampled for pH, dissolved oxygen, free carbon dioxide, hydrogen sulphide, total ammonia and nitrite determination[2] (Figure 2.7). The results of the analyses are presented in Table 2.5 and for comparison the tolerance limits for other teleost species for these water chemistry parameters are also presented in Table 2.6.

2. The water quality analysis was performed by Mr Vijai Srisuwantach of the National Inland Fisheries Institute, Bangkok.

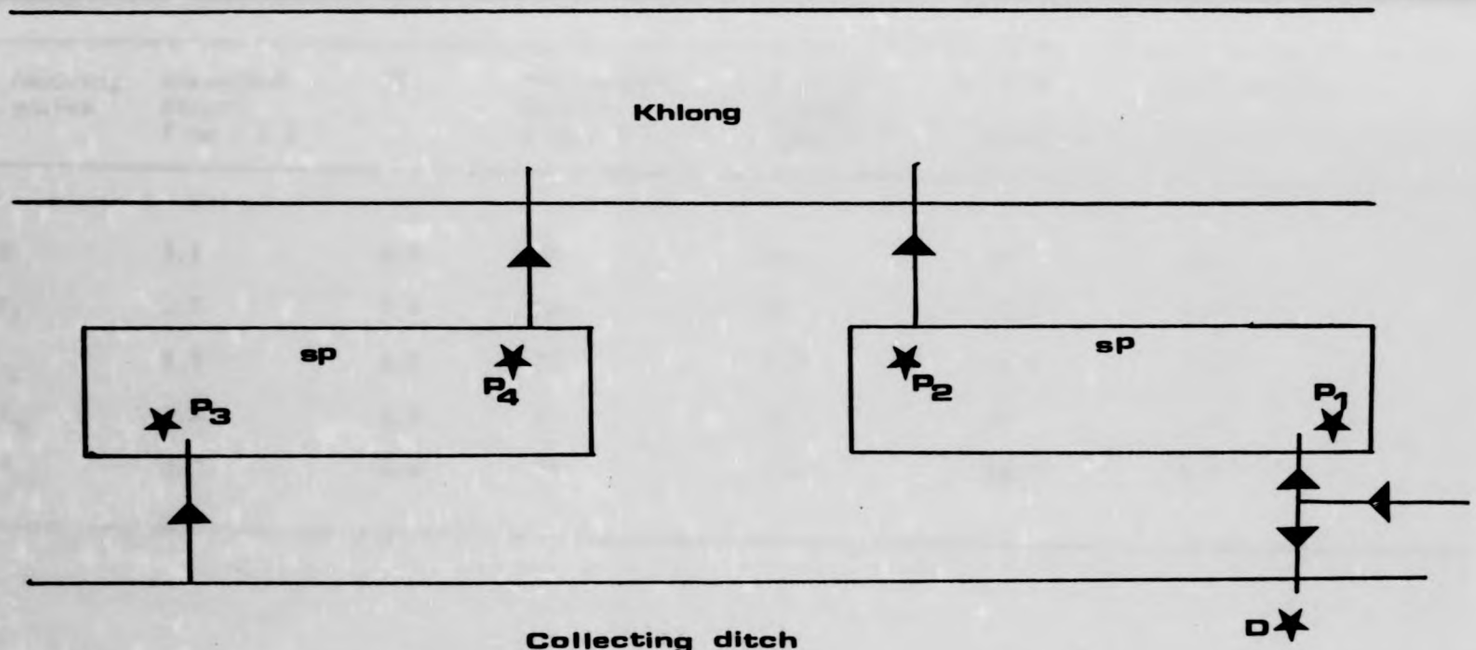


Figure 2.7 Sampling points for water quality analysis, Snakehead production unit, Palurak Farm
——▶——: direction of water flow; **sp** : snakehead pond

Table 2.5 Results of the water quality analysis at the C. striata production unit, Palurak Farm

Sampling points	Dissolved oxygen (mg / 1)	pH	Free carbon dioxide (mg / 1)	Hydrogen sulphide (µg/1)	Nitrite (µg/1)	Total ammonia (mg/1)
D	3.1	6.4	10	0.3	37	1.03
P_1	2.3	6.6	13	0	25.5	1.27
P_2	0.3	6.5	27	0.7	25.5	1.37
P_3	1.2	6.6	8	1.8	40	1.29
P_4	0.3	6.6	23	1.3	58.5	1.3

Table 2.6 Tolerance limits of various water chemistry parameters for certain fish species

Parameter	Tolerance level	Effects on fish
pH	5 - 9	lethal above and below these values for rainbow trout, Salmo gairdneri (Jordan and Lloyd, 1964., Lloyd and Jordan, 1964)
	3.4 - 11	upper and lower 24 hour LC50 for tilapia, Tilapia nilotica (Mahdi,1973a)
Ammonia (unionised)	1.6 - 3.1 mg/l	lethal for channel catfish, Ictalurus punctatus (Colt and Tchobanoglous, 1976.,1978., Knepp and Arkin, 1972)
	0.4 mg/l	lethal for rainbow trout (Colt et al, 1979)
	0.4 - 0.6 mg/l	lethal for carp, Cyprinus carpio (Flis, 1968., Kawamoto, 1961)
Nitrite (NO_2-N)	7 - 13 mg/l	lethal for channel catfish (Colt and Tchobanoglous, 1976., Konikoff,1975)
	0.19 - 0.55 mg/l	lethal for salmonids (Colt et al, 1979)
Hydrogen sulphide (H_2S)	1 - 1.4 mg/l	lethal for channel catfish (Bonn and Follis, 1967)
	0.0146 mg/l	reduced growth in bluegills, Lepomis macrochirus (Oseid and Smith, 1972)
Dissolved oxygen (DO)	0.89 mg/l @ 20°C	lethal for channel catfish (Moss and Scott, 1961)
	2.1 - 2.3 mg/l @ 20°C	lethal for coho salmon, Oncorhynchus kisutch (Herrmann et al , 1962)
	0.8 mg/l @ 20°C	lethal for carp (Askerov, 1975)
	1.4 mg/l	lethal for tilapia, Sarotherodon nilotica (Mahdi, 1973 b)
	0.9 mg/l	lethal for tilapia, Tilapia zillii (Saad and Ezzat, 1973)
Carbon dioxide (CO_2)	more than 60 mg/l	lethal for most species of fish (Colt et al, 1979)

From the results, it would appear that the levels of the constituents measured within the production ponds in Palurak Farm all lay within the tolerance limits of the majority of fish species. It must be pointed out that Palurak Farm has a relatively high continuous water exchange and thus a high flushing rate and low water retention time; and so this would help to keep the water quality within acceptable limits. Other C. striata farms, however may not have continuous water exchange because of the high cost of pumping or the lack of suitable water source. In these farms, the levels of these potentially harmful water chemistry parameters are likely to be higher than those recorded at Palurak Farm.

Although lethal levels were not apparently reached, the sublethal levels may have affected the growth. One important point to consider is that the lethal and sublethal effects of water chemistry parameters on growth and survival rate are not singular, they are all interrelated, in particular with low levels of dissolved oxygen and condition of the fish. In Palurak Farm, the main intake water supply is already low in dissolved oxygen and the water becomes anaerobic in the production ponds. Therefore, in view of the near anaerobic conditions in the production ponds, the sublethal effects of water chemistry parameters such as unionised ammonia, hydrogen sulphide, and nitrite on growth and survival rate may be significant. However, insufficient is known about the significance of these factors for a species as strongly air-breathing as the snakeheads.

2.3.3.8 Disease

There were numerous incidences of disease and fish kills described, though the causes were not known. The farmer did not have any basic diagnostic apparatus or training to ascertain the cause of death or perform routine screening procedures. The normal course of action taken when the mortality rate in the farm was adjudged to be higher than the normal rate was to use antibiotics. The antibiotics were incorporated into the feeds. A typical dosage used would be 2 kg of 'Sulpha' (Sulphonamide antibiotic) in 850 kg of trash fish and 35 kg of rice bran for 190 day fish, administered for two days. The high cost of the antibiotic ('Sulpha' cost 400 ₱ per kg, 1980 price) deters the farmer from using it, particularly when the high mortalities occur late in the production cycle where the biomass of the crop is high thus requiring a corresponding high level of 'Sulpha'. To prevent mortalities occurring early in the production cycle, in the 1980 crop the farmer gave a prophylactic treatment by incorporating 'Sulpha' into the feed at the beginning of the production cycle, the dosage used was not specified.

Observed mortalities (the number of dead fish recovered from the surface of the ponds) from the 1980 crop show that although there were mortalities throughout the production cycle, essentially there were two peaks (Figure 2.8). The first major peak occurred after approximately 60 to 80 days of culture and the minor peak after approximately 230 days of culture. Similar trends were reported for Clarias catfish operations (Muir, 1981). The cumulative effects of the stress from handling,

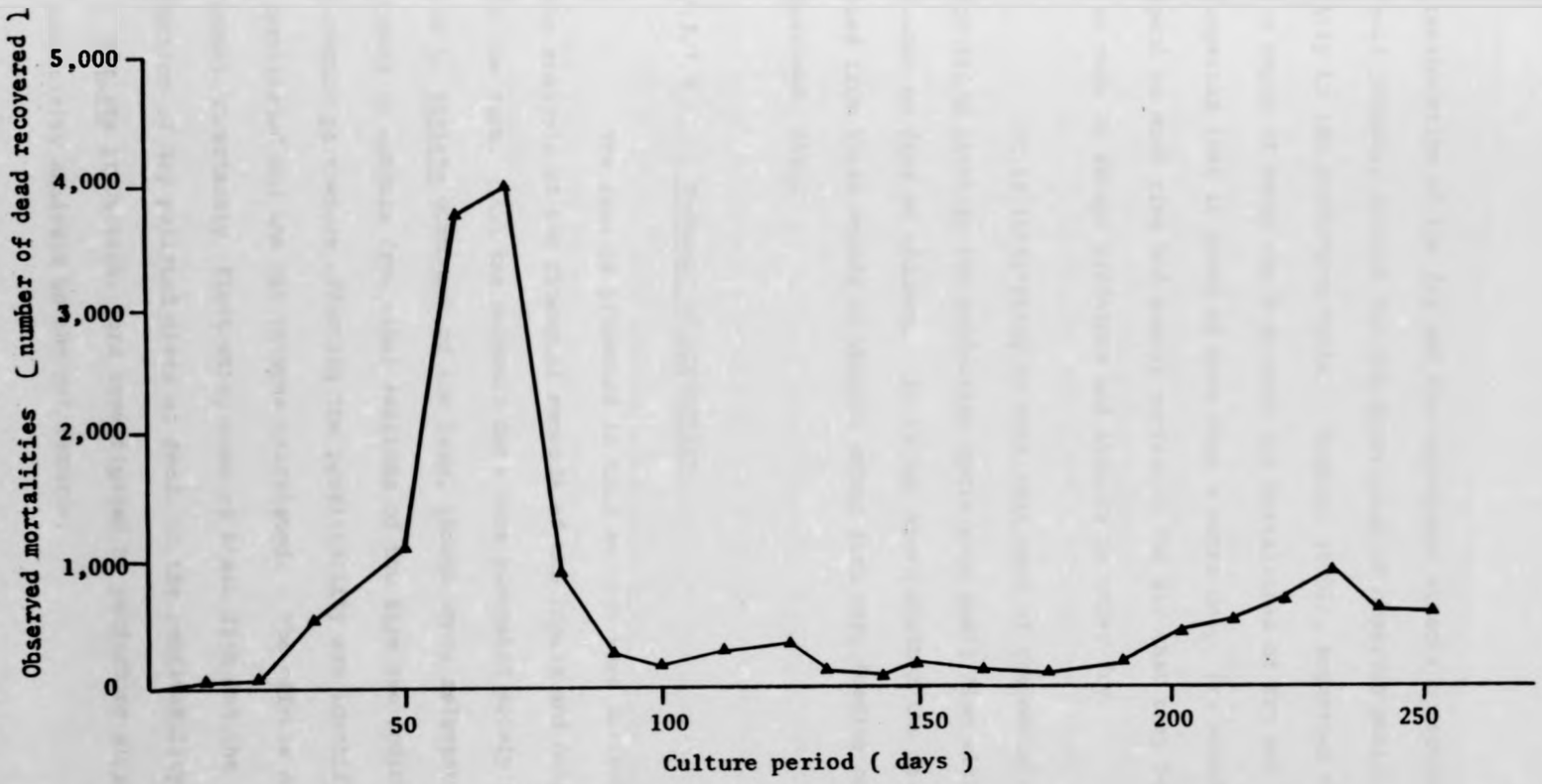


Figure 2.8 Observed mortalities of Channa striata over a production cycle (1980/81), Snakehead production unit, Palurak Farm

translocation of the fry and the subsequent attacks by ectoparasites could probably account for the major peak of observed mortalities early in the production cycle. Pandian (1981), suggested that the depth of ponds could account for mortalities of fry and suggested that in ponds of more than a metre deep, fry would spend so much time and energy surfacing for air that they become too weak to escape predators and attacks by other fry.

It is interesting to note that most of the mortalities occurring later in the production cycle were small fish with wounds on fins or abdomen. It is not known whether the fish died from these wounds or whether other fish were feeding on moribund fish.

2.3.3.9 Economic of production

The results presented in this section were derived from the analysis of the financial records of the inputs and outputs of the farm. All the economic data were generated solely from the C. striata component of the farm, though where relevant, inputs or outputs from other sections of the farm are indicated. Economic parameters affecting the profitability are identified, quantitated and the net returns calculated. The effects of market uncertainty, fluctuating cost of trash fish and the introduction of dry pelleted diets as feed, on the profitability of C. striata production, were investigated by performing simple sensitivity analysis on the net returns.

Economic data on the operating costs for two productions

were available, the 1979-80 crop where the single pond unit was in use, and the 1980-81 crop where the double pond unit was in use.

Tables 2.7, 2.8, 2.9 and 2.11 show the capital (establishment) cost, depreciation costs, fixed costs and variable costs of the C. striata production component of Palurak Farm respectively. Finance for the overall establishment costs was from the owner's capital funds, this totalled 668,666 ฿ for the one pond unit (682,816 ฿ for the two pond unit; Table 2.7). The total operating cost was secured as a loan from a bank at an interest rate of 16% per annum. Table 2.10 shows the total operating costs, where it is clear that the feed item was the major cost for both operations (1 and 2 ponds) accounting for 67.53% and 85.82% of the total operating costs respectively. The higher feed bill in the 1980-81 crop (two pond unit) was due to the poor feed conversion efficiency (as evident by the high FCR averaging 8.5 : 1, Table 2.4).

The next important cost for the single pond unit operation was the miscellaneous cost item at 11.14%; followed by the labour cost at 9%; fuel cost at 4.43%; disease control at 3.87% and seed fish cost at 2.36% of the total operating costs respectively. For the two pond unit operation, the next important cost was the fuel cost at 4.25%; followed by miscellaneous cost at 3.75%; labour cost at 2.24%; disease control at 1.93% and seed fish cost at 1.59% of total operating cost. It is apparent that the cost of seed fish currently

Table 2. 7 Capital cost of the Channa striata production unit, Palurak Farm
(all values are expressed as Bahts *1)

Item	Unit	Single pond operation	Two pond operation
Land @ 77,800 ฿ per rai *2	5 rai	389,000	389,000
Pond (cost of construction and materials)	1 (2)	58,333	72,483
Equipment			
pump	1	38,000	38,000
house and working shed	1	115,000	115,000
feed mixer	1	3,333	3,333
Vehicle			
pickup truck	1	40,000	40,000
motor cycle	1	25,000	25,000
Total		668,666	682,816

*1 Baht (Thai currency) = ฿ , US$ 1.00 = approximately 20 ฿

*2 1 rai = 1,600 m^2

Table 2.8 Depreciation cost of items in the Channa striata production unit, Palurak Farm *1

Item	Life expectancy (years)	Capital cost (฿)	Depreciation per annum (฿)
Buildings	20	115,000	5,750
Pickup truck	6	40,000	6,667
Motor cycle	6	25,000	4,167
Pump	6	38,000	6,333
Food mixer	6	3,333	556
Total			23,473

*1 Depreciation costs were calculated using the straight line method of dividing the capital cost by the life expectancy of the item, assuming that all the items have zero salvage value at the end of the life expectancy period

Table 2.9 Fixed costs of the Channa striata production unit, Palurak Farm (all values are expressed as Bahts)

Item	Operation	
	Single pond	Two pond
Interest on capital cost*1	106,987	109,251
Depreciation costs*2	23,473	23,473
Total	130,460	132,724

*1 Interest on capital cost was calculated at 16 % per annum for respective operations

*2 Depreciation costs as calculated in Table 2.8

Table 2.10 Total operating costs of the Channa striata production unit, Palurak Farm
(all values are expressed as Bahts)

Item	Single pond operation	% of total operating cost	Two pond operation	% of total operating cost
Seed fish	14,170	2.36	38,400	1.59
Feeds		67.53		85.82
- trash fish	379,290	63.22	1,978,052	82.07
- rice bran	25,877	4.31	90,446	3.75
Disease control	23,190	3.87	46,410	1.93
Fuel	26,569	4.43	102,500	4.25
Labour cost	54,000	9.0	54,000	2.24
Maintenance	10,030	1.67	10,242	0.42
Miscellaneous cost	66,874	11.14	90,168	3.75
Total	600,000	100	2,410,218	100

Note: Values for the maintenance items were stated by the farmer.

Table 2.11 Variable costs of the Channa striata production unit, Palurak Farm (all values are expressed as Bahts)

Item	Operation	
	Single pond	Two pond
Seed fish	14,170	38,400
Feeds	405,167	2,068,498
Fuel	26,569	102,500
Disease control	23,190	46,410
Labour cost	54,000	54,000
Maintenance cost	10,030	10,242
Interest on operating cost *1	96,000	385,635
Other miscellaneous cost	66,874	90,168
Total	696,000	2,795,853

*1 calculated based on an interest rate of 16 % per annum on the total operating costs (Table 2.10)

constitutes only a very small percentage of the total operating costs ranging from 1.59 to 2.36%. In contrast, in Clarias catfish farms, the cost of the seed fish is the next important cost after the feed item, accounting for up to 8% of the total variable costs (i.e. total operating costs plus the interest on the total operating costs, Wattanutcharya, 1982).

The total cost of production for the single pond operation with seven months rearing period was 33 ฿ / kg and for the two pond operation at more than 12 months rearing period was 46 ฿ / kg. Taking into account the average selling price of 32 ฿ / kg in 1980 and 26 ฿ / kgin 1981, a loss of 1 ฿ / kg and 19.5 ฿ / kg was incurred in 1980 and 1981 respectively (Table 2.12).

Aquaculture enterprises are subjected to risk factors which include market uncertainty and technological failure; these can be assessed to some extent by the analyses of the change in net returns in response to changing input and output conditions. Market uncertainty can be defined as the fluctuation in selling price or demand. Technological risks can be measured as partial or total stock loss resulting from system failures, incompetent management or natural phenomena, such as outbreaks of epizootics, toxic phytoplankton blooms or flooding. Implications also include the failure to realise anticipated production targets and/or an increase in operating costs.

In order to measure the profitability of the C. striata production component in Palurak Farm, when subjected to such risks, sensitivity analyses were performed on the net returns when the average selling price (farm gate price) and the cost of

Table 2.12 Production and Economic indicators of the performance of the Channa striata production unit, Palurak Farm

Indicators	Operation	
	Single pond	Two pond
Yield (kg / unit operation)	25,050	64,391
Average farm gate selling price (฿ / kg)	32	26
Gross revenues (฿)	801,600	1,674,166
Costs of production		
Variable costs (฿)	696,000	2,795,853
Fixed costs (฿)	130,460	132,724
Total (฿)	826,460	2,928,577
Returns		
Operating profits *1 (฿ / kg produced)	4.22	-17.42
Net profits *2 (฿ / kg produced)	-0.99	-19.5
Average rate of return on investment *3 (฿)	-4.14	-183.73
Average rate of return on operating costs *4 (฿)	-3.57	-52.05
Cost of production (฿ / kg produced)	32.99	45.48

*1 Operating profit = (Gross revenues - Variable costs) / production

*2 Net profit = (Gross revenues - Total costs) / production

*3 Average rate of return on investment = Total net profit / Total investment cost (capital cost)

*4 Average rate of return on operating cost = Total net profit / Total operating costs

trash fish was increased or decreased by 20%. The cost effectiveness of the use of dry pelleted feed was also analysed.

From the sensitivity analyses presented in Table 2.13, it can be seen that in the 1980 season, where the technological failure risk factor was low (as evident by the relatively acceptable FCR values averaging 6.5 : 1 and the low mortality observed, Table 2.3), the profit was affected by the selling price. An increase in the selling price by 20% (the new 'increased' selling price of 38.4 ฿ per kg lay within the range of the selling price for that year, Table 2.2) would have generated a profit of 5.4 ฿ per kg and needless to say a reduction by 20% in the selling price would have resulted in a heavier loss of -7.39 ฿/kg.

Profits for the 1980-81 crop was affected by both risk factors, the selling price in 1981 was low (averaging 26 ฿ per kg compared to 32 ฿ per kg in 1980) and there was some degree of technological failure as evident by the high FCR of 8.5 : 1 and the high mortality rates observed (Table 2.4). Hence an increase in the selling price by 20% would only alleviate the loss and not generate profit.

In the sensitivity analysis of the net returns to evaluate the cost effectiveness of the use of dry pelleted feeds in C. striata farming (based on the sensitivity analysis of the net returns of Palurak Farm), it was assumed that the diets to be provided are well formulated, containing adequate nutrients for

Table 2.13 Sensitivity analysis of the net returns of the Channa striata production unit, Palurak Farm in response to changes in the farm gate selling price*1, cost of trash fish*2 and the utilization of dry pelleted diet as feed*3

Item	Gross revenues (฿)	Operating profit (฿ / kg)	Net profit (฿ / kg)
Single pond operation			
Farm gate selling price			
@ 32 ฿ / kg	801,600	4.22	-0.99
A 20 % increase to 38.4 ฿ / kg	961,920	10.62	5.41
A 20 % decrease to 25.6 ฿ / kg	641,280	-2.18	-7.39
Cost of trash fish			
@ 2.8 ฿ / kg	801,600	4.22	-0.99
A 20 % increase to 3.36 ฿ / kg	801,600	0.70	-4.51
A 20 % decrease to 2.24 ฿ / kg	801,600	7.73	2.52
Utilization of dry pelleted feed			
@ 7 ฿ / kg	801,600	10.8	5.59

Table 2.13 (cont.)

Item	Gross revenues (฿)	Operating profit (฿ / kg)	Net profit (฿ / kg)
Two pond operation			
Farm gate selling price			
@ 26 ฿ / kg	1,674,015	-17.42	-19.5
A 20 % increase to 31.2 ฿ / kg	2,008,999	-12.22	-10.16
A 20 % decrease to 20.8 ฿ / kg	1,339,333	-22.62	-24.68
Cost of trash fish			
@ 3.72 ฿ / kg	1,674,015	-17.42	119.5
A 20 % increase to 4.46 ฿ / kg	1,674,015	-24.55	-26.61
A 20 % decrease to 2.98 ฿ / kg	1,674,015	-10.30	-12.36
Utilization of dry pelleted feed			
@ 7 ฿ / kg	1,674,015	7.66	5.6

*1 for detail working, see Appendix 3

*2 for detail working, see Appendix 4

*3 for detail working, see Appendix 5

maximal growth, and that the feed conversion ratio will be 1.5 or better (on a dry wet weight basis). It is known that the normal expected FCR values obtained with dry pelleted feeds in rainbow trout in Britain is approximately 1.5 or better (A. Tacon, pers. comm.). An even better FCR of less than 1 have been achieved by Clarias catfish in laboratory feeding trials (J. Muir, Pers. Comm.).

With this assumption, the amount of feed required was calculated by multiplying the production figures by a factor of 1.5 (assuming a FCR of 1.5 and complete consumption of the feed presented with no wastage). Hence the total amount of feed required in the 1 pond unit production was 25,050 X 1.5 = 37,575 kg and in the 2 pond unit production required 64,391 X 1.5 = 96,586.5 kg of feed. At the time of the survey, the only dry pelleted feed available were formulated for the Clarias catfish and Macrobrachium rosenbergii (the Giant Malaysian prawn), therefore the cost of dry pelleted feed for these two species was used to calculate the feed cost item in the analysis (this averaged 7 ₿ per kg in 1980).

From Table 2.13, it can be seen that both the 1979-80 and 1980-81 crops would have generated healthy net profits of 5.59 ₿ per kg and 5.6 ₿ per kg respectively, with the use of dry pelleted feeds.

In addition to the significant economic benefits to be gained from using dry pelleted feeds, there are other advantages such as the maintenance of the water quality and reduction in the risks of disease transmission from the wild trash fish fed to the

hatchery fish, although these advantages are not easily quantified in terms of improved production or reduction in mortality. Feeding trials in rainbow trout farms in Denmark comparing wet diet (trash fish) and dry pelleted diet have shown that production can be improved by up to 15% when dry pelleted feeds are used (Rasmussen, 1976).

The price of trash fish was also significant in affecting the profitability of the C. striata production component of Palurak Farm. In the 1979-80 crop, a 20% decrease in the price of trash fish would have generated a net profit of 2.52 ฿ / kg while a 20% increase would have caused an even bigger loss of 4.51 ฿ / kg. For the 1980-81 crop, a 20% decrease in the price of trash fish would only have reduced the loss to 12.36 ฿ / kg and a 20% increase would have increased loss to 26.61 ฿ / kg (Table 2.13).

2.4 DISCUSSION

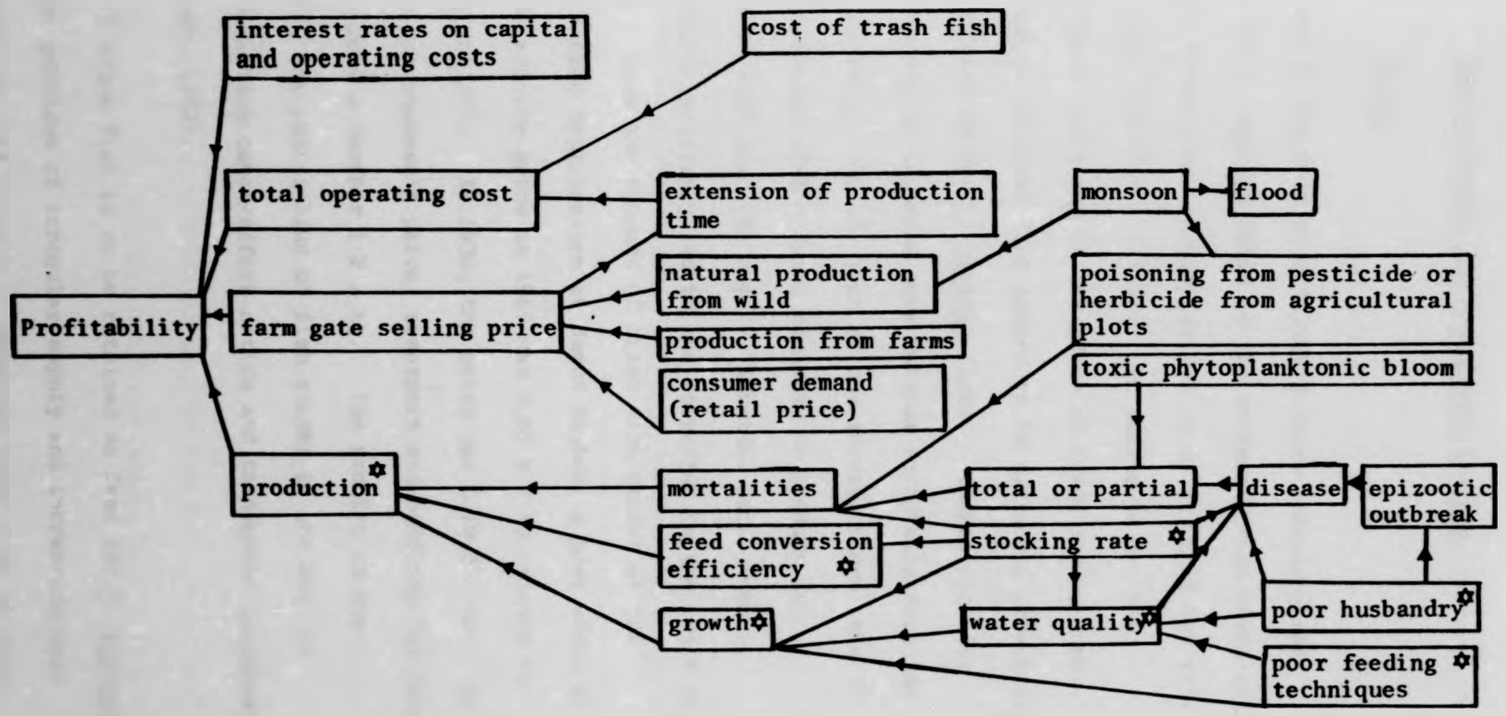
On the basis of the primary data generated from the visits to four representative farms, it is apparent that the expansion phase of C. striata farming industry as reported by Suraswadi (1980) may have ended and although the industry is still surviving with farms producing high yields using traditional methods, the margin for financial success is now very small. Results from the field survey of the four farms indicate that there are several factors whose effects are critical to the production and sale of fish stocks and the subsequent profits. These factors can be broadly classified into two categories:

1) Technological and Management factors
   These include the use of trash fish as feed, extreme stocking densities and stocking practices employed, supply of seed fish, disease and water management problems.

2) Market and Economic factors
   The main factor is the farm gate selling price which is governed by the natural production of C. striata in the wild and to a lesser extent on the production from C. striata farms.

These factors are interrelated and their relationships are summarised and represented as a flow diagram in Figure 2.9.

Figure 2.9 Flow diagram showing the inter-relationships of factors affecting profitability of Channa striata farms. Those marked with ✡ are within the control of farmers and could be manipulated.



2.4.1 Technological and Management factors

2.4.1.1 Feeds

One of the most significant factors affecting the profits of the C. striata farms is the extremely high costs of feed (mainly trash fish) accounting for up to 85.8% of the total operating costs (section 2.3.3.9). In recent years, the disproportionate increases in the price of trash fish and the irregular supply of trash fish appear to be seriously undermining the profitability of the C. striata farms. Competition for trash fish supply is becoming more intense especially from the fish meal industry. At the time of the survey, there were 79 fish meal producers with a total capacity to produce 280 metric tons of fish meal per day (P. Edwards, pers. comm.). The competition invariably led to the increases in the price of trash fish. From the records of trash fish prices at the Fish Marketing Organisation at Samut Sakhon, a port south of Bangkok, the average price in 1969 was 0.65 ฿ / kg, rising to 1.03 B / kg in 1975. In 1980, the price was 2.19 ฿ / kg. In addition to the wholesale price, transport and handling for farm supply will cost a further 1 ฿ / kg. the problem is now aggravated by the overfishing of fish stocks in the Gulf of Thailand, declining catch/effort ratios and consequent increased cost (Nitsmer, 1982).

If trash fish is to be retained as feed for C. striata farming, the problems of irregular supply and increasing cost may be reduced by cold storing or freezing trash fish in bulk,

this however, would require high capital costs to store as well as to provide for the building and running of the storage facilities and this is normally beyond the financial means of most of the C. striata farmers.

Many of the problems associated with the use of trash fish as feed might be solved by changing to feeding with dry pelleted feeds. Advantages in using dry pelleted ration include a continuous availability and uniformity of feed, ease of transport and storage, ease of feeding controlled ration, water stability (implying minimal nutrient loss by leaching) and maintenance of water quality and the reduction in the risks of transmitting disease by feeding wild trash fish to hatchery fish (Cowey and Sargent, 1972). The development and progress of the channel catfish farming industry in the United States of America and the salmonid farming industry in North America and Europe have been aided to a considerable extent by the development and availability of such dry pelleted feeds.

In Thailand, there are three major fish feed producers manufacturing pelleted feeds for Clarias catfish and M. rosenbergii prawn (Nitsmer, 1982). A campaign is being waged to persuade C. striata farmers to switch to pelleted feeds. However, the higher cost and the lack of proven quality performance of C. striata fed such pelleted feed, as well as the lack of experience on the farmers part, have discouraged the farmers from making the transition.

Sensitivity analyses of the net returns of a single farm using dry pelleted feeds (based on the production figures achieved using trash fish as feed and the prevailing price of the pelleted feed available at the time of the survey and assuming a FCR of 1.5 is obtainable), have shown that it could be a very cost effective proposition, greatly increasing the margin of profits and other unquantifiable advantages (section 2.3.3.9).

The introduction of pelleted feeds into C. striata farming may present problems which are not apparent. From a biological point of view, the nutritional requirements of C. striata are not known and the growth performance and production using pelleted feeds have not been evaluated. As such, for the immediate needs, C. striata farms will have to use pelleted feed formulated for other species. The use of pelleted feeds will also incur extra costs, for adequate storage facilities need to be provided as the high temperature and humidity greatly increases the risk of having mouldy feeds and poisoning by alflatoxins and rancid feeds. Capital costs will have to be secured for bulk purchase. The availability and the cost of pelleted feeds also needs to be assessed critically for long term planning as a very high proportion of the pelleted feed consists of fish meal as protein source.

2.4.1.2 Stocking practices

The stocking rates employed in the farms visited are not standardised and are extremely high. Information on

'optimal' stocking rates were developed empirically and passed from one farmer to another by word of mouth. The very high stocking densities used are likely to lead to high levels of stress from overcrowding, which in turn increases their susceptibility to diseases and increased mortalities (Roberts, 1978). In intensive culture of tilapia, high stocking densities may lead to stunting, runting and the formation of size hierarchy producing an overall smaller size fish, differential growth rates and inefficient feed conversion (Balarin & Hatton, 1979). Multiple cropping or grading exercises could eliminate most of these problems. However, in all the farms visited, no attempts were made to grade the stock fish nor was any form of multiple cropping practised, as such, the effects of the excessive stocking show up in the later part of the production cycle where the overcrowding is often acute and the smaller fish attacked by the bigger dominant fish. All four farmers were of the opinion that C. striata is extremely sensitive to stress and disturbance and that grading or netting exercises normally result in the fish not feeding for several days. In addition, the netting procedures would damage the stock fish because of their vigorous behaviour in trying to escape the net, exposing them to possible secondary infection by bacteria and/or fungus. Therefore, although grading and multiple cropping are obviously beneficial, it would be useful to evaluate the effects of handling stress during netting exercise and the cost effectiveness in terms of improvement in production against loss in damaged fish.

Current stocking practices involve the introduction of fry directly into production ponds without any preparatory nursery stage. In view of the extreme stress experienced during capture, transport and translocation, this part of the production cycle is considered to be the most critical and nursery facilities should be provided and quarantine procedures instituted whereby the fry can be maintained and allowed to recover and prophylactic treatment administered before release. Nursery ponds need not be specially constructed. Newly secured fry could be simply held in net cages suspended in production ponds, similar to the hapas used in carp culture in India, until they are in better condition for release.

2.4.1.3 Seed fish

The problems of seed fish supply need to be investigated though at present, the supply is still adequate. There are signs that seed fish are becoming increasingly more difficult to secure in the traditional localities, having to be collected further and further afield as spawning grounds are destroyed by pollution from industries situated within the spawning areas and the decimation of the natural stock by indiscriminate collection of fry in the past. This problem will become acute as the industry expands and more fry are required. Techniques for artificial propagation have been developed for several species of Channa (Parameswaran and Murugesan, 1976). These techniques now only need to be refined and standardised to allow for genetic selection of desirable traits such as better growth performance, disease

resistance and benefits from hybrid vigour. These advantages will produce better stocks as well as enabling the C. striata farmer to reduce his dependency on the natural production of wild C. striata fry and thus have control on the timing and the production of fry on site for stocking.

2.4.1.4 Water management

If it were to become highly successful C. striata farming would tend to attract more participation and in the more favourable areas, farms would tend to aggregate. Even now it is not uncommon to find a series of fish farms along a stretch of canal or river, only a few hundred metres or so downstream from each other. Pollution and disease transmittance problems are likely to increase, as all these farms would be utilizing the same water source and the discharge of one farm would become the intake of another without any precautionary treatment or testing for water quality before use. Another problem related to the usage of a common water source is the disruption of management and production strategy. When one farm harvests, because of the high stocking densities and feeding rates employed, the disturbed water which is discharged is heavily polluted, forcing the farmer downstream to harvest also. This has already been observed by Clarias farmers in similar congregated farm areas (Muir, 1981). In this respect, it is absolutely essential that more attention and effort be applied to investigating and monitoring changes in water quality with respect to the feeding regimes, stocking rates and duration of production cycle. It is also important

to establish links, if any, between outbreaks of disease and changes in water quality.

No information is available on the water chemistry parameters in C. striata farms. Muir (1981) in the only relevant report suggested that Clarias catfish farms have major problems relating production failure to the poor water quality and the effects of metabolic wastes on growth and reproduction.

Analysis of a single sampling exercise of production pond water at Palurak farm in the case study (section 2.3.3, Table 2.5) showed the poor water quality of th water source and a considerable deterioration of water quality in the ponds. Compared with known values of tolerance limits obtained for other teleost species (Table 2.6), it is obvious that the tolerance limits of C. striata must be higher. Thus, although the lethal level has apparently not been reached in this case, it is quite possible that growth might have been reduced. Unfortunately, there is little information on the quantitative effects of metabolic wastes such as NH_3, H_2S and NO_2 on growth in most teleosts let alone C. striata with its complication of air breathing. Metabolic waste such as NH_3 is probably the single most important factor with the high biomass (from the high stocking rate) and the breakdown products from uneaten trash fish. It has been shown that growth can be reduced significantly in channel catfish (Colt and Tchobanoglous, 1978) and rainbow trout (Burrows, 1964) with increasing levels of NH_3 (determined under controlled conditions in aquariums).

The water chemistry analysis at Palurak Farm was based on a single sampling and is obviously not adequate for consideration as the norm for typical C. striata ponds: furthermore Palurak Farm is situated in an area where there are few other fish farms and has an efficient water exchange system. In this respect, the water quality at Palurak Farm was thus likely to be better than the average farm in a congested area. In the absence of detailed information on the metabolic wastes produced and their effects on growth, these results can be used only as an estimate of the likely water quality characteristics and along with guidelines available for other species, be used for future practical water management in C. striata farms.

However, in view of the rigid constraint of reliance on water supply from river or canal, it is difficult to eliminate or minimise the effects of poor water quality without incurring high costs. This problem is now aggravated in Thailand by the shift in emphasis in public transport in favour of roads. The once efficient and extensive canal transport systems of Thailand are now being filled and converted into concrete roads, reducing the efficiency of water flow and causing drainage and flooding problems especially during the monsoon season.

Several methods of improving the water quality within aquaculture operations have been developed: a typical example is to reduce the stocking rate, thus reducing the biomass loading in the ponds and corresponding amount of metabolic wastes produced. Increasing the level of dissolved oxygen is another

way although this may not benefit C. striata directly in terms of increased available oxygen as it is an air breather (Vivekanandan, 1977). However, the toxicity of some metabolic wastes are often directly related to reduced dissolved oxygen levels (Colt et al, 1979). The costs involved in aerating pond water either by using mechanical aerators (run on diesel engines or powered by direct current from generators), supplying pure oxygen directly into the water, or pumping water to return to ponds via splash boards or baffled troughs, will have to be evaluated against gains in production before recommendation to farmers. Another method is to reuse the discharged water after filtration and treatment, this would involve the construction of large settling and oxidation pond complexes with mechanical and biological filters, and the allocation of land to build the pond complexes. Again this proposition needs to be evaluated for its cost effectiveness and the improvement in yield will have to offset the extra costs in the implementation of these measures. In areas where there is plentiful good quality water, the solution may be simply to increase the rate of water exchange through the ponds, however if the water has to be pumped then the extra costs of continuous pumping will need to be considered.

All the above mentioned methods for improving water quality will incur extra costs to establish and maintain, therefore the economic gains from improvement production need to be assessed and evaluated before the implementation of these measures. Muir (1981), has shown that the capital outlay for the establishment

and maintenance of such measures against the economic gains to be achieved was more critical for smaller farms than large farms in the Clarias catfish farming industry.

2.4.2 Market and Economic factors

Economic data from Palurak Farm presented in section 2.3.3 illustrate the extent market uncertainty and technological failure affect the profitability. Sensitivity analyses indicate that selling price (farm gate selling price) is the controlling factor in the level of profitability of the farm and that is beyond the control of the farmer. This is best exemplified by examining the sensitivity analyses of the net returns of the 1980 crop in Palurak Farm (section 2.3.3) where the technological aspects of the production were more or less fulfilled as evident by the fairly good FCR values and relatively low mortalities (a 20% increase in the selling price would have generated profits). On the other hand, in the 1981 crop, there was a slight technological failure with poor FCR and the high mortality experienced, coupled with a low selling price, culminated in a financially disastrous year.

The C. striata farming industry in Thailand suffers from the general reluctance of the banks to provide loans forcing the farmers to depend on other finance agencies who charge interest rates which are exorbitant to say the least. The high interest rates and unpredictable selling price seriously undermines the profitability of the farm and often the margin for

success is very narrow. The sensitivity analyses in section 2.3.3 also show the potential economic benefits of using dry pelleted feeds. However, it must be borne in mind that none of the dry pelleted feeds presently available in Thailand are formulated specifically for C. striata.

2.5 RECOMMENDATIONS FOR IMPROVEMENTS AND RESEARCH PRIORITY

It is clear from the preceding sections that numerous problems exist which are seriously undermining the profitability of the snakehead farms and the viability of the industry as a whole. These problems need to be surmounted to preserve the industry's potentially flourishing capacity. Some of the problems could be minimised if not eliminated, by the implementation of new or modified culture and management techniques. The following recommendations for improvement to the current practices are suggested:

1) Encouragement of the use of dry pelleted feed.
2) Evaluation of optimal stocking rates - a reduction in the stocking density from the present levels may be beneficial.
3) Optimal feeding rate and feeding frequency need to be evaluated and determined accurately.
4) Improvement in the feeding techniques - careful feeding and the removal of all uneaten feed to maintain water quality.

5) Development of sampling procedure - for stock assessment, calculation of feeding rate and grading.

6) Introduction of a nursery phase in the production cycle - to allow for an acclimatization period and prophylactic treatment before release, especially for anti-protozoal therapy.

7) Treatment of intake water - this would include screening for predators, simple filters to reduce suspended matter and biological oxygen demand and aeration of the pond water.

Of the recommendations presented above, the change to using dry pelleted feed would be the most beneficial. Sensitivity analysis (based on the net returns of a single farm) using dry pelleted feed has shown a significant potential financial gain (section 2.3.3.9). Other benefits include a reduction on the dependency on trash fish, improved feed conversion efficiency through less wastage and maintenance of good water quality. Based on results obtained with other species, it might be expected that the use of dry pelleted feed can improve production by up to 15% over that of trash fish fed snakehead farms.

However, at present, there is no such feed available and furthermore, there is no information or on-going research to ascertain the 'advantages' of using dry pelleted feed. Therefore, as the first step to the overall concept of introducing dry pelleted feed to C. striata farming industry, the formulation of a well balanced diet capable of providing essential nutrients

for maximal growth should be given top priority.

In view of these findings, and particularly the significance of feed costs to profitability, and the recognition of the complete absence of information on Channid nutrition, a laboratory study was performed to investigate the nutritional requirements of snakeheads by monitoring growth performances when fed semi-purified diets. The basic nutritional information generated being absolutely vital to the development and formulation of a least cost practical diet for snakehead farming.

CHAPTER 3

STUDIES ON THE NUTRITIONAL REQUIREMENTS OF SNAKEHEADS

GENERAL INTRODUCTION AND METHODOLOGY

At present the snakehead farming industry utilizes trash fish, alone or in combination with agricultural by-products, as feed and this represents up to 85% of the total operating costs involved in the production (Chapter 2). It has been suggested that in order to reduce feed costs and improve production, snakehead farmers should consider changing from using trash fish to using artificial dry pelleted feeds. The advantages and benefits in using this type of feed have been amply demonstrated by the successes of the channel catfish culture industry in the United States of America and salmonid farming industry in North America and Europe where their development and progress had been aided to a large extent by the development and availability of dry pelleted feeds.

The successful formulation of these pelleted diets were however, derived from the results of years of research on the nutritional requirements of these fish. At present, there is no information available on the nutritional requirements of snakeheads; other than physiological studies on growth and feed utilization in relationship to temperature, starvation, ration size, body weight, water depth and partial pressure of oxygen in water (Wee, 1982). It is therefore, the primary aim of this thesis, to secure basic information on nutritional requirements of snakehead and so enable a beginning to be made on the formulation of well-balanced and cost effective feeds.

At the onset of the study, it was intended to perform

such nutritional experiements on C. striata which is by far the most popular and economically important species of cultured snakeheads. However, owing to the failure of live fish dealers to fulfill their guarantees of supply of C. striata juveniles partly because of identification problems and partly due to air transport problems, experimental feeding trials had to be carried out with juveniles of both C. striata and C. micropeltes. It was fortuitous that C. micropeltes (plate 3.1) juveniles should be acquired because although it is a less commonly cultured species, popular only in Malaysia, Singapore and Indonesia, it has potential for culture by virtue of its reputation as a fast growing species and larger size that it can attain (K.J. Ang, pers. comm).

In this section, experimental feeding trials were designed to determine the dietary protein and quantitative essential amino acid requirements of both C. striata and C. micropeltes juveniles (Experiments 1 and 2); the effects of dietary lipids on growth and body fatty acid composition of C. micropeltes juveniles (Experiment 3); and finally the effects of dietary energy levels and energy sources on growth, feed conversion efficiency and carcass composition of C. striata juveniles (Experiment 4). The reasons for the usage of the particular species were entirely related to the aforementioned supply problems.

3.1 EXPERIMENT 1

THE DIETARY PROTEIN REQUIREMENT OF SNAKEHEADS

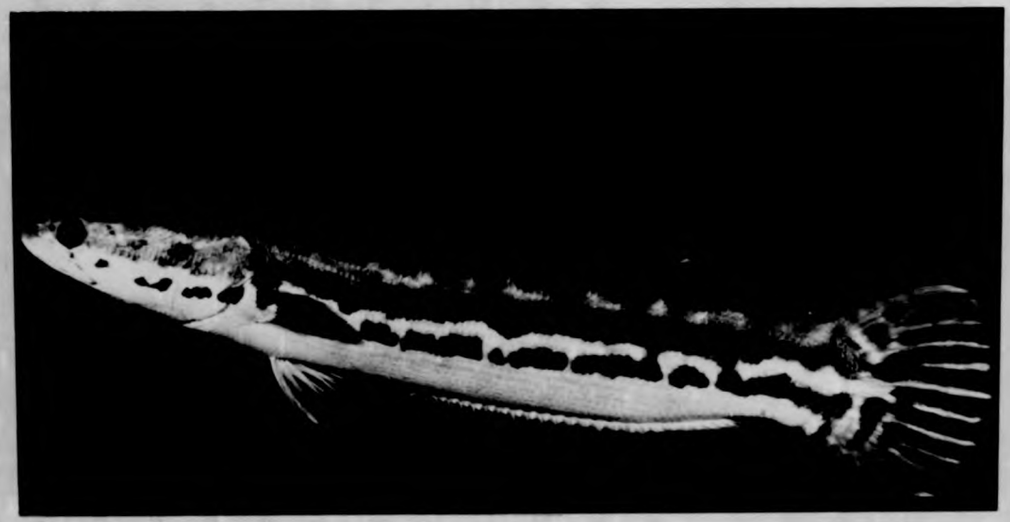


Plate 3.1 Channa micropeltes

This species can be identified by the following taxanomic features :

Vomer and palatine present with two series of teeth which are mostly canine or canninform. Lateral line scales 82 and 95 without an abrupt drop; scales transverse series 5.5 or 6.5 - 1 - 15 or 16 ; rows of scales on opercle 8; dark brown or dark blue above, white below; two narrow parallel black stripes extending from eye and angle of mouth to tip of caudal fin, interspace red (the stripes breaking up into irregular spots and blothces in older examples , see plate above).

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3.1.1 INTRODUCTION

As protein is the principal dietary component necessary for growth, its quality and quantity will markedly affect the growth response of fish. Its high cost also affects the economy of fish feed production. In view of the carnivorous habits of the snakehead and the high cost of good quality protein sources of fish (Tacon, 1981), it was considered essential that the dietary protein requirements be determined, and so enable the formulation of well balanced and cost effective feeds.

DeLong *et al.* (1958) were the first to introduce the concept of minimum dietary protein requirement giving optimal weight gain in fish. In their studies with chinook salmon (*Oncorhynchus tshawytscha*) they found that the minimum dietary protein level required was 40% and 55% at water temperatures of 8.3°C and 14.4°C respectively. Basically, the concept involves feeding fish with diets containing graded levels of a good quality protein source with sufficient energy density and an adequate balance of essential fatty acids, vitamins and minerals over a prolonged period. From the resulting dose response growth curves, the minimum protein requirement level is obtained by an Almquist plot. Since then, dose response growth curves have been used to determine the minimum dietary protein requirements for optimal weight gain in a number of fish species (Table 3.1.1).

Several factors may affect the protein requirements of fish; among these include temperature, salinity and quality of

Table 3.1.1 Estimated dietary protein requirement of certain fish

Species	Crude protein levels in diet for optimal growth (% by weight)	Reference
Rainbow trout (*Salmo gairdneri*)	40 - 46	Satia (1974), Zeitoun *et al.* (1976), Tiews, Gropp and Koops (1976)
Carp (*Cyprinus carpio*)	38	Ogino and Saito (1970)
Chinook salmon (*Oncorhynchus tschawytscha*)	40	DeLong, Halver and Mertz (1958)
Eel (*Anguilla japonica*)	44.5	Nose and Arai (1972)
Plaice (*Pleuronectes platessa*)	50	Cowey *et al.* (1972)
Gilthead bream (*Chrysophrys aurata*)	40	Sabaut and Luquet (1973)
Grass carp (*Ctenopharyngodon idella*)	41 - 43	Dabrowski (1977)
Channel catfish (*Ictalurus punctatus*)	22 - 32	Garling and Wilson (1976)
Tilapia aurea	36	Davis and Stickney (1978)
Sarotherodon mossambicus	40	Jauncey (1982)

protein source. DeLong et al (1958) showed that the protein requirement for optimal weight gain increased at higher temperature for chinook salmon. Zeitoun et al (1973), showed that at a salinity of 10 parts per thousand (ppt) the optimal protein level for maximal growth was 40% and at 20 ppt was 45% for rainbow trout. Coho salmon (Oncorhynchus kisutch) on the other hand, had the same dietary protein requirements (40%) at salinities of 10 and 20 ppt respectively (Zeitoun et al, 1974). The quality of the protein source may also affect the level of dietary protein required, hence Andrews (1979) reported that channel catfish fed a well balanced diet containing 28% dietary protein had a better feed efficiency than fish fed an unbalanced diet containing 36% dietary protein.

In this experiment, the dietary protein requirement of the juveniles of two species of snakehead (Channa micropeltes and Channa striata) were investigated in two separate feeding trials (Feeding trial 1 and Feeding trial 2 respectively) by monitoring the growth performance, feed conversion efficiency, protein utilization and carcass composition of fish fed semi-purified diets containing varying levels of dietary protein ranging from 25% to 60% by weight.

3.1.2 MATERIALS AND METHODS

3.1.2.1 Diets

The dietary protein requirements of C. micropeltes and C. striata were investigated in two separate feeding trials.

Trial 1

Eight semi-purified diets were formulated (Table 3.1.2). Within all diets, herring meal (71.6% crude protein, 9.6% crude lipid) was used as the sole source of dietary protein. The level of herring meal within the diets was varied with white dextrin and corn starch so as to provide the dietary crude protein (nitrogen, N x 6.25) concentrations ranging from 25% (diet CM1) to 60% (diet CM8) of dry diet by dietary increments of 5%. All diets were formulated to contain 10% lipid, of which 5% was supplied by adding corn oil and the remainder supplied by the addition of herring oil and the lipid contained within the herring meal. Within all diets, chromic oxide was included at the 0.5% inclusion level for the determination of digestibility coefficients. All diets were formulated to contain an estimated gross energy content of approximately 450 kcal / 100 g diet. No attempt was made to balance the ash, fibre or carbohydrate content of the diets.

The dry ingredients were mixed in a Hobart A 400 food mixer and blended for 10 minutes. The process was repeated with the addition of the oils and water, until the binder had been primed. The homogeneous paste was then extruded under pressure through a 2 mm die plate, forming long spaghetti-like strands; these were then dried by warm air currents in a constant temperature cabinet (37^{o}C), and subsequently broken into pellets approximately 0.5 - 1 cm in size. The dry pellets were then sealed in polythene bags and stored at -20^{o}C until fed. The proximate

Table 3.1.2 Composition of experimental diets used in feeding trial 1, Experiment 1. (% by weight)

Ingredient	Diet number							
	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8
Herring meal	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0
White dextrin	17.6	15.5	13.4	11.2	8.8	6.5	4.2	1.8
Corn starch, raw	35.2	31.0	26.8	22.3	17.7	13.0	8.3	3.7
Herring oil	1.7	1.0	0.3	0	0	0	0	0
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Binder *1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix *2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix *3	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

*1 carboxymethyl cellulose

*2 To supply / 100g diet : Thiamine HCL 6mg; Riboflavin 15.2mg; Pyridoxine HCL 3.6mg; Calcium pantothenate 40mg; Inositol 142mg; Biotin 2mg; Folic acid 1.5mg; para Aminobenzoic acid 30mg; Choline chloride 6448mg; Nicotinic acid 53.2mg; Cyanobalamin 0.01mg; Retinol palmitate 200 iu; Alpha tocopherol acetate 30mg; Ascorbic acid 200mg; Menadione 4mg .

*3 To supply / 100g diet : $Ca(H_2PO_4)_2.H_2O$ 1.37g; $CaCO_3$ 0.11g; $MgCO_3$ 0.48g; $FeSO_4.7H_2O$ 0.6g; KCL 0.10g; NaCL 0.16g; $AlSO_4.6H_2O$ 0.4mg; $ZnSO_4.7H_2O$ 8.0mg; $CuSO_4.5H_2O$ 2.0mg; $MnSO_4.4H_2O$ 5.4mg; $CaIO_3.6H_2O$ 0.5mg; $CoSO_4.^{4H_2O}$ 2.0mg .

composition of the experimental diets used in feeding trial 1 is shown in Table 3.1.3.

Trial 2

Five semi-purified diets were formulated (Table 3.1.4). The dietary formulations employed were as described previously for feeding trial 1, with the exception that the experimental diets in this feeding trial were formulated to contain 12% lipid and an estimated gross energy content of approximately 460 kcal / 100 g diet. The experimental diets were similarly prepared and stored as described previously in feeding trial 1. The proximate composition of the test diets used in this feeding trial is shown in Table 3.1.5.

3.1.2.2 Animals and Tanks

Trial 1

C. micropeltes juveniles (batch CM1, 120 - 140 g in weight) were obtained from the London Catfish Centre, London. Experimental fish were housed in eight 220 litre rectangular glass tanks, contained within a single recirculation system. A 95 litre header tank supplied freshwater by gravity via a main ring, to each holding tank at a rate of 2 litres / minute / tank. Each tank had a constant level overflow device which drained into a solids settling tank via a mechanical filter (cotton wool filter). From the solids settling tank, the water was then pumped up and allowed to percolate down a biological filter, from where the

Table 3.1. 3 Proximate composition and energy content of experimental diets used in feeding trial 1, Experiment 1 .

Component	Diet number							
	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8
Moisture (%)	5.4	6.6	6.4	5.2	5.7	5.2	4.7	5.6
Crude protein (N x 6.25) (%)	25.5	28.9	34.5	39.0	43.6	47.8	53.9	56.5
Crude lipid (%)	7.7	8.6	8.8	9.3	9.9	10.0	11.5	12.4
Ash (%)	7.7	8.7	9.5	11.0	12.0	13.2	14.3	15.6
Calculated available carbohydrate (%)	52.8	46.5	40.2	33.5	26.5	19.5	12.5	5.5
Gross energy *1 (kcal / 100g)	430	432	440	445	449	446	466	462
Gross protein energy (%)	33.1	38.9	44.5	50.0	55.4	61.2	65.8	69.7
Estimated digestible energy *2 (kcal / 100g)	303	315	332	346	360	368	398	405
Protein to gross energy ratio (mg protein/kcal)	59.30	66.90	78.41	87.64	97.10	107.17	115.67	122.29

*1 Calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g lipid; 4.0 kcal/g carbohydrate (Cowey et al, 1972)

*2 Calculated on an estimated 5.0 kcal/g protein; 9.o kcal/g lipid; 2.0 kcal/g carbohydrate.

Table 3.1.4 Composition of the experimental diets used in feeding trial 2, Experiment 1 (% by weight)

Ingredient	Diet number				
	CS1	CS2	CS3	CS4	CS5
Herring meal	36	51	65	80	87
White dextrin	16.4	11.9	7.7	3.2	0.8
Corn starch, raw	32.8	23.9	15.5	6.3	1.7
Herring oil	4.3	2.7	1.3	0	0
Corn oil	5.0	5.0	5.0	5.0	5.0
Binder *1	1.0	1.0	1.0	1.0	1.0
Vitamin premix *2	2.0	2.0	2.0	2.0	2.0
Mineral premix *2	2.0	2.0	2.0	2.0	2.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5

*1 carboxymethyl cellulose

*2 as in Table 3.1.2, Experiment 1 .

Table 3.1.5 Proximate composition and energy content of experimental diets used in feeding trial 2, Experiment 1 .

Component	Diet number				
	CS1	CS2	CS3	CS4	CS5
Moisture (%)	4.65	4.34	4.82	4.61	4.61
Crude protein (N x 6.25) (%)	26.30	36.23	45.82	55.44	60.36
Crude lipid (%)	13.30	12.93	11.58	11.03	12.63
Ash (%)	7.69	10.64	12.28	14.68	15.24
Calculated available carbohydrate (%)	49.2	35.8	23.2	9.5	2.5
Gross energy *1 (kcal / 100g)	473.03	472.55	463.98	458.80	474.04
Gross protein energy (%)	31.70	43.70	56.29	68.88	72.58
Estimated digestible energy *2 (kcal / 100g)	349.60	369.12	379.72	395.47	420.42
Protein to gross energy ratio (mg protein / kcal)	55.60	76.67	98.75	120.84	127.33

*1 calculated as defined in Table 3.1.3

*2 calculated as defined in Table 3.1.3

water was pumped (from the base of the biological filter tank) up to the header tank (Figure 3.1.1). The water temperature was maintained at 28°C ± 0.5°C by ten thermostatically controlled aquarium heaters, and the experimental tank system continuously aerated with compressed air. A 12 hour light cycle was provided by fluorescent lighting controlled by a time switch. Dissolved oxygen, total ammonia, and pH were monitored at fortnightly intervals within the experimental tanks over the course of the feeding trial and varied between 87% - 92% saturation, 0.4 - 1 mg / l, and 5.8 - 6.3 respectively.

From a random sample of 61 fish, seven fish were alloted per dietary treatment, and the order of the tanks assigned randomly. The fish were fed a standard commercial trout ration (Trout grower, Edward Baker Ltd., Bathgate, Scotland, U.K.) for four weeks prior to the start of the feeding trial so as to acclimatise the fish to the experimental tank system.

At the start of the feeding trial, five fish were sacrificed, killed by a sharp blow on the head, and stored at -20°C for subsequent proximate chemical analysis.

Experimental fish were weighed individually at the start of the feeding trial, and subsequently at fortnightly intervals throughout the course of the feeding trial. Fish were individually removed from their holding tank and anaesthetized using benzocaine (ethyl para-aminobenzoate, BDH Ltd, Poole, England.) at a concentration of 700 mg / l. Excess surface

Figure 3.1.1 Layout of the recirculating system used in feeding trials 1 and 2, Experiment 1.

a : Biological filter tank (containing gravel)

b : Header tank

c : Experimental tank (receiving an inflow of water at a rate of 2 litre/minute/tank)

d : Solids settling tank

P : Water pump

H : Aquarium heater

→ : Direction of water flow

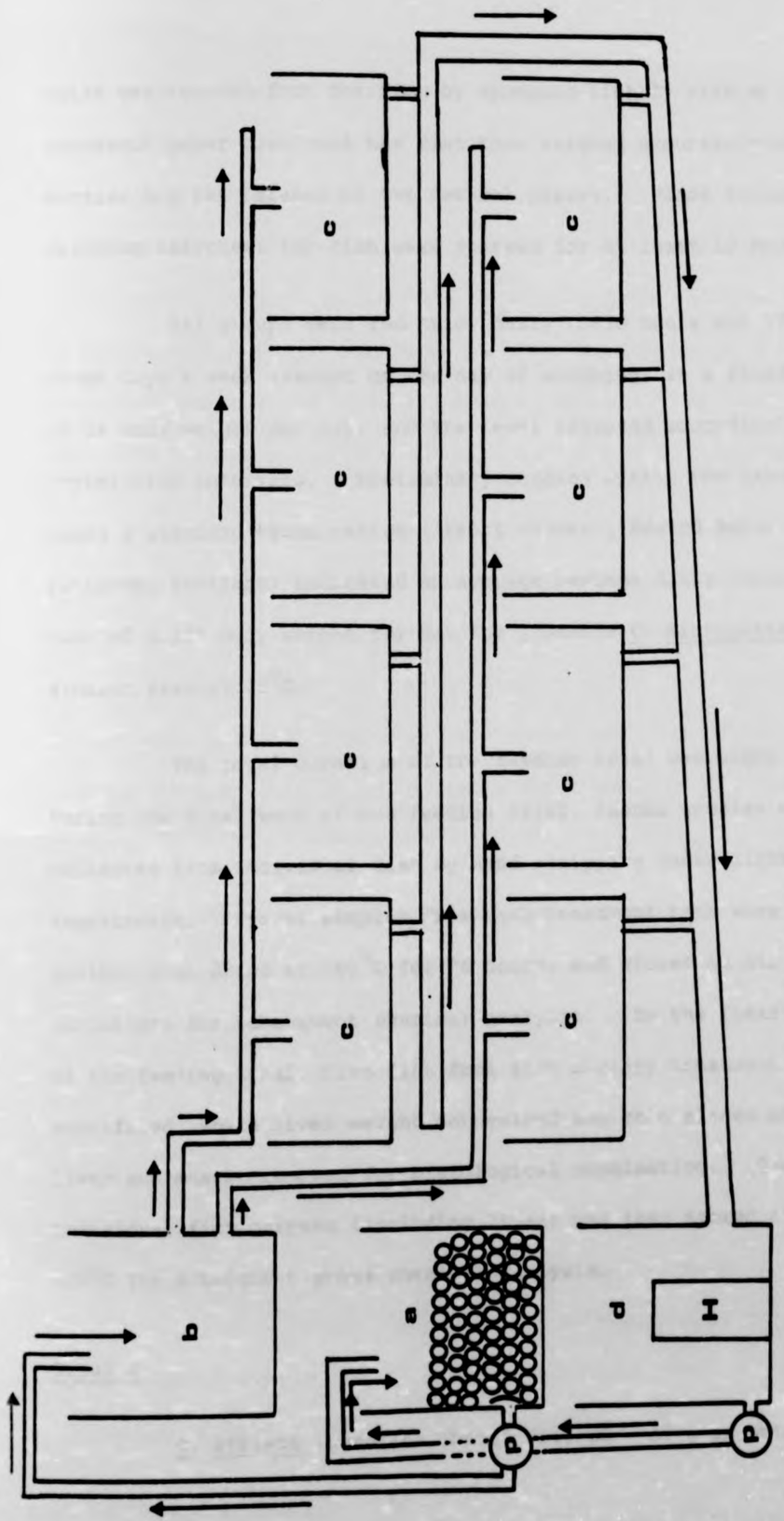

Figure 3.1.1

water was removed from the fish by sponging lightly with an absorbent paper towel and the fish then weighed accurately on a Mettler top pan balance to two decimal places. Prior to each weighing exercise, the fish were starved for at least 12 hours.

All groups were fed twice daily (0830 hours and 1700 hours), seven days a week (except on the day of weighing) at a fixed rate of 2% body weight per day, and the level adjusted accordingly at fortnightly intervals. Preliminary studies within the laboratory using a standard trout ration ('trout grower', Edward Baker, Bathgate, Scotland) indicated an average maximum daily feeding rate of 2.12% body weight per day for juvenile C. micropeltes of similar size at 28°C.

The total duration of the feeding trial was eight weeks. During the final week of the feeding trial, faecal samples were collected from individual fish by hand stripping under light anaesthesia. Faecal samples from each treatment tank were pooled, oven dried at 105°C for 24 hours, and stored in air tight containers for subsequent chemical analysis. On the final day of the feeding trial, five fish from each dietary treatment were sacrificed, whole liver weight determined and thin slices of liver and muscle removed for histological examination. The individual fish carcass (including liver) was then stored at -20°C for subsequent gross chemical analysis.

Trial 2

C. striata juveniles (batch CS1, 30 - 37 g in weight)

were obtained from the London Catfish Centre, London. Experimental fish were housed in five 220 litre rectangular glass tanks contained within a single recirculating system as described previously in feeding trial 1. The water temperature was maintained at 28°C ± 1°C and a 12 hour light cycle provided by fluorescent lighting. Dissolved oxygen, total ammonia, and pH monitored over the course of the feeding trial and varied between 85% - 95% saturation, 0.13 - 0.5 mg / l, and 6.35 - 7.1 respectively.

From a random sample of 55 fish, ten fish were allotted per dietary treatment, and the order of the tanks assigned randomly. The fish were fed a standard commercial trout ration ('trout grower') prior to the start of the feeding trial. At the start of the feeding trial, five fish were sacrificed, killed by a sharp blow on the head, and stored at -20°C for subsequent proximate chemical analysis.

Experimental fish were weighed at the start of the feeding trial and subsequently at fortnightly intervals throughout the course of the feeding trial. Experimental fish were individually weighed as described previously in feeding trial 1. All groups of fish were fed twice daily (0830 hours and 1700 hours), seven days a week (except on the day of weighing) at a fixed rate of 3% body weight per day, and the level adjusted accordingly at fortnightly intervals. The total duration of the feeding trial was eight weeks. During the final week of the feeding trial, faecal samples were collected and stored as previously described in feeding trial 1. On the final day of the feeding trial, five

fish from each dietary treatment were sacrificed, whole liver weight determined, and the individual fish carcass (including liver) then stored at -20°C for subsequent gross chemical analysis.

3.1.2.3 Chemical Methods

Replicate samples of dietary ingredients, individual whole fish carcass and faeces from feeding trials 1 and 2 were taken and analysed as follows:

Moisture: Samples were oven dried at 105°C for 24 hours.

Crude protein: Determined using the indirect method of Munro and Fleck (1969) by measuring the total nitrogen (N) within the sample and converting this value to a crude protein value by multiplication with an empirical factor 6.25. This factor is based on the assumption that the average protein contains about 16% N. In practice, however, a variation of between 12% - 19% is possible between individual proteins (Tacon, 1979).

Crude lipid: Moisture free samples were extracted with petroleum ether (40 - 60°C, boiling range) in a Soxhlet apparatus for four hours (AOAC, 1980).

Ash: Defined as the inorganic residue left after complete destruction of the organic matter. Samples (2 g) were ashed in a Muffler furnace at 450°C for 16 hours.

Chromic oxide: The chromic oxide content of the experimental diets and faeces were determined by the wet digestion method of

Furukawa and Tsukahara (1966). Apparent nutrient digestibility coefficients were determined using the formulae of Maynard and Loosli (1969).

Apparent nutrient digestibility =

$$100 \left(100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}} \right)$$

Apparent dry matter digestibility =

$$100 \left(1 - \frac{C}{B} \times \frac{1 - B}{1 - C} \right)$$

where C = Cr_2O_3 in diet (g / 100 g)

B = Cr_2O_3 in faeces (g / 100 g)

3.1.2.4 Histological Methods

Fish tissues from feeding trial 1 were sampled for histological examination. Tissue slices of liver and muscle from individual fish from each dietary treatment were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 µm, stained with haematoxylin and eosin, and examined under light microscopy.

3.1.2.5 Statistical Method

Statistical comparisons of the results were made using the analysis of variance for the results in both feeding trials.

Mean differences were determined using Duncan's Multiple Range Test (Duncan, 1955). Standard error (± SE) were calculated to identify the range of the means.

3.1.3 RESULTS

During trial 1 all fish soon became accustomed to the experimental diets within two days and fed aggressively for the duration of the trial. On the other hand, in trial 2, although all groups of fish became accustomed to the experimental diets within two days, the fish did not feed as actively as those fish in trial 1, and took approximately 10 - 15 minutes to consume all the feed presented, preferring to feed on the diets off the bottom of the tanks. As a result, nutrient leaching and a certain amount of feed loss was inevitable.

3.1.3.1 Weight gain and Feed conversion

Trial 1

The growth response is shown in Figures 3.1.2 and 3.1.3 and Table 3.1.6. Despite the differences in initial fish weights at the start of the feeding trial, percentage weight gain and specific growth rate ($\log_e$ final body weight - $\log_e$ initial body weight / time (days) X 100, SGR) increased proportionately with increasing dietary protein concentration (Figure 3.1.2). Percentage weight gain increased from 67.26% to 151.22% and SGR increased from 0.92 to 1.65 with progressive protein substitution of the experimental diets. Similarly, there was a progressive

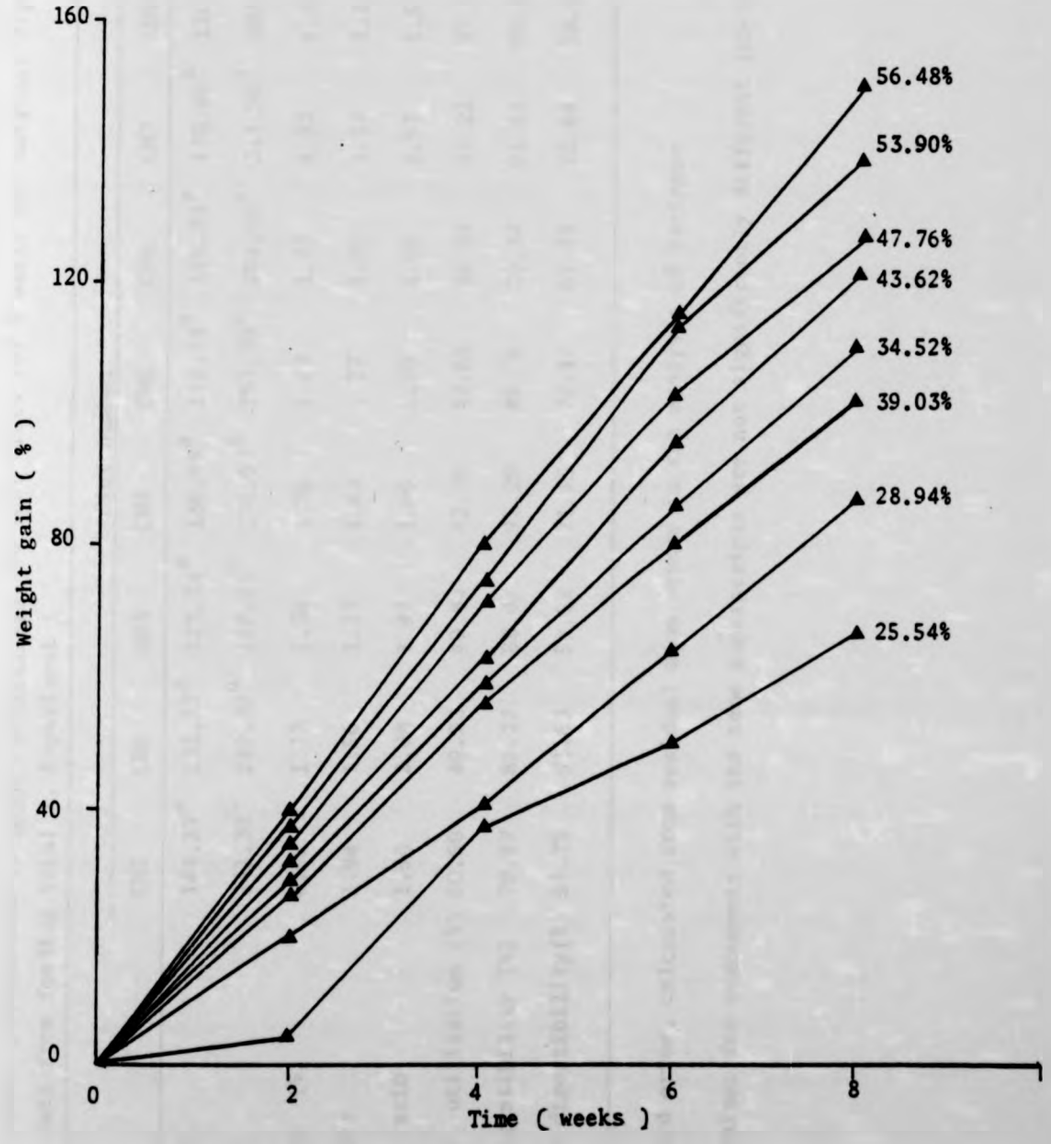

Figure 3.1.2 Growth responses of Channa micropeltes fed diets containing various levels of dietary protein. Percentage indicates the dietary protein level.

Table 3.1.6 Growth performance of Channa micropeltes fed test diets for 8 weeks and nutrient digestibility data from feeding trial 1, Experiment 1 .

Mean values	Diet number								± S.E *1
	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8	
Initial weight (g)	144.27[a]	132.27[a]	122.74[a]	136.68[a]	115.74[a]	128.04[a]	130.60[a]	120.85[a]	8.83
Final weight (g)	241.30[a]	249.02[a]	259.25[a]	276.97[a]	257.70[a]	292.16[a]	314.50[a]	303.60[a]	16.92
Specific growth rate (%)	0.92	1.13	1.34	1.26	1.43	1.47	1.57	1.65	
Food Conversion ratio	1.94	1.56	1.37	1.46	1.27	1.27	1.17	1.11	
Protein efficiency ratio	1.90	2.08	1.97	1.66	1.70	1.59	1.51	1.51	
Apparent Net protein utilization (%)	50.50	49.93	49.84	42.75	42.65	39.91	37.22	37.17	
Apparent protein digestibility (%)	79.97	85.37	88.02	91.29	89.76	89.34	91.41	90.91	
Apparent dry matter digestibility(%)	51.75	51.63	51.92	71.15	72.17	67.28	73.84	78.64	

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

ab Mean values for components with the same superscripts are not significantly different (P> 0.05)

improvement in food conversion ratio (Feed fed, dry weight/weight gain, fresh weight, FCR) with increasing dietary protein concentration. FCR decreased from 2.05 for fish fed diet CM1 (25.5% crude protein) to 1.17 for fish given diet CM8 (56.5% crude protein). Daily weight gain, on the other hand, increased progressively with increases in dietary protein level, reaching a maximum value at approximately 52% dietary protein level and decreased thereafter (Figure 3.1.3).

Trial 2

The growth response is shown in Figures 3.1.4 to 3.1.6 and in Table 3.1.7. The average fish weight at the start of the feeding trial was not significantly different ($P > 0.05$) and similarly, the average final fish weight at the end of the feeding trial was not significantly different ($P > 0.05$) between treatments. Figure 3.1.4 shows the growth curve of fish fed the test diets; it is clear that those fish given diet CS3 (45.82% dietary protein) achieved the best growth. Despite the differences in initial fish weight at the start of the feeding trial, percentage weight gain (Figure 3.1.5), daily weight gain and SGR (Figure 3.1.6) all increased proportionately with increased dietary protein concentrations to a level of 45.82% (diet CS3) and thereafter remained relatively constant or decreased slightly. Percentage weight gain and daily weight gain increased from 25.81% and 0.58 g / day (in fish fed diet CS1, 26.3% dietary protein) to a maximum of 93.0% and 0.56 g / day (in fish given diet CS3, 45.82% dietary protein) and remained relatively constant at approximately 91%

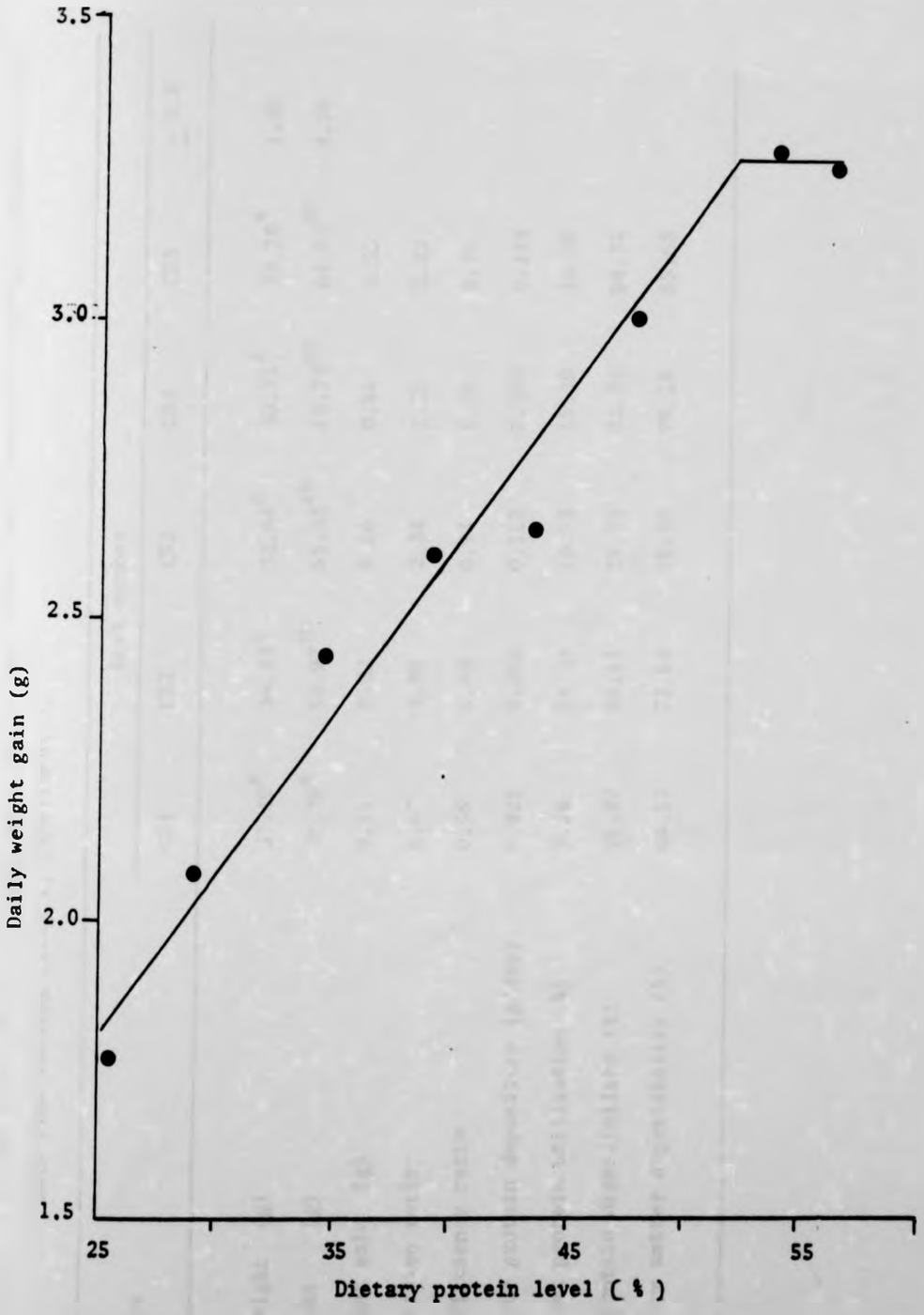

Figure 3.1.3 Relationship between daily weight gain of Channa micropeltes and dietary protein level.

Table 3.1.7 Growth performance of Channa striata fed test diets for 8 weeks and nutrient digestibility data from feeding trial 2, Experiment 1.

Mean values	Diet number					
	CS1	CS2	CS3	CS4	CS5	± S.E
Initial weight (g)	37.19[a]	36.13[a]	33.84[a]	30.71[a]	33.78[a]	1.89
Final weight (g)	46.79[a]	52.97[ab]	65.33[ab]	58.73[ab]	64.67[ab]	4.96
Daily weight gain (g)	0.17	0.32	0.56	0.50	0.55	
Food conversion ratio	6.47	3.40	2.34	2.28	2.22	
Protein efficiency ratio	0.56	0.43	0.94	0.79	0.75	
Daily tissue protein deposition (g/day)	0.021	0.056	0.112	0.100	0.114	
Apparent net protein utilization (%)	7.28	14.35	18.93	15.89	15.58	
Apparent protein digestibility (%)	75.87	86.11	89.72	92.34	94.74	
Apparent dry matter digestibility (%)	64.17	72.60	75.30	79.28	85.68	

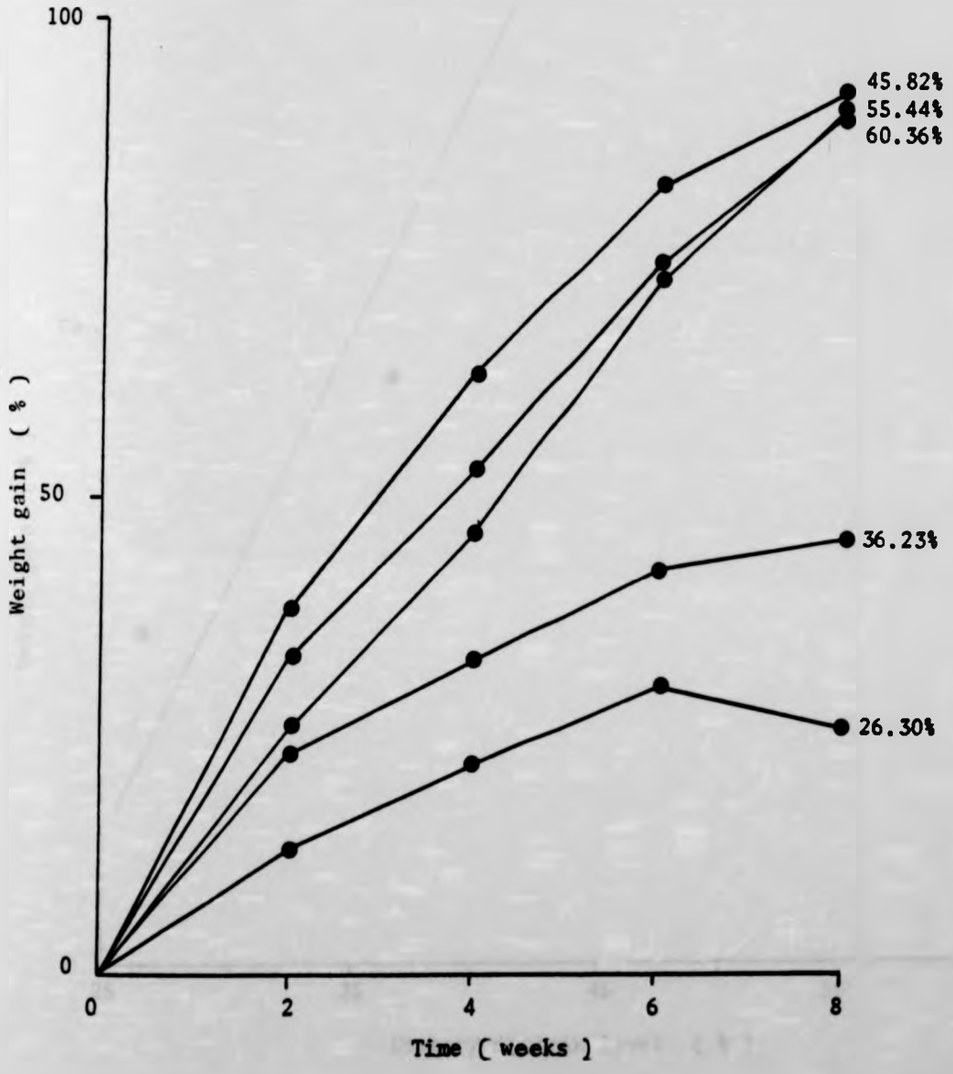

Figure 3.1.4 Growth responses of Channa striata fed diets containing various levels of dietary protein. Percentage indicates dietary protein level.

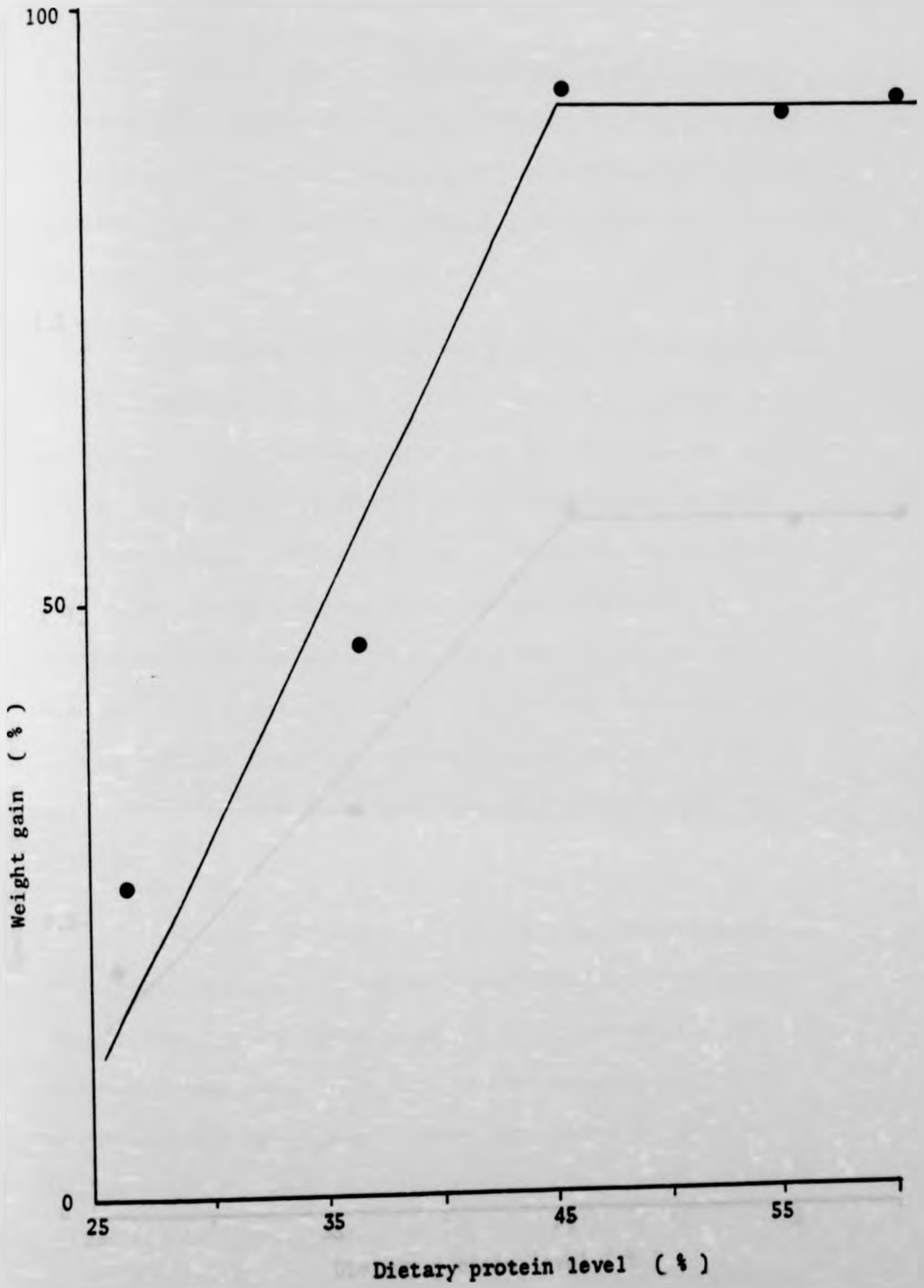

Figure 3.1.5 Effect of dietary protein content on the weight gain of Channa striata.

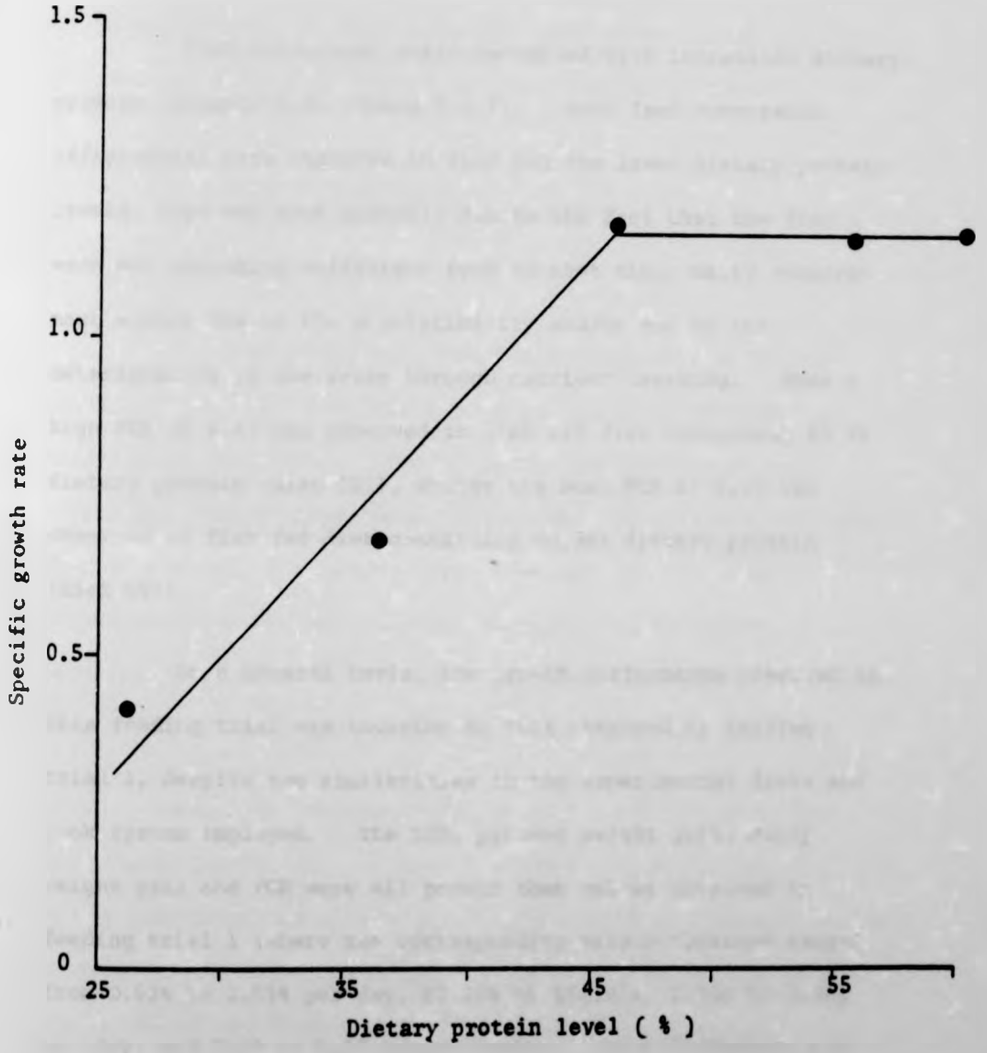

Figure 3.1.6 Relationship between specific growth rate of Channa striata and dietary protein level.

and 0.50 g / day respectively, thereafter. Similarly, SGR increased from 0.41% / day (in fish fed diet CS1) to a maximum of 1.18% / day (in fish given diet CS3) and thereafter remained constant at 1.16% / day with increasing dietary protein concentration.

Feed conversion ratio decreased with increasing dietary protein concentration (Table 3.1.7). Poor feed conversion efficiencies were observed in fish fed the lower dietary protein levels, this was most probably due to the fact that the fish were not consuming sufficient feed to meet their daily requirement either due to its unpalatibility and/or due to its deterioration in the water through nutrient leaching. Thus a high FCR of 6.47 was observed in fish fed diet containing 25.3% dietary protein (diet CS1), whilst the best FCR of 2.22 was observed in fish fed diet containing 60.36% dietary protein (diet CS5).

On a general basis, the growth performance observed in this feeding trial was inferior to that obtained in feeding trial 1, despite the similarities in the experimental diets and tank system employed. The SGR, percent weight gain, daily weight gain and FCR were all poorer than values obtained in feeding trial 1 (where the corresponding values obtained ranged from 0.92% to 1.65% per day, 67.26% to 151.22%, 1.73g to 3.28g per day, and 2.05 to 1.17 respectively). This difference was presumably a reflection of the feeding behaviour of the two species of snakehead. C. micropeltes in feeding trial 1 fed

aggressively consuming all the feed presented, whereas C. striata in the present study showed poorer appetite, preferring to feed off the bottom of the tank. It is believed therefore, that during the present feeding trial, the experimental fish were not consuming sufficient feed (through feed loss, nutrient leaching and poor appetite) to meet their energy demands as indicated by the poor growth and feed utilization performance (Table 3.1.7).

3.1.3.2 Protein Utilization

Trial 1

The efficiency with which fish were able to utilize dietary protein was determined by calculating the protein efficiency ratio (PER). PER is defined as the gain in fish weight per gram of crude protein consumed. PER values obtained in this feeding trial ranged from 1.51 to 2.08, the highest value occurring in fish fed diet containing 37.5% protein calories (diet CM2, 28.9% dietary protein), thereafter decreasing progressively as the percentage of protein calories increased.

Although PER values give a better indication of the nutritional quality of the diet than feed conversion ratio, they do not take into account the proportion of ingested protein which is used for the maintenance and are based on the assumption that the growth of the fish consists of tissue with identical composition in the case of all diets (Cowey and Sargent, 1972). A better assessment of dietary protein quality is the apparent efficiency of the deposition of dietary protein as body tissue,

the net protein utilization (NPU). During this investigation NPU values were determined by the carcass analysis method of Miller and Bender (1955). Since no corrections were made for endogenous nitrogen losses during the present experiment, the results are expressed as apparent NPU, and calculated as follows:

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

where Na is the body nitrogen at the start of the feeding trial and Nb the body nitrogen at the end of the feeding trial, and Ni is the amount of nitrogen consumed.

In this feeding trial, apparent NPU values decreased from 50.5% to 37.2% with increasing dietary protein calories indicating a poorer utilization of dietary protein with increasing dietary protein concentration. The correlation between the apparent NPU and percentage protein calories can be given by the regression equation Apparent NPU = .64.0 - 0.39 P, where P is the percent protein calories in the diet.

On the basis of daily tissue protein deposition, there was a progressive protein deposition to a maximum level of approximately 52% dietary protein concentration, beyond which the protein deposition rate remained relatively constant (Figure 3.1.7).

Trial 2

PER values obtained in this feeding trial ranged from

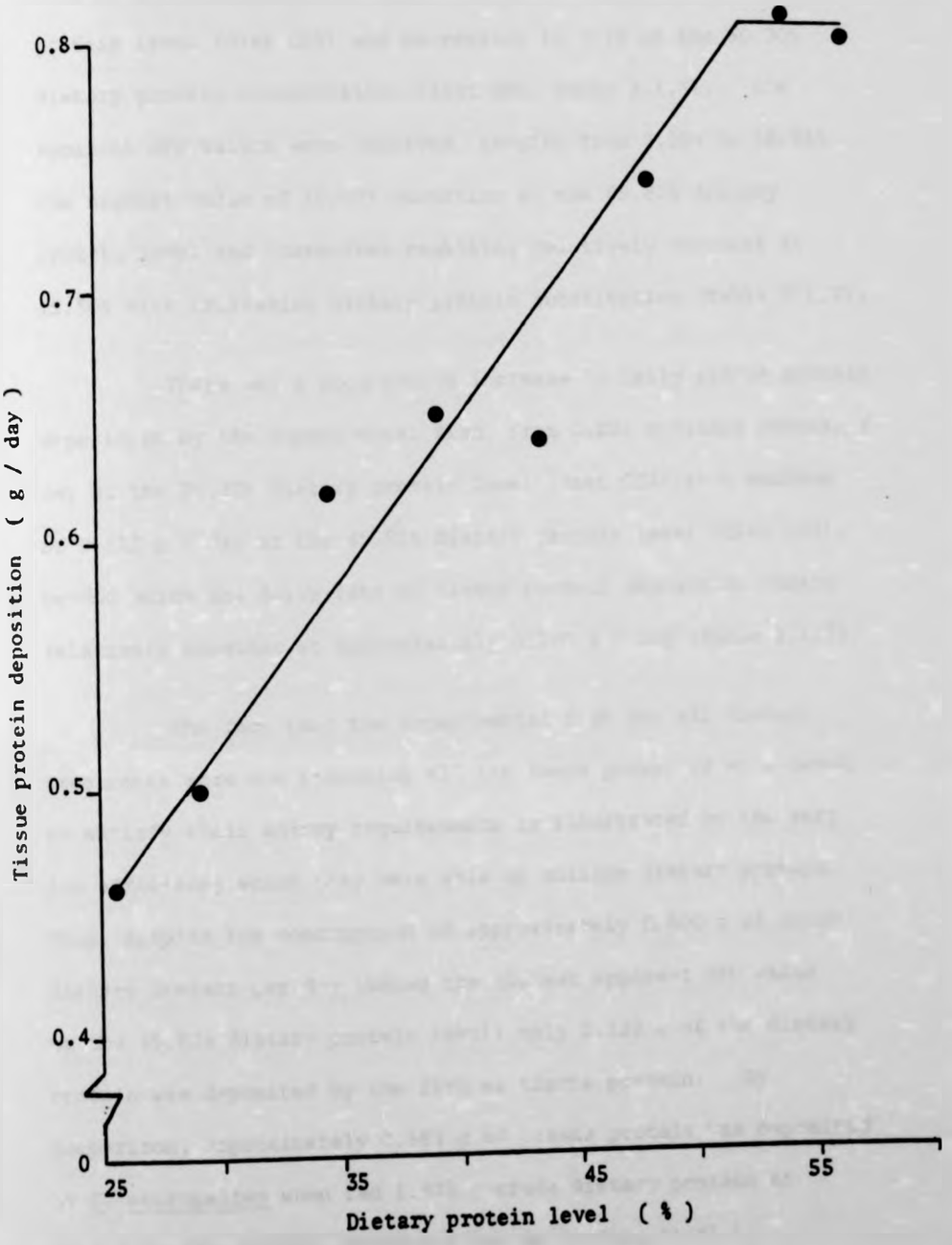

Figure 3.1.7 Relationship between daily tissue protein deposition of Channa micropeltes and dietary protein level.

0.56 to 0.94, the highest value occurring at the 45.82% dietary protein level (diet CS3) and decreasing to 0.75 at the 60.30% dietary protein concentration (diet CS5, Table 3.1.7). Low apparent NPU values were observed, ranging from 7.28% to 18.93%, the highest value of 18.93% occurring at the 45.82% dietary protein level and thereafter remaining relatively constant at 15.50% with increasing dietary protein substitution (Table 3.1.7).

There was a progressive increase in daily tissue protein deposition by the experimental fish, from 0.021 g tissue protein / day at the 26.30% dietary protein level (diet CS1) to a maximum of 0.112 g / day at the 45.82% dietary protein level (diet CS3), beyond which the daily rate of tissue protein deposition remain relatively constant at approximately 0.100 g / day (Table 3.1.7).

The fact that the experimental fish for all dietary treatments were not consuming all the feeds presented at a level to satisfy their energy requirements is illustrated by the very low efficiency which they were able to utilize dietary protein. Thus, despite the consumption of approximately 0.600 g of crude dietary protein per day (using the highest apparent NPU value at the 45.82% dietary protein level) only 0.122 g of the dietary protein was deposited by the fish as tissue protein. By comparison, approximately 0.583 g of tissue protein was deposited by C. micropeltes when fed 1.575 g crude dietary protein at 46.25% dietary protein concentration in feeding trial 1.

3.1.3.3 Nutrient Digestibility

Trial 1

The use of exogeneous inert indicators, which pass unaffected by digestion through the alimentary canal has provided a convenient method of measuring digestibility in a number of animals. This method has been successfully applied to fish digestion studies using chromium (III) oxide as the exogeneous inert indicator (Furukawa and Tsukahara, 1966).

In this feeding trial, the effects of dietary protein level on apparent protein and apparent dry matter digestibility showed interesting results. Surprisingly, despite the high apparent NPU values observed at the three lowest dietary protein levels tested, apparent protein digestibility increased from 80% to 91% between dietary protein levels of 25.5% and 39.0% and thereafter remained relatively constant at approximately 90%. By contrast, apparent dry matter digestibility remained relatively constant at 52.0% between dietary protein levels of 25.5% and 34.5%, sharply rose to 71% at a dietary protein level of 39.0% and subsequently increased to a level of 79% with progressive dietary protein substitution (Table 3.1.6).

Trial 2

The apparent protein and dry matter digestibility of this feeding trial is shown in Table 3.1.7. Apparent protein digestibility increased progressively with increasing concentration

of dietary protein, ranging from 75.9% at the 25.3% dietary protein level to 94.7% at the 60.30% level. Similarly, the values of the dry matter digestibility increased with progressive dietary protein substitution, ranging from 64.2 % at the lowest dietary protein level to 85.7 % at the highest.

3.1.3.4 Carcass composition and liver somatic index

Trial 1

The proximate carcass composition of C. micropeltes fed test diets in this trial is presented in Table 3.1.8. Dietary protein level had a profound effect on the fish carcass composition. There was a significant increase ($P < 0.05$) in carcass moisture content and a significant decrease ($P < 0.05$) in carcass lipid content with progressive dietary protein increase. A similar inverse relationship between carcass moisture and lipid contents have also been observed in channel catfish (Andrews and Stickney, 1973), sockeye salmon (Oncorhynchus nerka, Brett et al, 1969), and rainbow trout (Papoutsoglou and Papoutsoglou, 1978., Takeuchi et al, 1978d).

There was a significant decrease ($P < 0.05$) in the liver somatic index (whole liver weight, wet weight / body weight, wet weight X 100, LSI) of fish fed increasing dietary protein levels (Table 3.1.8).

Trial 2

The results of the proximate analysis of experimental

Table 3.1.8 Carcass composition of Channa micropeltes at the start and end of the 8 week feeding trial (trial 1, Experiment 1). (values are expressed as % by weight, wet basis)

Component	Diet number									
	Initial	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8	± S.E
Moisture	69.26	62.96^{a}	63.90^{ab}	63.92^{ab}	63.15^{a}	63.26^{a}	64.09^{ab}	84.84^{b}	65.00^{b}	0.37
Crude protein	18.54	21.77^{a}	21.08^{a}	22.13^{a}	22.20^{a}	22.14^{a}	22.43^{a}	22.08^{a}	22.19^{a}	0.44
Crude lipid	4.51	7.06^{a}	7.17^{a}	6.87^{a}	7.11^{a}	6.97^{a}	6.52^{a}	6.16^{b}	5.99^{b}	0.42
Ash	5.40	6.57^{a}	6.55^{a}	6.38^{a}	6.40^{a}	6.49^{a}	6.46^{a}	6.07^{a}	6.21^{a}	0.22
Total	97.71	98.36	98.70	99.30	98.86	98.86	99.50	99.15	99.39	
Liver somatic index		1.91^{bc}	2.31^{c}	2.17^{c}	1.92^{bc}	2.03^{bc}	1.59^{ab}	1.58^{ab}	1.15^{a}	

fish at the start and end of the feeding trial is presented in Table 3.1.9. The gross carcass composition was not greatly affected by varying dietary protein level. There was an increase in carcass moisture content and a decrease in carcass lipid content with progressive increase in dietary protein concentrations. However, these differences were not significantly different ($P > 0.05$). A similar inverse relationship between carcass moisture and carcass lipid content was also observed in feeding trial 1 with C. micropeltes. However, the carcass protein content increase marginally with increasing dietary protein level. Again the differences were not significant ($P > 0.05$) except for fish fed diet CS1 which had a significantly lower ($P < 0.05$) carcass protein content. Carcass ash content showed little variation with varying dietary protein concentration.

On the basis of the liver somatic index, only those fish fed diet CS4 (55.44% dietary protein) had a significantly lower index ($P < 0.05$) when compared with the other dietary treatments

3.1.3.5 Histopathology

Histopathological examination of fish tissues from feeding trial 1 revealed that there was proportionately less fatty infiltration within the liver, and to a lesser extent muscle tissues, of fish fed the higher dietary protein levels (plates 3.1.1 and 3.1.2).

Table 3.1.9 Carcass composition of Channa striata at the start and end of the 8 week feeding trial (trial 2, Experiment 1). (values are expressed as % by weight, wet basis)

Component	Diet number						
	Initial	CS1	CS2	CS3	CS4	CS5	± S.E
Moisture	64.99	65.87[a]	64.87[a]	65.40[a]	66.77[a]	66.95[a]	0.72
Crude protein	19.24	17.80[a]	18.97[a]	19.39[a]	19.49[a]	19.68[a]	0.21
Crude lipid	6.67	8.37[a]	8.14[a]	7.45[a]	6.58[a]	5.60[a]	0.66
Ash	6.95	6.41[a]	6.38[a]	5.94[a]	6.03[a]	6.34[a]	0.15
Total	97.80	98.46	99.38	98.26	98.77	98.62	
Liver somatic index		0.93[a]	1.15[a]	0.95[a]	0.77[a]	0.96[a]	0.07

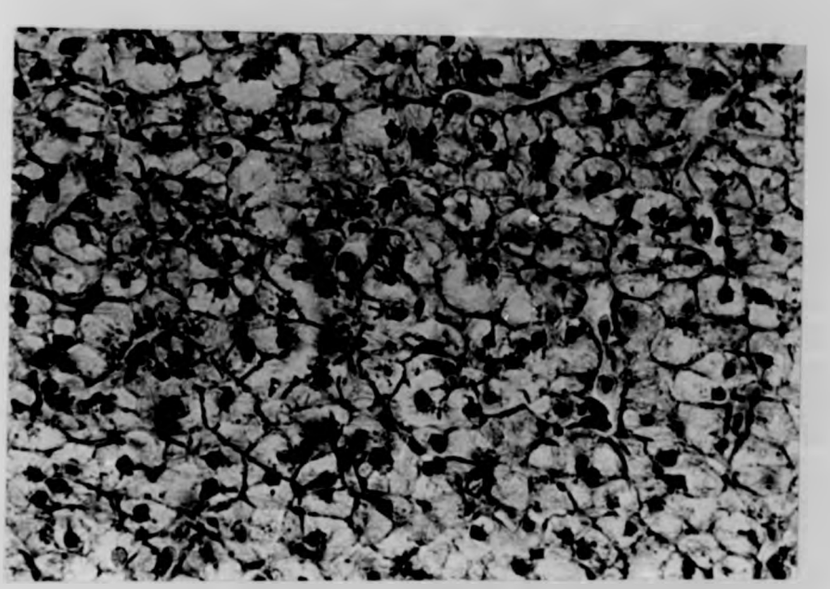

Plate 3.1.1 Section of liver from Channa micropeltes fed diet CM1 containing 25.5 % protein and 52.8 % carbohydrate. Note the severe fatty infiltration throughout the section
(x 80)

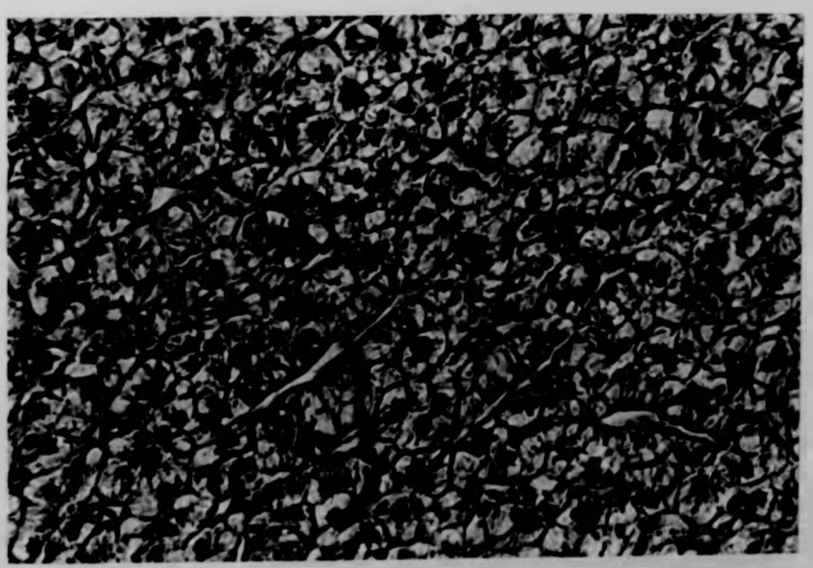

Plate 3.1.2 Section of liver from Channa micropeltes fed diet CM8 containing 56.5 % protein and 5.5 % carbohydrate . There is evidence of fatty infiltration but these are comparatively less than those observed in fish fed diet CM1
(x 80)

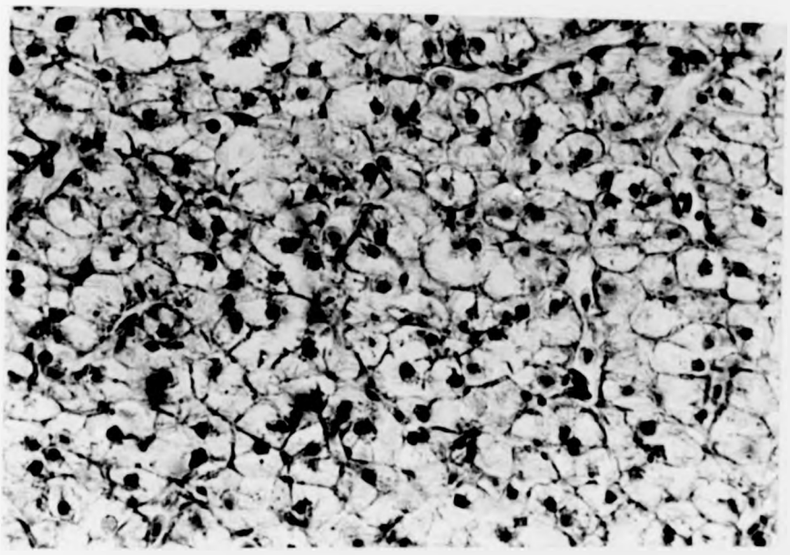

Plate 3.1.1 Section of liver from Channa micropeltes fed diet CM1 containing 25.5 % protein and 52.8 % carbohydrate. Note the severe fatty infiltration throughout the section
(x 80)

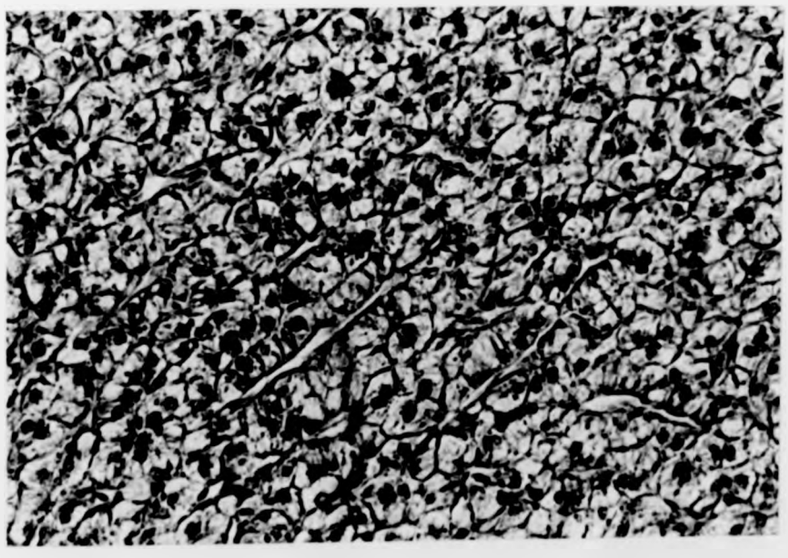

Plate 3.1.2 Section of liver from Channa micropeltes fed diet CM8 containing 56.5 % protein and 5.5 % carbohydrate . There is evidence of fatty infiltration but these are comparatively less than those observed in fish fed diet CM1
(x 80)

3.1.4 DISCUSSION

On the basis of daily weight gain and daily tissue protein deposition (Figures 3.1.3 and 3.1.4), the minimal dietary protein level for optimal growth for juvenile C. micropeltes was estimated to be approximately 52% (using herring meal as a protein source). On the other hand, on the basis of percentage weight gain, daily weight gain, SGR, FCR and daily tissue protein deposition, the minimum level of dietary protein producing maximum growth in C. striata juveniles was estimated to be approximately 45.82% (using herring meal as the sole protein source).

The estimated dietary protein requirement of juvenile C. micropeltes is similar to that obtained for other carnivorous fish species, for example, 50% for plaice (Cowey et al, 1972), grouper (Teng et al, 1978), and puffer fish (Kanazawa et al, 1980). On the other hand, the level observed for C. striata juveniles is in agreement with values (45%) obtained for rainbow trout (Satia, 1974., Zeitoun et al, 1976., Tiews et al, 1976) and eel (Nose and Arai, 1972).

The progressive increase in percentage weight gain, SGR and feed conversion efficiency with increasing dietary protein concentration, up to the highest concentration tested, observed for C. micropeltes in feeding trial 1 is similar to the dose response (growth) curve observed for carp (Ogino and Saito, 1970), plaice (Cowey et al, 1972) and gilthead bream (Sabaut and

Luquet, 1973). A possible explanation for the failure of the response curve to reach a plateau may have been that the calorific value of the experimental diets differed in their nutritional calorific value to the fish (i.e. the proportion of calories supplied by carbohydrate, lipid or protein (Cowey et al, 1972). This is in contrast to the apparent growth depressing effects of high protein diets observed for C. striata in feeding trial 2 and for other fish species such as milkfish (Chanos chanos, Lim et al, 1979), grouper (Teng et al, 1978), puffer fish (Kanazawa et al, 1980), carp (Ogino and Saito, 1970) and tilapia (Jauncey, 1982). It has been postulated that the decrease in SGR at protein levels above the optimum may be due to the reduction in dietary energy available for growth as energy is required to deaminate and excrete the excess amino acids absorbed (Jauncey, 1982). It should also be emphasised, however, that the highest level of protein fed in feeding trial 1 was only 56.5% by weight, as compared to dietary protein levels equal or in excess of 60% by weight in the majority of the other species studied.

PER values obtained in feeding trial 1 with C. micropeltes were similar to those observed in carp (Ogino and Saito, 1970), puffer fish (Kanazawa et al, 1980) and gilthead bream (Sabaut and Luquet, 1973), where PER was found to be highest at the lowest dietary protein level tested and fell almost linearly as dietary protein concentration increases. In contrast, PER values obtained in feeding trial 2 with C. striata, reached a maximum at the optimum dietary protein level and thereafter decreased. This

trend had similarly been observed in plaice (Cowey et al, 1972), grouper (Teng et al, 1978) and coho salmon (Zeitoun et al, 1974), where the PER values obtained reached a maximum level at the dietary protein concentration of 40% in all three instances and thereafter decreased with further increases in dietary protein concentrations.

Apparent NPU values obtained in feeding trial 1 was highest with the lowest dietary protein level tested and decreased linearly with increasing levels of dietary protein. A similar decrease with increase in dietary protein concentration has also been observed in carp (Ogino and Saito, 1970), plaice (Cowey et al, 1972) and grass carp (Dabrowski, 1977). In contrast, the relationship between apparent NPU values and varying dietary protein levels obtained in feeding trial 2 was in agreement with those obtained for coho salmon and rainbow trout (Zeitoun et al, 1973, 1974), where the values reached a maximum and thereafter decreased with increase in dietary protein levels.

Apparent protein digestibility increased with increasing dietary protein levels in both feeding trials 1 and 2 in this experiment. This trend has also been observed in studies with channel catfish (Page and Andrews, 1973), rainbow trout (Nose, 1967), carp (Ogino and Chen, 1973). It is believed that the lower apparent protein and dry matter digestibility observed at low dietary protein levels in feeding trials 1 and 2 in this experiment were due to the deleterious effect of the high carbohydrate content of these diets. The studies of Shimeno et al,

(1978) on the carbohydrate metabolism in the yellowtail (Seriola quinqueradiata), a marine carnivorous fish have shown that high dietary levels of purified carbohydrate (potato starch) have a deleterious effect on growth, feed efficiency, and resulted in reduced protein and carbohydrate digestibility. Similarly, the research of Singh and Nose (1967) and Nakamura et al (1973) with rainbow trout have shown that the digestibility of uncooked corn starch and dextrin varied with their dietary inclusion level, digestibility being lower at higher inclusion levels.

The gross carcass composition of C. striata at the end of the feeding trial 2 was not greatly affected by the dietary protein inclusion levels employed, fish fed lower dietary protein level tended to have a lower carcass protein, a higher lipid and a lower moisture content. On the other hand, the gross carcass composition of C. micropeltes was profoundly affected by varying dietary protein concentrations, there was a significant increase in carcass moisture content and a corresponding significant decrease in carcass lipid content, while the carcass protein and ash content remained relatively unaltered with increasing dietary protein substitution. A similar decrease in carcass lipid content with increasing protein level has also been reported in studies with mirror carp (Meske and Pfeffer, 1978) and plaice (Cowey et al, 1972). Cho et al (1976), however, found that the carcass lipid content was unaffected by varying dietary protein level in rainbow trout. By contrast, in eel, increasing dietary protein concentration led to higher carcass lipid content (Nose and Arai, 1972). It was postulated that the increase in carcass lipid

content with increasing dietary protein level may have been due to the 'spared' excess dietary protein ingested being metabolized to lipid for storage. The increase in carcass protein content with increasing dietary protein levels observed with C. striata in feeding trial 2 is in agreement with studies with plaice (Cowey et al, 1972) and gilthead bream (Sabaut and Luquet, 1973). As has been reported with other fish species, carcass ash content was found to be unaffected by dietary protein levels tested in both feeding trials 1 and 2 (Phillips et al, 1966., Cowey et al, 1972).

The increase in liver somatic index and elevated lipid deposition in the liver of C. micropeltes fed low dietary protein test diets in feeding trial 1 has also been observed within fish fed diets containing high dietary concentrations of carbohydrates (where carbohydrate replaced dietary protein, Shimeno et al, 1978). By contrast, there was no significant difference in the liver somatic index for C. striata fed varying dietary protein levels in feeding trial 2.

If the importance of a proper balance between protein and energy in the diets is to be included in the concept of growth potential of diets, then the ratio of dietary protein to gross energy within the diet must be evaluated. In this experiment, the maximum growth in feeding trial 1 was achieved with fish fed diet containing approximately 52% dietary protein and a protein to energy (P:E) ratio of 114.3 mg protein per kcal energy. In feeding trial 2, C. striata juveniles achieved maximum growth

with a diet containing approximately 45.82% dietary protein and a P:E ratio of 120.84 mg protein per kcal energy. Jauncey (1982) with tilapia obtained optimal growth on a diet containing 40% dietary protein (using white fish meal) with a P:E ratio of 97 mg protein per kcal energy (recalculated using energy values given in Table 3.1.3). Zeitoun et al (1973, 1974) in their studies with coho salmon and rainbow trout obtained maximum growth with fish fed 40% dietary protein (using casein and gelatin as protein source) and a P:E ratio of 85 mg protein per kcal energy for both species (recalculated using energy value given in Table 3.1.2).

Before any conclusion can be drawn from this study, it should be pointed out that interpretation of the data is subjected to a prior knowledge of the utilization of dietary protein, lipid and carbohydrate as energy sources in relation to varying protein energy levels (Cowey, 1975). At the outset of this study, it was assumed that relatively high dietary levels of digestible carbohydrates could be tolerated and assimilated by snakeheads. However, the low apparent dry matter digestibility and the reduced apparent protein digestibility of certain rations suggested that high concentrations of purified carbohydrates are not well tolerated and utilized by snakeheads. Consequently, although the gross energy content of all rations were relatively constant, on the basis of estimated digestible energy there was a progressive increase with increasing protein substitution, ranging from 308 kcal / 100 g diet to 410 kcal / 100 g diet (feeding trial 1, Table 3.1.2) and 349.6 kcal / 100 g diet to

420.47 kcal / 100 g diet (feeding trial 2, Table 3.1.5). In view of the poor digestibility of high concentrations of carbohydrates, therefore it may be advisable within such studies to use dietary lipid as a means of replacing or sparing dietary protein. In the present study, on the basis of daily weight gain and daily tissue protein deposition (when herring meal was used as a protein source) the optimum protein requirement for C. micropeltes juveniles was estimated to be approximately 52% and maximal growth was achieved with a P:E ratio of 114.4 mg protein per kcal energy within the diet. Similarly, on the basis of the growth performance, feed conversion efficiency and protein utilization the minimum dietary protein requirement producing optimum growth in C. striata juveniles was estimated to be approximately 45.82% with a P:E ratio of 120.84 mg protein per kcal energy (using herring meal as the sole source of protein).

3.2 EXPERIMENT 2

THE QUANTITATIVE ESSENTIAL AMINO ACID REQUIREMENTS OF SNAKEHEADS

3.2.1 INTRODUCTION

Several nutrition studies using amino acid test diets have shown that fish species require the same ten essential amino acids (EAA) for normal growth (Cowey and Sargent, 1979). These studies have involved the feeding of fish with amino acid test diets (containing a balanced mixture of crystalline amino acids equivalent to that contained in whole hens egg protein) deficient in the amino acid under test and monitoring the growth response before and after the inclusion of the deleted amino acid. By this method, it was found that fish fed diets deficient in arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan or valine respectively failed to grow until the deleted amino acid was replaced in the ration. This was true for chinook salmon, sockeye salmon, rainbow trout, channel catfish, eel and carp (Halver et al, 1957., Halver and Shanks, 1960., Shanks et al, 1962., Dupree and Halver, 1970., Arai et al, 1972., Nose et al, 1974).

Similarly, from dose response growth curves of fish fed amino acid test diets containing graded levels of the amino acid being investigated, the quantitative requirement for those ten essential amino acids in chinook salmon (Mertz, 1969), Japanese eel and carp (Nose and Arai, as cited by Cowey and Sargent, 1979) have been examined. The results showed important differences between species of fish and with omnivorous terrestrial mammals. The requirement of fish for many of the EAAs was shown to be more than twice that of the rat (Cowey and Sargent, 1979).

Recently, Ogino (1980) proposed a new method for determining the quantitative EAA requirements of fish by calculating the daily rate of EAA deposition within the whole body tissue of fish fed a good quality dietary protein source. This method is based on the assumption that the rate of deposition of an essential amino acid is similar to the requirement pattern for that essential amino acid. Results obtained using this method correlated well with results from dose response growth curves for other species of fish.

In view of the consistency of the qualitative EAA requirements of fish species, it is not unreasonable to assume that the same ten EAA would also be essential for snakeheads. Therefore for the purpose of this experiment, it is assumed that snakeheads also require arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine as EAAs.

With this qualification, the quantitative EAA requirements of two species of snakehead, C. micropeltes and C. striata, were estimated using the tissue amino acid deposition method of Ogino (1980). In this experiment, the amino acid composition of the whole body tissue of fish at the start and end of a feeding trial (Experiment 1) where fish were fed good quality dietary protein source were analysed; the daily rate of deposition of EAA calculated and the quantitative EAA requirement estimated on this basis.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Fish

Fish were taken at the following stages:

1) *C. micropeltes*: Fish at the start of trial 1 and those displaying optimal growth (fish fed diet CM7) at the end of feeding trial.

2) *C. striata*: Fish at the start of trial 2 and those displaying optimal growth (fish fed diet CS3) at the end of feeding trial. For comparison, wild and poly-cultured *C. striata* are also collected from Thailand for amino acid analysis.

Fish were killed by a sharp blow on the head, oven-dried at 105^{o}C for 24 hours, finely grounded into a homogeneous sample in a mortar and pestle and stored in air tight containers for subsequent amino acid analysis. The amino acid tryptophan was not analysed due to its destruction during acid hydrolysis.

3.2.2.2 Chemical Methods

Amino acid hydrolysis:

Samples of dried fish (100 mg) were hydrolysed by boiling under reflux in 420 ml 6N HCL for 18 hours. After cooling, the hydrolysate was filtered through Whatman No: 1 filter paper and diluted volumetrically to 500 ml. 10 ml aliquots were then withdrawn and the HCL removed by evaporation in rotary evaporator and the residue dissolved in distilled

water (this process was repeated three times) and the final residual solids dissolved in 10 ml 0.01 N HCL and stored at -20°C for subsequent amino acid analysis. Experimental diets (CM7 and CS3, Tables 3.1.2 and 3.1.4 respectively) were similarly hydrolysed and prepared for amino acid analysis.

Amino acid analysis

Individual amino acids were separated, identified and the concentrations measured on a JEOL JLO-6AH auto amino acid analyser (JEOL (UK) Ltd, JEOL House, Grove Park, Collingdale, London). The peak areas were integrated and compared with those of a known standard mixture of amino acids (Sigma AA-S18). Experimental diets (diet CM7 and CS3) were similarly treated and analysed for their amino acid contents. The amino acid tryptophan was not analysed due to its destruction during acid hydrolysis.

3.2.2.3 Estimation of quantitative EAA requirements

Ogino (1980), in his study on the quantitative EAA requirements of carp and rainbow trout, postulated that the rate of EAA deposited in the tissues of experimental fish fed a good quality dietary protein (g amino acid deposited / 100 g body weight / day) is equivalent to the EAA requirements pattern. It is assumed that the ingested amino acids are only used for protein synthesis and hence the rate of deposition is similar to the requirements pattern.

The quantitative EAA requirements pattern of snakeheads in the present study was similarly determined by calculating the daily rate of EAA deposition in whole body tissue from the EAA

content of the fish at the start and the end of the feeding trials. However, the concentrations of EAA within diets needed to satisfy the requirement rate would be dependent on the level and digestibility of dietary protein and the feeding rate (i.e. the available digestible dietary protein) within a feeding regime. Taking these factors into account, the EAA requirement rate can be calculated and expressed as % crude dietary protein within the diets by the following equation:

$$\frac{\text{Daily requirement pattern (g EAA/100g body weight/day)}}{\text{Available digestible dietary protein (g/100g body weight/day)}} \times 100$$

where available digestible dietary protein can be calculated by the following equation:

Available digestible protein = Level of dietary protein X Protein digestibility X Level of feeding.

3.2.3 RESULTS

The results of the amino acid analyses are presented in Tables 3.2.1 and 3.2.2. From Table 3.2.1, it would appear that the absolute concentrations (mg / 100 mg sample) of the essential amino acids (with the exception of tryptophan) in the initial and experimental C. striata were relatively similar quantitatively, with the exception of arginine where the initial fish contained a higher proportion (3.56 mg / 100 mg sample, compared to 2.76 mg / 100 mg sample in the experimental fish). This trend was also reflected in the whole fish carcass essential amino acid pattern

Table 3.2.1 Concentration of each of nine essential amino acids in whole body tissue of snakeheads and experimental diets (values are expressed as mg/100mg tissue sample on a dry weight basis)

Amino acid*1	Diet		Channa micropeltes		Channa striata			
	CS3	CM7	Initial	Fed diet CM7	Wild	Polyculture	Initial	Fed diet CS3
Arginine	2.62	3.66	5.84	3.42	4.49	4.63	3.56	2.76
Histidine	1.06	1.09	2.44	1.55	1.36	1.27	1.33	1.27
Isoleucine	1.82	2.35	3.51	2.06	2.45	2.44	2.23	2.08
Leucine	3.18	4.06	6.13	3.84	4.63	4.35	3.86	3.49
Lysine	3.10	3.87	6.91	4.33	5.13	4.87	4.04	3.86
Methionine	1.11	1.59	1.28	0.79	1.62	1.51	0.68	0.65
Phenylalanine	1.65	2.43	3.42	2.14	2.50	2.33	2.15	1.95
Threonine	1.91	2.55	3.86	2.41	2.88	2.79	2.62	2.40
Valine	2.01	2.72	3.96	2.34	2.65	2.67	2.45	2.40

*1 the essential amino acid tryptophan was not included, due to its destruction during acid hydrolysis

Table 3.2.2 Proportion of each of nine essential amino acids in whole body tissue of snakeheads. (values are expressed as % of the sum of all the essential amino acids)

Amino acid	*Channa micropeltes*		*Channa striata*			
	Initial	Fed diet CM7	Wild	Polyculture	Initial	Fed diet CS3
Arginine	15.64	14.95	16.20	17.24	15.53	13.23
Histidine	6.53	6.77	4.90	4.73	5.80	6.09
Isoleucine	9.40	9.00	8.83	9.08	9.73	9.97
Leucine	16.41	16.78	16.71	16.20	16.84	16.73
Lysine	18.50	18.92	18.56	18.13	17.63	18.50
Methionine	3.43	3.45	5.83	5.62	2.97	3.12
Phenylalanine	9.16	9.37	9.00	8.67	9.38	9.34
Threonine	10.33	10.53	10.40	10.39	11.43	11.51
Valine	10.60	10.23	9.57	9.94	10.69	11.51

where the proportion of each essential amino acid was expressed as percentage of total EAA (Table 3.2.2, again the exception was arginine where the proportion was slightly higher in the initial fish (15.53% compared to 13.23%)).

On the other hand, the wild and polycultured C. striata contained relatively higher amounts of all the nine EAA measured than did the initial or experimental fish (Table 3.2.1). However, when expressed as percentage of the total EAAs, the proportion of EAA in the wild and polycultured fish were quantitatively similar to the initial and experimental fish (Table 3.2.2, except for methionine representing 5.83% and 5.62% for wild and polycultured and 2.97% and 3.12% for initial and experimental fish respectively). This indicates that the proportions of the EAA were relatively constant despite differences in habitats and feeding regimes.

In contrast, in terms of absolute concentrations, C. micropeltes at the start of the feeding trial contained higher levels of EAAs in the whole fish carcass than experimental fish (Table 3.2.1). However, when expressed as percentage of total EAAs measured, the proportion of each EAA in the initial fish and experimental fish was quantitatively similar (Tabel 3.2.2).

The results of the quantitative EAA requirements are presented in Table 3.2.3 and 3.2.4. It would appear that although closely related and cultured under apparently similar experimental conditions and fed similar test diets, the EAA requirements of C. micropeltes and C. striata are different. In general the EAA requirements for C. striata are slightly

Table 3.2.3 Daily rate of essential amino acid deposition in whole body tissue of growing Channa micropeltes and its estimated essential amino acid requirement pattern

Amino acid	Mean initial fish weight (g)	Mean final weight of fish fed diet CM7 (g)	Amount of amino acid deposited over 56 days *1 (g)	Daily deposition rate *2 (g/100g body weight/day)	Essential amino acid requirement*3 (% dietary protein)	Essential amino acid requirement *4 (% diet)
Arginine	130.60	314.50	3.13	0.0303	3.08	1.66
Histidine	130.60	314.50	1.69	0.0164	1.66	0.89
Isoleucine	130.60	314.50	1.89	0.0184	1.87	1.01
Leucine	130.60	314.50	4.07	0.0395	4.01	2.16
Lysine	130.60	314.50	4.60	0.0446	4.53	2.44
Methionine	130.60	314.50	0.81	0.0079	0.80	0.43
Phenylalanine	130.60	314.50	2.26	0.0220	2.23	1.20
Threonine	130.60	314.50	2.54	0.0247	2.51	1.35
Valine	130.60	314.50	2.19	0.0212	2.15	1.16

*1 calculated using the equation EAAb - EAAa ; where EAAa is the amount of essential amino acid in whole body tissue at the start of feeding trial and EAAb is the amount of essential amino acid in whole body tissue at the end of feeding trial.

*2 these values correspond to the requirement pattern for each of the nine essential amino acids

*3 essential amino acid requirements calculated as defined in section 3.2.2.3 using these data : feeding level - 2g/100g body weight; dietary protein level - 53.90% by weight; and apparent protein digestibility - 91.41%

*4 calculated for a diet containing 53.90 % dietary protein

Note: The essential amino acid was assummed to be deposited within the whole body tissue at a linear rate.

Table 3.2.4 Daily rate of essential amino acid deposition in whole body tissue of growing Channa striata and its estimated essential amino acid requirement pattern

Amino acid	Mean initial fish weight (g)	Mean final weight of fish fed diet CS3 (g)	Amount of amino acid deposited over 56 days *1 (g)	Daily deposition rate*2 (g/100g body weight/day)	Essential amino acid requirement*3 (% dietary protein)	Essential amino acid requirement*4 (% diet)
Arginine	33.84	65.33	0.598	0.0388	3.15	1.44
Histidine	33.84	65.33	0.380	0.0246	2.00	0.92
Isoleucine	33.84	65.33	0.604	0.0392	3.19	1.46
Leucine	33.84	65.33	0.974	0.0631	5.13	2.35
Lysine	33.84	65.33	1.15	0.0748	6.08	2.79
Methionine	33.84	65.33	0.195	0.0126	1.02	0.47
Phenylalanine	33.84	65.33	0.546	0.0354	2.89	1.32
Threonine	33.84	65.33	0.681	0.0442	3.59	1.64
Valine	33.84	65.33	0.739	0.0479	3.89	1.78

*1 calculated using the equation EAAb - EAAa ; where EAAa is the amount of essential amino acid in whole body at the start of feeding trial and EAAb is the amount of essential amino acid in whole body at the end of feeding trial

*2 these values correspond to the requirement pattern for each of the nine essential amino acid

*3 essential amino acid requirement calculated as defined in section 3.2.2.3 using these data : feeding level - 3g/100g body weight; dietary protein level - 45.82% by weight; and apparent protein digestibility - 91.41 %

*4 calculated for a diet containing 45.82% dietary protein

Note: The essential amino acid was assumed to be deposited within the whole body tissue at a linear rate.

higher than those estimated for C. micropeltes.

3.2.4 DISCUSSION

The essential amino acid composition of the whole fish carcass of initial and experimental C. micropeltes and C. striata and wild and polycultured C. striata were analysed. Results showed that the proportions of the EAAs (expressed as percentage of total EAAs measured) were similar for all the different groups. The quantitative EAA requirements of C. micropeltes and C. striata were determined by the method of Ogino (1980).

The conventional method of determining the quantitative EAA requirements for fish and other organisms was based on the dose response growth curves of animals fed test diets containing graded levels of the EAA being investigated (DeLong et al, 1962., Nose, 1979., Wilson et al, 1978., Robinson et al, 1980). These amino acid test diets may contain the 'protein' component completely composed of free amino acids (Mertz, 1969) or the 'protein' component may contain a protein source low in a particular amino acid, and free amino acid (the same amino acid) added to provide a range of levels to be tested (Kaushik, 1977). Even though the method has been well used and tested, there are still some problems associated with the use of these test diets which reduced the accuracy of the method. The test diets are unacceptable to some species, in particular those containing free amino acids, for example, carp (Aoe et al, 1978) and tilapia (K. Jauncey, pers. comm.). Growth rate of several species given

a diet in which the protein component is composed of free amino acids is frequently inferior to that of fish given a diet of nearly identical amino acid composition but composed of protein (Murai et al, 1981., Walton et al, 1982). It should be emphasised however that salmonids have been observed to grow relatively well when fed test diets containing free amino acids as the dietary protein source (Mertz, 1969). Secondly, there is a danger of nutrient leaching, particularly those water soluble nutrients such as amino acids and certain vitamins, from the diet. The degree of loss will depend to a certain extent upon the feeding behaviour of the fish and the water stability of the test diets. Thirdly, the interpretation of the dose response curve is subjective. In particular, the point of intersection of the two portions of the growth curve (slope and plateau) cannot always be ascertained accurately.

Other methods of determining quantitative EAA requirements of fish have been developed using biochemical techniques. They include measuring the free amino acids in the whole blood, plasma, or other tissue (liver or muscle) of test animals fed test diets containing graded levels of the EAA in question. This method relies on the fact that once the requirement for the EAA being tested has been met, the concentration of the free amino acids pool will increase (Wilson, et al, 1978). However, it must be pointed out that this is not true for all the EAA examined (Robinson et al, 1980) and the method needs further evaluation before using the results for diet formulation. This method has been used for several species of fish (Kaushik, 1979.,

Plakas et al, 1980). The use of the rate of oxidation of radioactively labelled EAA in the test animals fed graded levels of these EAA being tested have also been reported (Cowey and Tacon, 1982). This method also relies on the fact that there will be a rapid increase in the rate of oxidation of the labelled EAA once the requirement has been met.

In the present study, the quantitative EAA requirements were determined by the method originally proposed by Ogino (1980). The accuracy and validity of the method are based on several assumptions. It was postulated that the rate of increase (deposition) of EAA in the fish approximate to the pattern required within the dietary protein. It was assumed that all the feed presented were completely consumed and no allowance was provided for any loss through uneaten feed or leaching. It was also assumed that all the ingested amino acids were assimilated and used solely for protein synthesis. The method did not take into account of the fate of the assimilated amino acids, i.e. the catabolism of these amino acids for maintenance requirements. There was no allowance for any loss of ingested amino acids through the faeces. Excretion of amino acids, though unlikely, is a possibility and should also be considered. The method also did not take into account the significance of the free amino acid pool in the body tissue. It has been shown that the total EAA level in the blood plasma can drop by as much as 50% in spawning male chinook salmon (Chance, 1962).

The merit of this method is the ease and speed at which

one can estimate the requirements and at the same time avoid the problems when using the conventional dose response curve method previously described.

With these qualifications, the results revealed differences in the EAA requirements pattern between C. micropeltes and C. striata, the requirements for C. striata being generally higher than that for C. micropeltes. The apparent differences could be due to genetical preference of one species requiring higher levels of EAA than the other, or it could be due to the different amounts of EAA present in the experimental diets provided. The levels of EAA measured in diet CM7 are higher than those in diet CS3 as it contained a higher level of dietary protein (Table 3.2.1).

When compared with the EAA requirements for other species, the EAA requirements estimated for C. micropeltes correlated well with that for common carp and rainbow trout (obtained by the EAA deposition method, Ogino, 1980), chinook salmon, eel and carp (determined by the dose response method; Mertz, 1969., Nose, 1979)except for methionine where the requirement was lower and leucine where the requirement was higher (Table 3.2.5). On the other hand, for C. striata the agreement was not so good and in general it requires higher levels of most of the EAAs except for arginine and methionine (Table 3.2.5).

The most significant difference in the EAA requirements of snakeheads when compared with those of other species is the very low requirement for methionine. Possible explanations for

Table 3.2.5 Comparison of the quantitative essential amino acid requirements of snakeheads (Channa striata and Channa micropeltes) and other species of fish (values are expressed as % of diet)

Amino acid	C. striata	C. micropeltes	Carp*1	Carp*2	Rainbow trout*1	Chinook salmon*3	Japanese eel*2
Arginine	1.44	1.66	1.52	1.60	1.40	2.40	1.70
Histidine	0.92	0.89	0.56	0.80	0.64	0.70	0.80
Isoleucine	1.46	1.01	0.92	0.90	0.96	0.90	1.50
Leucine	2.35	2.16	1.64	1.30	1.76	1.60	2.00
Lysine	2.79	2.44	2.12	2.20	2.12	2.00	2.00
Methionine	0.47[a]	0.43[a]	0.64[a]	1.20[b]	0.72[a]	1.60[a]	1.90[a]
Phenylalanine	1.32[c]	1.20[c]	1.16[c]	2.50[d]	1.24[c]	2.10[d]	2.20[d]
Threonine	1.64	1.35	1.32	1.50	1.36	0.9	1.50
Valine	1.78	1.16	1.16	1.40	1.24	1.30	1.50

*1 Data for carp and rainbow trout from Ogino (1980)

*2 Data for carp and Japanese eel from Nose and Arai (unpublished data as cited by Cowey and Sargent,1979)

*3 Data for chinook salmon from Mertz (1969)

a methionine and cystine

b in the absence of cystine

c in the presence of tyrosine

d in the absence of tyrosine

the low requirement value estimated include methionine loss from the experimental diets; some methionine may have been oxidised during the drying procedures when preparing the diets rendering them unavailable to the fish. Thus an insufficient amount of methionine was provided and a correspondingly low amount assimilated and deposited. Secondly, the levels of methionine measured may have been underestimated, as all fish and diets were oven dried at 105^{o}C for 24 hours prior to acid hydrolysis and subsequent amino acid analysis, the oxidative nature of methionine may have resulted in some loss through oxidation.

Alternatively, snakeheads may actually require lower levels of methionine especially if provided with excess of the amino acid cystine which can spare methionine for growth. However, the abnormally low values estimated, when compared with the requirement obtained for other fish species, are unlikely to be the correct even with the surplus cystine, and this is an area where study could be profitably expended.

When expressed as percentage of the total EAA required, the EAA requirements estimated showed close compatability with results obtained for other species (except for methionine and phenylalanine which were lower and leucine which was higher for both C. micropeltes and C. striata, Figure 3.2.1).

In young growing animals, the greatest proportion of body weight gain is in the form of muscle. Cowey and Tacon (1982) thus postulated that optimal dietary EAA requirements will be closely related or even governed by the pattern of amino acids

Figure 3.2.1 Comparison of the relative amounts of each of nine essential amino acids required for four species of fish and snakeheads. The proportion of each amino acid is represented as a percentage of the sum of all nine amino acids. Data for chinook salmon Oncorhynchus tshawytscha are from Mertz (1969), for Japanese eel Anguilla japonica and for carp Cyprinus carpio from Nose (1979) and for channel catfish Ictalurus punctatus from Wilson et al (1978) and Robinson et al (1980).

Data for snakeheads (Channa striata and Channa micropeltes) are from the present study.

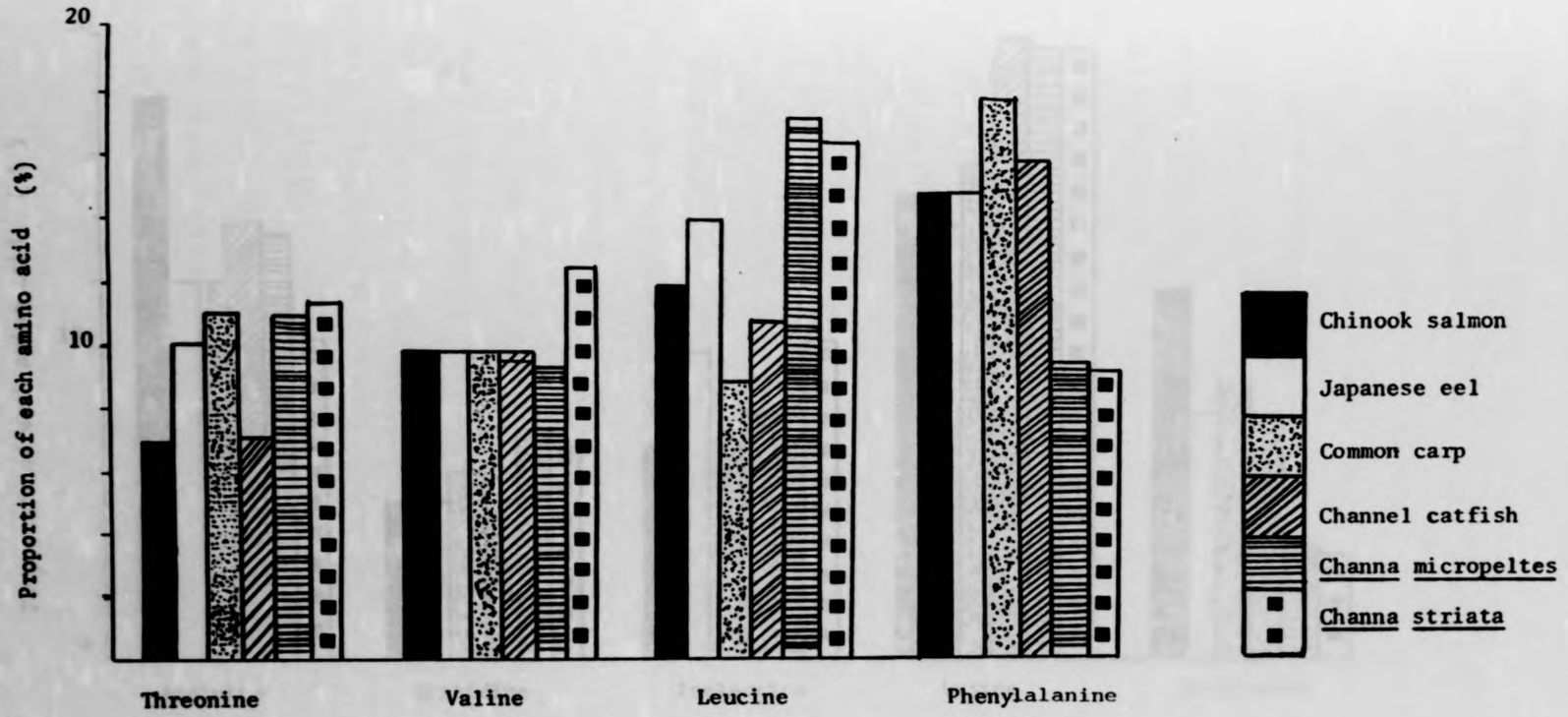

Figure 3.2.1 Comparison of the relative amounts of each of nine essential amino acids required for four species of fish and snakeheads.

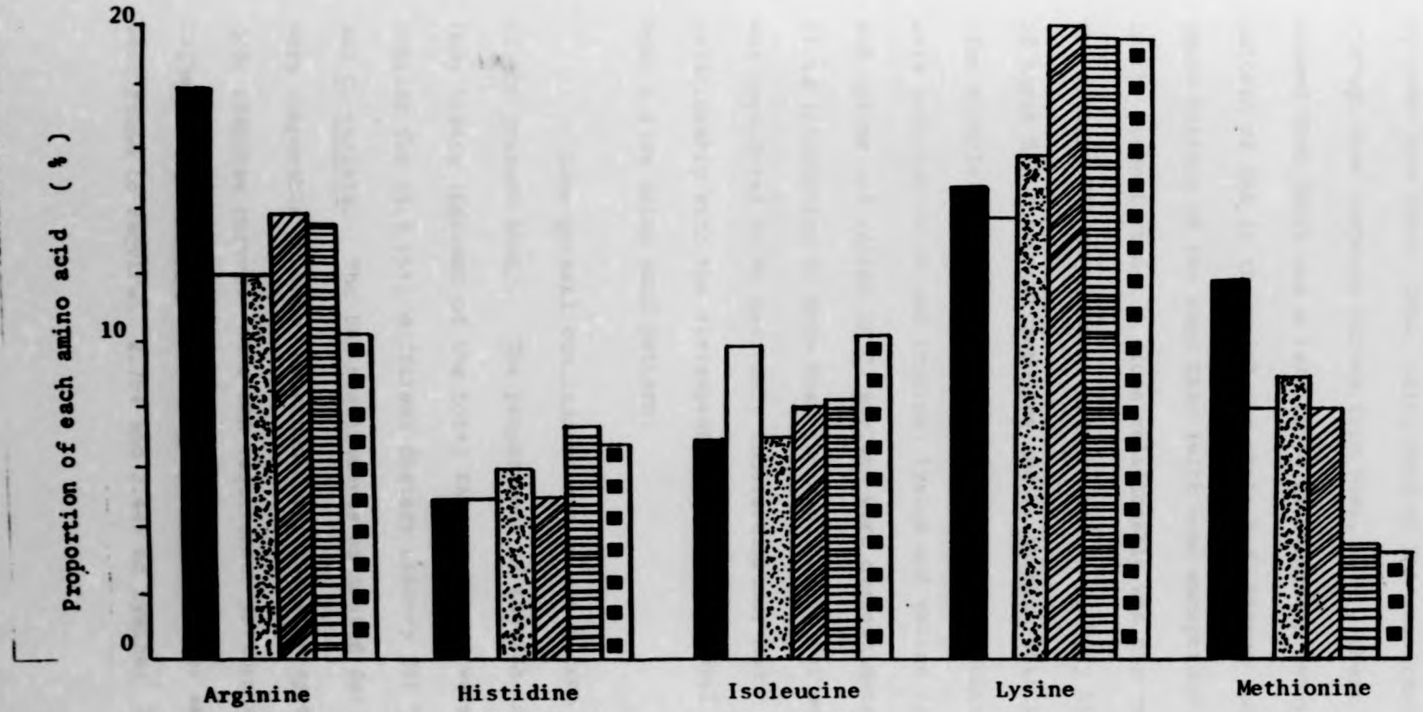

Figure 3.2.1 (cont.) Comparison of the relative amounts of each of nine essential amino acids required for four species of fish and snakeheads.

present in the muscle. This proposition has been examined in bird (chick) and mammal (rat) by Boorman (1980) and in common carp by Cowey and Tacon (1982, using data of EAA requirements obtained through dose response curves from Nose, 1979), their results showed that there was a very close relationship between the pattern of EAA in the muscle or whole body tissue and the requirements pattern of the same EAAs (with some exceptions). Similarly, in the present study, a close relationship was observed between the dietary EAA requirement pattern and whole body tissue pattern of these EAA for C. micropeltes and C. striata with some deviations (for example, arginine in C. micropeltes and threonine in C. striata were underestimated and leucine, lysine and valine in C. micropeltes and lysine and valine in C. striata were overestimated, Figure 3.2.2). It is interesting to note that the requirement for methionine, which was considered to be seriously underestimated, had a very close relationship with the corresponding methionine level in the whole body tissue amino acid pattern.

Some general conclusions can be drawn from the findings of the present study. The proportions of the EAA in the whole body tissue (percent of the total EAAs measured) were relatively similar for fish with different dietary history for C. micropeltes and C. striata. The EAA requirements estimated for both species were comparable to those obtained for other fish species from dose response curves. The EAA requirement pattern was slightly higher for C. striata than for C. micropeltes, for example, they were found to require: 2.79% and 2.44% of lysine; 0.92% and 0.89%

Figure 3.2.2 Relationship between pattern of EAA requirements found by the tissue EAA deposition method for lysine (lys), valine (val), methionine (met), histidine (his), threonine (thr), leucine (leu), phenylalanine (phe), arginine (arg) and isoleucine (iso) and the pattern of the same amino acids in the whole body of growing snakehead (Channa striata (●) and Channa micropeltes (■)). The level of each amino acid is represented as a fraction of the sum of all nine in each pattern. The line represents coincidence of requirement and tissue patterns.

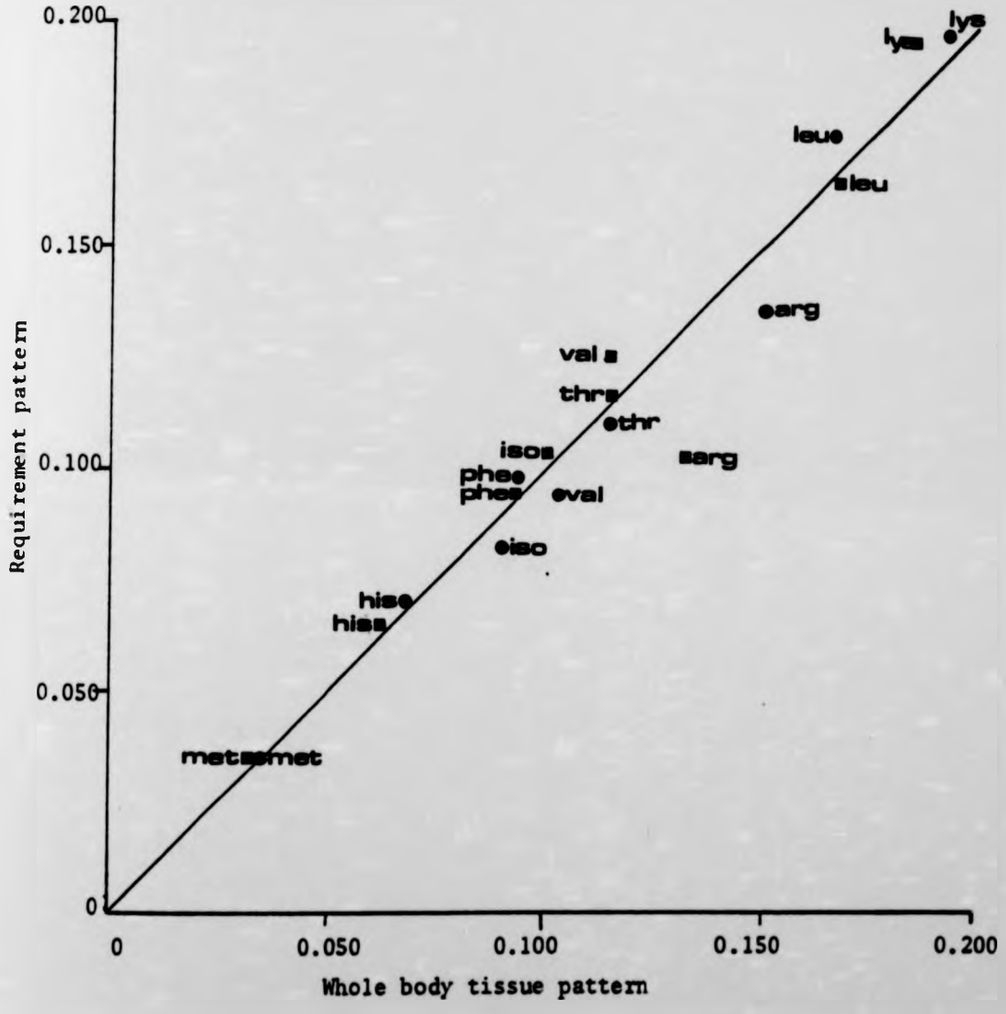

Figure 3.2.2 Relationship between pattern of requirements for nine essential amino acids and the pattern of the same amino acids in the whole body of growing snakeheads (Channa striata and Channa micropeltes)

of histidine; 1.44% and 1.66% of arginine; 1.64% and 1.35% of threonine; 1.78% and 1.16% of valine; 0.47% and 0.43% of methionine; 1.46% and 1.01% of isoleucine; 2.35% and 2.16% of leucine and 1.32% and 1.20% of phenylalanine respectively (values are expressed as percent diet).

3.3 EXPERIMENT 3

THE EFFECT OF DIETARY FATTY ACID INTAKE ON GROWTH AND FATTY ACID COMPOSITION OF LIVER AND MUSCLE OF SNAKEHEAD

3.3.1 INTRODUCTION

Fish require dietary lipid for two main reasons: as an energy source and secondly certain fatty acids are essential components of the cell in that they are necessary for the maintenance and functional integrity of membranes. In the absence of these essential fatty acids from the diets, deficiency diseases occur in fish (Cowey and Sargent, 1979).

Those fish that have so far been studied exhibit considerable variation in their requirement for essential fatty acids (EFA), some species being much more exacting than others. At one extreme are marine species such as turbot, Scophthalmus maximus (Owen et al, 1975), that have an absolute requirement for polyunsaturated fatty acids (PUFA) of the (n-3)[1] series as they cannot elongate and desaturate linolenic acid (18:3(n-3). Other marine species such as red sea bream, Chrysophrys major (Yamada et al, 1980), and yellowtail, Seriola quinqueradiata (Yone, 1980), appear equally demanding. At the other extreme are rainbow trout (Salmo gairdneri) which appear able to satisfy their EFA requirement with a dietary supply of 18:3(n-3); Yu et al, (1979) were able to grow this species through a generation with a diet containing 1% linolenic acid as the sole dietary EFA.

1. a short band designation for fatty acid will be used throughout the thesis, for example linolenic acid would be written 18:3(n-3). The first number identifies the number of carbons, the second number, the number of double bonds and the last number the position of the double bonds counting from the methyl end.

Some warm water fish such as channel catfish (Ictalurus punctatus, Stickney and Andrews, 1972) appear to have a much lower EFA requirement than do rainbow trout, while others such as eel (Anguilla japonica, Takeuchi et al, 1980) and Tilapia zillii (Kanazawa et al, 1980) seem to need EFA of both the (n-6) and (n-3) series.

The aim of the present experiment was therefore to examine the effect of fatty acid intake on growth and fatty acid composition of liver and muscle of the snakehead Channa micropeltes.

3.3.2 MATERIALS AND METHODS

3.3.2.1 Diets

Three isoenergetic and isonitrogenous experimental diets, supplemented with three different exogenous sources of dietary lipids, containing 50% dietary protein and an estimated gross energy content of approximately 450 kcal / 100 g diet were formulated. The composition of the experimental diets is presented in Table 3.3.1. Within all diets, lipid free herring meal (81% crude protein) and lipid free casein supplied 20% and 30% of the dietary protein respectively. The protein sources were defatted by continuous extraction with petroleum ether within a Soxhlet apparatus for five hours; subsequent analysis with "Freon" (trichloroflouromethane; Korn and Macedo, 1973) showed them to have lipid content of less than 0.05%. Diet 1 contained

Table 3.3.1 Composition of experimental diets used in Experiment 3
(% by weight)

Component	Diet number		
	1	2	3
Lipid free herring meal	25	25	25
Lipid free casein	33	33	33
White dextrin	20	20	20
Cod liver oil	10	0	0
Linolenic acid	0	1.5	0
Linoleic acid	0	0.5	2
Palmitic acid	0	8	8
Binder *1	1	1	1
Vitamin premix *2	2	2	2
Mineral premix *3	2	2	2
- cellulose	6.5	6.5	6.5

*1 carboxymethylcellulose

*2 To supply /100g diet : thiamine HCL 5mg; Riboflavin 5mg; Calcium pantothenate 10mg; Niacin 20mg; Pyridoxine HCL 4mg; Biotin 0.6mg; Folic acid 1.5mg; Cyanobalamin 0.01mg; Inositol 200mg; Ascorbic acid 100mg; Choline chloride 400mg; Menadione 4mg; paraaminobenzoic acid 5mg; tocopherol acetate 40mg; retinol palmitate 200 iu

*3 As in Table 3.1.1

cod liver oil as source of EFA and provided both long chain (n-3) series fatty acids as well as small amounts of linolenic and linoleic (18:2(n-6) acids. Diet 2 provided linolenic acid at a level approximating that of total (n-3) fatty acids in diet 1, it also provided 0.5% linoleic acid, finally diet 3 supplied linoleic acid only as EFA source. The linolenic, linoleic and palmitic acids used were obtained from Sigma Chemical Co. Ltd., London. The proximate composition and the measured amount of fatty acids in the diets together with those present in the pre-experiment trout pellets used are shown in Table 3.3.2 and 3.3.3.

The experimental diets were prepared and stored as previously described (Experiment 1, 3.1.2.1).

3.3.2.2 Animals and Tanks

C. micropeltes juveniles (batch CM2, 90 - 120 g in weight) were obtained from Cliff Murray Ltd, Tropical Fish Importer, Glasgow and randomly distributed among four self-cleaning 25 litre circular tanks at a stocking rate of three fish per tank. The experimental tanks were maintained within a single recirculation system (Figure 3.3.1). A 100 litre header tank supplied freshwater by gravity to each holding tank at a rate of 3 litres per minute per tank. Each tank had a constant level overflow device which drained into a series of four solids settling tanks. Water was then pumped up through a biological filter tank and allowed to overflow into the header tank. The water temperature was maintained at 28°C ± 0.5°C by ten thermostatically controlled aquarium heaters. Experimental tanks

Table 3.3.2 Proximate composition and energy content of experimental diets used in Experiment 3

Component	Diet number		
	1	2	3
Moisture (%)	5.3	5.6	5.4
Crude protein (N x 6.25) (%)	49.1	48.8	48.3
Crude lipid (%)	11.1	9.1	12.5
Ash (%)	5.8	6.0	5.5
Calculated available carbohydrate (%)	20	20	20
Calculated gross energy *1 (kcal / 100g)	466	445	474

*1 Calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g lipid and 4.0 kcal/g carbohydrate (Cowey et al, 1972)

Table 3.3.3 Principal fatty acids in the experimental diets and in diet given to the fish before Experiment 3 began (% by weight of total fatty acids)

Fatty acid	Diet number			Pre-experiment
	1	2	3	
16:0	18.8	76	74.8	22.9
16:1(n-9)	9.9	0.7	0	5.7
16:1(n-7)	2.7	0	0	0
18:0	2.7	1.2	1.1	3.5
18:1(n-9)	20.5	2.5	1.9	15.9
18:1(n-7)	4.8	0	0	0
18:2(n-6)	2.9	5	18.9	11.9
18:3(n-3)	1.1	13.0	0.8	2.3
20:1(n-9)	9.1	0	0.3	4.4
20:5(n-3)	6.8	0	0	6.3
22:1(n-11)	4.2	0	0.2	0
22:6(n-3)	6.5	0	0.6	7.9
Total (n-3)	14.4	13.0	1.4	16.5
Total (n-6)	2.9	5.0	18.9	11.9
(n-3) / (n-6)	5.0	2.6	0.1	1.4

Figure 3.3.1 Layout of the recirculating system used in Experiments 3 and 4

a : Solids settling tank

b : Biological filter tank (containing gravel)

c : Header tank

d : Experimental tank (receiving an inflow of water at a rate of 3 litre/minute/tank)

P : Water pump

H : Aquarium heater.

⟶ : Direction of water flow

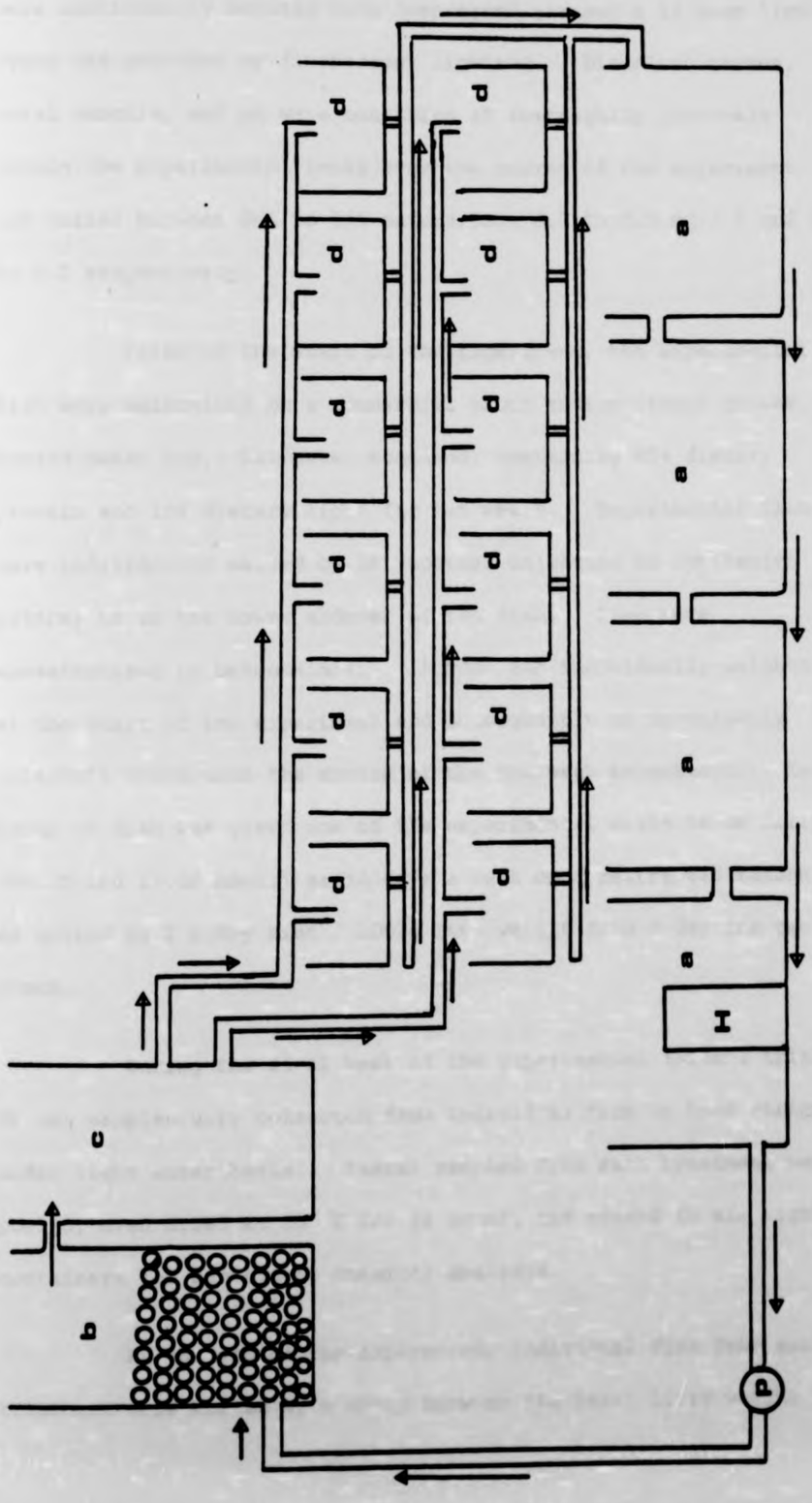

Figure 3.3.1

were continuously aerated with compressed air and a 12 hour light cycle was provided by fluorescent lighting. Dissolved oxygen, total ammonia, and pH were monitored at fortnightly intervals within the experimental tanks over the course of the experiment, and varied between 88% to 93% saturation, 0.2 to 0.5 mg / l and 5.7 to 6.2 respectively.

Prior to the start of the experiment, the experimental fish were maintained on a commercial trout ration (trout grower, Edward Baker Ltd., Bathgate, Scotland) containing 45% dietary protein and 10% dietary lipid for ten weeks. Experimental fish were individually marked by intradermal injection of dye (Panjet system) on to the lower abdomen of the fish. Fish were anaesthetized in benzocaine (1 : 15,000) and individually weighed at the start of the experiment and subsequently at fortnightly intervals throughout the course of the ten week experiment. Each group of fish was given one of the experimental diets twice daily (08.30 and 17.00 hours) seven days a week on a restricted ration amounting to 2 g dry diet / 100 g live weight fish / day for ten weeks.

During the final week of the experimental feeding trial, faecal samples were collected from individual fish by hand stripping under light anaesthesia. Faecal samples from each treatment were pooled, oven dried at 105^{o}C for 24 hours, and stored in air tight containers for subsequent chemical analysis.

At the end of the experiment, individual fish from each treatment were killed by a sharp blow on the head, liver weight

determined and liver and white dorsal muscle samples taken for subsequent chemical analyses.

3.3.2.3 Chemical Methods

Proximate chemical analysis

Replicate samples of dietary ingredients, experimental diets and faeces were taken and analysed for moisture, crude protein, crude lipid and ash and chromic oxide content, as described previously (Experiment 1, 3.1.2.3).

Lipid and Fatty acid composition analysis

Lipids were extracted with chloroform and methanol from whole livers and muscle tissue of individual fish from each treatment using the method described by Bligh and Dyer (1959).

In order to examine fatty acid composition of polar lipids and triacylglycerols in the liver and muscle extracts, samples of these extracts were streaked along the starting line of a thin layer silica gel (0.25 mm thick) plate which was then developed with a hexane:diethyl ether:acetic acid (90:10:1 by volume) solvent mixture. After developing the plate, the relevant lipid bands were individually identified by spraying with a 2% (weight/volume) iodine chloroform solution. The silica gel containing the bands themselves was then scraped with a spatula, transferred to a sintered glass funnel, and the triacylglycerols or polar lipids then eluted from the silica gel into a tube with ground glass neck and side by means of gentle suction. The solvent used in the elution process was methanol:water (95:5 by

volume). Fatty acid methyl esters of the triacylglycerols and polar lipids were prepared by H_2SO_4 catalysed methylation (Christie, 1973). Two ml of methanol H_2SO_4 (1% H_2SO_4 in methanol) was added to the separated polar lipids and triacylglycerols and allowed to stand overnight at 50°C, after which time 4 ml of distilled water and 2 ml of $KHCO_3$ were added and the methyl esters extracted with 5 ml hexane. This was repeated with 5 ml hexane:diethyl ether (1:1 by volume). The extracts were then washed with 5 ml distilled water and allowed to separate overnight. The organic phase was then carefully removed and evaporated to dryness under nitrogen, the residual solids were then dissolved in dichloromethane and applied to a gas liquid chromatograph (Fractovap, 2150., Erba Science, Swindon, England) for fatty acid separation, identification and quantification. A 50 metre capillary column, 0.35 mm internal diameter, coated with liquid phase silar 5 CP was used. Hydrogen was used as the carrier gas at a flow rate of 4 ml/minute. The gas chromatograph was equipped with a hydrogen flame ionisation detector and a splitless injection system was employed. The injector and detector temperatures employed were 250°C, temperature programming being from 150°C to 250°C at 2°C per minute. Individual fatty acid methyl esters were identified by comparison of retention times with those of known standards.

Throughout the entire process of extraction and separation of lipids, a nitrogen atmosphere was employed wherever practical and the solvents used in the extraction and transmethylation of polar lipids and triacylglycerols contained the phenolic lipid antioxidant BHT (butylated hydroxytoluene at 0.00125%) to prevent

oxidation of the lipid samples.

Total lipids of the experimental diets were similarly extracted by the method of Bligh and Dyer (1969). Portions of these extracts were transmethylated and the individual fatty acid methyl esters separated, identified and quantitated as described in the preceding paragraphs.

3.3.2.4 STATISTICAL METHOD

Statistical comparisons were made using the analysis of variance. Mean differences were determined using Duncan's Multiple Range test (Duncan, 1955). Standard error (± SE) were calculated to identify the range of the means.

3.3.3 RESULTS

In all groups, the fish accepted the experimental diets within two days and fed aggresively for the duration of the experiment. However, occasionally (in some treatments) the daily fixed ration was not completely consumed and the uneaten feed was subsequently weighed and the quantity actually consumed thus ascertained.

3.3.3.1 Weight gain, Feed conversion efficiency and dietary protein utilization

The growth response is shown in Figure 3.3.2 and Table 3.3.4. Fish grew well on all treatments, those given diet 2 more

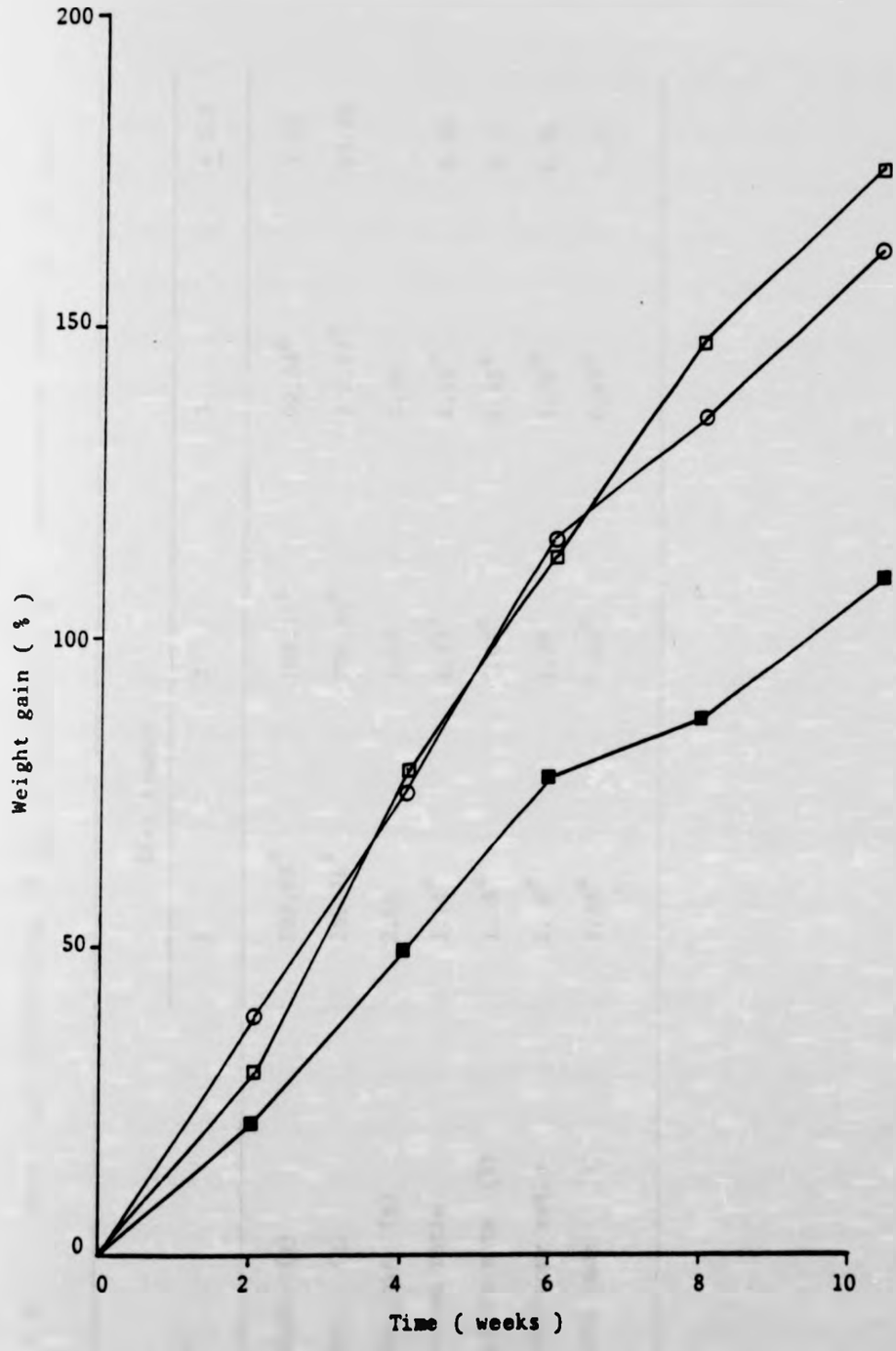

Figure 3.3.2 Growth responses of Channa micropeltes fed diets containing various dietary lipid source.
(—●— : cod liver oil ; —■—: linoleic acid;
—■— : linolenic and linoleic acids)

Table 3.3.4 Growth and related data of Channa micropeltes given experimental diets for ten weeks

Mean values	Diet number			
	1	2	3	± S.E
Initial weight (g)	109.99[a]	108.51[a]	99.04[a]	7.24
Final weight (g)	289.16[a]	226.84[a]	272.57[a]	43.88
Daily weight gain (g)	2.56	1.69	2.49	
Food conversion ratio	1.14[a]	1.47[a]	1.15[a]	0.08
Specific growth rate (%)	1.38[a]	1.05[a]	1.45[a]	0.12
Protein efficiency ratio	1.80[a]	1.39[a]	1.79[a]	0.35
Liver somatic index (%)	1.34[a]	0.62[a]	0.67[a]	0.19

than doubled their weight during the experiment while the response of those given diets 1 and 3 was even greater, final weight being almost three-fold that of initial weight. Differences in weight gain were not however significant ($P < 0.05$) nor were the differences in specific growth rate. Good feed conversion efficiency and protein efficiency ratio values were obtained in treatments 1 and 3, while the values for diet 2 were appreciably poorer.

3.3.3.2 Nutrient Digestibility

The dietary lipid type did not have any noticeable effect on apparent protein or dry matter digestibility of the rations fed, remaining relatively constant at approximately 94% and 79% respectively.

3.3.3.3 Fatty acid composition of experimental diets and fish

3.3.3.3.1 Fatty acid composition of experimental diets

The fatty acid composition of the experimental diets is shown in Table 3.3.3. The commercial trout ration used to maintain the experimental fish prior to the start of the experiment contained a relatively high proportion of the long chain (n-3) PUFAs (20:5(n-3) and 22:6(n-3)) consisting 14.20% of the total fatty acids and linoleic acid consisting 11.90% of the total fatty acids. Diet 1 formulated to provide long chain (n-3) PUFAs contained 13.29% of

these fatty acids (20:5(n-3) and 22:6(n-3)) in the dietary lipid. Diet 2 which was formulated to provide a mixture of linolenic and linoleic acids contained 11.87% and 3.41% of the total fatty acids as linolenic and linoleic acids respectively. Diet 3 contained 18.91% of the total fatty acids as linoleic acid. Palmitic acid was added at a level of 8 g/100 g diet to diets 2 and 3 so as to keep the experimental diets isocaloric. This is shown by the high concentrations found in the test diets totalling 72.63% and 74.18% of the total fatty acids respectively.

3.3.3.3.2 Fatty acid composition of experimental fish

The proportions of fatty acids in the polar lipids of liver and muscle are shown in Tables 3.3.5 and 3.3.6 respectively, and those in liver triacylglycerols in Table 3.3.7. The fatty acid composition of the muscle triacylglycerols was not analysed.

Before considering the results of the fatty acid composition of the fish at the end of the experiment, it is interesting to note that at the start of the experiment, fish had relatively high levels of linoleic acid totalling 5.8%, 18.4% and 8.5% of the total fatty acids in the polar lipids and triacylglycerols of the liver and the polar lipids of the muscle respectively. No arachidonic acid 20:4(n-6) was detected by the method used in the present experiment. Pre-experiment fish also had large amounts of PUFA of the (n-3) series present totalling 29.7%, 4.8% and 31.0% of the total fatty acids in the polar lipids and triacylglycerols of the liver and the polar lipids of the muscle respectively. These values must reflect the previous dietary input of the fish

Table 3.3.5 Principal fatty acids in hepatic polar lipids of *Channa micropeltes* given different experimental diets in Experiment 3 (% by weight of total fatty acids)

	Diet number			
Fatty acid	1	2	3	Fish at start
16:0	17.3	14.4	14.8	18.6
16:1(n-7)	2.8	3.3	0	1.5
18:0	10.8	12.9	15.2	15.6
18:1(n-9)	10.0	10.9	13.3	9.7
18:1(n-7)	5.6	0	0	2.7
18:2(n-6)	1.7	4.3	11.0	5.8
18:3(n-3)	0.2	10.6	6.2	2.0
20:1(n-9)	5.2	3.8	3.7	4.2
20:3	3.1	0	7.4	4.4
20:5(n-3)	9.2	6.5	0	7.8
22:1(n-11)	0	3.2	0	1.3
22:5(n-3)	3.8	4.9	0	2.6
22:6(n-3)	20.8	20.3	15.6	19.3
20:5(n-3) + 22:5(n-3) + 22:6(n-3)	33.8	31.7	15.6	29.7
Total (n-3)	34.0	42.3	21.8	31.7
Total (n-6)	1.7	4.3	11.0	5.8

Table 3.3.6 Principal fatty acids in polar lipids from muscle of Channa micropeltes given different experimental diets (% by weight of total fatty acids)

Fatty acids	Diet number 1	2	3	Fish at start
16:0	14.5	16.7	14.7	12.9
16:1	2.3	1.4	1.4	1.1
18:0	10.2	11.0	12.6	12.9
18:1)n-9)	9.7	13.0	9.6	9.5
18:1(n-7)	3.6	0.9	1.8	2.7
18:2(n-6)	1.7	4.0	12.8	8.5
18:3(n-3)	0.3	4.6	0.1	0.9
20:0	1.3	0.4	1.0	1.5
20:1(n-9)	5.6	1.6	1.4	4.0
20:2	0.5	0.8	2.9	2.1
20:3	1.3	1.4	4.7	2.9
20:4(n-6)	0	0.3	0	0
20:5(n-3)	6.8	4.4	1.7	3.7
22:1(n-11)	1.3	0.8	0.2	2.3
22:5(n-3)	3.2	2.5	1.6	3.3
22:6(n-3)	23.8	17.6	14.1	24.0
20:5(n-3) + 22:5(n-3) + 22:6(n-3)	33.8	24.5	17.4	31.0
Total (n-3)	34.1	29.1	17.5	31.9
Total (n-6)	1.7	4.3	12.8	8.5

Table 3.3.7 Principal fatty acids in hepatic triacyglycerols of *Channa micropeltes* given different experimental diets (% by weight of total fatty acids)

	Diet number			
Fatty acid	1	2	3	Fish at start
16:0	15.7	19.2	24.2	17.7
16:1(n-7)	9.9	1.8	2.5	5.9
18:0	2.8	5.8	7.4	4.8
18:1(n-9)	24.2	19.1	22.5	22.6
18:1(n-7)	5.6	1.4	0	1.2
18:2(n-6)	3.0	10.4	16.7	18.4
18:3(n-3)	0	13.9	4.6	3.5
20:1(n-9)	9.2	3.7	0	3.0
20:5(n-3)	4.4	1.9	1.0	1.0
22:1(n-11)	0	1.3	0	3.2
22:1(n-9)	1.4	0	0	0.4
22:6(n-3)	8.0	6.8	2.5	3.8
20:5(n-3) + 22:6(n-3)	12.4	8.7	3.5	4.8
Total (n-3)	12.4	20.7	8.1	8.3
Total (n-6)	3.0	10.4	16.7	18.4

when they were fed trout pellets.

Fish given diet 1 had similar levels of most poly-unsaturated fatty acids (PUFA) in the liver polar lipids to those present in fish at the start of the experiment; there was some reduction in the level of linoleic acid. The levels of 20:5(n-3), 22:5(n-3) and 22:6(n-3) in liver polar lipids of fish given diet 2 were again similar both to those of diet 1 fish and to those of fish at the start of the experiment. As there was no dietary input of these fatty acids and the fish had more than doubled their weight it may be inferred that some synthesis of these fatty acids from 18:3(n-3)had occurred. By contrast with (n-3) series fatty acids there was no evidence of modification of dietary linoleic acid as arachidonic acid was not detected. This conclusion is borne out from the analysis of treatment 3 hepatic polar lipids where it was shown that dietary linoleic acid was incorporated as such with no evidence of conversion to arachidonic acid (Table 3.3.5). Levels of 20:5(n-3) and 22:6(n-3) in liver polar lipids from this treatment fell by comparison with levels at the start of the experiment, this may be related to the increase in weight.

In muscle polar lipids little change occurred in PUFA of treatment 1 fish compared with those of fish at the start other than a fall in the proportion of linoleic acid present. There was again no evidence of any conversion of linoleic acid to arachidonic acid in either treatment 2 or 3, nor was there in this tissue any sign of modification of 18:3(n-3) to higher more unsaturated acids of the same series. The total amount of (n-3)

PUFA (20:5(n-3) + 22:5(n-3) + 22:6(n-3) decreased in treatment 2 as well as treatment 3 - but considerably more in treatment 3 which may simply reflect the greater growth in those fish.

Hepatic triacylglycerols in treatment 1 were appreciably higher in 20:5(n-3) + 22:6(n-3) and substantially lower in 18:2(n-6) than were fish at the start. This is in line with the expectation from the dietary input. There was an increase in the amounts of both 20:5(n-3) and 22:6(n-3) in fish given diet 2, taken together with the weight gain over the ten weeks this indicates strongly that some synthesis of these fatty acids has occurred although substantial amounts of linolenic acid are also accumulated. Little change occurred in the PUFA of liver triacylglycerols of fish given diet 3 when compared with the pre-experimental fish.

3.3.4 DISCUSSION

The growth data indicate that C. micropeltes is not very demanding in EFA requirement during its on-growing phase. Highest specific growth rate actually occurred in fish given a diet lacking (n-3) series fatty acids. In these fish the sum of (n-3) PUFA (20:5(n-3) + 22:5(n-3) + 22:6(n-3)) in polar lipids of the liver declined from 29.7% at the start to 15.6% at the end of the experiment. The corresponding figures for muscle are 31% and 17.4%. Conventionally it is considered that long chain PUFA may comprise 50% of the fatty acids in polar lipids as a PUFA is normally esterified at the two position of the phosphoglyceride

(C.B. Cowey, pers. comm.). Such a provision is clearly not a limitation to growth in warm water fish. The sum of (n-3) and (n-6) fatty acids in polar lipids of diet 3 fish is 32.8% in liver and 30.3% in muscle so that an appreciable amount of monosaturated acids are esterified at the 2 position of the phosphoglyceride in these fish.

The total (n-3) fatty acid component of diets 1 and 2 was similar although this was largely in the form of linolenic acid in diet 2 while virtually all those present in diet 1 were longer chain fatty acids. While the tissue fatty acids of liver and muscle in these treatments largely reflected the dietary input, comparisons of fatty acids in liver lipids of diet 2 fish with those of fish at the start of the experiment showed distinct evidence of chain elongation and desaturation of 18:3(n-3). This did not extend to muscle lipids which appeared to incorporate dietary lipid directly into polar lipids.

In marked contrast to the apparent modification of dietary linolenic acid in treatment 2 there was no evidence of any modification of dietary linoleic acid in either treatment 2 or 3. This is surprising as similar Δ 4, Δ 5 and Δ 6 desaturations are involved in the modification of the two acids, the ability to anabolise one and not the other implies substrate specificity toward them (Gurr and James, 1975).

Evidence that other tropical freshwater carnivorous fish distinguish between the acyl structures of linoleate and linolenate was reported recently (Bandyopadhyay et al, 1982). These authors

showed that in the livers of two species of catfish little oxidation of intraperitoneally injected linoleic acid occurred while there was profuse oxidation of (1-^{14}C) linolenic acid shown by the presence of extensive radioactivity in saturated fatty acids. The authors were also able to show that in both species of catfish (Heteropneustes fossilis and Clarias batrachus) some capacity to desaturate and elongate linoleic and linolenic acids was present. In the context of the present experiments it is of interest that the radioactivity present in hepatic fatty acids four hours after intraperitoneal injection of (1-^{14}C) linolenic acid 16.8% (H. fossilis) and 11.4% (C. batrachus) were found in longer chain more unsaturated members of the (n-3) series. The corresponding figures for linoleic acid were much lower 4.3% (H. fossilis) and 5.8% (C. batrachus). These results parallel the present results on C. micropeltes in that there is a greater capacity to modify linolenic than linoleic acid.

Some general conclusions may be drawn from the findings of the present study. On the basis of growth response, C. micropeltes are not very demanding in their EFA requirement during their on-growing phase. Fish grew equally well when fed test diets containing long chain PUFA of the (n-3) series, linolenic and linoleic acids or linoleic acid alone. The fatty acid composition of experimental fish reflected the fatty acid profiles of the experimental diets and previous dietary history. C. micropeltes possess a limited capacity to chain elongate and desaturate linolenic acid but seem unable to similarly modify linoleic acid. Dietary linolenic acid was however incorporated

directly into muscle polar lipids without modification.

However, in view of the strong influence of the previous dietary treatment on the fatty acid composition of experimental fish and the capacity of large fish to selectively retain EFA, it would be advisable to repeat such experiments using fish of a much smaller size with short dietary history and longer duration of experimentation.

3.4 EXPERIMENT 4

THE EFFECT OF DIETARY ENERGY LEVEL AND DIETARY ENERGY SOURCE ON GROWTH, FEED CONVERSION EFFICIENCY AND CARCASS COMPOSITION OF SNAKEHEAD

3.4.1 INTRODUCTION

Cowey and Sargent (1972) showed that most farmed fish require higher levels of dietary protein than most conventional livestock such as pig and poultry.

They were of the opinion that the majority of fish species normally use carbohydrate to only a limited extent and when provided with a natural protein rich diet, have a tendency to metabolize protein for their energy needs. As protein is usually the single most costly component of the diet, it is uneconomical as well as nutritionally wasteful to oxidise protein for provision of energy.

Several studies have shown that the provision of adequate non-protein energy sources within the diet can spare the dietary protein for growth. The variation in the ratio of total energy to protein in the diet on feed conversion, protein conversion and weight gain has been examined in several species of fish (Ringrose, 1971., Lee and Putnam, 1973., Page and Andrews, 1973., Cowey *et al*, 1975., Takeda *et al*, 1975). In most of these studies, certain trends or findings are common, thus an increase in the ratio of digestible energy / protein energy in the diet resulted in an increase deposition of lipid in the fish; an increase in dietary energy level at constant dietary protein level resulted in improved feed efficiency; and PER was positively correlated with the ratio digestible energy / protein energy in the diet, indicating the protein sparing action by dietary carbohydrate and/or dietary lipid. The improved growth and feed efficiency

would however need to be balanced by the acceptability of the fattier fish produced.

This experiment was thus designed to investigate the effects of varying dietary gross energy levels (by varying levels of dietary carbohydrate and lipid) at a constant dietary protein concentration on growth, feed efficiency and carcass composition in C. striata juveniles. Fish were fed test diets containing 45% dietary protein and three levels of dietary lipid (5%, 10% and 20%) and within each dietary lipid concentration three levels of dietary carbohydrate (5%, 10% and 20%).

3.4.2 MATERIALS AND METHODS

3.4.2.1 Diets

The composition of the experimental diets is shown in Table 3.4.1. Herring meal and casein were used as the dietary protein source within the experimental diets, supplying 25% and 20% of the dietary protein respectively. Nine experimental diets were formulated to contain 45% dietary protein and to provide three dietary lipid levels (5g / 100g diet., 10g / 100g diet and 20g / 100g diet) by supplementing the lipid present within the herring meal with a mixture of cod liver oil and corn oil (1:1 by weight). Within each dietary lipid level, the concentration of dietary carbohydrate (white dextrin) was also varied (with α-cellulose) to provide one of three levels (5g / 100g diet., 10g / 100g diet and 20g / 100g diet). Within all diets, chromic oxide was included at the 0.5% inclusion level for the

Table 3.4.1 Composition of the experimental diets used in Experiment 4
(values are expressed as % by weight)

Ingredient	Diet number								
	1	2	3	4	5	6	7	8	9
Herring meal	30	30	30	30	30	30	30	30	30
Casein	25	25	25	25	25	25	25	25	25
Lipid *1	2.5	2.5	2.5	7.5	7.5	7.5	17.5	17.5	17.5
White dextrin	5	10	20	5	10	20	5	10	20
Binder *2	1	1	1	1	1	1	1	1	1
Vitamin premix *3	2	2	2	2	2	2	2	2	2
Mineral premix*3	2	2	2	2	2	2	2	2	2
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
α-cellulose	32	27	17	27	22	12	17	12	2

*1 Dietary lipid supplied by cod liver oil and corn oil (1:1, by weight)

*2 Carboxymethylcellulose

*3 As in Table 3.1.1

determination of nutrient digestibility coefficients. The experimental diets were formulated to contain an estimated gross energy content ranging from 316.9 kcal / 100g diet (diet 1) to 530.0 kcal / 100g diet (diet 9) and dietary protein to gross energy ratios (P:E ratios) varying from between 138.28 to 82.58 mg protein per kcal energy.

The experimental diets were prepared and stored as previously described (section 3.1.2.1, Experiment 1) and the proximate composition of the test diets is presented in Table 3.4.2.

3.4.2.2 Animals and Tanks

C. striata juveniles (15g to 21g in weight) were obtained from Cliff Murray Ltd., Tropical Fish Importer, Glasgow (batch CS3) and randomly distributed among ten 25 litre circular plastic tanks at a stocking rate of four fish per tank. The experimental tanks were housed within a recirculating system as described previously (section 3.3.2.2). The water temperature was maintained at 28°C ± 0.5°C. Experimental tanks were continuously aerated with compressed air and a 12 hour light cycle was provided by fluorescent lighting. Dissolved oxygen, total ammonia and pH were monitored at fortnightly intervals within the experimental tanks over the course of the experiment and varied between 85% and 95% saturation, 0.3 to 0.6 mg / l and 5.5 to 6.3 respectively.

Prior to the start of the experiment, the experimental fish were maintained on commercial trout ration (trout grower,

Table 3.4.2 Proximate composition and energy content of the experimental diets used in Experiment 4

Mean values	Diet number								
	1	2	3	4	5	6	7	8	9
Moisture (%)	3.51	4.74	4.36	4.66	4.55	3.59	3.08	3.87	3.09
Crude protein (%)	43.82	42.76	43.94	43.88	43.86	43.64	43.56	43.94	43.77
Crude lipid (%)	4.96	6.60	5.90	11.00	10.31	10.56	21.47	20.91	21.11
Ash (%)	6.38	5.38	5.49	6.32	6.45	6.06	6.32	5.88	6.19
Calculated available carbohydrate (%)	5	10	20	5	10	20	5	10	20
Gross energy *1 (kcal/100g)	316.89	346.43	386.51	374.62	387.95	429.07	472.49	489.10	530.03
Gross protein energy (%)	78.82	70.35	64.80	66.77	64.44	57.97	57.97	51.21	47.07
Estimated digestible energy (kcal/100g)	273.74	293.20	312.80	328.40	332.09	353.24	421.03	427.89	448.84
Protein to energy ratio (mg protein/kcal)	138.28	123.19	113.68	117.13	113.05	101.71	92.19	89.84	82.58

*1 calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g fat; 4.0 kcal/g carbohydrate .

Edward Baker Ltd., Bathgate, Scotland). At the start of the experiment, an initial sample of four fish were sacrificed, killed by a sharp blow on the head and stored at -20°C for subsequent chemical analysis. Four fish were allotted per dietary treatment and the order of tanks assigned randomly. Fish in all groups were weighed individually at the start of the experiment and subsequently at fortnightly intervals throughout the course of the experiment as previously described (Experiment 1, section 3.1.2.2). All groups were fed twice daily (0830 hours and 1700 hours), seven days a week (except on the day of weighing) at a fixed rate of 2% body weight per day, and the level adjusted accordingly at fortnightly intervals. The total duration of the experimental feeding trial was seven weeks. During the final week of the experiment, faecal samples were collected from individual fish as previously described (section 3.1.2.2, Experiment 1). Faecal samples from each dietary treatment were pooled, oven dried at 105°C for 24 hours and stored in air tight containers for subsequent chemical analysis.

On the final day of the experiment, all four fish from each dietary treatment were sacrificed and whole liver weight determined. The individual fish carcass (including liver) was then stored at -20°C for subsequent gross chemical analysis.

3.4.2.3 Chemical and Statistical Methods

All chemical and statistical analyses were performed as described previously (sections 3.1.2.3 and 3.1.2.5, Experiment 1).

3.4.3 RESULTS

All groups of fish accepted the experimental diets within two days and fed aggressively for the duration of the experiment. However, occasionally in some instances, the daily fixed ration was not completely consumed and the uneaten feed was subsequently weighed and the quantity actually consumed thus ascertained.

3.4.3.1 Weight gain and Feed conversion

The growth response is shown in Figures 3.4.1 to 3.4.6 and Table 3.4.3. Despite large differences in the mean final body weights of fish, given different test diets, at the end of the seven week feeding trial, statistical analysis revealed that the differences were not significant ($P > 0.05$) (Table 3.4.3). In general, experimental fish fed the higher gross energy content diets grew better and had a better feed conversion ratio than fish given the lower dietary energy containing diets.

Figures 3.4.1 to 3.4.3 show the effects of increasing lipid concentration, at each of the dietary dextrin levels employed, on the percent weight gain. On the basis of percent weight gain, feed conversion ratio (FCR) and specific growth rate (SGR), the growth improvement observed with increasing dietary of lipid within those groups fed the lower dietary dextrin concentration of 5% (diet 1, 4 and 7) and higher dietary dextrin concentration of 20% (diet 3, 6 and 9) was less marked than that observed for fish fed the 10% dietary dextrin rations (diet 2, 5 and 8) (Table 3.4.3). The poorer growth shown by fish fed diet 5 was believed in part to be due to their lower feed intake (Table 3.4.3).

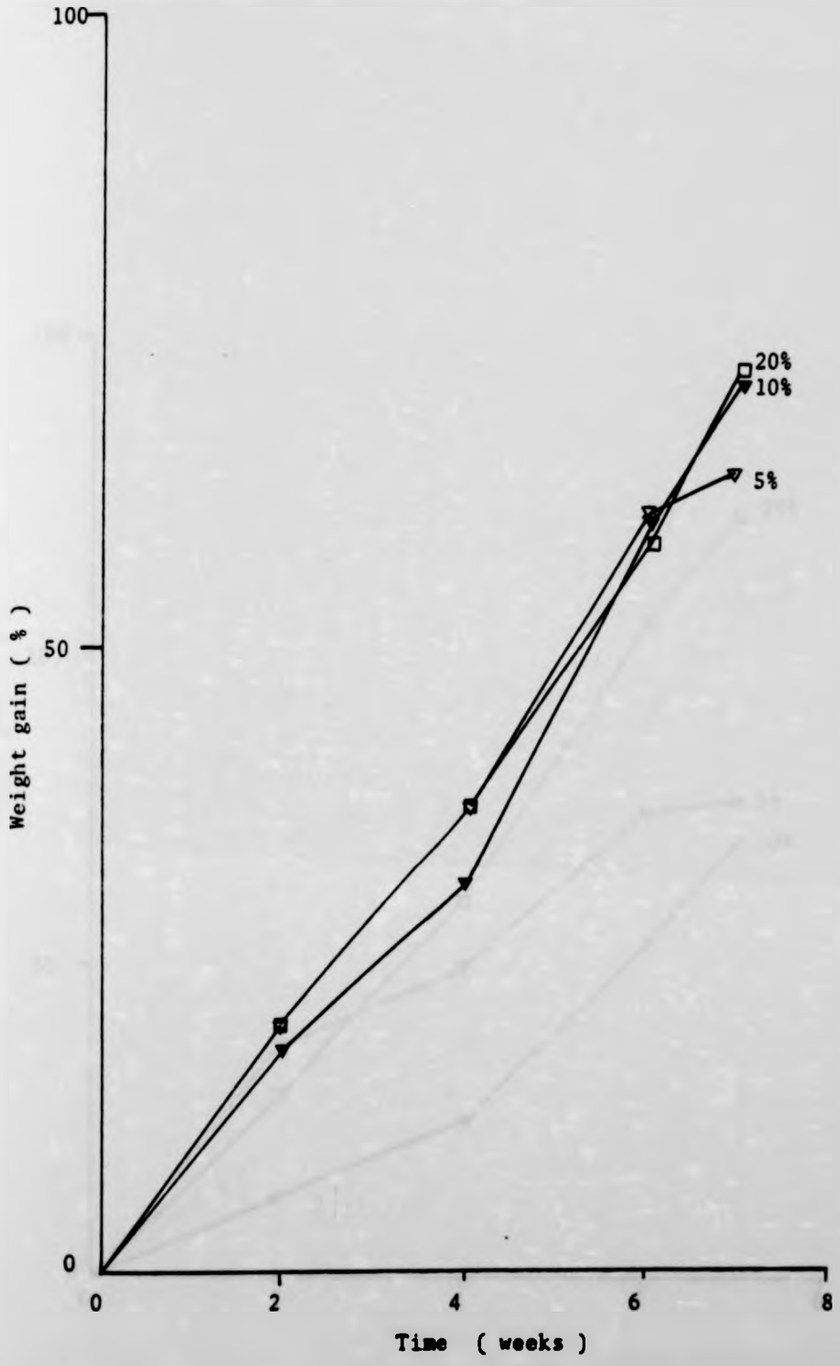

Figure 3.4.1 Growth responses of Channa striata fed 5% dietary dextrin and three levels of dietary lipid. Percentage indicates level of dietary lipid.

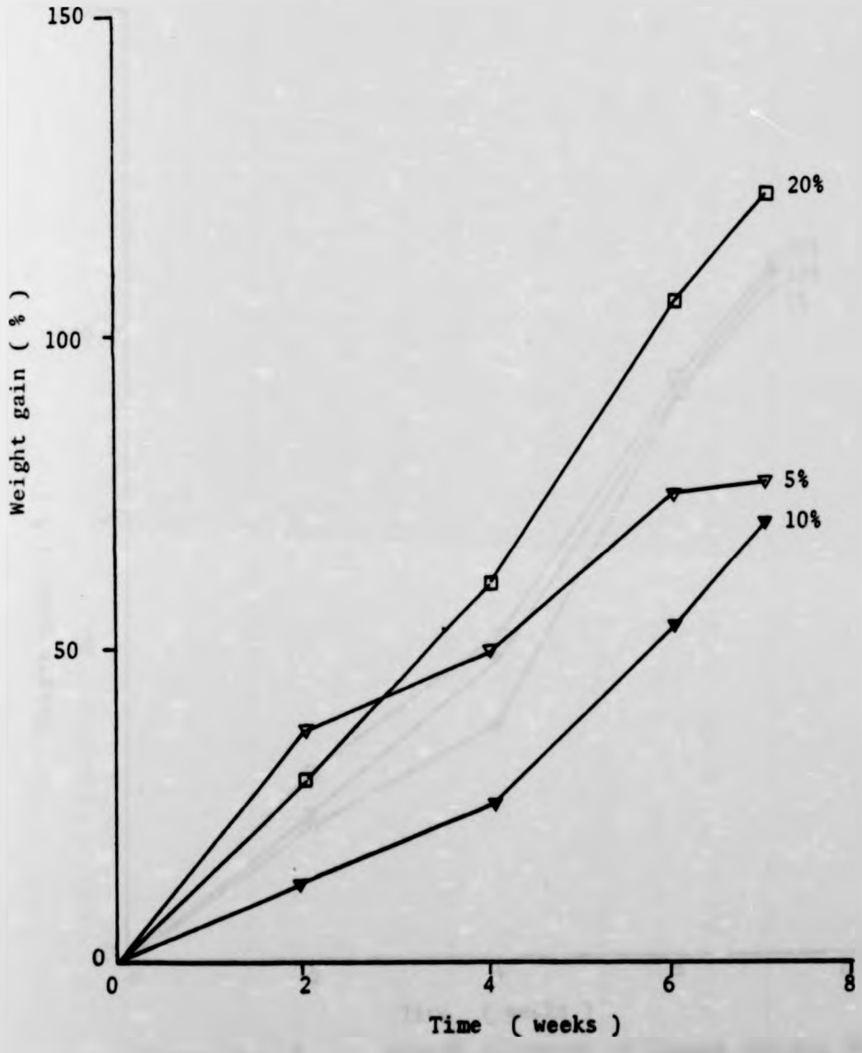

Figure 3.4.2 Growth responses of Channa striata fed 10% dietary dextrin and three levels of dietary lipid. Percentage indicates level of dietary lipid.

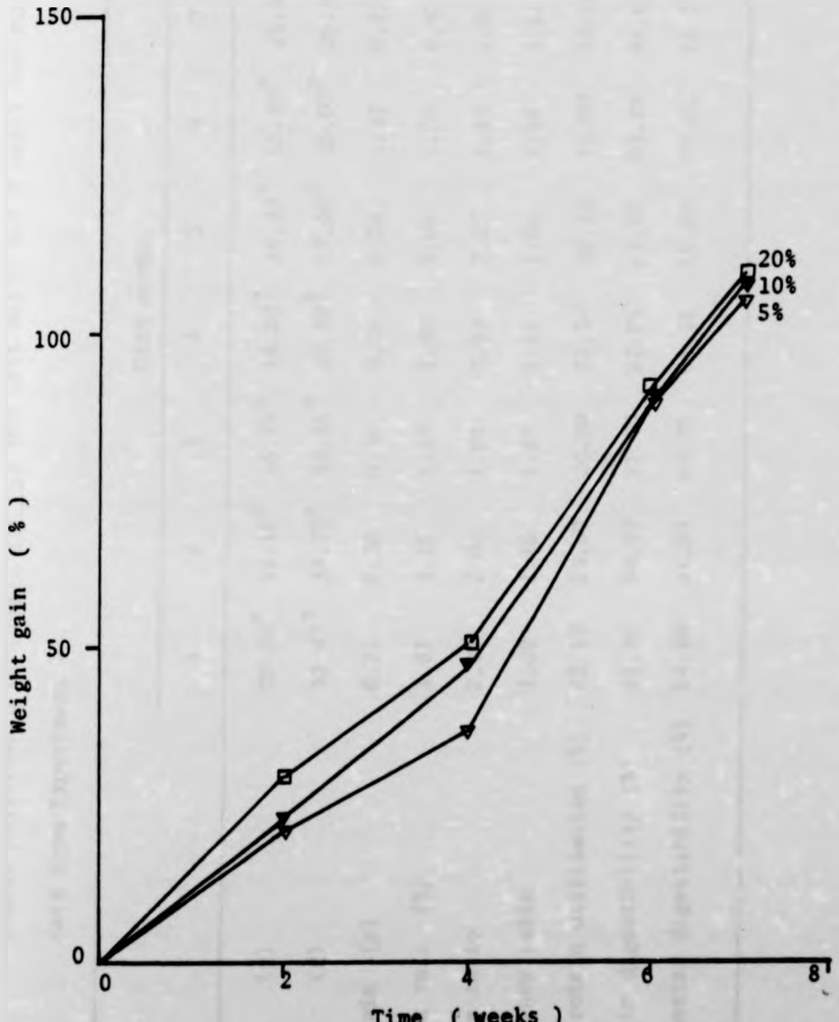

Figure 3.4. 3 Growth responses of Channa striata fed 20% dietary dextrin and three levels of dietary lipid. Percentage indicates the level of dietary lipid.

Table 3.4.3 Growth performance of Channa striata fed test diets for 8 weeks and nutrient digestibility data from Experiment 4 .

Mean values	Diet number									
	1	2	3	4	5	6	7	8	9	± S.E
Initial weight (g)	20.40[a]	19.19[a]	16.79[a]	18.07[a]	16.38[a]	16.83[a]	15.36[a]	17.77[a]	20.78[a]	2.18
Final weight (g)	33.47[a]	33.79[a]	34.46[a]	30.89[a]	27.90[a]	35.03[a]	26.39[a]	39.54[a]	43.58[a]	7.58
Daily weight gain (g)	0.27	0.30	0.36	0.26	0.24	0.37	0.23	0.44	0.47	
Specific growth rate (%)	1.01	1.15	1.47	1.09	1.09	1.50	0.92	1.63	1.51	
Food conversion ratio	2.07	2.00	1.80	2.03	2.13	1.40	1.96	1.34	1.40	
Protein efficiency ratio	1.09	1.16	1.27	1.12	1.06	1.64	1.15	1.73	1.62	
Apparent net protein utilization (%)	22.17	23.89	24.99	22.34	26.10	31.00	20.91	34.18	30.73	
Apparent protein digestibility (%)	95.45	94.77	91.50	92.95	91.99	93.29	94.62	88.65	87.73	
Apparent dry matter digestibility (%)	54.20	61.91	63.35	61.26	65.00	70.85	74.73	72.49	76.80	

Figures 3.4.4 to 3.4.6 show the effects of increasing dietary carbohydrate (white dextrin) concentration, at each of the dietary lipid levels, on the percent weight gain. At the 5% dietary lipid level, progressive substitution of α-cellulose with white dextrin from 5% to 20% resulted in a stepwise improvement in percent weight gain, FCR and SGR (Table 3.4.3). Similarly, at the 10% dietary lipid level, maximum percent weight gain, FCR and SGR were achieved at the 20% dietary dextrin level (diet 6) however, fish fed diet 4 (5% dietary dextrin) showed better growth than fish given diet 5 which contained 10% dietary dextrin. As previously mentioned, the poorer growth of fish fed diet 5 was partly due to its lower feed intake. At the 20% dietary lipid level, maximum percent weight gain and optimal FCR and SGR were achieved at the 10% dietary dextrin level (diet 8), despite a higher feed intake by fish fed the 20% dietary dextrin ration (diet 9) (Table 3.4.3).

The best growth response in terms of maximal percent weight gain and optimal FCR and SGR was achieved in fish fed diet 8 (122.51%, 1.34 and 1.63 respectively).

3.4.3.2 Protein Utilization

Determinations of protein efficiency ratio (PER) and apparent net protein utilization (NPU) provided indications of the dietary protein sparing activity of the test diets. In the present experiment, PER and apparent NPU values observed increased progressively as the gross energy content of the experimental diets increased with either dietary lipid or carbohydrate concentration

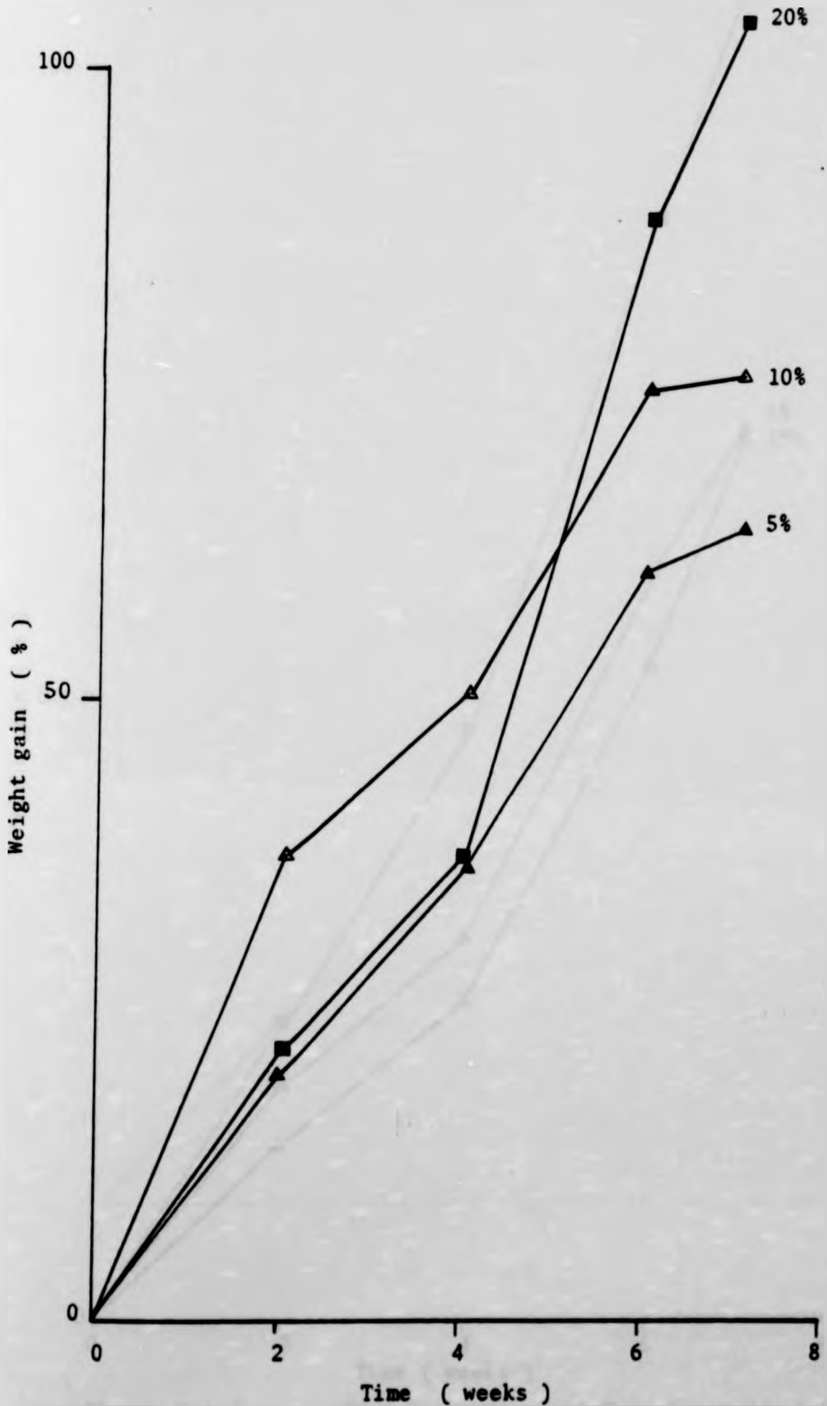

Figure 3.4. 4 Growth responses of Channa striata fed 5% dietary lipid and three dietary dextrin levels. Percentage indicates level of dietary dextrin.

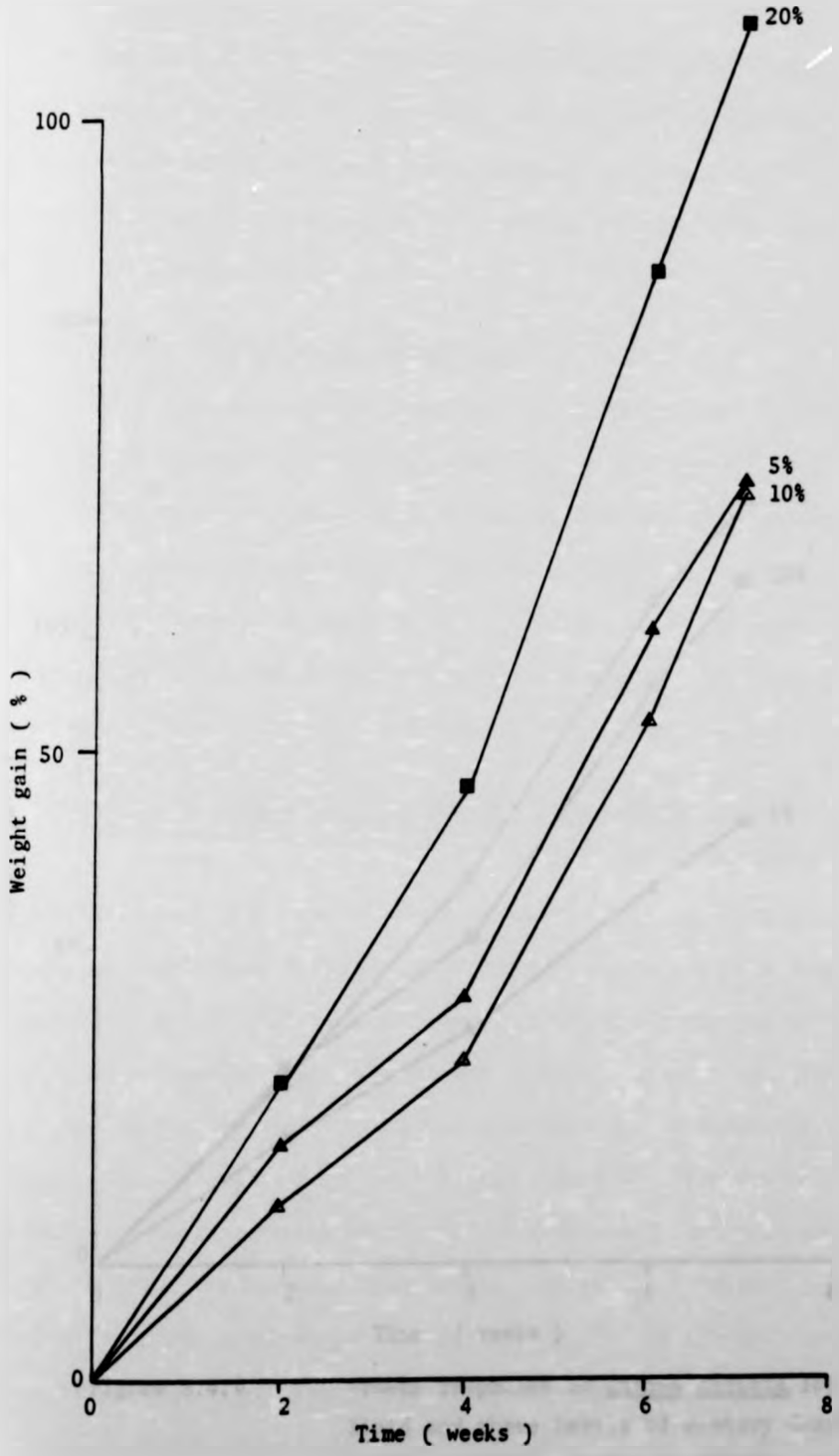

Figure 3.4.5 Growth responses of Channa striata fed 10% dietary lipid and three levels of dietary dextrin. Percentage indicates level of dietary dextrin.

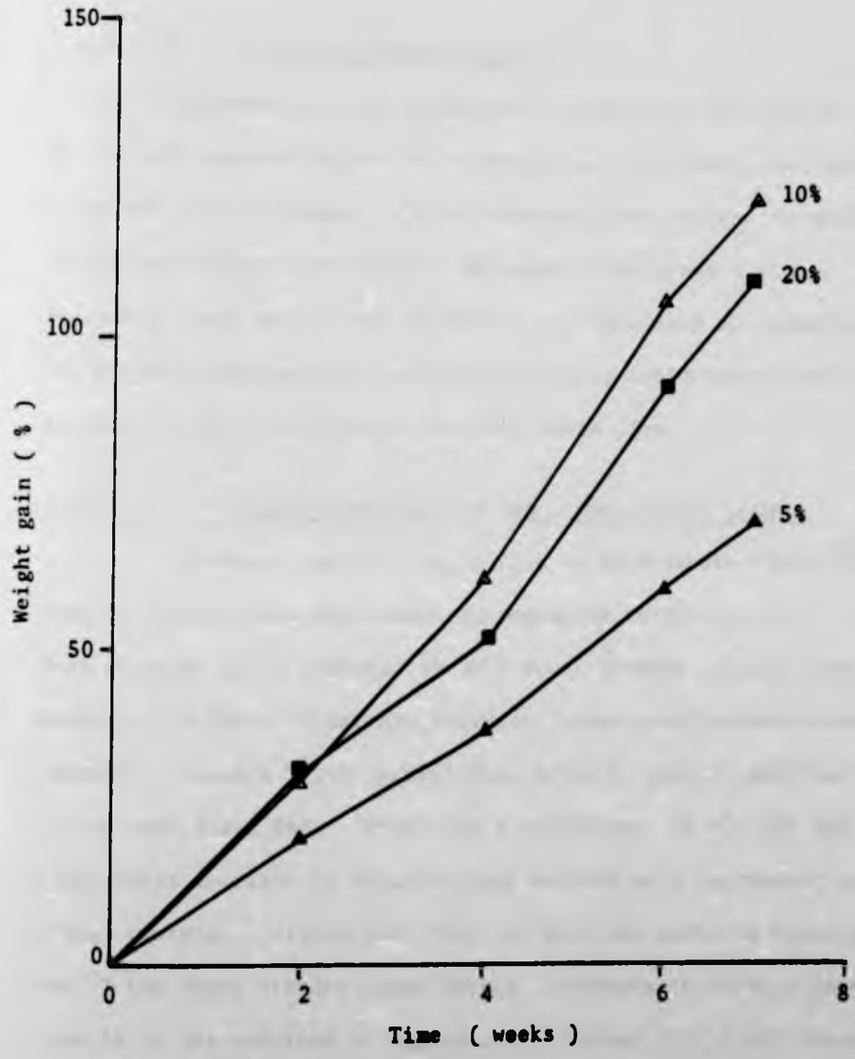

Figure 3.4.6 Growth responses of Channa striata fed 20% dietary lipid and three levels of dietary dextrin. Percentage indicates level of dietary dextrin.

(ranging from 1.06 to 1.73 and 20.91% to 34.18% respectively). Highest PER and apparent NPU values were observed in fish fed diet 8 containing 20% lipid and 10% dextrin (1.73 and 34.18% respectively) at a protein to gross energy ratio of 89 mg protein per kcal energy (Table 3.4.3).

3.4.3.3 Nutrient Digestibility

Apparent protein digestibility decreased from 95.45% to 87.73% and apparent dry matter digestibility increased from 54.20% to 76.80% with increasing dietary dextrin concentration at each of the three dietary lipid levels employed in the present study. Similarly, apparent protein digestibility decreased and apparent dry matter increased with increasing dietary lipid concentration at each of the three dietary dextrin levels (Table 3.4.3).

3.4.3.4 Carcass composition and liver somatic index

Proximate carcass composition of experimental fish at the start and end of the experiment is presented in Table 3.4.4. There were no significant differences ($P > 0.05$) between dietary treatments on the basis of carcass moisture content and carcass protein content. Carcass lipid content was, however, greatly affected by the test diets fed. There was a significant ($P < 0.05$) and progressive increase in carcass lipid content with increasing gross energy content. Within each group of fish fed diets containing one of the three dietary lipid levels, increases in dietary dextrin from 5% to 20% resulted in significantly higher ($P < 0.05$) carcass lipid content. Similarly, increases in dietary lipid concentration from 5% to 20% at each one of the three dietary dextrin levels employed also resulted in significantly higher carcass lipid

Table 3.4.4 Carcass composition of *Channa striata* at the start and end of the 7 week feeding trial in Experiment 4. (values are expressed as % by weight, wet basis)

Component	Fish at the start	Diet number 1	2	3	4	5	6	7	8	9	± S.E
Moisture	69.50	70.52^{a}	69.52^{a}	69.45^{a}	70.83^{a}	69.43^{a}	68.13^{a}	69.00^{a}	66.05^{a}	66.42^{a}	2.85
Crude protein	19.40	19.50^{a}	19.70^{a}	19.35^{a}	19.48^{a}	18.86^{a}	19.00^{a}	18.73^{a}	19.46^{a}	19.01^{a}	0.34
Crude lipid	7.85	5.77^{a}	5.87^{a}	6.66^{b}	5.68^{a}	7.17^{b}	8.82^{bc}	8.83^{bc}	10.28^{bc}	10.43^{c}	0.60
Ash	3.51	5.23^{b}	4.96^{ab}	4.82^{ab}	4.92^{ab}	4.78^{ab}	4.58^{ab}	4.73^{ab}	4.51^{a}	4.51^{a}	0.13
Total	99.90	101.02	100.05	100.28	100.91	100.24	100.53	100.79	100.30	100.37	
Liver somatic index		1.12^{a}	1.36^{b}	1.46^{c}	1.39^{b}	1.64^{d}	1.94^{e}	1.94^{e}	1.90^{e}	2.19^{f}	0.02

content ($P < 0.05$, Table 3.4.4). As observed previously in Experiment 1, there was an inverse relationship between carcass moisture and carcass lipid content.

The liver somatic index (LSI) of the fish at the end of the experiment was markedly affected by the test diets fed (Table 3.4.4). Within each treatment group, at each dietary lipid level employed, an increase in dietary dextrin concentration from 5% to 20% resulted in a significantly higher LSI ($P < 0.05$). Similarly, within each treatment group of fish fed one of three dietary dextrin concentration an increase in dietary lipid level resulted in a significantly higher LSI ($P < 0.05$). For example, LSI values obtained ranged between 1.12 to 1.46 in fish fed the 5% dextrin ration, 1.39 and 1.94 for fish fed the 10% dextrin ration and between 1.94 and 2.19 in fish fed the 20% dietary dextrin level (Table 3.4.4).

3.4.4 DISCUSSION

In general, growth response and dietary protein utilization were positively correlated to the gross energy content of the test diets fed; maximum growth and optimal protein utilization occurred in fish given diet 8 (gross energy content of 489.10 kcal / 100 g diet) and the poorest response was observed in fish fed diet 1 (gross energy content of 316.89 kcal / 100 g diet). On the basis of percent weight gain, FCR, SGR, PER and apparent NPU, increasing the dietary lipid concentration within each of the three dietary dextrin levels employed, and increasing

dietary carbohydrate (white dextrin) within each of the three dietary lipid levels employed resulted in improved growth, feed conversion efficiency and protein utilization. Increase in the gross energy content of the test diets also resulted in significantly higher ($P < 0.05$) carcass lipid content and liver somatic index. Carcass protein, moisture and ash content on the other hand, were relatively unaffected by dietary treatment.

The protein sparing action of dietary lipid was evident by comparisons of the PER and apparent NPU values obtained for diets 1, 4 and 7., 2, 5 and 8., and 3,6 and 9 which contained 5%, 10% and 20% dietary lipid at dietary dextrin levels of 5%, 10% and 20% respectively. Feeding progressively higher levels of dietary lipid to groups of fish led to higher PER and apparent NPU values at each of the dietary dextrin level tested (. Table 3.4.3). This finding is in broad agreement with reports by other authors. Jauncey (1979) observed increased growth and improved protein utilization in common carp fed increased dietary lipid concentrations up to 18% at four dietary protein concentrations (21%, 29%, 37% and 45%) and concluded that it is possible to reduce the protein contents of diets containing 18% lipid from 45% to 29% with no diminution in weight gain or dietary protein utilization. On the other hand, Murray *et al.* (1977) found that in channel catfish fed a diet containing 35% protein, increasing dietary lipid from 5% to 12% resulted in increased weight gain but that there was no apparent increase in weight gain in fish fed diet containing 25% protein with similar increase in dietary lipid levels. Similar observations on the protein sparing action of dietary lipid have

also been reported in mirror carp (Sin, 1973a, b) where it was found that dietary protein concentration could be reduced from 38% to 33% by increasing the dietary energy content from 2.8 to 3.1 kcal/ g diet (by the addition of 5% soybean oil). Similarly in plaice it has been possible to reduce the dietary protein content from 50% to 35% with 9% dietary lipid with no loss in weight gain or dietary protein utilization using diet containing 3 kcal / g diet gross energy (Adron et al, 1976). Reinitz et al (1978) were also able to demonstrate that dietary lipid can spare protein for growth in practical diets for rainbow trout. Takeuchi, (1978b) and Watanabe (1977) were able to reduce dietary protein level from 48% to 35% by the addition of 15% to 20% dietary lipid, provided the dietary lipid sources contained fatty acids of the linolenic type (n-3) to satisfy the essential fatty acid requirement of the rainbow trout.

On the contrary, Buhler and Halver (1961) found in chinook salmon that growth rate was relatively unaffected by increasing dietary lipid levels from 7% to 14% in diets containing 36% protein and thus concluded that the additional calories as supplied by the corn oil (replacing dietary dextrin) was not utilised by the fish.

The protein sparing action of dietary carbohydrate (white dextrin) in the present study was evident by the comparisons of the PER and apparent NPU values obtained for fish fed diets 1, 2 and 3., 4, 5 and 6., and 7, 8 and 9 (. Table 3.4.3). There was an increase in weight gain and improvement in protein

utilization with increase in dietary dextrin levels at each of the dietary lipid tested. This finding is in broad agreement with studies of Lee and Putnam (1973) with rainbow trout and Cowey et al. (1975) and Adron et al.(1976) with plaice, in that a protein sparing action by dietary carbohydrate was observed. Protein sparing action by dietary carbohydrate is perhaps best shown in the studies with chinook salmon by Buhler and Halver (1961). Fingerling chinook salmon were fed a series of diets of constant protein content (37.5%) in which increasing amounts of dextrin (0 to 48%) were substituted for α-cellulose. Weight gains of fish increased with dietary dextrin concentration up to a level of 20%; thereafter a further increase in dextrin content had little effect on weight gain. PER was highest on diets containing 20 to 30% dextrin, indicating that in these diets less protein was being catabolized for energy provision.

In the present study, growth performance and feed conversion efficiency improved with increasing dietary energy concentration, reaching an optimum level in fish fed diet 8 (containing 20% lipid and 10% dextrin). Excellent growth and feed efficiency of fish fed test diets containing high levels of dietary lipid (up to 35%) have been reported by other authors. No growth depressing or pathological effects have been observed in fish fed rations containing high dietary lipid concentrations of up to 18% (herring oil) in common carp (Jauncey, 1979) and 18% to 35% (a mixture of soybean oil and pollock oil, 3:2) in rainbow trout (Higashi et al, 1964., Higuera et al, 1977., Takeuchi et al, 1978d). On the other hand, growth was found to be depressed in

channel catfish when dietary lipid levels were raised from 12% to 16% (corn oil and beef tallow)(Dupree, 1969).

The apparent protein digestibility of the test diets in the present study tended to decrease with increasing dietary lipid levels and with increasing dietary dextrin levels. This is in contrast to studies with rainbow trout where increased dietary lipid had no noticeable effect on apparent protein digestibility (Higuera et al, 1977., Takeuchi et al, 1978a). The apparent dry matter digestibility in the present study however increased with increasing dietary dextrin concentrations at all levels of dietary lipid tested. This is in contrast to studies with yellowtail where it was found that high dietary levels of purified carbohydrate (potato starch) had a deleterious effect on growth and resulted in reduced protein and carbohydrate digestibility (Shimeno et al, 1978). It should be pointed out however that the highest level of dietary dextrin used in the present study was only 20% (compared to 45% in the studies with yellowtail).

An increase in the gross energy content of the experimental test diets (by the increase in dietary lipid or dietary carbohydrate levels) did not greatly affect the carcass moisture or protein content of fish at the end of the experiment. Carcass lipid content was however, significantly higher ($P < 0.05$) in fish fed diets containing the higher dietary lipid or dextrin concentrations. There was an inverse relationship between the carcass moisture and carcass lipid content of the experimental fish. This has been reported for other species of fish (Andrews and Stickney, 1972.,

Brett et al, 1969., Papoutsoglou and Papoutsoglou, 1978., Takeuchi et al, 1978c). Increased carcass lipid content with increasing dietary lipid concentrations have also been reported for rainbow trout (Lee and Putnam, 1973., Austreng, 1976., Watanabe, 1977., Takeuchi et al, 1978., Reinitz et al, 1978), channel catfish (Page and Andrews, 1973., Garling and Wilson, 1976., Murray et al, 1977), mirror carp (Sin, 1973a, b), plaice (Cowey et al, 1975., Adron et al, 1976) and chinook salmon (Buhler and Halver, 1961). Carcass lipid content was also observed to increased with increased dietary dextrin concentrations. This finding is in agreement with studies with chinook salmon (Buhler and Halver, 1961). On the other hand, Dupree (1969) and Garling and Wilson (1976) observed that increased dietary dextrin did not result in an increased carcass lipid content in channel catfish.

In the present study, there was no consistent trend to suggest that dietary treatments affected the carcass protein content. This is contrary to the studies with rainbow trout by Watanabe (1977) and Reinitz et al (1978) where it was observed that carcass protein content decreased with increasing dietary lipid content.

The concept of optimum dietary protein to energy (P:E) ratio for maximal growth is dependent on the level of dietary protein and energy present in the test diets, and should be restricted to experiments where the test diets provide adequate levels of protein and energy for normal growth. It has been shown by Garling and Wilson (1976) and Tiemeier et al (1965) that in channel catfish fed diets with an approximately similar P:E

ratio but differing greatly in dietary protein concentrations and total energy contents, resulted in significantly different growth and carcass composition. Garling and Wilson (1976) showed that channel catfish fed diets containing similar P:E ratios of 82 mg protein / kcal energy had an average daily weight gain of 12.4 g when the diet contained 28% dietary protein and 341 kcal / 100 g diet total energy, and only 6.1 g when the test diet contained 17.2% dietary protein and 209 kcal / 100 g diet total energy.

In the present study, the test diets contained approximately 45% dietary protein, this level has been determined as the optimum dietary protein for maximal growth (feeding trial 2, Experiment 1). Maximal growth and optimal protein utilization was shown by fish fed diet 8 containing 20% dietary lipid and 10% dietary dextrin at a P:E ratio of 89 mg protein / kcal energy. Further increases in dietary energy density did not result in an improvement in growth or protein utilization, thus indicating that the optimum P:E ratio had apparently been reached. This finding is in broad agreement with the results obtained in Experiment 1 (feeding trial 2) where maximum growth was achieved in fish fed a diet containing 45.82% dietary protein, a gross energy content of 467 kcal / 100 g diet and a P:E ratio of 98.75 mg protein / kcal energy.

Other studies where optimum P:E ratio had been examined, the results were determined with diets containing a limited number of protein levels (which was considered to be optimal for each species studied) and an expanded number of energy levels

(Tiemeier et al, 1965., Hasting, 1967., Page and Andrews, 1973., Prather and Lovell, 1973., Ringrose, 1971., Takeda et al, 1975). In order to facilitate a proper comparison of the results of this study with the others, the gross energy contents of the diets have been recalculated using metabolizable energy values of 4 kcal / g for protein, 9 kcal / g for fat and 4 kcal / g for carbohydrate (these values were the most commonly used in all the other studies). Using these energy values, the optimum P:E ratio obtained in the present study recalculated as 108.8 mg protein / kcal energy and a total energy content of 403.95 kcal / 100 g diet. Table 3.4.9 shows the optimal P:E ratio for maximal growth and feed utilization obtained for other fish species.

The recalculated value of the P:E ratio for optimal growth and feed utilization obtained for juvenile snakehead in the present study is in broad agreement with those values obtained for other carnivorous fish species including brook trout (Salvelinus fontinalis) (Ringrose, 1971), yellowtail (Takeda et al, 1975) and rainbow trout (Watanabe, 1977) (Table 3.4.5).

Studies on the effects of dietary P:E ratio in fish fed test diets containing varying protein concentrations and gross energy contents have produced differing results in channel catfish (Table 3.4.9). It was believed that the observed differences in the optimal P:E ratios may have resulted from differences in diet compositions, experimental designs and cultural methods employed. On this qualification, the optimal P:E ratio obtained for snakehead juvenile in the present study is in agreement with the studies of

Table 3.4.5 Optimum P:E ratio and related data of experimental diets giving maximal growth and feed conversion efficiency for some species of fish

Species	Dietary protein level (%)	Gross energy content of of diet *1 (kcal/100g)	Optimum P:E ratio (mg protein/kcal)	Reference
Brook trout	32	211.2 - 253.4	105.0 - 133.5	Ringrose, 1971
Yellowtail	32	380	107.9	Takeda et al, 1975
Rainbow trout	35	301.2	115.54	Watanabe, 1977
Channel catfish	25.3	297	84.8	Nail, 1962
Channel catfish	24 - 28	275 - 341	88	Garling and Wilson, L976
Channel catfish	25	187	133.7	Tiemeier et al, 1965
Channel catfish	32	264.4	121	Hasting, 1967
Channel catfish	29 - 42	220 - 286.3	131.6 - 146.7	Prather and Lovell, 1973
Channel catfish	25 - 35	403	133.1	Page and Andrews, 1973

*1 Calculated on an estimated 4 kcal/g protein; 9 lcal/g fat; 4 kcal/g carbohydrate

Nail (1962) and Garling and Wilson (1976) although the P:E ratio obtained in the present study was lower than that reported by Tiemeier et al (1965), Hasting (1967), Prather and Lovell (1973), Page and Andrews (1973).

The improved growth and dietary protein utilization with increasing gross energy content of diets (or conversely decreasing P:E ratios) in the present study was however achieved at the expense of increasing carcass lipid content. The carcass lipid content of fish fed the optimal P:E ratio diet (diet 8) was 10.28% (wet basis), this is several times greater than the value obtained for wild snakehead (C. striata, 0.85%, wet basis) and polycultured C. striata (3.5% wet basis, Wee, 1981). The high fat content of the fish fed the test diets containing high levels of dietary lipid and dietary dextrin observed in the present study would be of some significant consequences when formulating practical diets for snakehead farming, since for culinary purposes, snakehead is favoured for its low fat flesh.

Therefore, the benefits of improved growth and better protein utilization of a high fat and/or carbohydrate diet over the low fat diet (at a constant dietary protein level) need to be balanced by the acceptability of the consequently fatter fish produced, the difficulty in the mechanics of extruding and pelleting rations containing more than 18% fat (Jauncey, 1979) and also the increased risks of oxidative rancidity of the dietary lipid within the diets during prolonged storage. The risk of oxidative rancidity of the dietary lipids however, can be reduced

by the incorporation of saturated fat into the diets. Saturated animal fats (lard) have successfully replaced normal supplement of fish oil without any growth depressing effects in channel catfish (Andrews, 1978). Yu et al (1977), reported that 50% of the fish oil, in a diet containing 22% dietary lipid, can be successfully replaced by swine fat (lard) for rainbow trout with no apparent ill effects.

Some general conclusions can be drawn from the findings in the present study. Based on the percent weight gain and PER and apparent NPU, it would appear that both dietary lipid and dietary carbohydrate (white dextrin) can spare protein for growth. It was shown that increasing the concentrations of dietary lipid at each of the three dietary dextrin levels tested and the increasing concentration of the dietary dextrin at each of the three dietary lipid level tested both resulted in improved growth and protein utilization although at the expense of increasing carcass lipid content. Maximal growth and protein utilization was achieved in fish fed diet containing 45% dietary protein, 20% dietary lipid (1:1 cod liver oil and corn oil) and 10% dietary dextrin at a P:E ratio of 89 mg protein / kcal energy. Fish fed this ration were adjudged to have reached the optimum P:E ratio for maximal growth, as a further increase in the gross energy content did not result in the improvement of growth or protein utilization despite a higher feed intake.

CHAPTER 4

DESCRIPTION OF DISEASE PROBLEMS ASSOCIATED WITH TRANSLOCATION OF JUVENILE SNAKEHEADS

4.1 INTRODUCTION

There are very few reports on the diseases of snakeheads, apart from those reports of the incidence of parasitic infestations in routine screening exercises on wild snakeheads (Wee, 1982). As the snakehead culture industry progresses and operations become more capital intensive, so it becomes more imperative that information is available concerning the disease problems which occur so that they may be identified and rectified where possible. This chapter reports on the disease problems encountered during quarantine procedures and subsequent screening exercises when monitoring the condition of the stock fish for the principal experiments described in the previous pages. These problems were invariably associated with transportation, overcrowding or poor water quality. Under normal experimental circumstances, the fish remained remarkably healthy.

4.2 MATERIALS AND METHODS

4.2.1 Fish

The experimental fish used for the studies detailed in Chapter 3 were obtained directly from fish dealers and exporters in Hong Kong and Malaysia or else were obtained from British live fish importers who brought them from Thailand for sale as aquarium species. Full details of the different batches acquired, their country of origin and the methods and duration of transportation are presented in Table 4.1.

Table 4.1 Clinical history of the batches of fish acquired for experimentation

Batch	Source	Species	Country of origin	Mode of transport (No. of hours of journey)	Time of year at importation	Packing density*1	Size of of fish (cm)
CM1	The London Catfish Centre, London	Channa micropeltes	Thailand	Rail from London (8 hrs)	November	200 fish in two plastic bags	3 - 5
CS1	The London Catfish Centre, London	Channa striata	Thailand	Rail from London (8 hrs)	February	500 fish in four plastic bags	2 - 3
CMA	Leu Keung Aquarium, Hong Kong	Channa maculatus	Hong Kong	Air freighted direct from Hong Kong (24 hrs)	June	500 fish in four plastic bags	2 - 3
CS2	Ng Aquatic Farm, Malaysia	Channa striata	Malaysia	Air freighted direct from Malaysia (24 hrs)	July	500 fish in four plastic bags	1 - 2
CM2	Cliff Murray Ltd Glasgow	Channa micropeltes	Thailand	Collected in van from Glasgow (1 hr)	August	16 fish in one plastic bag	10 - 15
CS3	Cliff Murray Ltd Glasgow	Channa striata	Thailand	Collected in van from Glasgow (1 hr)	August	50 fish in one plastic bag	5 - 6

*1 all the plastic bags used to transport fish were of 12 litre in volume

4.2.2 Quarantine procedures

In view of the major losses experienced by importers and experience over some years at Stirling with introduction of tropical fish from overseas, a very closely monitored quarantine procedure was developed for new batches of snakeheads. The holding tanks for quarantine consisted of 220 litre rectangular glass tanks. These were always sterilised with an iodophor disinfectant (1% FAM, Evans Vanodine International Ltd., Manchester, England) prior to use. Clean freshwater was provided, maintained at 28°C ± 1°C and kept aerated continuously. The water quality was maintained by the use of Eheim recirculating filters with freshly cleaned and sterile filter medium for each batch of fish. On arrival at the Institute of Aquaculture, the fish were allowed to acclimatize to the temperature of the holding tanks by immersing the bag holding the fish in the tanks for three hours, after which time, the fish were released into the holding tanks.

Sample fish were necropsied for screening for parasites by microscopic examination of skin and gill smears. This was initiated within 24 hours of arrival and subsequent screening exercises were performed at regular intervals and whenever the fish showed signs of distress. Prophylactic treatments were not always automatically administered with every batch of fish on arrival, since they are in themselves traumatic. The timing for such treatments was decided after an evaluation of the condition of the fish and the screening exercise had been carried out. It was not known whether any prophylactic treatments were given prior

to transportation in the country of origin.

4.2.3 Screening procedures

The screening procedures for parasites, which were carried out during quarantine and also regularly throughout experiments, consisted of microscopic examination of wet smear preparations of skin and gill of sacrificed specimens. Sample fish were killed by a cut through the cranium. Skin smears were prepared by scraping of the skin and fins, gill smears were prepared from primary gill lamellar squashes. The preparations in wet mount form, with a cover glass, were examined under light microscopy using direct illumination.

4.2.4 Sampling for Bacteriology and Virology

Where a microbial infection was suspected, samples were taken aseptically for bacteriological or virological examinations as follows: Bacteriology: Lesions or renal vascular tissue (for suspected septicaemias) were seared by means of a heated spatula and then a sterile inoculating loop introduced into the tissue. Samples of the lesion material or of renal blood were then inoculated onto triple soy agar or nutrient agar plates and incubated at 25°C for up to one week. Suspected colonies were subcultured and Gram smears, as well as biochemical analysis carried out to determine their taxonomic identity.

Virology: Since no tissue culture cell lines for channids exist, primary C. micropeltes cell culture, or RTG - 2 cell lines

(rainbow trout gonad - 2: Wolf and Quimby, 1962)were used. Suspected material was ground up with sterile sand in a sterile pestle and mortar, suspended in tissue culture medium, filtered through a Millpore filter and layered over the tissue culture maintained in a plastic tissue culture flask which was then incubated at 20°C with Eagles medium overlay, for up to two weeks. Tissue cultures were examined daily, and subculture carried out of any suspected flasks. (This virological work was carried out by Professor R.H. Johnson, Visiting Professor, University of Queensland, Australia.)

4.2.5 Histological Method

Fish tissue samples were cut up into small pieces measuring less than 1 cm by 1 cm. These tissues were fixed in 10% buffered formalin solution, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin for examination under light microscopy.

4.2.6 Treatments

Appropriate treatments were administered where indicated as recommended by Hoffman and Meyer (1974). The treatments include dip treatments in formalin solution or in a mixed malachite green: formalin solution or else in continuous methylene blue baths. Full details of the dosage and exposure times are presented in Table 4.2.

Table 4.2 Summary of the findings from screening procedures during quarantine and regular monitoring of the condition of stock fish and treatments administered

Species	Source	Country of origin	Days after arrival	Screening finding	Treatment administered	Efficacy
Batch CM1 *Channa micropeltes*	London Catfish Centre	Thailand	1	*Ichthyobodo* sp.; *Chilodonella* sp.; *Trichodina* sp.; *Ichthyopthirius multifilis* - on skin and gill	125 ppm 40% formalin bath for 30 minutes repeated every other day for a week	successful for all the ectoparasites except *I. multifilis*
			3	heavy infection of *I. multifilis* on skin and gill	- 25 ppm malachite green / 40% formalin solution (14g in 4 l) bath for 2 hours repeated every other day for a week	both treatments were successful
					- permanent bath of methylene blue at 0.3 c.c (1% methylene blue stock solution) per litre tank water for 3 weeks	
Batch CS1 *Channa striata*	London Catfish Centre	Thailand	5	heavy infection of *I. multifilis* on skin and gill	permanent bath of methylene blue at 0.3 c.c (1% methylene blue stock solution) per litre tank water for 3 weeks	successful

Table 4.2 (cont.)

Species	Source	Country of origin	Days after arrival	Screening findings	Treatment administered	Efficacy
Batch CMA *Channa maculatus*	Direct from Hong Kong	Hong Kong	1	*Gyrodactylus* sp.; *Trichodina* sp.; *Chilodonella* sp.; *I. multifilis*	125 ppm 40 % formalin bath for 30 minutes repeated every other day for a week	not successful
					150 ppm 40 % formalin bath for 30 minutes repeated every other day for a week	successful for all the ectoparasites except for *I. multifilis*
				heavy infection of *I. multifilis* on skin and gill	permanent methylene blue bath at 0.3 c.c (1% methylene blue solution) per litre tank water for 3 weeks	successful
			28	myxosporidian infection in the interstitial tissue of trunk kidney subsequently identified as *Thellohanellus* sp.	-	-

4.3 RESULTS

The findings of the screening procedures carried out during quarantine and subsequent monitoring exercises and treatments administered are summarised and presented in Table 4.2. However, despite the screening exercises and efforts to maintain adequate husbandry standards, outbreaks of disease occasionally occurred. The different kinds of infections observed are presented as follows:

4.3.1 Parasitic infections

Results presented in this subsection include the findings of routine screening exercises during quarantine as well as major outbreaks of parasitic diseases which were all mainly ectoparasitic infections. These infections always occurred very soon after arrival at the Institute of Aquaculture, for example, in two of the batches of fish (batch CM1 and CMA) the infection were observed within 24 hours of arrival and in batch CS1 after five days.

Ectoparasitic infection by Ichthyobodo sp., Chilodonella sp., and Trichodina sp. were observed in C. micropeltes (batch CM1). The ectoparasites were mainly found on the gills of infected fish and to a lesser extent on the skin. Although at the time of sampling for screening procedures there were significant mortalities (approximately 20% of a total of 200 fish), it was not possible to ascertain with certainty whether all of the deaths could be ascribed to the severe ectoparasitic infection or if many were associated with the stress of the translocation procedures. Nevertheless,

treatments were administered and the infections were successfully controlled by dip treatments in 125 ppm 40% formalin for 30 minutes, once a day for three days.

Gyrodactylus sp. infection was recorded only from C. maculatus (batch CMA from Hong Kong). There was an approximately 20% mortality, out of 500 fish, within 24 hours of arrival. Skin and gill smear preparations examinations revealed heavy infection by Gyrodactylus sp. and low level infection of Trichodina sp., Chilodonella sp. and Ichthyopthirius multifiliis. Dip treatment in 125 ppm 40% formalin for 30 minutes once a day was initiated. After three days of treatment, Trichodina sp., Chilodonella sp. and I. multifiliis infection were controlled. Mortalities were however, still very high and subsequent examination of skin and gill smears showed that the infection by Gyrodactylus sp. was not alleviated. The dosage of the dip treatment was then increased to 150 ppm for 30 minutes. This had therapeutic effect and the infection was controlled within three days. The mortality rates (number of dead fish out of 100 fish treated) for the duration of the treatment is given in Table 4.3.

Infections of I. multifilis were observed in three species of snakeheads in batches CM1, CS1 and CMA (C. micropeltes, C. striata and C. maculatus respectively). The infections occurred within 3 - 8 days of arrival (Table 4.2). The infection in C. micropeltes (batch CM1) was successfully controlled by either one of two treatments: 1) a short term bath treatment in 25 ppm malachite green / formalin solution (1.84 g malachite green in

Table 4.3 Mortality rate during dip treatment of Channa maculatus for Gyrodactylus spp. infection (values expressed are number of dead fish per 100 fish treated).

Treatment	Treatment period (days)				
	1	2	3	4	5
125 ppm 40 % formalin for 30 minutes	2	10	20	-	-
150 ppm 40 % formalin for 30 minutes	20	7	2	0	0

500 ml 40% formalin) for two hours, every other day for a week.

2) a continuous bath in methylene blue (at a dosage of 0.3 ml 1% methylene blue (weight / volume) stock solution per litre tank water) with regular change of freshly prepared 'medicated' water, for three weeks.

The I. multifilis infection in C. striata (batch CS1) was successfully controlled by the continuous methylene blue bath treatment using similar dosage and exposure times as for the treatment in batch CM1 described previously.

Stock C. maculatus recovering from the effects of ectoparasitic infections by Ichthyobodo sp., Trichodina sp., and Chilodonella sp. (described previously) had low level of I. multifilis infection, therefore a prophylactic treatment of continuous methylene blue bath (at a dosage of 1.5 ml of 1% stock methylene blue solution per litre tank water) was administered. The fish appeared healthy and fed very well on frozen earthworms while under treatment. However, on the ninth day of the treatment, there were high mortalities (35 fish out of 300 fish) and examination of skin and gill smears revealed that the fish were very heavily infected with I. multifilis at all stages of the life cycle (Plate 4.1), histological examination of the head and gill sections confirmed the results of the skin and gill smear examinations (Plate 4.2). A short term bath of 25 ppm malachite green / formalin solution (1.84 g malachite green in 500 ml 40% formalin) for two hours was started. The treatment was repeated every other day for a week, the fish being transferred to clean

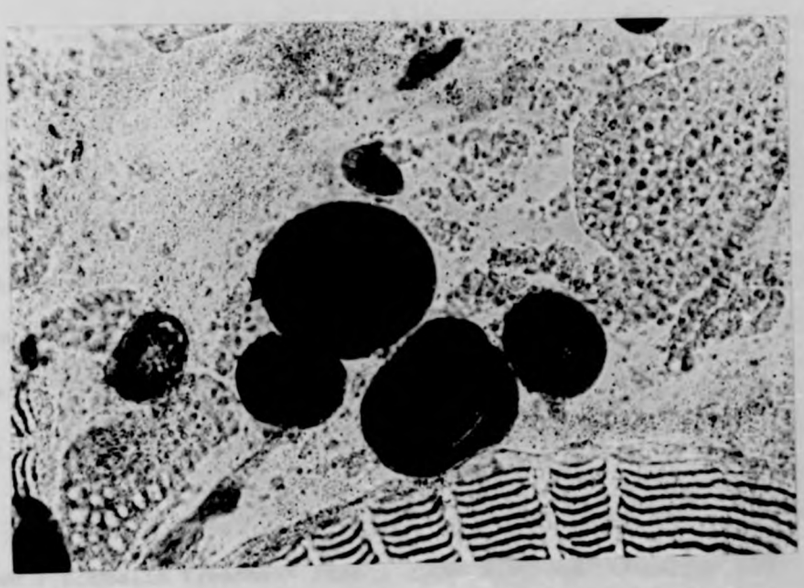

Plate 4.1 Ichthyopthirius multifilis infection on Channa maculatus, demonstrating the protozoa with typical horse shoe nucleus

(x 80)

Plate 4.2 Ichthyopthirius multifilis encysted within the mucosa of the buccal cavity of Channa maculatus . Note the horse shoe shape nucleus.

(x 80)

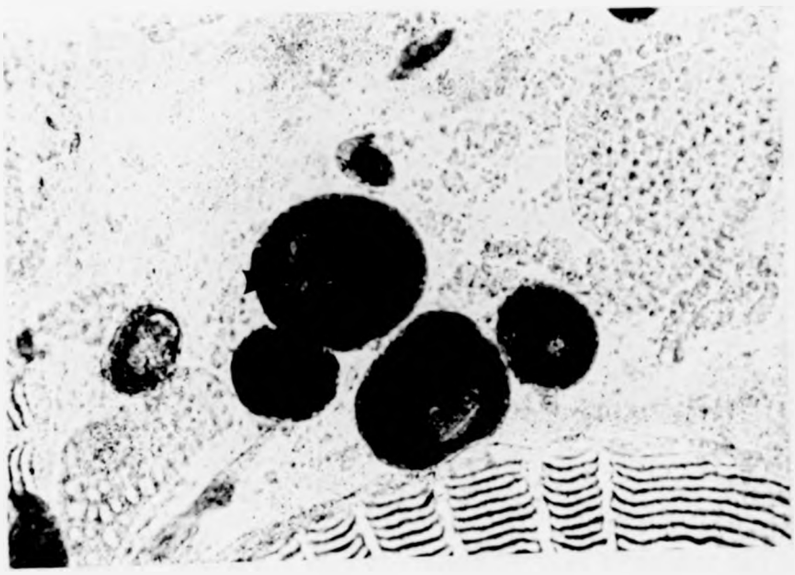

Plate 4.1 Ichthyopthirius multifilis infection on Channa maculatus, demonstrating the protozoa with typical horse shoe nucleus

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(x 80)

sterilised tanks after each bath. Out of a total of 100 fish treated in this manner, 51 died during the duration of the treatment (the majority succumbed in the first two days after treatment, 27 in day 1 and 17 in day 2). The infection was apparently under control after a week of treatment as there was no mortality recorded, however the remainder of the fish were observed to have 'white spots' (trophont stage of the life cycle of I. multifilis) on their body and fins.

It was postulated that the effects of the malachite green / formalin treatment itself might have been traumatic and may even play a contributory role in the mortalities, especially to very stricken fish. Thus a short term study was initiated to evaluate the effects and efficacy of other treatments. It was decided to treat 10 fish with short term baths of 200 ppm 40% formalin for two hours, repeated every day for eight days, with the treated fish transferred to clean sterilised tanks; a similar number of fish (10) in a continuous bath of methylene blue (0.3 ml of 1% methylene blue stock solution per litre tank water) and a control group of 10 fish were left untreated. The mortality rates for the three groups of fish are presented in Table 4.4.

In the control group, where the fish were not treated, there was 100% mortality after nine days. The short term bath treatment with formalin was not effective, it was repeated for eight days during which time there were seven mortalities. Examination of skin and gill smears showed that even after eight

Table 4.4 Mortalities observed during treatments for *Ichthyopthirius multifilis* infection in *Channa maculatus**1 (number of dead fish)

Treatment	Days of treatment										
	1	2	3	4	5	6	7	8	9	10	(11 - 21)
200 ppm 40 % formalin bath for 2 hours, repeated every other day for a week	0	1	1	1	1	1	1	1	1	1	0
Long term continous methylene blue bath for 3 weeks	0	0	0	0	0	0	1	1	1	0	0
Control (untreated)	1	1	1	1	2	1	1	1	1	0	0

*1 for each of the treatments 10 fish were treated

days of repeated treatment, the fish were still very heavily infected. It is apparent that there was reinfection as the entire life cycle of I. multifilis lasts for less than five days at 28°C (Sommerville, pers. comm.).

On the other hand, the continuous methylene blue bath treatment was very effective in controlling the infection, there was no mortality until day 7 and the overall mortality rate after three weeks was three fish. At the start of the study, most fish were heavily infected with the parasite as reflected by the large numbers of 'white spots' which covered the whole of the body and fins. These trophonts started to disappear after 12 days in the continuous methylene blue bath and after 18 days of treatment, the trophonts were completely eliminated and subsequent skin and gill smear examinations showed that the infection had been controlled.

As mentioned in the preceding pages, C. maculatus fingerlings from Hong Kong (batch CMA) were seriously affected by ectoparasites which probably accounted for the very high mortalities recorded. In addition to the skin and gill smears, the kidneys of moribound fish were also examined. The interstitial tissue was squashed and examined, in wet mount form with cover glass, under light microscopy. It was found that 2 out of 50 fish examined harboured myxosporidian parasites.

Description of the parasite:

The spore was pyriform with a broadly rounded posterior and a tapered anterior end. The shell valve was uniformly thick with a prominent sutural ridge. The single polar capsule was

turned into 8 - 10 coils without touching the sides of the capsule wall. The wet preparations of the tissue containing the spores indicated a halo around the posterior end (Plate 4.3). Spores were observed to appear singularly or in groups of up to eight within the tissue. In most cases, there was a lot of dark pigments close to the parasites and often covering the parasites. Dried preparations were stained and mounted with PVLP-Iodine (polyvinyl lactophenol - Iodine). The polar filament was not extruded after this treatment and no iodinousphilous vacoule could be seen, but most of the spores were observed to be enclosed in a distinct sac or envelope attached to the posterior end which was stained brownish orange by PVLP-Iodine.

Dimensions of 20 spores measured in wet squash preparations:

	(mm)
Length of the spore	1.9 - 2.1
Width of the spore	1.0 - 1.1
Length of the polar capsule	0.8 - 0.9
Width of the polar capsule	0.45 - 0.6
Thickness of the sac	0.1
Length of the sac	2.2 - 2.9
Width of the sac	1.5 - 1.9

The parasite was subsequently identified as Thellohanellus spp. by virtue of its possession of a pyriformous spore with only one capsule (this identification was carried in conjunction with Dr. Elisabeth Degener , Hanover, West Germany).

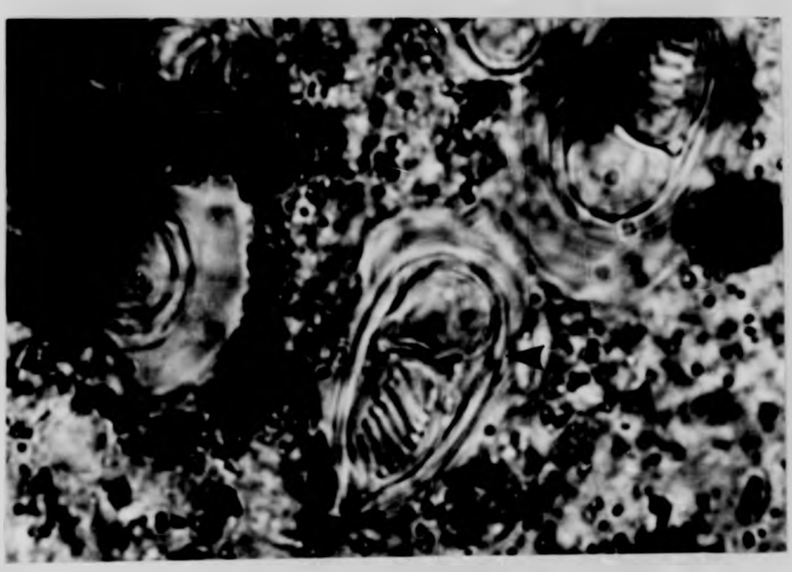

Plate 4.3 Thellohanellus spp. from the kidney of Channa maculatus

(x 80)

Plate 4.4 Putative viral infection in Channa striata

Note the symmetrical pattern of ulceration on the head

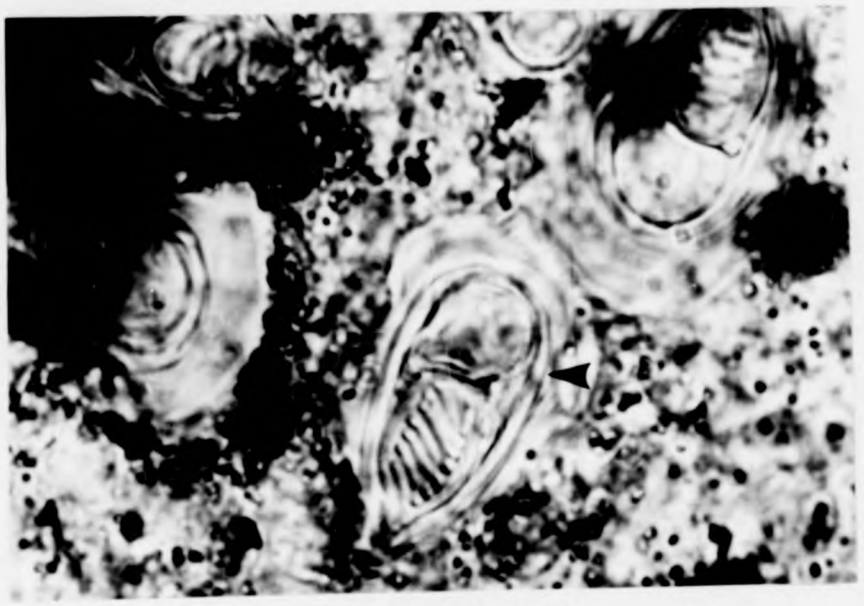

Plate 4.3 Thellohanellus spp. from the kidney of Channa maculatus

(x 80)

Plate 4.4 Putative viral infection in Channa striata

Note the symmetrical pattern of ulceration on the head

4.3.2 Bacterial infection

Bacterial infection was observed only in C. micropeltes (batch CM1). Fish from this batch suffered from severe ectoparasitic infections very soon after arrival, but these infections were successfully controlled and the stock fish recovered (section 4.3.1).

However, on the 24th day after arrival, evidence of a septicaemic infection was observed. Clinical signs observed included

1) Loss of appetite, lethargic movements and 'floating' on the surface, unresponsive to external stimuli.
2) Lesions on the tail region of the body, particularly around the caudal peduncle, caudal fin and the posterior parts of the dorsal fin.
3) Haemorrhages around the eye, head region and operculum.

Examination of wet preparations of the gill lamellae showed swollen secondary lamellae, necrosis of the lamellar epithelial cells and in some cases actual sloughing of the gill epithelium.

Inoculation of material from lesions and from renal blood from the infected fish onto triple soya agar plates produced profuse growth of a microorganism subsequently identified as Aeromonas hydrophila by the methods of Buchanan and Gibbons (1974).

Antibiotic treatment was initiated. As the fish were not feeding, it was not possible to administer the drug through

incorporation into the feed. The infected fish were therefore treated in permanent bath of the antibiotic Terramycin (Pfizer Ltd., Sandwich, Kent) at a level of 55 mg (containing 3 mg oxytetracycline hydrochloride as the active ingredient) per litre tank water. The bath was exchanged for freshly prepared medicated water every other day and the treatment lasted for a week. The bacterial infection was controlled with a drop in mortalities (less than 1% per day during the treatment).

4.3.3 Viral infection

C. striata (batch CS1) were acquired from the London Catfish Centre, London. Routine screening procedures on arrival revealed no evidence of any parasitic infection. However, by the fifth day, there were some mortalities and examination of skin and gill smears showed that there were heavy infection of I. multifilis which was successfully controlled by the continuous methylene blue bath treatment (section 4.3.1).

The stock fish were then housed in a recirculating system prior to use in the principal nutrition experiments. They were fed commercial trout rations (Trout grower, Edward Baker Ltd., Bathgate, Scotland). An unusual erosion of the skin was then observed five months later in approximately 20% of the fish, and detailed clinical pathological and microbiological examination carried out. The clinical signs observed were most unusual: small regular round ulcers were observed appearing in regular rows on the shield like upper part of the head. These ulcers appeared

to have occurred symmetrically on the upper surface as well as the underside along the lower jaw (Plate 4.4). In advanced conditions, the ulcers fused to form deep trenches. There was very little evidence of haemorhage and the affected fish were still feeding actively and there were no acute mortalities.

Histopathological examinations showed that the infected fish had encephalitis and nerve damage related most probably to erosion of nerve endings. There was also evidence of brain and liver focal necrosis (R.J. Roberts, pers. comm.). There was no evidence of general bacterial involvement although Aeromonas hydrophila was isolated on triple soya agar plates and the most likely cause was assumed to be a virus although attempts to isolate the causative virus were not successful (R.H. Johnson pers. comm.).

No attempts were made to treat for the infection, all infected fish were killed and the holding tanks completely sterilised with 1% FAM. No further infection was observed after this course of action.

4.4 DISCUSSION

From the findings of the screening procedures and the case histories presented (section 4.3, Table 4.2), it would appear that ectoparasitic infestations are most significant in the maintenance of snakehead fish stocks for experimentation. Ectoparasitic infestation of translocated fish stocks occurred very soon (normally within days) after arrival at the Institute of

Aquaculture. Ectoparasites such as Ichthyobodo sp., Chilodonella sp. and Trichodina sp. were commonly found in C. micropeltes and C. maculatus and I. multifilis commonly found in all three species of snakeheads acquired (including C. striata). While Gyrodactylus sp. was only found in C. maculatus.

The high mortalities observed very soon after arrival of the batches of snakeheads were postulated to be in part due to the stress from handling and transportation and temperature changes (in particular those batches from the Far East where they were transported over long distances and the temperature change was drastic) and in part due to the ectoparasitic infections.

It is no doubt highly significant that the size of fish and the packing density used in transferring fish may affect the survival rate. For example, fish in batch CM2 and CS2 were fairly large fish of between 10 - 15 cm and 5 - 6 cm in size respectively, packed at a lower density than in other batches had no ectoparasitic problems (Table 4.1). Thus, it may be that it would be more beneficial to transport small numbers of large fish than a large number of small fish. It was not possible to ascertain whether prophylactic treatments were given prior to the transfer of the fish, it is however, obvious from the findings in this chapter that providing such treatments would be of significant advantage. The timing of the transfer of fish stocks is also important, particularly of tropical fish stocks. It is of obvious advantage to transport fish at times when the prevailing air temperature lies within the range of temperature that the fish inhabits. In the case of

tropical fish, the spring and summer would be the ideal season for the transport of fish stocks to temperate countries, as the temperature changes would be less drastic. However, the availability of fish fry may complicate matters as it may not always be possible to obtain fry at these times of the year.

The ectoparasites Ichthyobodo sp., Chilodonella sp. and Trichodina sp. can as has been shown be successfully treated by a dip treatment in 125 ppm 40% formalin for 30 minutes, repeated once a day for three days. However, a higher level of treatment (150 ppm 40% formalin for 30 minutes, repeated once a day for five days) was more effective in controlling Gyrodactylus sp.. These dip treatments were however not effective in controlling the I. multifilis infections. A short term bath of malachite green / formalin or a long term continuous methylene blue bath were used successfully to control I. multifilis infections (Table 4.2).

However, the effects of the treatments themselves need to be investigated in greater depth, it is quite possible that the treatments themselves were traumatic to a degree i.e. the treatment may actually have brought forward the death of the already weakened infected fish or it may have injured the fish during the process of treating (particularly the dip or short term bath treatments where the fish were subjected to frequent netting and handling) and thereby exposing them to possible secondary infections by bacteria and fungus. The bacterial infection in C. micropeltes (bath CM1) appeared most likely to have been a secondary infection brought on by the traumatic effects of handling during the treatment

for ectoparasites. As such, the long term continuous methylene blue bath may be preferable to the malachite green / formalin short term bath treatment for controlling I. multifilis by virtue of less handling and lesser risks of reinfection.

The myxosporidian Thelohanellus sp. was found to infest C. maculatus although the level of infection detected was not likely to cause any mortalities. However, in intensive culture conditions in earthen ponds, there is an increased likelihood that the infection could be critical.

Similar ectoparasites such as those described in this chapter have been reported to infest wild snakeheads in Thailand (S. Chinabut pers. comm.) and Malaysia (S. Mohamad pers. comm.). Therefore, the results of these incidental studies on the disease problems associated with the translocation of juvenile snakeheads illustrate the harmful effects of stresses induced by the translocation processes. It is highly probable that the high mortalities of fry early in the production cycle experienced in C. striata farms in Thailand (Chapter 2) are caused by the combined effects of stress induced by the translocation processes and the subsequent ectoparasitic and bacterial infections.

CHAPTER 5

FINAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

FINAL CONCLUSIONS

It is clear from the results of the field survey of C. striata farming in Thailand that the snakehead farming industry is experiencing difficulties which could seriously undermine the production rates and profits of the farms. These difficulties or problems are affected by several factors which can be broadly classified into two categories:

1) Technological and Management factors;
   These include problems associated with the use of trash fish as feed, extreme stocking densities and stocking practices employed, supply of seed fish and disease and water quality deteriorations.

2) Market and Economic factors;
   These include fluctuating farm gate selling price and increases in the cost of trash fish.

The three most important factors affecting the profitability of C. striata farming appear to be the high mortalities experienced at an early stage of the production cycle, the disproportionate increases in the price of trash fish in the recent years and the fluctuating farm gate selling price.

The high mortalities experienced during the first two months of production could be attributed to the combined effects of stress from the translocation of fry during stocking procedures and the poor husbandry techniques employed prior and after

stocking. This is wasteful in terms of seed fish resources and in economic terms as feed cost will have been incurred during the two months of growth.

The farm gate selling price is dependent on the wholesale price at auction sales in the Central Fish Market for distribution (Bangkok), which is in turn governed by the supply of wild C. striata from natural production and to a lesser extent on the production from the culture industry. The natural production of wild C. striata is affected by a number of factors including the unpredictable onset of monsoon and the amount of rainfall and flooding during the monsoon. A large supply encourages competition and subsequent low wholesale and retail prices and the corresponding low farm gate selling price. The sensitivity of the level of profits of a C. striata farm (C. striata production unit, Palurak farm) was examined by analysing the net returns of a production in 1979-80 with changes in farm gate selling price. There was a net loss of 0.99 ฿ / kg fish produced when the average farm gate selling price was 32 ฿ / kg. By extrapolation, a 20% increase in the farm gate selling price to 38.4 ฿ / kg would have improved the net profits by six and a half fold to 5.41 ฿ / kg profit.

The cost of trash fish is the most important cost item of the total operating costs; accounting for up to 67.53% in the 1979-80 production, thereafter any fluctuation would have significant impact on the profits. This was illustrated by analysing the net returns for the 1980 crop from the C. striata production unit,

Palurak Farm, where it was found that a 20% increase in the price of trash fish would have increased the total net loss by over three fold to 4.51 ฿ / kg produced.

However, it should be pointed out that the net profits of each production also depended on the level of technological achievement (i.e. obtaining high production rates with good feed conversion efficiency and low mortalities). For example, in 1980-81 in the C. striata production unit at Palurak Farm, high mortalities and poor feed conversion ratios (FCR values obtained averaged 8.5 : 1 as compared to 6.5 : 1 in the 1980 production) were experienced. This coupled with the extended raising period to more than 12 months increased the feed costs to 85.82% of the total operating costs and incurring a net loss of 19.5 ฿ / kg fish produced. With this qualification, extrapolation of the net returns by a 20% increase in average farm gate selling price or a 20% increase in the cost of trash fish would have reduced the net loss to 12.21 ฿ / kg or incurring a higher net loss to 26.61 ฿ / kg produced respectively.

Technological and management problems can potentially be solved by corrective measures. These consist of modifications of current culture and management practices as well as implementation of new procedures as described in detail in Chapter 2. The marketing and economic factors are more problematical mainly because these are governed by forces which are beyond the control of the farmers.

With the reduction in the supply of trash fish from

diminishing marine fish stocks and increased competition from fish meal producers, the price of trash fish is likely to continue to increase. Therefore, as a short term strategy, there is an urgent need to seek alternative to replace trash fish as feed. The use of dry pelleted feeds is the most obvious answer. Advantages of dry pelleted diets as feed include reliability and uniformity of supply, ease of feeding and storage and the maintenance of water quality. More importantly, it has been shown with other fish species that very good FCR values of between 1 - 1.5 can be achieved with dry pelleted feed, thus reducing feed cost when compared to using trash fish as feed for the same production rate. Sensitivity analysis of the net returns of a single farm (C. striata production cycle, Palurak Farm) when using dry pelleted feeds indicate potential economic gains, results showed a more than five fold increase in the net profits for the 1980 and 1981 productions. However, at present owing to the lack of information and proven performance of C. striata on this type of feed, there is still an almost absolute requirement for trash fish as feed. Furthermore there is no such feed available which is formulated specifically for snakeheads and almost no information on the basic nutritional requirements of snakeheads.

Therefore, as a first step to the overall concept of formulating a least cost well balanced practical diets for use in snakehead farming industry, it has been the principal aim of this study to secure basic information on nutritional requirements. Such information was derived from results of experimental feeding trials designed to monitor the growth responses of fish fed

semi-purified diets over a prolonged period.

It was shown in Experiment 1 that C. micropeltes and C. striata juveniles, as expected for carnivorous species, have high dietary protein requirements, approximately 52% and 46% of the diet respectively, for maximal growth. These values are comparable with those obtained for other carnivorous fish species (Table 3.1.1). Similarly, the quantitative essential amino acid requirements for C. striata and C. micropeltes juveniles determined in Experiment 2 are comparable with the requirements for other fish species, with the exception of the amino acids methionine and phenylalanine (Table 3.2.5). The effects of dietary essential fatty acid intake on growth and fatty acid composition of the liver and muscle were investigated with C. micropeltes in Experiment 3. Results showed that C. micropeltes juveniles are not very demanding in their essential fatty acid requirements during the on-growing phase. Fish fed either dietary linoleic acid, a combination of linolenic and linoleic acids or cod liver oil all grew equally well. Analysis of the fatty acid composition of liver and muscle indicate that C. micropeltes juveniles possess some capacity to chain elongate and desaturate dietary linolenic acid producing long chain (n-3) polysaturated fatty acids but seem unable to similarly modify linoleic acid. Dietary lipid, and to a lesser extent dietary carbohydrate, were shown to spare protein for growth in C. striata in Experiment 4. However, the improvements in growth and feed conversion efficiency with increasing dietary lipid concentrations at constant dietary protein level, were achieved at the expense of fattier fish carcass. The optimum dietary

protein to gross energy (P:E) ratio, within the diets, for maximal growth of C. striata ranged from 89 mg protein / kcal (Experiment 1) to 98 mg protein / kcal (Experiment 4) and for C micropeltes was 114 mg protein / kcal (Experiment 1). When compared with the optimal P:E ratio for maximal growth obtained for other fish, it is clear that the values for snakeheads obtained in the present studies are comparable with those of carnivorous species (Table 3.4.9).

In conclusion, it would appear that the nutritional requirements of snakeheads are similar to that of carnivorous fish species, for example rainbow trout, in that they require high levels of dietary protein, a high P:E ratio for optimum growth and exhibit a requirement for dietary linolenic acid.

On the basis of the nutritional studies conducted with C. striata and C. micropeltes a practical diet, using locally available ingredients (in South East Asia), was formulated for the snakehead farming industry. The proximate composition of the ingredients used and the composition of the practical diet formulated are shown in Tables 5.1 and 5.2 respectively. With knowledge of only the basic nutritional requirements of these species, the formulation of feeds is, out of necessity, fairly simplistic in nature. As can be seen from Table 5.2, the diet contains a relatively high proportion of fish meal (45%, by weight). It is necessary, at this early stage in diet development, to include such high levels of fish meal (or animal protein) so as to provide an adequate supply and balance of essential amino acids and to ensure that the diet is palatable. It may be possible in future to reduce

Table 5.1 Proximate composition of dietary ingredients for use in a Snakehead diet formulation *1

(all values are expressed as % by weight)

Ingredient	Moisture	Crude protein	Crude fat	Crude fibre	Ash	Nitrogen free extracts
Fish meal (Thailand)	8.0	65.0	10.0	0.8	9.6	6.6
Soyabean meal, solvent extracted (China)	12.0	45.0	2.0	7.0	6.0	28.0
Meat and bone meal	6.5	47.0	14.5	0.2	30.0	1.8
Blood meal	10.7	80.0	1.0	1.0	4.4	2.9
Rice bran	13.0	15.0	1.0	13.0	16.0	42.0
Wheat middling	12.0	15.0	3.6	6.0	6.0	57.0

*1 The proximate composition data for these ingredients were provided by Sahapatana Kaset Company Limited, Pathunam Pra-ni, Ayuddha, Bangkok.

Table 5.2 Composition of a proposed practical Snakehead ration

(all values are expressed as % by weight)

Ingredient	Content (wet basis)
Fish meal	45
Soybean meal	15
Meat and bone meal	15
Blood meal	5
Rice bran	5
Wheat middling	5
Soy oil / fish oil (1 : 1)	8
Binder *1	1
Vitamin and mineral premix *2	1

Component	Nutrient content (dry basis)
Crude protein	44.4
Crude lipid	14.73
Calculated available carbohydrate	9.6
Gross energy *3 (kcal / 100g)	431.42
Protein to energy ratio (mg protein/kcal)	102.9

*1 Basfin (Behn Meyer (Singapore) Pte Ltd, Singapore)

*2 As supplied for the experimental diets in Experiment 3 (Table 3.3.1) with the addition of the anti-oxidant BHT (Butylated Hydroxytoluene) at 200 mg/kg and a mould inhibitor.

*3 Calculated on an estimated 5.7 kcal / g protein; 9.5 kcal/g fat and 4.0 kcal/g carbohydrate

the amounts of fish meal by isonitrogenous substitution with alternative, possibly lower cost, protein sources such as soybean meal. However, this can only be carried out after proper evaluation of the utilization of such materials as feedstuff by snakeheads. It may also be possible to replace some of the dietary energy sources employed with cheaper materials such as saturated animal fats for example lard and beef tallow.

In order to estimate the cost of the production of this feed, a simple economic analysis (based on current prices of raw materials) was performed. Details of the costs of raw materials (available in Thailand) are presented in Table 5.3. The total costs of raw materials amount to ฿ 9,200 / metric tonne. As a general rule, an additional 15% on top of the total raw materials costs should be included as an overhead incurred by the cost of production (pers. comm., K. Koh, Area Sales Manager, CPM (California Pellet Mill) Pte. Ltd., Singapore), giving a final cost of ฿ 10,580 / metric tonne or 10.58 ฿ / kg feed. (1982)

However, it should be pointed out that it is uneconomical to enter into 'in-house' independent feed production unless the feed requirements are more than 100 metric tonnes per month (K. Koh, pers. comm.) because of high capital and overhead costs and high dead time (time when the processing machine is not in use). However, an established feed manufacturer could, due to the scale of production, have lower overhead charges, produce the snakehead feed specified in the formulation. This would significantly reduce the cost of producing snakehead feeds thus making them available

Table 5.3 Cost of dietary ingredients and the estimated cost of Snakehead feed production *1

Ingredient	Amount required in feed (kg / metric tonne feed)	Price (฿ / kg)	Cost (฿ / metric tonne feed)
Fish meal	450	10	4,500
Soybean meal	150	8	1,200
Meat and bone meal	150	6	900
Blood meal	50	6	300
Rice bran	50	3	150
Wheat middling	50	3	150
Soy oil	40	22	880
Fish oil	40	10	400
Basfin *2	10	22	220
Vitamin and mineral premix *3	10	50	500
Total			9,200

*1 all prices for the dietary ingredients were provided by Sahapatana Kaset Company Limited, Pathunam Pra-ni, Ayuddha, Bangkok (except for *2 and *3)

*2 price for Basfin was from Behn Meyer (Singapore) PTE Ltd, Singapore

*3 price for the vitamin and mineral premix was estimated by Mr. Roy van der Pol, Roche Food Products Ltd, Dunstable , England.

at a price comparable with that currently quoted for feeds for Clarias catfish at 7 ฿ / kg.

Incidental studies were carried out on the disease problems associated with translocation of juvenile snakeheads from the tropics for experimentation. Despite the rigorous quarantine and husbandry procedures taken, high mortalities were experienced especially very soon after arrival in the Institute of Aquaculture. It was postulated that the high mortality rates were caused directly by the traumatic effects of the stress induced by the translocation process or by the attacks of ecto-parasites on fish weakened by the induced stress. Successful treatments were developed to control these ectoparasitic infections. In view of the high mortalities experienced in farm conditions early in the production cycle (Chapter 2), the results derived from these studies will contribute to the better understanding and development of a better stocking procedure.

SUGGESTIONS FOR FUTURE WORK

The experimental diets formulated in the present study contained a high proportion of fish meal as the protein source and since, as mentioned previously, fish meal is expensive and in short supply, it would be beneficial to investigate whether it is possible to replace it with other, cheaper, protein sources for example, soybean meal. It has been shown that growth rate, feed efficiency and mortality were not adversely affected by reducing the level of herring meal from 35% to 18%, and increasing the level of soybean meal from 10% to 39%, in an open formula dry-pelleted diet fed to rainbow trout (Cho et al, 1974).

In the present study, it was also shown that dietary lipid can effectively spare dietary protein for growth, therefore it would be useful to consider increasing the energy content of the diet by replacing the protein component with dietary lipid. Cheap animal fats such as lard and beef tallow have been used successfully as dietary lipid for channel catfish (Dupree, 1969., Andrews, 1978). In view of the fact that C. micropeltes are not demanding in their EFA requirements during the on growing phase, the use of such saturated animal fat for snakeheads should also be considered.

Growth performances of snakeheads fed experimental diets in the present study were all obtained under laboratory conditions; further investigations are thus necessary to evaluate the growth performances and subsequent production of snakeheads fed dry

pelleted rations in farm conditions. This would also allow for definitive calculation of the cost effectiveness of the use of dry pelleted feeds in snakehead farming.

It was postulated from the evidence obtained in the study that the stress induced during the transfer of seed fish for stocking greatly increased the vulnerability of the snakeheads to pathogens and this, coupled with the inadequate husbandry provided in most of the farms, was the direct cause for the high mortalities observed throughout the industry. In view of this finding, further investigations are required to develop a less stressful transfer system and better husbandry techniques. In particular, attention should be given to providing quarantine, a period for acclimatization and prophylactic treatment of seed fish prior to their release into the production ponds.

In view of the extremely high stocking rates used and the poor quality of the water resources in the farms, the effects of the metabolic waste buildup, in particular ammonia and nitrite levels, on growth need to be investigated. Although their air breathing capabilities may have allowed snakeheads to evolve a higher tolerance level to these water chemistry parameters, the sublethal levels attained could still have significant effects on the growth.

APPENDIX

Appendix 1.

Questionnaire used in the survey of C. striata farming in Thailand (Adapted from Shang , 1976)

A. General information

(1) Location of farm :
(2) Name and Address of respondent :
(3) Farm ownship :
Owned _____ Area (ha) _____

Leased from (private)
(Government) _____ Area (ha) __ annual rent ___

Duration of lease (yrs) _____ renewable : Yes ___ No _____

Percent of owner's income from pond _____

Source of other income _____

(4) Experience :
Years farm in operation _____ Years experience of operator _____

Where fishpond experience acquired _____

(5) Species cultured :

Major species _____
Minor species _____
Reason for choice of major species _____

(6) Pond area :

Nursery pond (s) _____ Area (ha) _____ Depth _____
Rearing pond(s) _____ Area (ha) _____ Depth _____

(7) Type of pond :
Excavated _____ Ditch & Levee _____ Other _____

B. Management

Growing period :
Water source: How water supplied: pump Other
Frequency of water change :
Days per year without cultured organisms in pond :
Reason for inactivity (specify) :
Is pond bottom dried : Frequency : Duration :

Appendix 1 (cont)

C. Stocking

(1) Stocking rate of rearing pond :

Species	Fry	Fingerling	No. of crops/yr.

(2) Factors influencing the quantity and frequency of purchase of fry/fingerling:

Price of fry / fingerling _____
Availability of fry _____
Anticipated supply of fry / fingerlings _____
Anticipated demand for species at harvest _____
Weather conditions _____ Others (specify) ____

(3) How is price of fry determined ?

prevailing price _____ bidding _____
dictated by seller _____ others _____
bargaining

(4) Source of stock :

Location _____ pick-up _____ delivered _____
distance travelled _____

D. Feeds

(1)

Types of feed	Feeding rate	Cost of feed

(2) Factors affecting the quantity and feeding techniques employed :

Price of feed _____
Stocking rate _____
Anticipated demand for crop at harvest _____
Others (specify) _____

(3) How knowledge of feeding technique acquired

self _____ extension agent _____
neighbours _____ reading _____
other _____

E. Labour

(1) Labour required for pond area

	Family	Caretaker	Hired	Others
pond preparation				
stocking				
feeding				
weeding				
repairing and maintenance				
harvest				
others (specify)				

Appendix 1 (cont)

(2) Payment

	Rate/man/day	Food	Share of cropping	other
caretaker				
hired				
others				

F. Harvesting

(1) production /ha/crop

Sale

	kg	price received / kg	Eaten	Given	Others
major species					
minor species					
others (specify)					

(2) How do you crop your stock ?
Section_____ Total_____ Other_____

(3) What cropping method do you use ?
Seining _____ gill net_____ pond draining _____
Others (specify)_____

(4) Number of harvests per crop ?_____

(5) Reason for harvesting schedule

To optimize production _____
Marketing strategy to get highest price _____
Availability of fry for restocking _____
Need for capital _____
Other (specify) _____

G. Mortality rate

(1) What is the mortality rate :

	Average	Highest	Lowest
From purchase to stocking			
From stocking to harvesting			

(2) What are the possible causes of mortality ?

Sudden change of weather____ Lack of proper food _____
Water quality (specify)____ Overstocking _____

H. Marketing

(1) Selling arrangement

	Pick-up or delivered	Marketing cost or commissions	Location of outlet place	distance
Direct sale				
Wholesale retail				
Cooperative sale				
contract				
consignment				
others (specify)				

(2) Method of payment

Cash _____

Credit _____ for how long _____ interest _____

Installment _____ How many _____

Others (specify) _____

I. Finance

(1) Need loan for

Expansion _____ purchase of ; fry _____
supplies _____
Repairs and maintenance _____ food _____

(2) Sources of loans

	Amount	Annual interest	Maturity	Purpose
Relatives				
Brokers				
Government				
Banks				
Others (specify)				

J. Problems and other information

(1) What problems are encountered in the industry ?

Unfavourable price structure _____ Lack of adequate infrastructure _____
Unavailability of credit _____ Shortage of fry _____
Unavailability of supplies _____ High price of supplies
Fuel : _____
Feed : _____
Unavailability of technical support _____

(2) Have you been reached by extension worker ? Yes No
If yes , what information was provided ?

(3) Do you attempt to improve your culture practices ? Yes No
If so , How ?

(4) What problems do you encounter when attempting to improve your culture practices ?

Appendix 2

Types of loans available to fish farmers from Bank for Agriculture and Agricultural Co-Operatives, Bangkok

(a) Types of loan available :

- personal loan
- group loan

(b) Collaterals required :

Collaterals are required for any loans, With personal guarantee, loans granted will be within the range of Baht 60,000, whilst with immovable assets, the amount granted will not exceed 50 % of the assessed amount of the assets.

(c) Interest Rates :

The rates will vary according to the size of the loans :

- less than Baht 300,000	:	14% per annum
- Baht 300,000 - less than 600,000	:	15% per annum
- in excess of 600,000	:	16% per annum

(based on 1982 rates)

(d) Repayment periods :

(1) long term loan , repayable within 5-15 years (average 7 years) during the early period of investment, only the interest are required, principal can be repaid within the contracted period.
(2) medium term loan, repayable within 3 years.
(3) short term loan, repayable within 1 year.

(e) Criteria for assessment of loan

The assessment is largely based on the productivity of each customer in the past years, this is because the Bank normally only grant loans for expansion purposes or as operating capital. Generally, the Bank is reluctant to grant loan for new establishment.

These notes were provided by courtesy of Mr. Pairote Varophas, Manager, The Thai Farmers Bank Limited , London Branch. 80 Cannon Street, London EC4N 6HH.

Appendix 3.

Calculation of Costs and Returns of C. striata production unit , Palurak Farm in response to changes in farm gate selling price . (all values are expressed in Bahts)

Item	Changes in farmgate selling price			
	20 % increase in farm gate selling price		20 % decrease in farm gate selling price	
	1 pond	2 pond	1 pond	2 pond
Variable costs *1	696,000	2,795,853	696,000	2,795,853
Fixed costs *2	130,460	132,724	103,460	132,724
Total costs	826,460	2,928,577	826,460	2,928,577
Gross revenues	961,920 @ 38.4 ฿/kg	2,008,999 @ 31.2 ฿/kg	641,280 @ 25.6 ฿/kg	1,339,332.8 @ 20.8 ฿/kg
Yield (kg)	25,050	64,391	25,050	64,391
Operating profits *3				
฿ / crop	265,920	-786,854	-54,720	-1,456,520
฿ / kg	10.62	-12.22	-2.18	-22.62
Net profit *3				
฿ / crop	135,460	-919,578	-185,180	-1,589,244
฿ / kg	5.41	-14.28	-7.39	-24.68
Cost of production ฿ / kg produced	32.99	45.48	32.99	45.48

*1 calculated as defined in Table 2.10

*2 calculated as defined in Table 2.9

*3 calculated as defined in Table 2.12

Appendix 4

Calculation of Costs and Returns of C. striata production unit , Palurak Farm , in response to changes in cost of trash fish (all values are expressed in Bahts)

Item	Changes in cost of trash fish			
	20 % increase in cost of trash fish		20 % decrease in cost of trash fish	
	1 pond	2 pond	1 pond	2 pond
Cost of trash fish	455,148 @ 2.24 ฿/kg	2,373,662 @ 2.98 ฿/kg	303,432 @ 3.36 ฿/kg	1,582,442 @ 4.48 ฿/kg
Total operating costs	675,858	2,805,828	524,142	2,014,608
Interest on total operating costs @ 16 % p.a.	108,137	448,933	83,863	322,327
Variable costs *1	783,995	3,254,761	608,005	2,336,945
Fixed costs *2	130,460	132,724	130,460	132,724
Total costs	914,455	3,387,485	738,465	2,469,669
Gross revenues*3	801,600	1,674,015	801,600	1,674,015
Yield (kg) *4	25,050	64,391	25,050	64,391
Operating profit*3 ฿ / crop 17,605	17,605	-1,580,746	193,595	-662,930
฿ / kg	0.70	-24.55	7.73	-10.30
Net profit *3				
฿ / crop	-112,855	-1,713,470	63,135	-795,654
฿ / kg	-4.51	-26.61	2.52	-12.36
Cost of production ฿ / kg produced	36.51	52.61	29.48	38.35

*1 calculated as defined in Table 2.10

*2 calculated as defined in Table 2.9

*3 calculated as dedined in Table 2.12

*4 as presented in Table 2.3

Appendix 5

Calculation of Costs and Returns of C. striata production unit , Palurak Farm , using dry pelleted feeds . (all values are expressed in Bahts)

Item	Operations	
	1 pond	2 pond
Feed cost @ 7 ฿/kg	263,025	676,106
Total operating costs	457,858	1,017,826
Interest on total operating costs at 16 % p.a.	73,257	162,852
Variable costs *1	531,115	1,180,678
Fixed costs *2	130,460	132,724
Total costs	661,575	1,313,402
Gross revenues *3	801,600	1,674,015
Yield *4	25,050	64,391
Operating profit *3		
฿ / crop	270,485	493,338
฿ /kg	10.8	7.66
Net profit *3		
฿ / crop	140,025	360,614
฿ / kg	5.59	5.6
Cost of production		
฿ / kg produced	26.41	20.40

*1 calculated as defined in Table 2.10

*2 calculated as defined in Table 2.9

*3 calculated as defined in Table 2.12

*4 as presented in Table 2.3

REFERENCES

ADRON J.W., BLAIR A., COWEY C.B., SHANKS A.M. (1976) Effects of dietary energy level and dietary energy source on growth, feed conversion and body composition of Turbot (Scophthalmus maximums L.). Aquaculture 7, 125-132.

ALDABA V.C. (1931) The Dalag Fishery of Laguna de Bay. Phil. Jour. Sci. 45 (1), 41-58.

ALIKUNHI K.H. (1953) Notes on the bionomics, breeding and growth of the murrel, Ophiocephalus striatus (Bloch). Proc. Indian Acad. Sci. B) 38 (1), 10-20.

ANDREWS J.W. (1979) Protein requirements. In: Nutrition and Feeding of Channel Catfish (ed. by R.R. Stickeny and R.T. Lovell) pp. 10-13. Southern Cooperative Series, Bulletin 218. 67pp.

ANDREWS J.W & STICKNEY R.R. (1972) Interactions of feeding rates and environmental temperature on growth, food conversion and body composition of Channel Catfish. Trans. Am. Fish. Soc. 101, 94-99.

ANON (1979) Culture of Channa striata. Leaflet produced by the National Inland Fisheries Institute, Bangkok, Thailand. 1p.

ANON. (1976) Monoculture of snakehead in Hong Kong. Leaflet produced by the Au Tau Fisheries Office, Hong Kong. 1p.

ANON. (1972) Culture of snakehead. FAO Aquaculture Bulletin 5 (1), 7.

ANON. (1975) Seed production and Culture of Air Breathing fishes. FAO Aquaculture Bulletin 7 (3-4), 4.

ANON (1982) Thai Fish Crisis. World Fishing. Jan/February, pp.25.

AOAC. (1980) Official Methods of Analysis of the Association of Official Analytical Chemists (ed. William Horwitz). 13th Ed. Association of Official Analytical Chemists. Washington.

AOE, H. MASUDA, I., ABE, I., SAITO, T., TOYODA, T & KITAMURA, S. (1978) Nutrition of protein in young carp I. Nutritive value of free amino acids. Bull. Jap. Soc. Sci. Fish 36, 407-413.

ARAI, S., NOSE, T & HASHIMOTO, Y. (1972) Amino acids essential for the growth of eels Anguilla anguilla and A. japonica. Bull. Jap. Soc. Sci. Fish. 38, 753-759.

ASKEROV, T.A. (1975) Survival Rate and Oxygen Consumption of Juvenile wild carp maintained under different conditions. Hydrobiological Journal 11 (3), 67-68.

AUSTRENG, E. (1976) Fat and Protein in diets for Salmonid Fishes II. Fat content in dry diets for rainbow trout (Salmo gairdneri). Meld. Nov. Landbrukshoegsk 55 (6), 1-18.

BANDYOPADHYAY, G.K., DUTTA, J. & GHOSH, S. (1982) Preferential Oxidation of Linolenic Acid Compared to Linoleic Acid in the Liver of Catfish (Heteropneustes fossillis and Clarias batrachus) Lipids 17, 733-740.

BALARIN, J.D. and HATTON (1979) Tilapia: A Guide to Their Biology and Culture In Africa. Unit of Aquatic Pathobiology, University of Stirling, Stirling U.K. 174pp.

BANERJI, S.R. (1974) Hypophysation and life history of Channa punctatus (Bloch). J. Inland Fish. Soc. India 4, 62-73.

BARDACH, J.E., Ryther, J.H., McLarney, W.D. (1972) Aquaculture: The Farming and Husbandry of Freshwater and Marine Organisms. Wiley-Interscience, New York, London. 868pp.

BLIGH E.G. & DYER W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. 37, 911-917.

BONN, E.W. & FOLLIS, B.J. (1967) Effects of Hydrogen Sulfide on Channel Catfish, Ictalurus punctatus. Trans. Am. Fish. Soc. 96 (1), 31-36.

BOORMAN, K.N. (1980) Dietary constraints on nitrogen retention. In: Protein Deposition in Animals (ed. P.J. Buttery and D.B. Lindsay) pp. 147-166. Butterworth and Co. (Publishers) Ltd., London.

BOYD, C.E. (1979) Water Quality in Warmwater Ponds. Auburn University Agricultural Experiment Station, Auburn, Alabama, U.S.A. 359pp.

BRETT, J.R., SHELBOURNE, J.E. & SHOOP, C.T. (1969) Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Bd. Can. 26, 2363-2394.

BROWN, M.E. (1946) The growth of brown trout (Salmo trutta Linn.) I. Factors influencing growth of trout fry. J. Exp. Biol. 22, 118-129.

BUCHANAN, R.E. & GIBBONS, N.E. (Eds.) (1974) Bergey's Manual of Determinative Bacteriology. pp.345-348. Eight Edition. Williams and Wilkins Company, Baltimore, U.S.A. 1268pp.

BUHLER, D.R. & HALVER, J.E. (1961) Nutrition of salmonid fishes. IX. Carbohydrate requirements of chinoon salmon. J. Nutr. 74, 307-318.

BURROWS, R.E. (1964) Effects of Accumulated Excretory Products on Hatchery-Reared Salmonids. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 66. 12pp.

CHANCE, R.E. (1962) I. Amino Acids Requirements of Chinook Salmon. II. Free Amino Acids and Related Compounds in the Plasma of Chinook salmon and swine. Ph.D. Thesis. Purdue University, Lafayette, Indiana, U.S.A.

CHEN, T.P. (1976) Aquaculture Practices in Taiwan. Fishing News (Books) Limited, Farnham, England, U.K. 161pp.

CHO, C.Y., BAYLEY, H.S. & SLINGER S.J. (1974) Partial replacement of herring meal with soybean and other changes in a diet for rainbow trout (Salmo gairdneri). J. Fish Res. Bd Canada 31, 1523-1528.

CHO, C.Y., SLINGER, S.J. & BAYLEY, H.S. (1976) Influence of level and type of dietary protein and level of feeding on feed utilisation by rainbow trout. J. Nutr. 106 (11), 1547-1556.

CHRISTIE, W.W. (1973) Lipid Analysis pp. 87-92. Pergamon Press, Oxford, U.K.

COLT, J. & TCHOBANOGLOUS G. (1976) Evaluation of the short term toxicity of nitrogenous compounds to Channel Catfish, Ictalurus punctatus. Aquaculture, 8 (3), 209-224.

COLT, J. & TCHOBANOGLOUS G. (1978) Chronic exposure of Channel catfish, Ictalurus punctatus to ammonia: Effects of growth and survival. Aquaculture 15 (4), 353-372.

COLT, J., MITCHEL S., TCHOBANOGLOUS G. & KNIGHT A. (1979) The Use and Potential of Aquatic Species for Wastewater Treatment Appendix B. The Environmental Requirements of Fish. California State Water Resources Control Board, Sacramento, California. 239pp.

COWEY, C.B. (1975) Aspects of protein utilization by fish. Proc. Nutr Soc. 34, 57-63.

COWEY, C.B. & SARGENT J.R. (1972) Fish Nutrition. Adv. Mar. Biol. 10, 383-492.

COWEY, C.B. & SARGENT J.R. (1979) Nutrition. In: Fish Physiology (ed. W.S. Hoar & D.J. Randall) Vol. VIII pp. 1-69. Academic Press Inc., London.

COWEY, C.B. & TACON A.G.J. (1982) Fish Nutrition - Relevance to Invertebrates. Proceedings of the 2nd International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Invertebrate Nutrition (in Press).

COWEY, C.B., ADRON, J.W., BROWN D.A. & SHANKS, A.M. (1975) Studies on the nutrition of marine flatfish. The metabolism of glucose

by plaice (Pleuronectes platessa) and the effect of dietary energy source on protein utilization in plaice. Br. J. Nutr. 33, 219-231.

COWEY, C.B., POPE, J.A., ADRON, J.W. & BLAIR, A. (1972) Studies on the nutrition of marine flatfish. The protein requirement of plaice (Pleuronectes platessa). Br. J. Nutr. 28, 447-456.

COWEY, C.B. ADRON, J.W., WALTON, M.J., MURRAY, J., YOUNGSON, A. & KNOX D. (1981) Tissue Distribution, Uptake and Requirement for α-tocopherol of Rainbow trout (Salmo gairdneri) Fed Diets with Minimal content of Unsaturated Fatty Acids. J. Nutr. III, 1556-1567.

DABROWSKI, K. (1977) Protein requirements of grass carp fry (Ctenopharyngodon idella). Aquaculture, 12, 63-73.

DAS, B.K. (1927) The bionomics of certain air-breathing fishes of India, together with an account of the development of their air-breathing organs. Phil. Trans. Roy. Soc. Lond. 216, 183-218.

DAVIS, A.T. & STICKNEY, R.R. (1978) Growth responses of Tilapia aurea to dietary protein quality and quantity. Trans. Am. Fish. Soc. 107, 479-483.

DELONG, D.C., HALVER, J.E. & MERTZ, E.T. (1958) Nutrition of salmonoid fishes. VI. Protein requirements of chinook salmon at two water temperatures. J. Nutr. 65, 589-661.

DELONG, D.C., HALVER, J.E. & MERTZ, E.T. (1962) Nutrition of

salmonoid fishes X. Quantitative threonine requirements of chinook salmon at two water temperatures. J. Nutr. 76, 174-178.

DUNCAN, D.B. (1955) Multiple Range and Multiple F-Tests. Biometrics 11, 1-42.

DUPREE, H.K. (1969) The influence of corn oil and beef tallow on growth of channel catfish. Tech. Pap. U.S. Bur. Sport Fish. Wildl. 27. 13pp.

DUPREE, H.K. & HALVER J.E. (1970) Amino acids essential for the growth of channel catfish, Ictalurus punctatus. Trans. Am. Fish. Soc. 99, 90-92.

FAO (1980) Outline Research Programmes for the Regional Aquaculture Lead Centres in Asia. Part II. Aquaculture Development and Coordination Programme Report ADCP/REP/80/14(EN); 40pp.

FLIS, J.R. (1968) Anatomicohistopathological changes induced in carp (Cyprinus carpio) by ammonia water. Part I. Effects of toxic concentration. Acta Hydrobiol. 10 (1-2), 205-224.

FRANK, S. (1970) Acclimatization experiments with Amur Snakehead, Ophiocephalus argus warparchowskii (Berg, 1909) in Czechoslovakia. Cesk Spolecneosti Zool. 35, 277-283.

FURUKAWA, A. & TSUKAHARA H. (1966) On the acid digestion method for determination of chromic oxide as an indicator substances in the study of digestibility in fish. Bull. Jap. Soc. Sci. Fish. 32, 502-504.

GARLING, D.L. & WILSON R.P. (1976) Optimum dietary protein to energy ratio for channel catfish fingerlings, Ictalurus punctatus. J. Nutr. 106, 1368-1375.

GURR, M.I. & JAMES A.T. (1975) Lipid Biochemistry - An Introduction. Second Edition. Chapman and Hall Ltd., London. 244pp.

HALVER, J.E. & SHANKS W.E. (1960) Nutrition of salmonoid fishes. VIII. Indispensable amino acids for sockeye salmon. J. Nutr. 72 (3), 340-346.

HALVER, J.E., DELONG, D.C. & MERTZ, E.T. (1957) Nutrition of salmonoid fishes. V. Classification of essential amino acids for chinook salmon. J. Nutr. 63 (i), 95-105.

HASTING, W.H. (1967) Feed Formulation. In: Progress in Sport Fisheries Research in 1966. Bur. Sport Fish. Wildl. Res. Publ 39, 137-139.

HERRMANN, R.B., WARREN, C.E. & DOUDOROFF, P. (1962) Influence of oxygen concentration on the growth of Coho Salmon. Trans. Am. Fish. Soc. 91, 155-167.

HIGASHI, H., KANEKO, T., ISHII, S., MASUDA, I. & SUGIHASHI, T. (1964) Effect of dietary lipid on fish under cultivation. I. Effect of large amounts of lipid on health and growth rate of rainbow trout. Bull. Jap. Soc. Sci. Fish. 30, 778-785.

HIGUERA, M. DELA., MURILLO, A., VARELA, G., & ZAMORA, S. (1977) The influence of high dietary fat levels on protein

utilization by the trout (Salmo gairdneri). Comp. Biochem. Physiol. 56 (1), 37-41.

HOFFMAN, G. & MEYER, F.E. (1974) Parasites of Freshwater Fishes - a review of their control and treatment. T.F.H. Publications Inc. Ltd. Neptune, N.J., U.S.A.

HUGHES, G.M. & MUNSHI, D.J.S. (1973) Nature of the air breathing organs of the Indian fishes, Channa, Amphipnous, Clarias and Saccobranchus as shown by electron microscopy. J. Zool. Lond. 170, 245-270.

JAUNCEY, K. (1979) Growth and Nutrition of Carp in Heated Effluents. Ph.D. Thesis. University of Aston in Birmingham, U.K.

JAUNCEY, K. (1982) The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia (Sarotherodon mossambicus). Aquaculture, 27. 43-54.

JAVAID, M.Y. (1970) Diurnal periodicity in the feeding activity of some freshwater fishes in West Pakistan. I. Studies on Channa punctatus and Mystus vittatus. Pakistan J. Zool. 2 (1), 101-111.

JORDAN, D.H.M. & LLOYD R. (1964) The resistance of rainbow trout (Salmo gairdneri Richardson) and roach (Rutilus rutilus) to alkaline solutions. Int. J. Air Wat. Poll. 8, 405-409.

KANAZAWA, A., TESHIMA, S., SAKAMOTO, M., & AWAL, M.A. (1980) Requirements of Tilapia zillii for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 46, 1353-1356.

KAUSHIK, S. (1977) Influence de la salinite sur le metabolisme azote et le besoin en arginine chez la truite arc-en-ciel (Salmo gairdneri). These de Doctorat d'etat. Univ. Bretagne, Brest, France. 230pp.

KAWAMOTO, N.Y. (1961) The influence of Excretory substances of Fishes on their growth. Prog. Fish Cult. 23 (2), 70-75.

KNEPP, G.L. & ARKIN, G.F. (1972) Ammonia Toxicity Levels and Nitrate Tolerance for Channel Catfish (Ictalurus punctatus). In: Annual Meeting American Society of Agricultural Engineers, Hot Springs, Arkansas. June, 27-30.

KONIKOFF, M. (1975) Toxicity of Nitrite to Channel catfish. Prog. Fish Cult. 37 (2), 96-98.

KORN, S. & MACEDO, D. (1973) Determination of Fat Content in Fish with a Non-toxic, Non-inflammable solvent. J. Fish. Res. Bd Canada 30, 1880-1882.

LAWSON, S. (1981) Inland Fish Culture in Thailand: A Case Study. Systems Group. Open University, U.K.

LEE, D.J. & PUTNAM, G.B. (1973) The response of rainbow trout to varying protein/energy ratio in a test diet. J. Nutr. 103, 1339-1346.

LIM, C., SUKHAWONGS, S., & PASCUAL, F.P. (1979) A preliminary Study on the protein requirements of Chanos chanos (Forskal) fry in a controlled environment. Aquaculture 17, 195-201.

LING, S.W. (1977) Aquaculture in South East Asia - A Historical Overview. Washington Sea Grant Publication. Washington University Press, Seattle and London. 198pp.

LLOYD, R. & JORDAN D.H.M. (1964) Some factors affecting the resistance of rainbow trout (Salmo gairdneri Richardson) to acid waters. Int. J. Air Wat. Poll. 8, 393-403.

MAHDI, M.A. (1973a) Studies on factors affecting survival of Nile fish in the Sudan. I. The effect of hydrogen ion concentration. Mar. Biol. 18 (2), 89-92.

MAHDI, M.A. (1973b)Studies on factors affecting survival of Nile fish in the Sudan. III. The effect of oxygen concentration. Mar. Biol. 18 (2), 96-98.

MAYNARD, L.A. & LOOSLI, J.K. (eds.) (1969) Animal Nutrition. 6th Edition. McGraw-Hill Book Company.

MEHROTA, B.K. & KHANNA, S.S. (1969) Histomorphology of the oesaphagus and the stomach in some Indian teleosts with inference on their adaptational features. Zoologische Beitrage 15 (2-3), 375-391.

MERTZ, E.T. (1969) Amino Acid and Protein Requirement of Fish In: Fish in Research. (eds. O.W. Neuhaus & J.E. Halver). pp. 233-244. Academic Press. London, New York, San Francisco.

MESKE, C.H. & PFEFFER, E. (1978) Influence of source and level of dietary protein in body composition of carp. E.I.F.A.C. Symp. Fin Fish Nutr. and Feed Technol.

MILLER, D.S. & BENDER, A.E. (1955) The determination of the net protein utilization by a shortened method. Brit. J. Nutr. 9, 382-388.

MITTAL, A.K. & BANERJI, T.K. (1975) Histochemistry and the structure of the skin of a Murrel, Channa striata (Bloch) 1797. I. Epidermis. I. Dermis and Subcutical. Can. J. Zool. 53 (6), 833-853.

MOORKEJEE, H.K. GANGULY, D.N. & BHATTACHARYA, R.N. (1948) On the biochemics, breeding habits and development of Ophiocephalus striatus Bloch. Proc. Zool. Soc. Bengal 1 (1), 58-64.

MOSS, D.D. & SCOTT, D.C. (1961) Dissolved oxygen requirements of three species of fish. Trans. Am. Fish. Soc. 90 (4), 377-393.

MUIR, J.F. (1981) Management in Clarias Culture in Thailand. Working Paper THA/75/012/WP3. Programme for Development of Pond Management Techniques and Disease Control (DOF-UNDP/FAO/THA/75/012) Thailand. 30pp.

MUNRO, H.N. & FLECK A. (1969) In: Mammalian Protein Metabolism. Volume III (ed. H.N. Munro). Academic Press, Inc. London. 423pp.

MURRAY, M.W., ANDREWS, J.W. & DELOACH, H.L. (1977) Effects of dietary lipids, dietary protein and environmental temperature on growth, feed conversion and body composition of Channel catfish. J. Nutr. 107 (2), 272-280.

MURAI, T., AKIYAMA, T. & NOSE, T. (1981) Use of crystalline amino acid coated with casein in diets for carp. Bull. Jap. Soc. Fish. 47, 523-527.

NAIL, M.L. (1962) The protein requirement of channel catfish, Ictalurus punctatus (Rafinesque). Proc. S. E. Assoc. Game Fish Commrs. 16, 307-316.

NAKAMURA, T., HAGIWARA, M. & HIGUCHI, T. (1973) Determination of nutritive value of fish feeds. I. DE and DCP of feeds for rainbow trout. (in Japanese). Research Annual, Nihon Nosan Kogyo 4, 35-42.

NITSMER, S. (1982) An Analysis of the Freshwater Fish Marketing System in Lower Central Thailand. M. Sc. Thesis. Asian Institute of Technology, Bangkok. Thailand.

NOSE, T. (1967) On the metabolic faecal nitrogen in young rainbow trout. Bull. Freshwat. Fish. Res. Lab. 17 (2), 97-105.

NOSE, T. (1979) Summary report on the requirements of essential amino acids for carp. In: Finfish Nutrition and Technology Feed Vol. I (eds. J.E. Halver & K. Tiews). pp. 145-156. I.H. Heenemann Gumblt & Co., Berlin.

NOSE, T. & ARAI, S. (1972) Optimal level of protein in purified diet for eel, Anguilla japonica. Bull. Freshwater Fish. Res. Lab. Tokyo 22 (2), 145-155.

NOSE, T., ARAI, S., LEE, D.L. & HASHIMOTO, Y. (1974) A note on amino acids essential for growth of young carp. Bull. Jap. Soc. Sci. Fish. 40 (9), 903-908.

OGINO, C. (1980) Requirements of carp and rainbow trout for essential amino acids. Bull. Jap. Soc. Sci. Fish. 46 (2), 171-174.

OGINO, C. & SAITO, K. (1970) Protein nutrition in fish. I. The utilization of dietary protein by young carp. Bull. Jap. Soc. Sci. Fish. 36, 250-254.

OGINO, C. & CHEN, M.S. (1973) Protein nutrition in fish. III. Apparent and true digestibility of dietary protein in carp. Bull. Jap. Soc. Sci. Fish. 39 (6), 649-651.

OJHA, J., MISHRA, A., SAHA, M.P. & MUNSHI, J.S.D. (1979) Bimodal oxygen uptake in juveniles and adults amphibious fish Channa (= Ophiocephalus) marulius. Hydrobiologia 63 (2), 153-159.

ONDARA, T. (1979) Percoba'an pemelihara'an ikan Toman (O. micropeltes) dalam sangkar dan pengembara'an nya di-peaira'an sepanjang Sungai Lempung. Pewarta LPPD. 1, 15.

OSEID, D. & SMITH, L.L. (1972) Swimming endurance and resistance to copper and Malathion of Bluegills treated by long term exposure to sublethal levels of Hydrogen sulphide. Trans. Am. Fish. Soc. 101 (4), 620-625.

OWEN, J.M., ADRON, J.W., MIDDLETON, C. & COWEY, C.B. (1975) Elongation and desaturation of dietary fatty acids in turbot, Scophthalmus maximus L. and rainbow trout (Salmo gairdneri Rich.). Lipids, 10, 528-531.

PAGE, J.W. & ANDREWS, J.W. (1973) Interactions of dietary levels of protein and energy on channel catfish (Ictalurus punctatus). J. Nutr. 103, 1339-1346.

PANDAY, J.P. & DWIVEDI, A.S. (1974) Studies of morphology and physiology of olfactory organs in a murrel Channa punctatus (Bloch). Indian J. Zootomy 14 (1), 59-66.

PANDIAN, T.J. (1981) Culture of air-breathing fishes. In: Tropical ecology and development: Proceedings of the Vth International Symposium of Tropical Ecology, 16-21, April, 1979, Kuala Lumpur, Malaysia (ed. J.I. Furtado), 1223-1227.

PANDIAN, T.J. & VIVEKANANDAN, E. (1977) Effects of feeding and starvation on growth and swimming activity in an obligatory air-breathing fish, Ophiocephalus striatus. Hydrobiologia 49 (1), 33-39.

PANTULU, V.R. (1976) Floating cage culture of fish in the Lower Mekong Basin. In FAO Technical Conference on Aquaculture, Kyoto, Japan, 26 May - 2 June 1976, FIR:AQ/CONF/76/E10.

PAPOUTSOGLOU S.E. & PAPOUTSOGLOU, E.G. (1978) Comparative studies on body composition of rainbow trout (Salmo gairdneri) in relation to type of diet and growth rate. Aquaculture 13 (3), 235-244.

PARAMESWARAN, S. & MURUGESAN, V.K. (1976) Observations on the hypophysation of murrels (Ophiocephalidae) Hydrobiologia 50 (1) 81-82.

PHILLIPS, A.M. Jr., LIVINGSTON, D.L. & POSTON, H.A. (1966) Use of calorie sources by brook trout. Prog. Fish Cult. 28 (2), 67-72.

PLAKAS, S.M., KATAYAMA, T., TANAKA, Y. & DESHIMARU, O. (1980) Changes in the levels of circulating plasma free amino acids of carp (Cyprinus carpio) after feeding protein and an amino acid diet of similar composition. Aquaculture 21, 307-322.

PRATHER, E.E. & LOVELL, R.T. (1973) Response of intensively fed channel catfish to diets containing various protein-energy ratios. Proc. S.E. Assoc. Game Fish Commrs. 27, 455-459.

RAMUSSEN, C.J. (1976) Experiments on the efficiency and cost of dry food in relation to wet food in Danish trout culture. EIFAC/T9 pp.55-62.

REINITZ, G.L., ORME, L.E., LEMM, C.A. & HITZEL, F.W. (1978) Influence of Varying Lipid Concentrations with Two Protein Concentrations in Diets for rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc. 107 (5), 751-754.

RINGROSE, R.C. (1971) Calorie-Protein Ratio for Brook trout (Salvelinus fontinalis) J. Fish. Res. BD. Canada 28, 1113-1117.

ROBERTS, R.J. (1978) Fish Pathology. Balliere Tyndall, London. 278pp.

ROBINSON, E.H., WILSON, R.P. & POE, W.E. (1980) Total aromatic amino acid requirement, phenyalanine requirement and tyrosine replacement value for fingerling channel catfish. J. Nutr. 110, 1805-1812.

SAAD, M.A.H. & EZZAT, A. (1973) Effect of pollution on the blood characteristics of Tilapia zillii G. Water, Air and Soil Pollution 2, 171-179.

SABAUT, J.J. & LUQUET, P. (1973) Nutritional requirements of gilthead bream, Chrysophrys aurata. Quantitative protein requirement. Mar. Biol. 18, 50-54.

SAMPATH, K. & PANDIAN, T.J. (1980) Effects of density on food utilization and surfacing behaviour in the obligatory air-breathing fish Channa striatus. Hydrobiologia 68 (2), 113-117.

SATIA, B.P. (1974) Quantitative protein requirements of rainbow trout. Prog. Fish Cult. 36 (2), 80-85.

SHANG, Y.C. (1976) Follow-up Programme on Economics of Aquaculture in the South China Sea Region. South China Sea Fisheries Development and Coordinating Programme Working Paper SCS/76/WP/39. July 1976, Manila. 19pp.

SHANKS, W.E., GAHIMER, G.D. & HALVER, J.E. (1962) The indispensable amino acids for rainbow trout. Prog. Fish Cult. 24, 68-73.

SHIMENO, S., HOSOKAWA, H. & TAKEDA, M. (1978) The importance of carbohydrate in the diet of a carnivorous fish. In: Proceedings of the Symposium on Finfish Nutrition and Feed Technology, 20-23 June. Hamburg pp.31 (Eds. Tiews K. and Halver, J.E.).

SIN, A.W. (1973a) The dietary protein requirements for growth of young carp (Cyprinus carpio). Hong Kong Fish. Bull. 3, 77-81.

SIN, A.W. (1973b) The utilization of dietary protein for growth of young carp (Cyprinus carpio) in relation to variations in fat intake. Hong Kong Fish. Bull. 3, 83-88.

SINGH, R. (1976) Studies on the morphology and histology of alimentary canal of Ophiocephalus marulius. Agra Univ. J. Res. 16, 27-38.

SINGH, R.P. & NOSE, T. (1967) Digestibility of carbohydrates in young rainbow trout diet. Bull. Freshwater Fish. Res. Lab. 17, 21-25.

SMITH, H.M. (1965) Freshwater fishes of Siam or Thailand. Bulletin 188, United States National Museum, pp. 465-474.

STICKNEY, R.R. & ANDREWS, J.W. (1972) Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. J. Nutr. 102, 249-258.

SURASWADI, P. (1980) Freshwater Fisheries of Thailand. Technical paper No: 4/1980. National Inland Fisheries Institute, Freshwater Fisheries Division, Department of Fisheries, Thailand.

TACON, A.G.J. (1979) The Use of Activated Sludge as a Supplementary Protein Source in the Diet of Rainbow Trout. Ph.D. Thesis. Department of Zoology, University College, Cardiff, U.K.

TACON, A.G.J. (1981) The possible substitution of fish meal in fish diets. In: Proceedings of the SMBA/HIDB Fish Farming Meeting, 26-27 February, 1981. Oban. pp. 46-56.

TAKEDA, M., SHIMENO, S., HOSOKAWA, H., KAJIYAMA, H. & KAISYO, T. (1975) The effect of dietary calorie to protein ratio on the growth, feed conversion, and body composition of young

yellowtail (Seriola quinqueradiata). Bull. Jap. Soc. Sci. Fish. 41, 433-447.

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978a) Supplementary effect of lipids in high protein diets for rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (6), 677-681.

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978b) Optimum ratio of protein to lipid in diets of rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (6), 683-688.

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978c) Use of hydrogenated fish oil and beef tallow as a dietary energy source for carp and rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (8), 875-881.

TAKEUCHI, T., YOKOYAMAMA, M., WATANABE, T. & OGINO, C. (1978d) Studies on the nutritive value of dietary lipids in fish. The optimum ratio of dietary energy to protein for rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (7), 729-732.

TAKEUCHI, T., ARAI, S., WATANABE, T. & SHIMMA, Y. (1980) Requirement of eel Anguilla japonica for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 46, 345-353.

TENG, S.K., CHUA, T.E. & LIM, P.E. (1978) Preliminary observation on the dietary protein requirement of estuary grouper, Epinephelus salmoides Maxwell. cultured in floating net cages. Aquaculture 15, 257-289.

TIEMEIER, O., DEYOE, C.W. & WEARDON, S. (1965) Effects on growth of fingerling channel catfish of diets containing two energy and two protein levels. Trans. Kansas Acad. Sci. 68, 180-186.

TIEWS, K., GROPP, J. & KOOPS, H. (1976) On the development of optimal rainbow trout pellet feeds. Arch. FischWiss. 27 (Bech. 1), 1-29.

UKKATEWEWAT, S. (1980) The taxanomic characters and biology of some important freshwater fishes. Technical Paper No: 4/1980. National Inland Fisheries Institute. Freshwater Fisheries Division, Department of Fisheries. Thailand.

VARMA, B.R. (1979) Studies on the pH tolerance of certain freshwater teleosts. Comp. Physiol. Ecol. 4 (2), 116-117.

VIVEKANANDAN, E. (1977) Ontogenetic development of surfacing behaviour in the obligatory air-breathing fish Channa (Ophiocephalus) striatus. Physiol Behav. 18, 559-562.

VIVEKANANDAN, E. & PANDIAN, T.J. (1977) Surfacing activity and food utilization in a tropical air-breathing fish exposed to different temperatures. Hydrobiologia 54 (2), 145-160.

WALTON, M.J., COWEY, C.B. & ADRON, J.W. (1982) Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr. in press.

WATANABE, T. (1977) Sparing action of lipids on dietary protein in fish - Low protein diet with high calorie content. Technocrat. 10 (8), 34-39.

WATTANUTCHARYA, S. (1982) Economic Analysis of Clarias Culture in Thailand. Presented at the Food and Fertilizer Technology Center for the Asian and Pacific Region/ASPAC and Kasetsart University Seminar on Inland and Coastal Aquaculture. Bangkok, 6-12 April, 1982.

WATTANUTCHARYA, S. & PANAYATOU, T. (1981) The Economics of Aquaculture: The Case of Catfish in Thailand. Presented at the Economics of Aquaculture Research Workshop held by the International Development Research Center (LDRC) and the International Center for Living Aquatic Resources Management (ICLARM) in Singapore, 2-5 June, 1981.

WEE, K.L. (1981) Proximate composition of wild and cultured snakeheads. Internal report Institute of Aquaculture, University of Stirling, Stirling. 8pp.

WEE, K.L. (1982) Snakeheads - Their Biology and Culture. In: Recent Advances in Aquaculture. (eds. J.F. Muir & R.J. Roberts) Croom Helm, London. 446pp.

WILLEY, A. (1910) Observations on the nests, eggs and larvae of Ophiocephalus striatus. Spol. Zeyl. 6, 108-123.

WILSON, R.P., ALLEN, O.W., ROBINSON, E.H. & POE, W.E. (1978) Tryptophan and threonine requirements of fingerling channel catfish. J. Nutr. 108, 1595-1599.

WOLF, K. & QUIMBY, M.C. (1962) Established Eurythermic Line of Fish Cells in vitro. Science 135, 1063-1066.

YAMADA, K., KOBAYASHI, K. & YONE, Y. (1980) Conversion of linolenic acid to 3-highly unsaturated fatty acids in marine fishes and rainbow trout. Bull. Jap. Soc. Sci. Fish. 46, 1231-1233.

YAPCHIONGO, J.V. & DEMONTEVERDE, L.C (1959) The Biology of Dalag (Ophiocephalus striatus Bloch). The Philippine Journal of Fisheries 7 (1-2), 105-140.

YONE, Y. (1980) In: Dietary Lipids in Aquaculture (ed. Japan Soc. Sci. Fish.) pp. 43-49. Koseisha-Koseikaku, Tokyo.

YU, T.C., SINNHUBER & HENDRICKS, J.D. (1979) Reproduction and Survival of Rainbow Trout (Salmo gairdneri) Fed Linolenic Acid as the Only Source of Essential Fatty Acids. Lipids 14, 572-575.

YU, T.C., SINNHUBER, R.O. & PUTNAM, G.B. (1977) Use of swine fat as an energy source in trout relations. Prog. Fish Cult. 39 (2), 95-97.

ZEITOUN, I.H., HALVER, J.E., ULLREY, D.E. & TACK, P.I. (1973) Influence of salinity on protein requirements of rainbow trout (Salmo gairdneri) fingerlings. J. Fish. Res. Bd. Canada 30, 1867-1873.

ZEITOUN, I.H., ULLREY, D.E., HALVER, J.E., TACK, P.I. & MAGEE, W.T. (1974) Influence of salinity on protein requirements of coho salmon (Oncorhynchus kitsutch) smolts. J. Fish. Res. Bd. Canada 31, 1145-1148.

ZEITOUN, I.H., ULLREY, D.E., MAGEE, W.T., GILL, J.L. & BERGEN, W.G. (1976) Quantifying nutrient requirements of fish. J. Fish. Res. Bd. Canada 33, 167-172.