TR84-44

INVESTIGATIONS INTO THE DIETARY REQUIREMENTS OF *CLARIAS GARIEPINUS* LARVAE (PISCES : CLARIIDAE) AND THE FORMULATION AND MANUFACTURE OF AN ARTIFICIAL DRY FEED FOR USE IN INTENSIVE LARVAL REARING

Submitted in partial fulfilment of the
requirements for the Degree of
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ERRATA

1. p.1, para 2: the natural distribution of *Clarias gariepinus* extends northwards to Turkey.
2. p.8, para 2, line 5: for 'similate' read 'simulate'.
3. p.10, Table 2: for '----' read 'no data available'.
4. p.16, para 4, line 3: for 'liter' read 'litre'.
5. p.21, line 5 of text: The number of samples for which the mass : length relationship was calculated was 272. The total number of larvae included in these samples 4138.
6. p.25, para 1, lines 10-12: Bowen (1980, Science 207: 1216-1218) has shown that free amino acids may be important in the diet of certain cichlid fishes.
7. p.25, para 4, line 4; p.26, para 1, line 4 and p.27, para 1, line 3: for 'Millikin 1983' read 'Millikin 1982'.
8. p.58, para 2, line 1: for 'deducted' read 'deduced'.
9. p.91, Table 23, legend: for'weigth' read 'weight'.
10. p.107, penultimate line: for '*Chaeborus*' read '*Chaoborus*'.
11. p.108, line 2: for 'neccessary' read 'necessary'.

CONTENTS

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ABSTRACT

The need for developing an artificial dry feed for Clarias gariepinus larvae was identified. Dry larval fishfeed requires certain physical properties. Techniques for manufacturing feeds which comply with these requirements were evolved and are recommended for practical use. The techniques that were used for artificial reproduction of C. gariepinus larvae are briefly discussed. The larvae were fed on various dietary formulations and their growth responses were monitored in order to determine their nutritional requirements. A feed was developed which gave superior results to those obtained with natural food organisms. This feed consisted of Torula yeast(69,8%), fishmeal(23,3%), vitamin supplements(0,9%), methionine supplement (150mg/kg), bactericide and preservatives. The feed particles were coated with a lipid supplement which was added at a ratio of 6% to the weight of the feed. The optimum particle size (diameter) for C. gariepinus larvae is 2,2% of their mean total length. The larvae should be given a feed ration of 25% of body weight per day at a frequency of once every four hours. A favourable feed conversion ratio of 1.1g of feed consumed per gram of larval weight gain was experienced. The established dry feed is suitable for use in commercial hatcheries and can be used as a standard and proven food source for further research on the intensive rearing of C. gariepinus larvae.

CHAPTER 1. INTRODUCTION

Fishes of the genus Clarias are widely distributed in Africa and Asia, where they are commonly known as catfish. The culture of C. batrachus and C. macrocephalus is a large and growing industry in Taiwan (Sidthimunka 1972). In Africa, C. gariepinus is the most important freshwater fish in traditional subsistance and modern commercial fisheries. In East and Central Africa it makes up 20% of the fish consumed by the populace and it is also one of the most sought after fish in Africa (Clay 1977). C. lazera is taken to be a synonym of C. gariepinus (Teugels 1982a,1982b; Bruton & Teugels In press) (where reference is made to C. lazera in this project, it is because the authors concerned used this name).

Natural history studies on C. gariepinus have shown that it is a remarkably hardy and adaptable fish (Greenwood 1966; Van der Waal 1972; Clay 1977; Bruton 1978,1979a). The suprabranchial organs of post larval C. gariepinus enable it to utilize atmospheric oxygen and survive in water with low levels of dissolved oxygen (Greenwood 1955). C. gariepinus is able to withstand diurnal temperature fluctuations ranging between 22 and 34°C (Greenwood 1955). It was shown to be highly adaptable in its feeding habits (Van der Waal 1972; Pham 1976; Bruton 1979b) and its abilty to utilize animal as well as plant protein is of importance when considering it for aquacultural purposes (Clay 1979,1981). C. gariepinus is widely distributed in Africa, occurring from South Africa to Asia Minor (Teugels 1982b; Bruton & Teugels In press). Furthermore, the high fecundity of C. gariepinus (Bruton 1979a; Clay 1979), its tolerance of parasitic infection (Van der Waal 1972) and its potentially high growth rate in the first year (Van der Waal 1972; Clay 1977; Bruton 1979a; Bruton & Allanson 1980) suggest that it is a prime candidate for aquaculture.

The culture of C. gariepinus has in recent years received much attention (Micha 1972,1973,1975; Van Der Waal 1972; De Kimpe & Micha 1974; Kouassi & Ville 1975; Pham 1975; Richter 1976; Clay 1977,1979,1981; Pham & Raugel 1977; Hogendoorn 1977,1979,1980,1981,1983; Hogendoorn & Vismans 1980; Schoonbee, Hecht, Polling & Saayman 1980; Christensen 1981a,1981b; Msiska 1981; Hecht 1981,1982; Hecht, Saayman & Polling

1982; Hogendoorn, Jansen, Koops, Machiels, Van Ewijk & Van Hees 1983; Hogendoorn & Koops 1983). These studies all confirm that C. gariepinus has great culture potential. The viability of catfish culture in Africa has been shown on an experimental basis but the lack of adequate propagation techniques has for some time remained the major obstacle (Coche 1982 In:Vincke 1982). Part of this constraint has been overcome by the development of improved hatchery procedures for C.gariepinus by Schoonbee et al. (1980) and Hecht et al. (1982). Since then it has been possible to produce sufficient numbers of larvae in the hatchery. This has facilitated further research on the intensive rearing of C.gariepinus larvae under controlled conditions (Hecht 1981, 1982).

Though endeavours to rear C. gariepinus larvae under hatchery conditions have so far been somewhat limited in success, the following studies have provided background information in planning this project. Van der Waal (1972) found that C. gariepinus larvae started feeding three to four days after hatching and that zooplankton, especially Daphnia, finely minced liver and ground trout pellets were consumed actively. Hogendoorn (1980) and Msiska (1981), working on C. lazera, found live Artemia and zooplankton to give good results in contrast with dry feeds such as trout starter feed. Inactive yeast and chickenfeed were found to be unsuitable. Hecht (1981) found Torula yeast in combination with either fishmeal or zooplankton to be an excellent feed as well as supplement for the first 10 days of feeding.

The majority of authors concerned with the culture of C. gariepinus stress that special attention be given to the large scale rearing of larvae, since an adequate supply of larvae is critical to the development of catfish farming as a whole. Commercial hatcheries should, therefore, not only produce enough larvae, but should also rear the larvae up to a stage where they are fit for transportation and stocking into ponds. Even for extensive culture purposes, production of larvae will have to remain an intensive operation (Hogendoorn 1979; Hecht 1982).

Intensive rearing of fish requires an understanding of genetics, breeding, disease control, environmental control as well as nutrition and feeding (Stickney 1977). Each of these areas is intimately related to the others, and it is only through proper manipulation and control of all aspects that success can be achieved.

The understanding of fish nutrition has advanced slowly because the quantity of fish feed required by hatcheries and commercial fish farms has not been sufficient to justify feed companies spending money on larval nutrition research. Neither has there been as much emphasis on rearing cultured fish as a major human food source as there has been on other livestock (Piper, McElwain, Orme, McCraren, Fowler & Leonard 1982).

The question of fish nutrition for culture purposes was first approached by investigating natural food organisms as a complete diet (Piper et al. 1982). In most cases this did provide the solution despite considerable effort to provide artificially prepared complete diets as an alternative. Intensive larval rearing of non-salmonids presently relies to a large extent on the culture of live food organisms (Horvath 1979; Bryant & Matty 1980). Horvath (1979) went as far as saying that no suitable alternatives exist. The difficulty involved in the composition of artificial diets which contain all the elements required, lies in the fact that enzyme systems of larvae are underdeveloped (Horvath 1979). There is, however, a small, but worldwide school of workers engaged in investigating the formulation of ready-to-use feeds for the mass rearing of fish larvae (Appelbaum 1976a,1976b, 1978,1979a,1979b,1980a,1980b; Barahona-Fernandes, Girin & Metailler 1977; Dabrowski 1977; Appelbaum & Dor 1978; Dabrowski, Dabrowska & Grudniewski 1978; Dabrowski & Kozak 1979; Girin 1979; Metailler, Manant & Depierre 1979; Meyers 1979a; Sen & Chatterjee 1979; Van Limborgh 1979; Reinitz 1980; Hecht 1981,1982; Hecht & Viljoen 1982).

Artificially prepared diets for larval fish, if suitably nutritious and acceptable, have definite advantages over live food organisms. Natural food is subject to seasonal variation in supply and composition. The culture of live food organisms such as Daphnia and Brachionus, or their collection from natural sources, is an unneccessary encumberance. Artificial diets can be quality controlled during fabrication, can be manufactured on a large scale and can be distributed with ease so as to ensure regular supplies. Artificial feeds can be sterilized whereas natural live food cannot be sterilized by conventional methods, resulting in the risk of importing disease or parasites into the hatchery. Because of the reliance on Artemia as a first food for fish larvae and the escalating demand for this crustacean in the growing world fish culture industry, drastic shortages have resulted (Meyers 1979a; Appelbaum 1976b). In a review paper on formulation of diets for larval fish and in a summary report on the discussions and recomendations during the proceedings of the EIFAC Symposium on Finfish Nutrition and Fishfeed Technology held in Hamburg in 1978, Meyers (1979a,1979b) stressed the importance of developing larval feeds, since: "The lack of satisfactory products is severely limiting the development of aquaculture in many parts of the world and for many fish species".

The object of this research project was to investigate some of the dietary requirements of Clarias gariepinus larvae during their first two weeks of feeding and to formulate and manufacture an artificial dry diet for use in intensive culture in the hatchery. The artificial diet, apart from having the advantage over natural food as stated, should also give a superior growth rate and survival of the larvae.

In the following chapters, experimental feed preparation is discussed (Chapter 2), followed by a description of the artificial reproduction techniques and types of rearing systems that were used (Chapter 3). In Chapter 4, the methods by which the growth responses of the larvae were monitored and the statistical analysis involved therein is discussed. All the feeding experiments appear in Chapter 5 and, finally, the conclusions and recommendations are made in Chapter 6.

CHAPTER 2. FEED PREPARATION

INTRODUCTION

Either moist or dry artificial feeds are currently used in fish culture. Moist pellets have the advantage of being more palatable to various fish species (Nose 1979), and are in some cases more nutritious (Ghittino 1979). Wastes generated by the fishing industry and scrap fish unusable for human consumption can also be used for the manufacture of moist feeds without any supplemental processing. Moist feeds, however, have the disadvantage of being perishable and difficult to store (Ghittino 1979; Piper et al. 1982). They are used mostly in salmonid culture but have also been found to be of use in other species where dry feed is not readily taken (Nose 1979).

Dry feed has the advantage of stability during storage. This in turn has economic advantages and a regular supply can be ensured (Girin 1979; Van Limborgh 1979). Technical requirements, such as small particle size as well as the homogeneity of individual particles, are easier to comply with when manufacturing dry feeds. If the problem of acceptability to the fish can be overcome, dry feeds certainly lend themselves better to application under African conditions, where the warm climate and irregular transport to remote areas might cause problems with moist feed.

A third alternative is the use of microencapsulated diets. This involves manufacturing miniature packaging assemblages in which liquids or particulate dietary components are enclosed within a biochemically engineered wall (Meyers 1979a). This allows each food particle to be presented to the fish in a complete form with no loss of nutrients to the water. Encapsuled rations also facilitate more rigid control over water quality. This is especially important under intensive culture conditions where water quality may dictate the success or failure of the operation (Meyers 1979a). Techniques of microcapsule manufacture such as those given by Gabbot, Jones & Nichols (1975) are extremely complex and involve the use of large quantities of expensive organic solvents and reagents. Most important from a commercial point of view is that the time and cost involved in manufacturing even small quantities are exorbitant.

All facts considered, dry feed, if acceptable to the larvae and adequately nutritious, seems to be best suited as a larval feed for use in commercial fish culture. It was, therefore, decided to work exclusively on dry feed for the purposes of this project.

There are ethological and technical considerations involved in larval feeding which dictate certain qualities that a suitable dry larval feed should have. These can be listed as follows:

1. The feed must have the right particle size for ingestion by the larvae (Meyers 1979a; Van Limborgh 1979; Webber & Huguenin 1979).

2. The larvae must be able to identify the feed as food. It must, therefore, cater for the particular senses the larvae utilize in finding and selecting food. Chemical and/or optical recognition of food play an important role with fish larvae (Appelbaum 1978,1979b, 1980b). The physical shape and hardness of feed particles might make them unacceptable to some species of fish larvae (Meyers 1979a).

3. The feed particles must be water stable to restrict nutrient leaching and they should not disintegrate once they are exposed to water (Csávás, Majoros & Váradi 1979; Ghittino 1979; Goldblatt, Conklin & Brown 1979; Luquet & Rumsley 1979; Meyers 1979a; Randall-Robinette 1977; Van Limborgh 1979).

4. The particles must have a suitable specific gravity so that they sink slowly through the water column to allow the larvae enough time to have access to the feed (Appelbaum 1976b; Lovell 1977b; Meyers 1979a). If the larvae prefer feeding on the surface or from the bottom of the tank the buoyancy properties of the feed should be adjusted accordingly.

5. The feed should not promote water pollution by organic buildup in the rearing system (Meyers 1979a).

6. It should have a low moisture content to promote stability during storage (Randall-Robinette 1977; Csávás et al. 1979; Ghittino 1979; Van Limborgh 1979).

7. The feed should be free of bacteria, fungi and toxins (Meyers 1979a).

8. Each feed particle must have the complete composition of the feed as a whole. If not, the particles may differ in degree of acceptability to the larvae and result in selective feeding which will in turn unbalance their nutrient intake and cause water pollution.

9. During the manufacturing process, the feed must not be exposed to excessive and prolonged heat, or chemicals that may denature dietary components or reduce the biological value of heat labile compounds (Meyers 1979a).

10. The complete range of nutrients must be present in controlled, optimum levels and ratios and must be in forms that are biologically available through the normal digestive processes of the larvae concerned (Appelbaum 1976b;Meyers 1979a; Nose 1979).

11. The cost and time involved in manufacturing must be reasonable.

After selecting ingredients for the experimental feeds to comply with the theoretical nutritional requirements, suitable processing techniques had to be devised with which a dry feed that complies with the physical and chemical requirements as listed, could be produced.

METHODOLOGY

One of the most problematic requirements of dry larval feed is the small particle size of the final product. All solid ingredients must be finely pulverized so that the feed particles which are finally produced have equal proportions of all the ingredients. Furthermore, the high surface to volume ratio of such small particles increases their susceptibility to leaching. As stated before, microencapsulation seems to be the ultimate technical solution to the problem but the cost and time involved disqualified its use in this project. An alternative and simpler method was to process the ingredients in such a way as to produce very dense particles which restrict water penetration and thus leaching.

The basic feed preparation technique that was used was based upon finely pulverizing the solid ingredients, mixing them with water and the water soluble ingredients to form a dough, drying the dough and then pulverizing it to form the feed particles. The particles were sieved into required size ranges and the lipid component of the feed was added at the end. Variations in the feed composition and processing techniques were investigated in order to limit the rate of leaching of the final product.

The principle that water conductivity rises with increased concentrations of ionised solutes was applied to measure the rate and extent of leaching of the various experimental feeds: 500ml of water at 25°C in a glass beaker was kept turbulent by aeration in order to similate conditions in a larval rearing tank. A 1g feed sample of standard nutrient composition and particle size was introduced into the water. The conductivity was continuously measured (in mS/m) and noted at short intervals. The rate at which the conductivity rose in each case, was taken as a measure of the rate of nutrient leaching from the feed sample.

The conductivity values normally stabilized after approximately six minutes. In order to establish the total leachable content, some samples were agitated for 24 hours.

Leaching experiments were conducted with the initial test feed formulations and were repeated at the end of the project with the most successful feed formulation (only the results of the final leaching experiments are shown). The formulation of the standard feed (ingredients as % of total weight) is as follows:

Torula yeast	:	69,8%
Fishmeal	:	23,3%
Ascorbic acid	:	0,1%
"21 Plus"	:	0,8%
Methionine	:	6,0%
"Endox"	:	250ppm
"Furanace"	:	5ppm

The oil supplement consisted of an equal mixture of cod liver oil and soya bean oil.

RESULTS AND RECOMMENDATIONS

Those variations in the processing techniques which had marked effects on leaching are listed in Table 1 and the leaching rates of the feeds thus prepared are shown in Table 2. The leaching rates are shown graphically in Figure 1.

It became evident that the volume of water used during preparation influenced the rate of leaching of the final product. 700ml of water per kilogram of feed is the ideal volume.

It appeared that gelatin had little effect on leaching properties. Gelatin is often used as a binder in fishfeeds, but due to the minimal effect it had on leaching in this experiment, it is not included in the feed formulation that is finally recommended.

The oil supplements which were used in Feeds 8 to 11 (Table 1), had the most important effects on leaching. It was found that Feed 8 (4% oil added), only lost 20% of its total leachable content after two minutes of immersion. This is satisfactory, since it was later found that the duration of the larvae's feeding response was only two minutes.

TABLE 1.

DIFFERENT FEED PREPARATION TECHNIQUES USED IN THE LEACHING EXPERIMENT

Feed no.	Particle size	Treatment
1	<100µm	Control; Ingredients pulverized, no processing.
2	125-212	Using 150ml of water in liquid component.
3	125-212	Using 350ml of water in liquid component.
4	125-212	Using 500ml of water in liquid component.
5	212-250	Same as "3", but larger particle size.
6	250-355	Same as "3", but larger particle size.
7	355-710	Same as "3", but larger particle size.
8	125-212	Same as "3", but adding 4% oil to final product.
9	125-212	Same as "3", but adding 8% oil to final product.
10	212-355	Same as "3", but larger particles and 4% oil.
11	212-355	Same as "3", but larger particles and 8% oil.
12	212-355	Same as "3", but dissolving 10% gelatin in liquid.
13	212-355	Same as "3", but dissolving 15% gelatin in liquid.

TABLE 2.

EFFECT OF PREPARATION TECHNIQUES ON LEACHING RATES OF FEEDS.
Conductivity in mS/m

Feed no.	Initial conductivity	After 15 sec.	After 30 sec.	After 1 min.	After 2 min.	After 4 min.	After 6 min.	After 24 hours
1	35.3	46.9	49.9	52.1	53.5	55.6	56.3	58.5
2	35.3	45.6	49.2	50.8	52.1	52.9	53.0	58.5
3	35.3	38.3	40.2	42.7	46.6	49.6	51.2	56.2
4	35.3	38.4	40.7	42.9	46.8	50.1	51.1	----
5	35.3	37.9	38.1	38.9	40.9	45.1	47.2	----
6	35.3	37.0	38.0	38.8	41.0	46.0	47.5	----
7	35.4	37.1	38.2	38.8	41.3	45.5	47.1	55.4
8	35.1	35.4	36.1	37.2	39.9	44.4	48.6	56.0
9	35.1	35.5	36.2	37.8	40.1	44.6	48.0	----
10	35.0	35.6	36.5	37.2	40.2	44.2	45.5	----
11	35.0	35.5	35.9	37.8	40.2	43.4	45.1	----
12	35.3	35.7	37.2	39.0	41.0	46.2	51.5	----
13	35.3	35.6	37.0	38.9	41.1	46.0	51.6	----

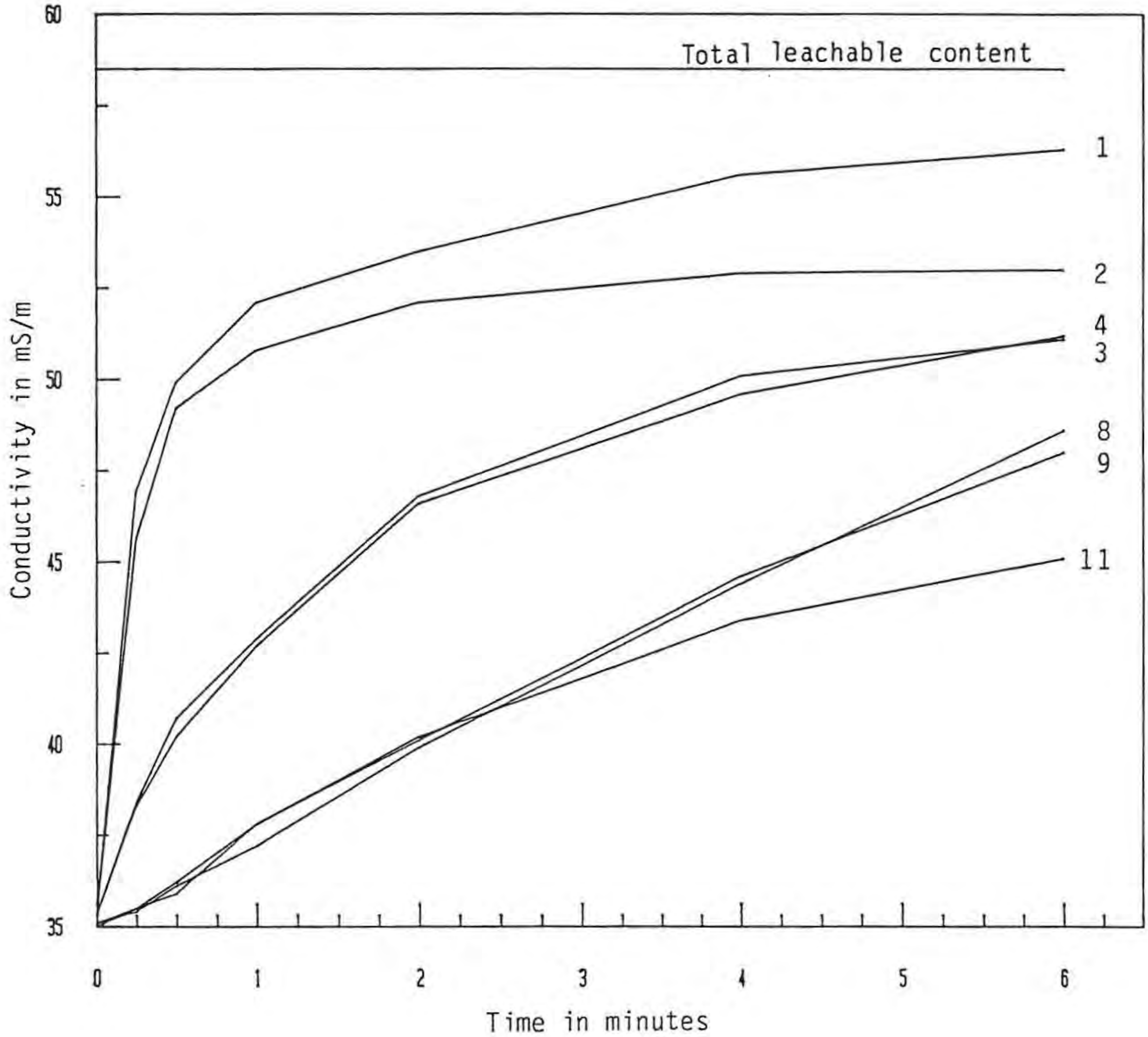


FIGURE 1. LEACHING RATES OF LARVAL FEEDS PROCESSED IN THE DIFFERENT WAYS AS LISTED IN TABLE 1

After several feed preparation trials, a standard manufacturing process was evolved which came closest to producing the required properties of larval feeds. This process is as follows:

1. Solid ingredients were dried until the moisture content was less than 10 percent.

2. All solid ingredients were pulverized with a high speed laboratory hammer mill (Retch type ZM1). Particles were repeatedly passed through the hammer mill until they were less than 100μm in diameter.

3. For each particular feed, the required amounts of solid ingredients were measured out on a proportional basis, so as to make up 500g of feed in total and were mixed thoroughly.

4. The water soluble ingredients were dissolved in 350ml of water.

5. The solution was added to the pulverized solids and then mixed and kneaded thoroughly to produce a thick, smooth dough. (Torula yeast imparted viscous properties to the dough, resulting in a certain degree of gelatinization. No binder was needed when Torula yeast exceeded 50% of the dry ingredients by weight)

6. The dough was then rolled out to form a number of thin cakes 5 - 10mm thick. These were dried in a drying oven with adequate ventilation at a temperature not exceeding 45°C. Drying at this temperature took at least 24 hours but drying at higher temperatures caused porosity of the mixture. As stated previously, the texture must remain smooth and dense. Destruction of heat-labile vitamins also occurs at higher temperatures.

7. The dried cakes were then fractionated using the high speed hammermill without grater sieves into particles 112-750μm in diameter.

8. By using a series of laboratory sieves the particles were then separated into the required sizes. The unusable larger particles were refractionated in the hammermill and sieved again until sufficient quantities of the required particle sizes were produced.

9. The antioxidant, which is oil soluble, was added to the oil supplement. Because of the high density of the particles, the oil supplement did not penetrate but only coated the particles with a thin layer of oil. This limits leaching to a great extent.

Feeds prepared in this manner can be stored for several weeks if the moisture content is maintained at less than 5% and if the feed is kept in a dark and airtight environment at 5°C. The oil supplement must be stored separately and added just prior to the start of a 10-14 day feeding trial.

CHAPTER 3. HATCHERY PROCEDURES AND REARING SYSTEMS

INTRODUCTION

Artificial propagation techniques for C. gariepinus have recently been investigated by several workers (Micha 1972,1975; Van der Waal 1972; Pham 1975; Pham & Raugel 1977; Hogendoorn 1977,1979; Hogendoorn & Vismans 1980; Schoonbee et al. 1980; Hecht et al. 1982). Briefly, this entails collection of broodstock, the induction of spawning with hormonal treatment, treatment and incubation of eggs, and finally hatching and nursing of the larvae.

COLLECTION AND PREPARATION OF BROODSTOCK

The experimental phase of the project was conducted over two breeding seasons. The work during the first season was carried out at the University of the North in the Northern Transvaal. Broodstock was obtained from either Turfloop Dam near the university, or from the Nylstroom municipal reservoir, Donkerpoort Dam. During the second season, the work was continued in the Department of Ichthyology and Fisheries Science, Rhodes University. Broodstock was obtained from the Amalinda Warmwater Fish Station near East London. Some broodstock was also obtained from the P.K. le Roux Dam (30°S / 25°E).

Both male and female broodstock ranged from one year old fish (400-800g) to large (8kg) fish of unknown age. Fish were normally caught by means of gill nets. The nets were patrolled hourly, since high mortalities were encountered if C. gariepinus were trapped in nets for longer periods. Broodstock were transported in 200 litre drums, half filled with water and were then transferred to 1000 litre plastic containers in the hatchery. Due to the distances and often high temperatures involved in transporting fish to the hatchery, conditions in many instances were far from ideal. This again shows how tolerant these fish are of extreme environmental conditions which is a definite advantage when considering C. gariepinus for aquaculture.

INDUCED SPAWNING

Spawning was induced by injecting female fish intramuscularly with 500 IU/kg human chorionic gonadotrophin (tradename Pregnyl). Eight hours later, male and female fish were injected with *C. gariepinus* pituitary gland homogenate. Each fish was administered one pituitary gland from a donor fish of similar size. The eggs were stripped when they became free running, normally 12 hours after the second injection, working at 24°C. At this stage the animal pole was adjacent to the micropyle (Hecht *pers. comm.* 1984).

Eggs were stripped into a dry, plastic bowl. To effect fertilization, semen was obtained by sacrificing males and removing their testes. It was found that semen could be stored for at least two hours, diluted 1 in 10 in a solution of 3g urea and 4g NaCl per litre of water.

TREATMENT AND INCUBATION OF EGGS

Eggs were fertilized by gently mixing in the fresh or diluted sperm for at least three minutes. The eggs were then washed with either a urea-salt solution (Woynarovich 1962) and/or fresh milk or powdered milk solution (concentration equivalent to fresh milk) to remove the adhesiveness from the eggs. The best and most consistant results were obtained by washing the eggs with Woynarovich solution for 15-20 minutes and then with fresh full cream milk for a further 40-60 minutes. During the entire washing time, the respective solutions were decanted and replaced every five minutes.

Eggs were incubated in plastic Zűger funnels (1,5 litre) as described by Hecht *et al.* (1982). On two occasions eggs were not washed, but placed directly on mosquito gauze, suspended in a tank with recirculating hatchery water and left to incubate. A 10-20% higher hatching success was obtained using this method than with eggs of the same batch that were treated for adhesiveness and incubated in funnels. This was presumably due to less mechanical manipulation and resulting damage of the eggs during the early developmental stages. Similar techniques and results were used and obtained by Jongbloed (*pers comm.* 1982) at the Amalinda Warmwater Fish Station, East London. The funnel technique is, however, preferred when dealing with large volumes of eggs because considerably less hatchery space is required and the use of funnels simplifies prophylactic treatment of eggs.

In order to overcome fungal infection, the following prophylactic treatment was found to be effective: Malachite green was applied for 20 minutes every 10 hours at a final concentration of five parts per million. Malachite green application was discontinued at the onset of hatching due to its toxicity to C. gariepinus larvae (Schoonbee et al. 1980). For this reason, it was found that larvae had to be separated from the dead eggs soon after hatching. The most effective separation technique was decanting the contents of a funnel into a tray, with a 1mm mosquito gauze bottom, suspended in a larval rearing tank until all the larvae had swum through the gauze, leaving dead eggs, empty egg cases and deformed larvae behind.

At the University of the North, catfish were spawned successfully from late November (1981) to March (1982). Animals from Amalinda Fish Station were spawned from early October (1982) until February 1983. The early spawning was effected by raising the water temperature of the broodstock ponds with plastic tunnels.

REARING SYSTEMS

During the first breeding season, two different types of larval rearing systems were used. The first type of system (Figure 2) consisted of a 90x35x40cm glass aquarium filled with 70 liters of matured tap water. The water in the aquarium was continuously recirculated by using an "Eheim" filter-pump. A sponge filter medium was used in the filter-pump and the flow rate was 300 litres per hour. A corner of each tank was partitioned off with fine nylon gauze to facilitate the intake of the filter-pump. Water returning from the filter-pump was showered into the aquarium to maintain a high level of dissolved oxygen. Half of the water was replaced every third day.

The second type of system (Figure 3) consisted of a 60x30x36cm glass aquarium containing 25 liters of matured tap water. Maintenance of water quality was effected by using an undergravel filter with air uplift recirculation and by replacing half of the water every third day.

At the start of the second breeding season at Rhodes University, a recirculating fish culture system was adapted for larval rearing purposes (Figure 4). Essentially this system consisted of 40 rectangular fibreglass rearing tanks with a 50 litre capacity each. The water was recirculated through a sand and gravel biological filter (900 litre capacity). A 2500 litre header tank was used to gravity feed the water into the rearing tanks. Aeration was effected at the inflow of each tank where water from the supply pipes under considerable pressure was jetted into the tanks at a rate of 120 litres per hour. Dissolved oxygen was always found to be in excess of 85% saturation. Water temperature was maintained at 23-24°C using immersion heaters. The system was continuously topped up with municipal tap water at a rate of 12 litres per hour. The pH of the system was adjusted to pH 7-8 by using sodium hydroxide when required.

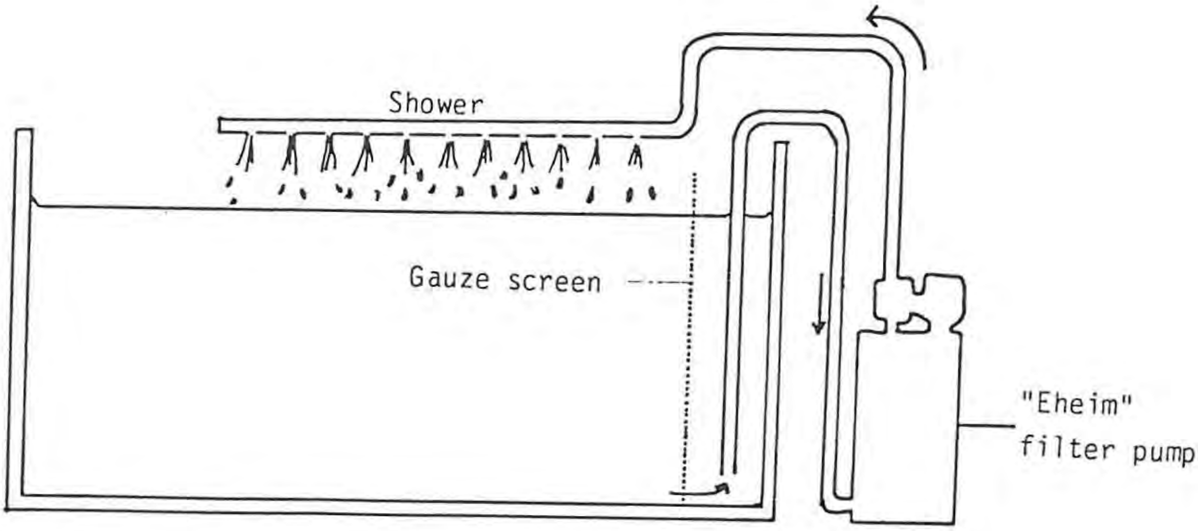


FIGURE 2. TYPE 1 LARVAL REARING TANK WITH "EHEIM" FILTER PUMP

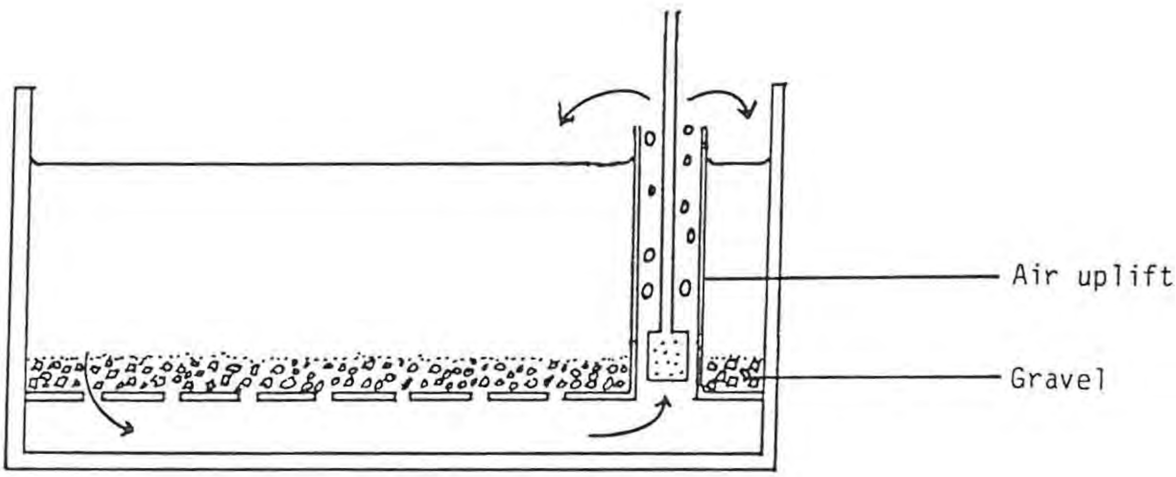


FIGURE 3. TYPE 2 LARVAL REARING TANK WITH UNDERGRAVEL FILTER

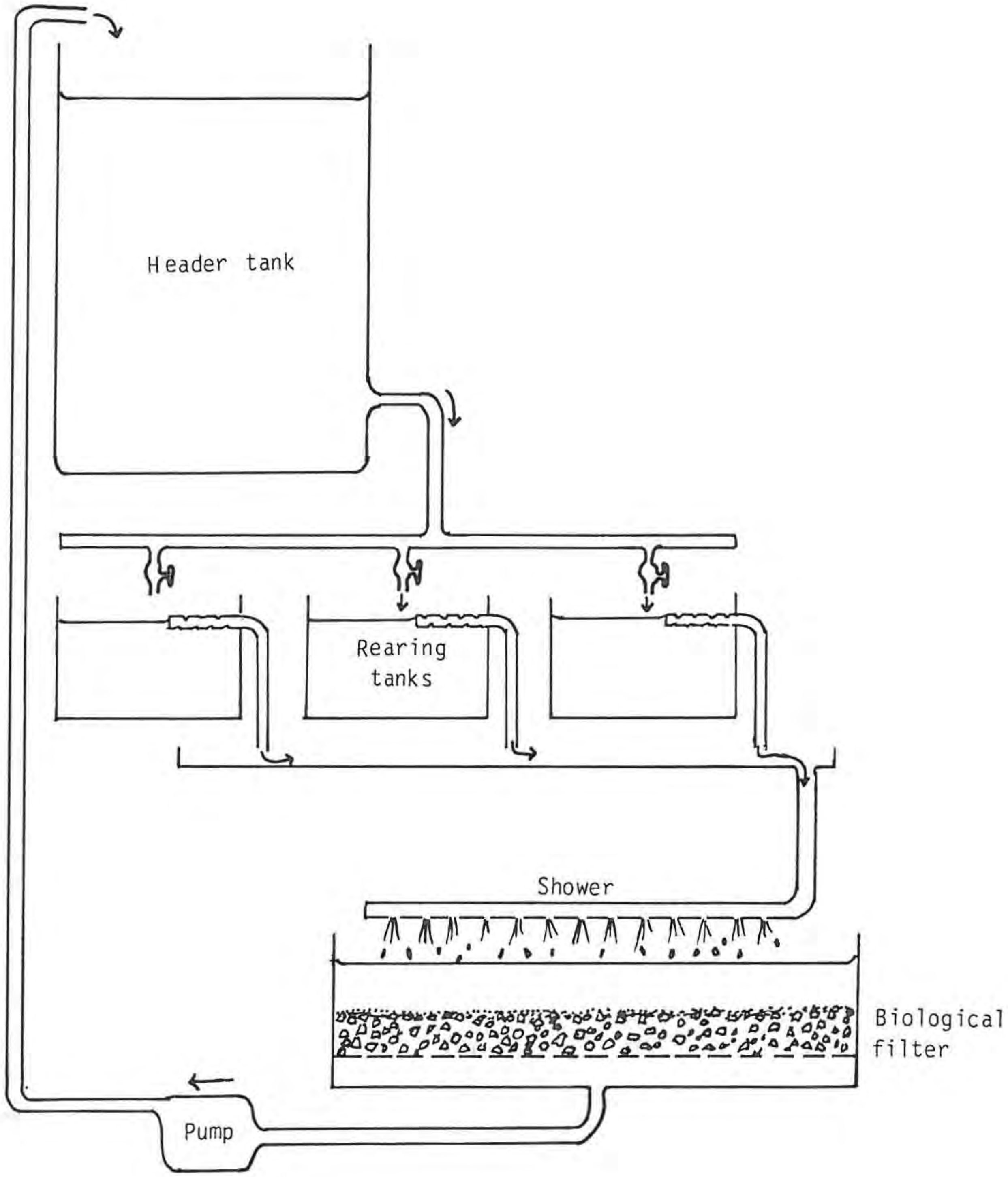


FIGURE 4. RECIRCULATING LARVAL REARING SYSTEM WITH BIOLOGICAL FILTER

CHAPTER 4. MONITORING RESPONSES AND INTERPRETATION OF RESULTS

INTRODUCTION

From a commercial point of view, there are three main parameters by which the degree of success of a larval rearing operation can be measured. These are growth, survival and fitness of the larvae. The physical means of measuring these parameters and the interpretation of data so as to compare responses between different groups of larvae are discussed in this chapter.

METHODOLOGY

When larvae were obtained from more than one female for a particular experiment, they were all placed together in a single container to effect random mixing. The larvae were then divided into separate experimental tanks. In cases where sufficient larvae were available, experimental groups were replicated. If not, the effect of changes in the experimental feeds on the larvae was verified in a subsequent experiment.

In order to measure growth response, the gain in length and mass of an experimental group was determined at intervals during the course of a feeding trial. This was done as follows: A sample of ±15 larvae was taken from each experimental tank. The larvae were placed in a petri dish and anaesthetised by dissolving a pencil tip measure of MS222 in the water. After placing the petri dish on a piece of 1mm graph paper, their individual lengths were determined with the aid of a stereo microscope. The mean total length of a sample and the standard deviation in length, were computed using the formula: $s=\frac{\sqrt{n\Sigma x^2-(\Sigma x)^2}}{n(n-1)}$ (Ferguson 1976). Because of their small size, larvae were not weighed individually. All the larvae of a sample were placed on blotting paper for 30 seconds and then transferred to a balance where the whole sample was weighed to 0,1mg accuracy. Mean mass was thus calculated for each sample.

Where the mean lengths of two samples from groups with different feeding treatments were tested for significant difference, the t-test was applied where: $t=\frac{\bar{L}_1+\bar{L}_2}{\sqrt{s_1^2/n_1+s_2^2/n_2}}$ and $df=n_1+n_2-2$ (Ferguson 1976).

Where: $\bar{L}_1$ = mean length of first sample (in mm)
$\bar{L}_2$ = mean length of second sample (in mm)
s_1^2 = variance of first sample (variance=s^2)
s_2^2 = variance of second sample
n_1 = number of individuals in first sample
n_2 = number of individuals in second sample
df = number of degrees of freedom

The mass-length relationship of a sample of larvae was also taken into account in order to evaluate their condition. A condition factor formula was established by a regression analysis of the mass-length relationship of all the samples of larvae that were taken over the two year period. The mass-length regression formula was found to be $114,5M = L^{2,87}$ (where M= mass in mg and L= length in mm). The condition factor of a sample of larvae could therefore be determined by applying the condition factor formula $CF = \frac{114,5M}{L^{2,87}}$. Where CF>1, it means that the larvae have a higher than normal mass for their particular length and are considered to be in good condition.

The total mortalities of a test group, accumulated over the duration of an experiment, was taken into account when comparing different feeds. This was determined by removing and counting dead larvae on a daily basis.

CHAPTER 5. FEEDING TRIALS

INTRODUCTION

The feeding experiments that follow in this chapter can briefly be described as follows: Aided with the knowledge that Torula yeast is a suitable feed for the first 10 days of feeding (Hecht 1981), an attempt was made to establish a simple feed formulation which could be used as a basis for further experimentation. The most suitable feed formulation that emerged from this first experiment was used in the second experiment to determine the optimal feed particle size.

This being established, it was decided to investigate specific nutrient categories. In the third experiment the adequacy of the mineral content of the basic feed was investigated.

In Experiment 4 and 5 it was attempted to determine the optimum level of lipid supplementation to the basic feed.

In Experiment 6 the optimum level of lipid supplementation was determined more accurately. The use of a commercial antioxidant to preserve the feeds was also investigated. Also, the effect of a more complete vitamin supplement as well as the supplementation of methionine, which was suspected to be a limiting amino acid in the basic feed, were investigated.

Both the methionine and more complete vitamin supplements were found to have beneficial effects. In Experiment 7 the larvae's quantitative requirements for these two supplements were, therefore, investigated.

Further dietary lipid tests were done in Experiment 8, in combination with the new vitamin and methionine supplements. Considering that the lipid supplement had a limiting effect on the nutrient leaching rate, the true nutritional value of the lipid was investigated by using pre-leached feeds.

In Experiment 9 the sufficiency of carbohydrate levels in the feed, using the established level of oil supplementation, was examined. The effects of substituting 6 or 10% of the yeast in the diet with pure starch, were investigated.

In Experiment 10 a wide spectrum bactericide was included in the experimental feeds and the effect thereof investigated. Because of the favourable results achieved with the higher levels of vitamin supplementation in Experiment 7, even higher levels of vitamins were investigated. The effects of replacing some of the yeast with fishmeal as an alternate protein source, to possibly improve amino acid ratios, was also investigated in Experiment 10. The formulation which gave the best results in this experiment, was regarded as the optimal feed and finally recommended.

In Experiment 11 the established dry feed was evaluated by determining conversion efficiencies. Food retention time and the size of a single meal was also determined to serve as guidelines for determining the optimum ration and feeding frequency.

In Experiment 12 the established feed was further evaluated by testing it against natural live food organisms. The effect of different feeding frequencies was also investigated.

EXPERIMENT 1. BASIC EXPERIMENTAL FEED FORMULATION

INTRODUCTION

Although work has been done on the food and feeding of C. gariepinus adults and larvae (Van der Waal 1972; Bruton 1979b; Kouassi and Ville 1975; Pham 1975; Clay 1979, 1981; Hogendoorn 1980; Christensen 1981b; Msiska 1981; Hecht 1981, 1982 and Hogendoorn et al. 1983), data on actual nutritional requirements of C. gariepinus adults and larvae is non-existent. Knowledge of the nutritional requirements of larval fish in general is very limited. This is largely due to the difficulty involved in handling larvae and measuring weight increments to monitor growth, not to mention asessment of food intake and feed efficiency (Van Limborgh 1979). However, even if it is not very reliable, one may obtain some indication of the nutritional requirements of larvae by extrapolating data from bigger fish (Horvath 1979). Indications of nutritional requirements can also be obtained from the nutrient composition of natural food organisms (Dabrowski and Rusiecki 1983). It was, therefore, necessary to consider the available data on the requirements of fishes in general for the specific nutrient categories and the basic metabolic processes involved, to form a theoretical basis from which the practical aspects of this research project could be approached.

As an introduction to this experiment, the role and requirement of protein and vitamins in fish nutrition are briefly discussed. Other nutrient requirements eg. minerals, lipids and carbohydrates are discussed in the introductions to subsequent experiments. Fish digest proteins into amino acids which, after absorption, are used first to form the functional body proteins, namely, enzymes and hormones, and then as "building blocks" in tissue repair and growth (Piper et al. 1982). Protein is the principal dietary component for growth and has been given priority in nutritional studies (Millikin 1982).

The quality, or amino acid ratios, is the most important factor in optimizing utilization of dietary proteins (Andrews 1977). Fish can synthesize some amino acids but usually not in sufficient quantities to satisfy the total requirement (Piper et al. 1982) and certain amino acids are considered indispensible in their food. If a feed is grossly deficient in any of the essential amino acids, poor growth and an increase in food conversion will result even if the food has a high protein level (Piper et al. 1982). The same ten amino acids have been identified as essential amino acids (EAA) for all fish species that have been investigated to date (Cowey 1979; Piper et al. 1982; Millikin 1982). These are listed in Table 3, together with quantitative requirements of four fish species. When formulating test diets and even if EAA ratio requirements are known, the matter is complicated by the fact that amino acids have to be presented to the fish as intact proteins. It seems that free amino acids cannot be utilized equally well and the addition of such free amino acids has provided little or no benefit to fish feeds (Andrews 1977; Cowey 1979; Piper et al 1982). Therefore, a combination of intact plant and animal proteins with different EAA ratios must be used to try and establish the correct EAA balance in the diet.

Further optimization of dietary protein utilization can be achieved by determining the correct protein to energy ratio required in the feed. If insufficient energy is provided in the form of lipids and carbohydrates, dietary protein will to a certain extent be used as an energy source (Andrews 1977; Cowey 1979; Millikin 1982 and Piper et al. 1982). Energy derived from protein by deamination is on a volume and mass basis, less than the energy derived from lipids and carbohydrates. A typical figure for protein/energy ratio is 21mg protein/kJ gross energy as required by Ictalurus punctatus (Andrews 1977).

Although certain patterns are evident in Table 3, significant differences exist between fish species in their requirement for certain EAA's. Cowey (1979) suggested that a possible solution when formulating practical diets for those fish species whose EAA requirements are not yet known, is to use for each amino acid, the highest level required by any of those fish species for which data is available.

In considering the data in Table 3, it should be noted that the figures of the methionine requirement for I. punctatus and Cyprinus carpio were determined in the absence of cystine in the diets. Cystine is a non- essential amino acid but it can replace 50 to 60% of the methionine requirement (Harding et al. In: Milliken 1983). The methionine figures for the salmonids and Anguilla japonica are actually the requirement for methionine plus cystine. The high phenylalanine figures for C. carpio, A. japonica and the salmonids were determined in the absence of tyrosine. Tyrosine can replace approximately 50% of phenylalanine (Robinson et al. In: Millikin 1983). The phenylalanine figure given for I. punctatus is the combined phenylalanine and tyrosine requirement.

Table 4 shows the amino acid composition of some common food organisms. The EAA ratios show some correlaton with the requirements of fishes shown in Table 3.

The total dietary protein requirement of adult freshwater fish species varies from 35 to 55%. It should be borne in mind, however, that dietary protein requirement for most fishes is size dependant i.e. smaller fish of the same species require higher levels of protein in their diet (Piper et al. 1982).

It was decided to use dried Torula yeast (Candida utilis, a type of alkan yeast), as a major ingredient for the experimental feeds since the nutrient composition of Torula yeast and especially the amino acid ratios compare favourably with the amino acid requirements of fishes as described above. Torula yeast also has a high protein content and in addition is an excellent source of vitamins and minerals. These values are shown in Tables 5 and 6. Furthermore, Torula yeast has a proven potential as a first feed for C. gariepinus larvae (Hecht 1981, 1982). Appelbaum (1976b) found another alkan yeast type, Candida lipolytica, very suitable as a substitute for Artemia for C. carpio larvae. Appelbaum and Dor (1978), working with C.carpio larvae, also found that C. lipolytica used as a dry feed, gave a significantly superior growth response to a mixture of boiled egg yolk and Artemia. C. lipolytica was also found to be a suitable dry feed for Coregonus albula fry (Appelbaum 1978,1979a).

Commercially produced synthetic vitamins have to be added to fish feeds as most ingredients of fish feeds do not supply adequate levels of vitamins. Synthetic vitamins can be added to feeds with great precision as a premix (Millikin 1982).

Vitamins have no nutritive value as such, but are dietary essentials, required in small quantities which act as catalysts in enzymatic processes and systems (Piper et al. 1982). Qualitative vitamin requirements of fishes and their physiological functions have been shown to be similar to those of terrestrial animals (Millikin 1983). Most fishes tested have requirements for 11 water soluble vitamins and for at least three of the four fat soluble vitamins. These vitamins and their respective qualitative requirements by freshwater fishes are listed in Table 7.

It should be taken into account that vitamins in fish feed are destroyed by heat, moisture, rancidity and interaction with other ingredients. The oil soluble vitamins A,D,E and K as well as the water soluble vitamins C, thiamine and folic acid are particularly susceptable to destruction during processing and storage of the feed (Dupree 1977; Slinger Razzaque & Cho 1979; Piper et al. 1982). All the water soluble vitamins are naturally subject to leaching and it is, therefore, necessary to supply higher levels in the feed than is required in the diet. Commercial synthetic vitamins covering the whole spectrum were found to be freely available in pharmacies and health food retailers.

The object of this experiment was to establish a simple basic diet which could be used for further experimentation, as well as to identify deficient basic nutrients in Torula yeast.

TABLE 3.

QUANTITATIVE DIETARY AMINO ACID REQUIREMENTS
OF FOUR FISH SPECIES
Expressed as percentage of dietary protein (Millikin 1982)

AMINO ACID	ICTALURUS PUNCTATUS	ONCORHYNCHUS TSHAWYTSCHA	ANGUILLA JAPONICA	CYPRINUS CARPIO	HIGHEST VALUES	MEAN VALUES
Arginine	4.3	6.0	4.5	4.2	6.0	4.33
Histidine	1.5	1.8	2.1	2.1	2.1	1.88
Isoleucine	2.6	2.2	4.0	2.3	4.0	2.25
Leucine	3.5	3.9	5.3	3.4	5.3	4.03
Lysine	5.1	5.0	5.3	5.7	5.7	5.37
Methionine	2.3	4.0	5.0	3.1	5.0	3.10
Phenylalanine	5.0	5.1	5.8	6.5	6.5	5.80
Threonine	2.3	2.2	4.0	3.9	4.0	2.80
Tryptophan	0.5	0.5	1.1	0.8	1.1	1.10
Valine	3.0	3.2	4.0	3.6	4.0	3.40

TABLE 4.

AMINO ACID COMPOSITION OF FOOD ORGANISMS.
Expresssed as percentage of crude protein
(Dabrowski & Rusiecki 1983; Watanabe, Kitajima & Fujita 1983)

AMINO ACID	BRACHIONUS SP.	DAPHNIA SP.	MOINA SP.	ARTEMIA SALINA
ESSENTIAL:				
Arginine	7.62	3.76	5.10	5.00
Histidine	5.96	1.62	1.60	1.30
Isoleucine	5.47	2.42	2.50	2.60
Leucine	9.23	3.94	6.00	6.10
Lysine	10.13	4.70	5.80	6.10
Methionine	2.79	1.32	1.00	0.90
Phenylalanine	7.75	3.25	3.60	3.20
Threonine	5.34	3.62	3.80	1.70
Tryptophan	////	////	1.20	1.00
Valine	8.42	3.44	3.20	3.20
NON ESSENTIAL:				
Cystine	3.51	1.58	0.60	0.40
Tyrosine	7.32	4.80	3.30	3.70
	(Dabrowski & Rusiecki 1983)		(Watanabe *et al*. 1983)	

TABLE 5.

THE AMINO ACID COMPOSITION OF CANDIDA UTILIS, LIVE FOOD ORGANISMS AND THE REQUIREMENT OF FISHES AS SHOWN IN TABLE 3
Expressed as percentage of total protein

AMINO ACID	TORULA YEAST	HIGHEST VALUES REQUIRED BY FISH	MEAN OF VALUES REQUIRED BY FISH	BRACHIONUS SP.	ARTEMIA SALINA	DAPHNIA SP.
ESSENTIAL:						
Arginine	4.78	6.00	4.75	4.61	5.00	3.76
Histidine	2.86	2.10	1.88	1.51	1.30	1.62
Isoleucine	7.37	4.00	2.78	3.60	2.60	2.42
Leucine	8.10	5.30	4.03	5.99	6.10	3.94
Lysine	10.74	5.70	5.28	5.94	6.10	4.70
Methionine	1.41	5.00	3.60	0.86	0.90	1.32
Phenylalanine	4.10	6.50	5.60	3.86	3.20	3.25
Threonine	4.80	4.00	3.10	3.44	1.70	3.62
Tryptophan	0.55	1.10	0.73	1.16	1.00	?
Valine	5.75	4.00	3.45	3.96	3.20	3.44
NON ESSENTIAL:						
Cystine	0.32	?	?	0.78	0.40	1.58
Tyrosine	1.41	?	?	3.03	3.70	4.80
	(Hecht 1981)	(Millikin 1983)		(Watanabe *et al*. 1983)		(Dabrowski &Rusiecki 1983)

TABLE 6.

PROXIMATE ANALYSIS AND VITAMIN COMPOSITION OF CANDIDA UTILIS (Hecht 1981), BRACHIONUS, ARTEMIA, AND DAPHNIA (Watanabe *et al*.1983) AND THE GENERAL REQUIREMENTS OF WARMWATER FISHES (Piper *et al*. 1982)

NUTRIENT		TORULA YEAST	REQUIREMENTS OF WARMWATER FISHES	ARTEMIA SALINA	DAPHNIA	BRACHIONUS
Protein	%dry wt.	50-52	35-55	58	70	45-73
Fat	%dry wt.	2-3	3.5-12	19	13	15
Ash	%dry wt.	8-11	-----	10	6.5	10.8
Carb.hydr.	%dry wt.	20-22	-----			
Vitamins:						
Thiamin	mg/kg	25-30	20			
Riboflavine	"	50-80	20			
Niacin	"	300-370	100			
Pantothenic ac.	"	100-140	50			
Biotin	"	2-2.5	0.09			
Folic acid	"	20-25	5			
Choline	"	5500	550			
Vitamin D	I.U./kg	1500	1000			

TABLE 7.

DIETARY VITAMIN REQUIREMENTS OF FISHES
Expressed as mg/kg of dry diet unless otherwise indicated

	SALMONIDS	WARMWATER FISHES	*CYPRINUS CARPIO*	*ICTALURUS PUNCTATUS*
Vitamin A (I.U.)	2000	5500	2000	500-1000
Vitamin D (I.U.)	---	1000	---	1000-4000
Vitamin E (I.U.)	30	50	100	essential
Vitamin K	80	10	required	very low
Thiamine	10	20	2-3	1
Riboflavine	20	20	7-10	9
Pyridoxine	10	20	5-10	4.2
Pantothenic acid	40	50	30-40	10-250?
Biotin	1	0.09	1-15	8
Choline	3000	551	500-600	essential
Vitamin B12	0.02	0.02	---	---
Niacin	150	100	30-50	14.4
Ascorbic acid	100	100	30-50	50
Folic acid	5	5	---	essential
Inositol	400	100	200-300	---
	Piper *et al*.(1982)		Halver (1972)	Millikin (1982)

METHODOLOGY

Parental material was obtained from Turfloop Dam. One thousand, four day old larvae were placed in each of the six 70 litre glass aquaria (Type 1) described in Chapter 3.

The physico-chemical properties of the water and stocking density were as follows:

Temperature	: 22-24 °C
pH :	: 8,2-8,5
Oxygen saturation	: 95%
Flow rate	: 5 litres/min.
Stocking density	: 13 larvae/litre

The larvae were fed three times daily on an overfeeding basis (in excess of 25% of body wt./day). Uneaten food and faeces were siphoned from the tanks daily. A feed particle size of 180-250µm was used throughout the experiment. The formulation of the six feeds are given in Table 8 and their nutrient compositions in Table 9.

The Group 1 -larvae were fed pure Torula yeast, as a first control diet.

Group 2 had vitamins A, D, E, thiamine, riboflavine, B_6, cyanocobalamine, C, biotin, choline, folic acid, inositol, niacin and pantothenic acid added to the yeast to ascertain whether the yeast alone has an adequate vitamin content.

In Group 3, the same vitamin supplement was added, but two other protein sources, namely gluten flour and "Weider High Protein" (health beverage), were included at the cost of yeast. These two protein sources were included to alter the amino acid ratios of the feed without affecting the crude protein composition as a whole. Gluten flour is an effective binder and is also a rich source of methionine. It was suspected that the methionine levels in Torula yeast might not be sufficient, because of the relatively low level of this amino acid in Torula yeast as compared to the requirements of warmwater fishes (See Table 3). Gluten flour has different amino acid ratios from those of Torula yeast, but its proximate analysis is essentially the same (Crampton and Harris, 1969).

The feed for Group 4 was more complicated as it was an attempt to mix a Torula yeast based feed to conform to the nutritional requirements of warmwater fish in general. Minerals were added in the form of pulverized, dried kelp seaweed and bonemeal. Carbohydrate was supplemented in the form of pure soluble starch. The protein source was mainly yeast and gluten flour. The vitamin premixes were added as before, but more vitamin E was included.

The composition of the diet fed to Groups 5 and 6 was essentially the same as that fed to Group 4, except that 12 and 16% dietary lipids were added to the feeds respectively. The lipid supplement consisted of an equal mixture of cod liver oil and soya bean oil.

TABLE 8.

FORMULATION OF FEEDS USED IN EXPERIMENT 1.
values represent percentage of total weight

INGREDIENTS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
Torula	100.00	94.96	57.96	66.61	58.47	56.10
"Nutri B"	---	0.40	0.40	0.44	0.39	0.37
Ascorbic acid	---	0.14	0.14	0.15	0.14	0.13
"Weider H.P."	---	4.50	16.50	---	---	---
Bonemeal	---	---	---	4.40	3.90	3.70
Sol. starch	---	---	---	5.30	4.70	4.40
Gluten flour	---	---	25.00	17.60	15.60	14.70
Kelp	---	---	---	5.5	4.80	4.60
Soya bean oil	---	---	---	---	6.00	8.00
Cod liver oil	---	---	---	---	6.00	8.00

TABLE 9.

NUTRIENT COMPOSITION OF FEEDS USED IN EXPERIMENT 1.

PROXIMATE ANALYSIS (% dry weight)

NUTRIENT	GROUP 1	GROUP 2	GROUP 3	GROUP 4	REQUIRED VALUES (Piper *et al*. 1982)
Protein	55	55	52	48	35-55
Lipids	2.7	2.7	2.5	2.3	3.5-12
Carbohydrates	28	28	34	32.5	?
Minerals	10.3	10.3	8	12.7	?

AMINO ACID COMPOSITION
(% of dietary protein)

AA.	GROUP 1	GROUP 2	GROUP 3	GROUP 4	MEAN REQUIREMENT (From Table 1) (Millikin 1982)
Arginine	4.80	4.80	3.20	4.10	4.33
Histidine	2.90	2.90	2.00	2.50	1.88
Isoleucine	7.40	7.50	6.50	6.40	2.25
Leucine	8.10	8.40	9.40	8.20	4.03
Lysine	10.70	10.80	8.50	8.70	5.37
Methionine	1.40	1.50	2.00	2.10	3.10
Phenylalanine	4.10	4.30	4.80	3.90	5.80
Threonine	4.80	4.90	4.40	3.90	2.80
Tryptophan	0.60	0.70	0.90	0.52	1.10
Valine	5.80	5.90	5.70	5.10	3.40
Cystine	0.30	0.30	0.30	0.38	?
Tyrosine	1.40	1.40	1.40	1.82	?

VITAMINS (mg/kg unless indicated otherwise)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	REQUIRED VALUES (Piper *et al*. 1982)
A (IU/kg)	---	9000	30000	35000	5500
D (IU/kg)	15000	15100	11900	10000	1000
E (IU/kg)	---	27	136	150	50
Thiamine	25	36	33	25	20
Riboflavine	75	83	65	59	20
Pyridoxine	--	12+	21+	20+	20
Pantothenic.	100	278	280	186	50
Biotin	2	2.3	1.6	1.8	0.09
Choline	5500	5250	3330	3770	551
B12	---	0.10	0.71	0.54	0.02
Niacin	300	480	476	385	100
Ascorbic acid	---	1400	1300	1000	100
Folic acid	20	27.7	22.5	22	5
Inositol	---	40+	40+	44+	100

RESULTS AND DISCUSSION

The results of Experiment 1 are shown in Table 10 and Figures 5 and 6. All feeds were taken readily from the onset of feeding at 100 hours after hatching. This showed that the larvae already have well developed sensory systems at this stage and that they were able to identify the feed particles as food (one of the important required properties of larval fish feed (Appelbaum 1978,1979b,1980b)).

The Group 2 (yeast & vitamins) and Group 4 (yeast, minerals, starch, vitamins & alternate protein) larvae were significantly larger in length than the other groups from the 11th day onwards ($p<0.001$). Even though Group 4 fared well, Group 2 grew significantly faster than any other group ($p<0.001$) from as early as the fourth day. The control group (Group 1, fed pure yeast) showed good initial growth, but started levelling off between the 8th and 11th days. Between the 11th and the 13th day, no additional growth was recorded for this group. This indicated that Torula yeast alone, after being processed into feed particles, was lacking in dietary vitamins.

The feeds to which oil was added (Groups 5 & 6) showed the poorest results, especially during the last three days when weight loss occurred. High mortalities were also encountered with these two feeds. At this stage it seemed that dietary oil supplement had a detrimental effect on the larvae (Compare Groups 5 & 6 with Group 4 which received the same diet except the oil supplement. In subsequent experiments it was shown that dietary oil supplement was required provided that it was suitably preserved).

The use of gluten flour as an alternate protein source was shown to be unsuccessful: The Group 2 larvae were significantly larger in length than those of Group 3 by as early as the 4th day ($p<0.001$). It therefore seemed that the altered amino acid ratios in the feed given to Group 3 had a detrimental effect on the larvae.

The most important item of information that came to light in this experiment was the deficiency of vitamins in Torula yeast. As can be seen in Table 6, the vitamin composition of Torula yeast when compared to the levels of vitamins required by warmwater fishes in general, is deficient in vitamins A, E, B6(pyridoxine), B12(cyanocobalamine), C(ascorbic acid), and inositol. It can also be seen that the increased vitamin levels of of the Group 2 feed (which showed the best results), correspond closely with the levels required by warmwater fishes in general.

TABLE 10.

GROWTH IN LENGTH AND MASS OF <u>C. GARIEPINUS</u> LARVAE FED ON SIX DIFFERENT DIETS IN EXP. 1.

DAY 1:

GROUP	N	L	S	M	M+L	CF
All	19	6.0	0.21	2.1	8.1	1.41

DAY 4:

GROUP	N	L	S	M	M+L	CF
1	15	6.8	0.25	2.5	9.3	1.17
2	15	7.2	0.29	3.0	10.2	1.19
3	16	6.7	0.36	2.4	9.1	1.17
4	14	6.8	0.22	2.7	9.5	1.26
5	15	6.8	0.28	2.6	9.4	1.22
6	17	6.7	0.25	2.3	9.0	1.12

DAY 8:

GROUP	N	L	S	M	M+L	CF
1	15	9.0	0.44	4.2	13.2	0.88
2	14	9.8	0.75	7.7	17.5	1.26
3	17	8.7	0.39	3.9	12.6	0.90
4	15	9.1	0.74	6.2	15.3	1.26
5	15	7.7	0.42	3.8	11.5	1.24
6	15	7.8	0.50	3.7	11.5	1.17

DAY 11:

GROUP	N	L	S	M	M+L	CF
1	15	9.2	0.48	4.4	13.6	0.86
2	15	11.0	0.59	8.9	19.9	1.05
3	13	9.0	0.58	4.2	13.2	0.88
4	16	10.3	0.58	7.7	18.0	1.09
5	15	8.4	0.52	4.1	12.5	1.05
6	15	8.7	0.48	4.5	13.2	1.04

DAY 13:

GROUP	N	L	S	M	M+L	CF	ACCUMULATED TOTAL MORTALITY
1	13	9.2	0.52	4.4	13.6	0.86	10%
2	17	11.8	0.50	11.0	22.8	1.06	5%
3	15	9.2	0.70	4.5	13.7	0.88	10%
4	16	11.0	0.57	9.3	20.3	1.09	5%
5	17	8.4	0.58	4.0	12.4	1.02	30%
6	17	8.9	0.51	4.1	13.0	0.89	40%

N = number of larvae in sample
L = mean total length in mm.
S = standard deviation of L
M = mean mass in mg.
CF= condition factor

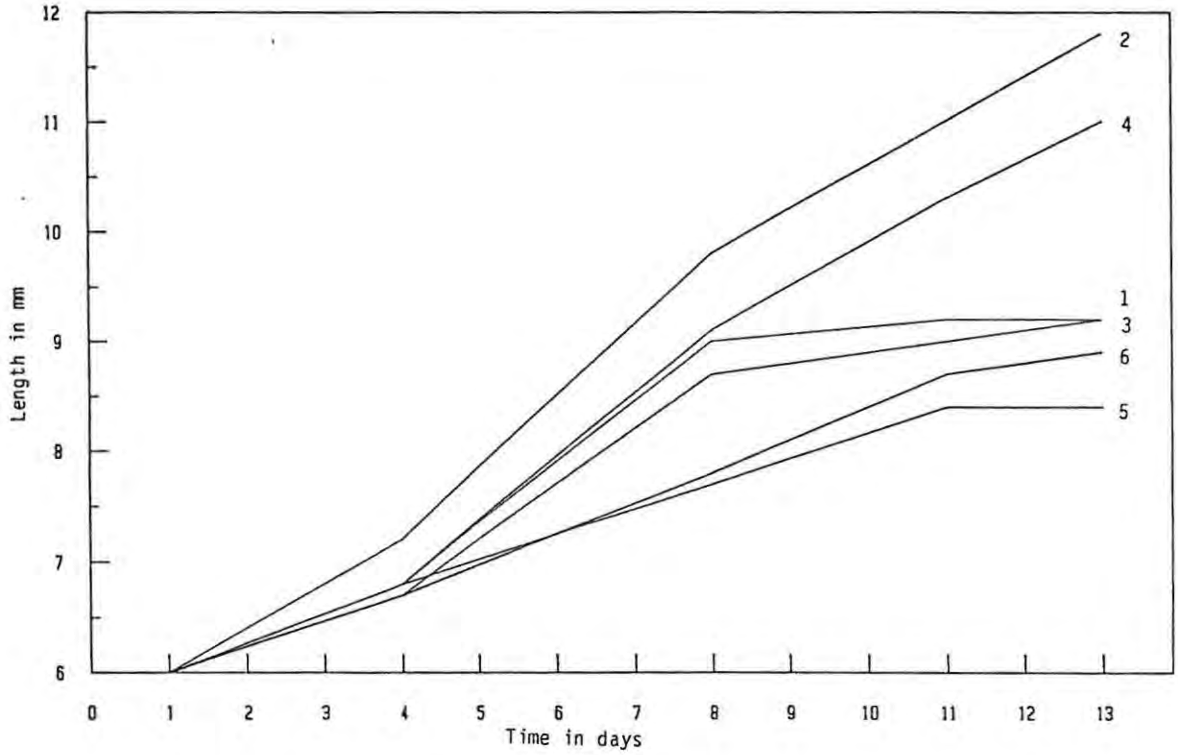


FIGURE 5. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXP. 1

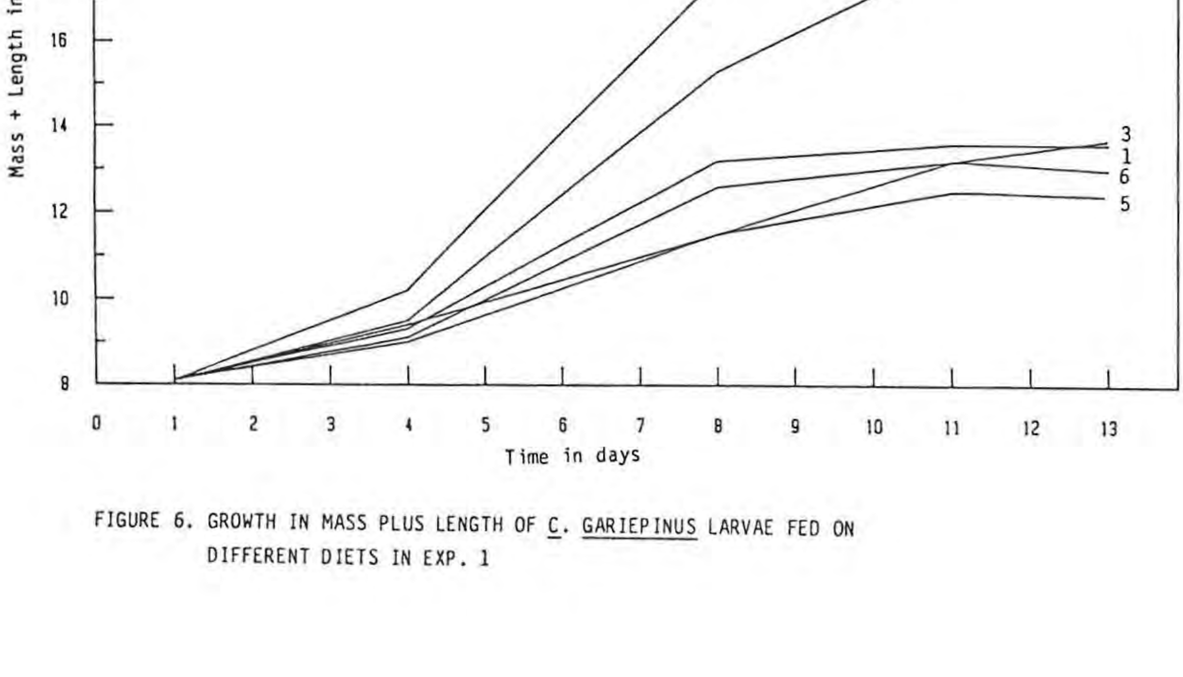


FIGURE 6. GROWTH IN MASS PLUS LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXP. 1

EXPERIMENT 2. OPTIMUM FEED PARTICLE SIZE

INTRODUCTION

Since a suitable experimental feed formulation had been established in Experiment 1 (yeast + vitamins), the next step was to investigate one of the more important physical requirements of larval feed, namely optimum particle size (Van Limborgh 1979; Webber & Huguenin 1979). The reasons for investigating optimum feed particle size were firstly to determine the feed particle size which would optimize growth and survival of the larvae. Secondly, efficient feed collecting by the larvae will minimize the duration which feed particles are immersed before being ingested. Finally, it was important to establish the optimum particle size in order to reduce nutrient leaching from smaller particles due to their high surface to volume ratio.

METHODOLOGY

Approximately 3000 newly hatched larvae were kept in a 1000 litre plastic bin containing 400 litres of matured tap water. The six 70 litre glass aquaria (Type 1) as described in Chapter 3 were also used. The physico- chemical conditions were essentially the same as in Experiment 1:

Temperature : 22-24 °C
pH : : 8,2-8,5
Oxygen saturation : 95%
Flow rate : 5 litres/min.
Stocking density : 13 larvae/litre

For the first four days of feeding, the six aquaria were stocked with 100 larvae each. The six groups of larvae were then fed on different feed particle sizes. On the fourth day the six groups of larvae were taken out, weighed and measured and then replaced by new groups of larvae from the plastic bin. The same procedure was carried out for the 9th to 12th and the 13th to 16th days. The stock of larvae in the plastic bin were fed on a wide range of feed particle sizes (180-315µm) throughout the experiment. The established control diet formulation (yeast & vitamins) was used.

The feed particle sizes fed to the six groups were as follows:
GROUP 1: 180-315μm (wide range, control)
GROUP 2: 125-180μm
GROUP 3: 180-200μm
GROUP 4: 200-250μm
GROUP 5: 250-315μm
GROUP 6: 315-355μm

RESULTS AND DISCUSSION

The results of the experiment are shown in Table 11 and Figure 7. Mortalities were negligible in all cases. Observations showed that all feed particle sizes were actively ingested by larvae of all sizes. During the first four days and to some extent also during days five to eight, the large particles (250-315μm and 315-355μm) could not be ingested whole. The larvae did, however, succeed in breaking pieces off the large feed particles after about two minutes of the feed being immersed. Normal duration of feeding behaviour was two to three minutes. However, larvae fed on particles too large to ingest whole continued their feeding behaviour for six to eight minutes. This must be avoided, since excessive nutrient leaching takes place if the feed is immersed too long before being ingested.

Overall, the size of feed particles did not have a strong influence on the growth rates of the various groups of larvae. Statistical differences were not very significant, except for Group 4 on the 12th day which was significantly larger in length than any other group at that stage ($p<0.05$) and Group 5 on the 16th day which also was significantly longer than any other group at that stage ($p<0.01$).

Considering the mean lengths, masses and condition factors of the groups of larvae used in this experiment, the following recommendations could be made as to the optimum feed particle size ranges:

Age or size of the larvae	Feed particle size
1st to 4th day of feeding or total length 6-8.5mm.......	125-200μm
5th to 8th day of feeding or total length 8.5-10mm......	200-250μm
9th to 16th day of feeding or total length 10-15mm......	250-350μm

This substantiates the recommendation of Van Limborgh (1979), that initial feed particle size for non-salmonids that hatch from eggs approximately 1mm in diameter (as in the case of C. gariepinus) should not exceed 100-200µm.

A rule of thumb would be to multiply the total length of the fish in mm, by a factor of 22 to give the required feed particle diameter in µm. These particle size ranges were used in all subsequent experiments.

TABLE 11.

GROWTH IN LENGTH AND MASS IN 4 FEEDING TRIALS WITH C. GARIEPINUS LARVAE FED ON DIFFERENT FEED PARTICLE SIZES IN EXPERIMENT 2

FIRST 4 DAYS OF FEEDING:

On 1st day:

N	L	S	M	M+L	CF
10	8.0	0.12	3.4	11.4	1.00

On 4th day:

PARTICLE SIZE(um)	GROUP	N	L	S	M	M+L	CF
180-315	1	15	8.3	0.10	3.9	12.2	1.03
125-180	2	15	8.3	0.12	3.9	12.2	1.03
180-200	3	15	8.2	0.13	3.8	12.0	1.04
200-250	4	15	8.2	0.10	3.9	12.1	1.07
250-315	5	15	8.3	0.13	3.8	12.1	1.00
315-355	6	15	8.2	0.10	3.7	11.9	1.01

5TH TO 8TH DAYS OF FEEDING:

On 5th day:

GROUP	N	L	S	M	M+L	CF
	10	8.4	0.18	4.0	12.4	1.02

On 8th day:

PARTICLE SIZE(um)	GROUP	N	L	S	M	M+L	CF
180-315	1	15	9.3	0.14	5.7	15.0	1.09
125-180	2	15	9.2	0.18	5.7	14.9	1.12
180-200	3	15	9.5	0.14	6.0	15.5	1.07
200-250	4	15	9.6	0.23	6.2	15.8	1.08
250-315	5	15	9.3	0.22	5.6	14.9	1.07
315-355	6	15	9.5	0.24	5.9	15.4	1.06

9TH TO 12TH DAYS OF FEEDING:

On 9th day:

N	L	S	M	M+L	CF
11	9.9	0.22	6.8	16.7	1.08

On 12th day:

PARTICLE SIZE(um)	GROUP	N	L	S	M	M+L	CF
180-315	1	15	10.9	0.30	9.0	19.9	1.09
125-180	2	15	10.6	0.24	8.4	19.0	1.10
180-200	3	15	10.4	0.18	7.5	17.9	1.04
200-250	4	15	11.0	0.12	9.4	20.4	1.11
250-315	5	15	10.9	0.13	8.9	19.8	1.07
315-355	6	15	10.7	0.22	8.4	19.1	1.07

13TH TO 16TH DAYS OF FEEDING:

On 13th day:

N	L	S	M	M+L	CF
10	11.9	0.21	11.9	23.8	1.12

On 16th day:

PARTICLE SIZE(um)	GROUP	N	L	S	M	M+L	CF
180-315	1	15	13.2	0.34	15.4	28.6	1.07
125-180	2	15	13.0	0.24	15.5	28.5	1.13
180-200	3	15	13.0	0.30	15.4	28.4	1.12
200-250	4	15	13.3	0.39	16.2	29.5	1.10
250-315	5	15	13.8	0.28	18.5	32.3	1.13
315-355	6	15	13.4	0.32	16.3	29.7	1.09

N = number of larvae in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

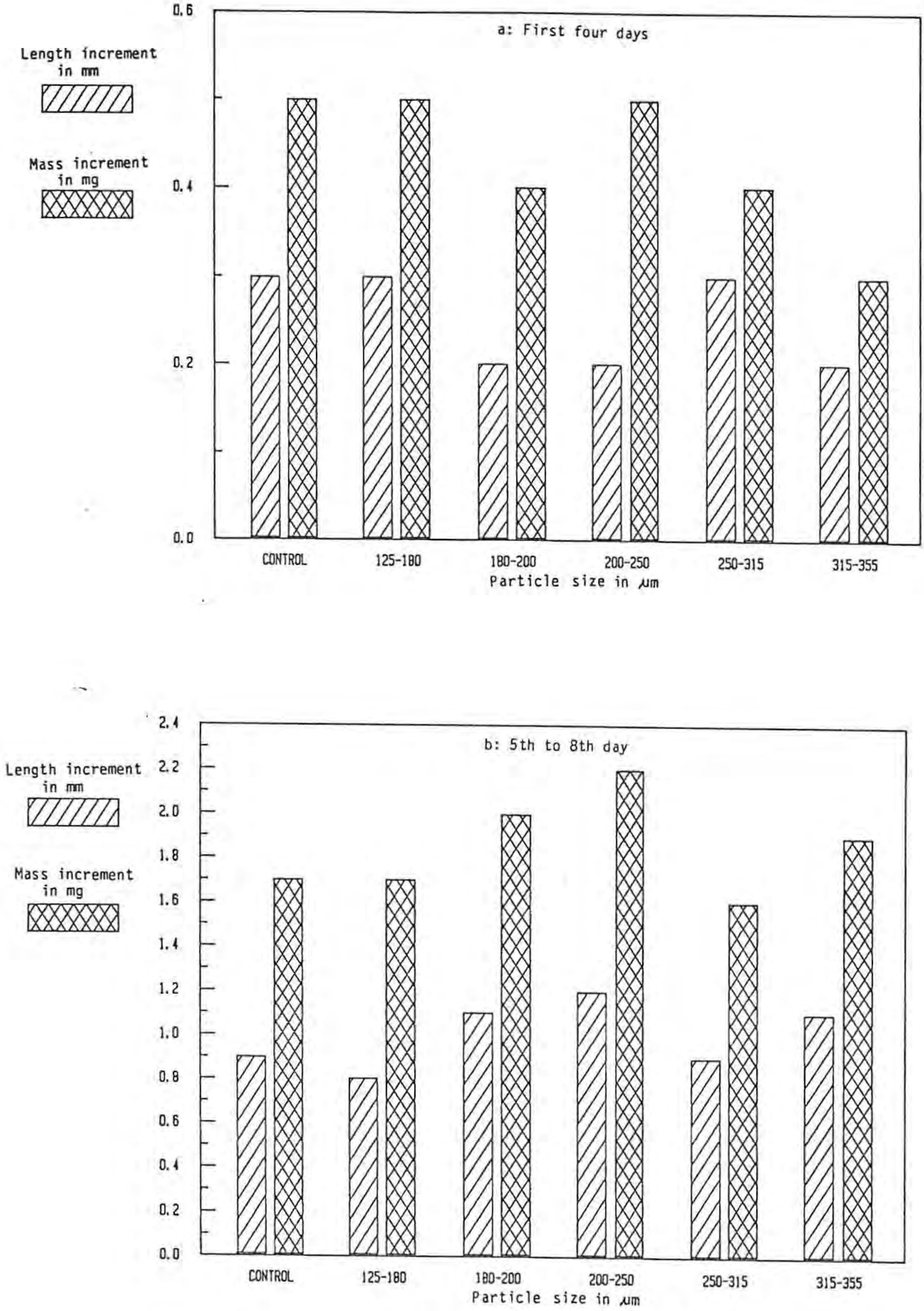
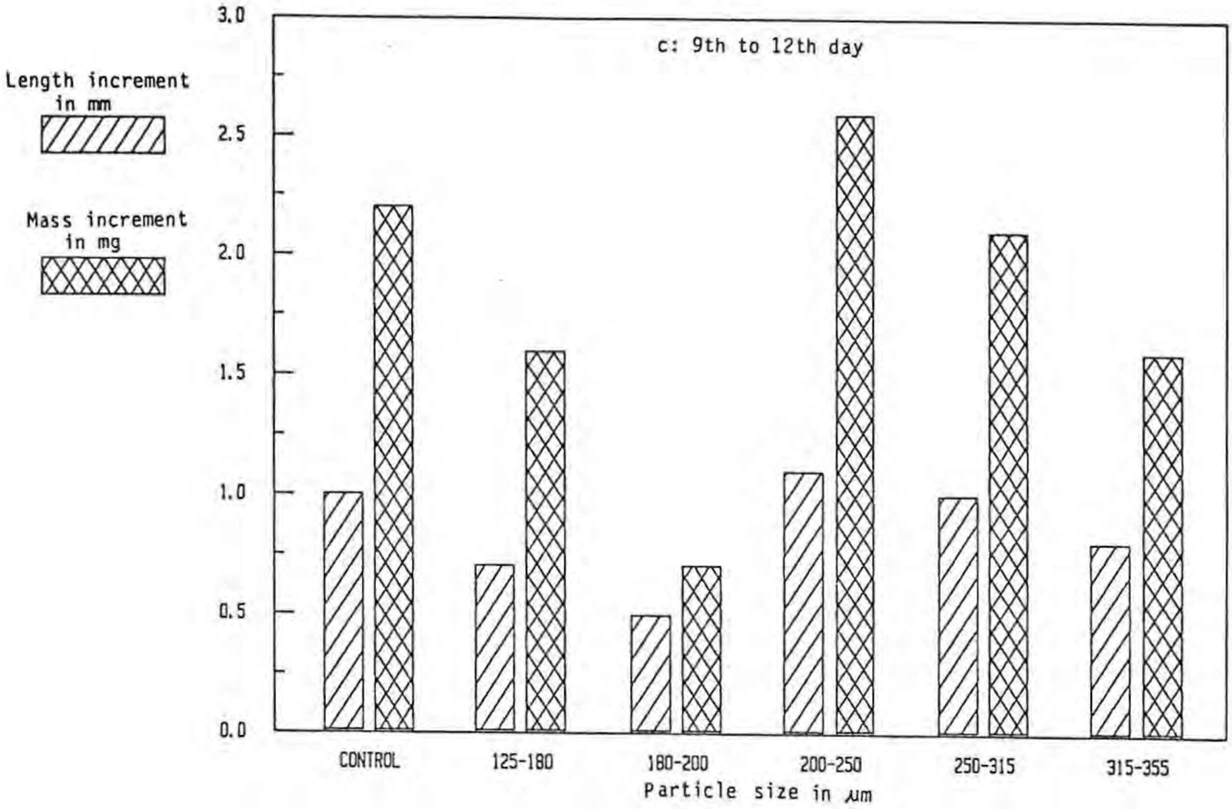


FIGURE 7a & b. INCREMENTS IN LENGTH AND MASS OVER FOUR DAY PERIODS OF C. GARIEPINUS LARVAE FED ON DIFFERENT FEED PARTICLE SIZES IN EXPERIMENT 2



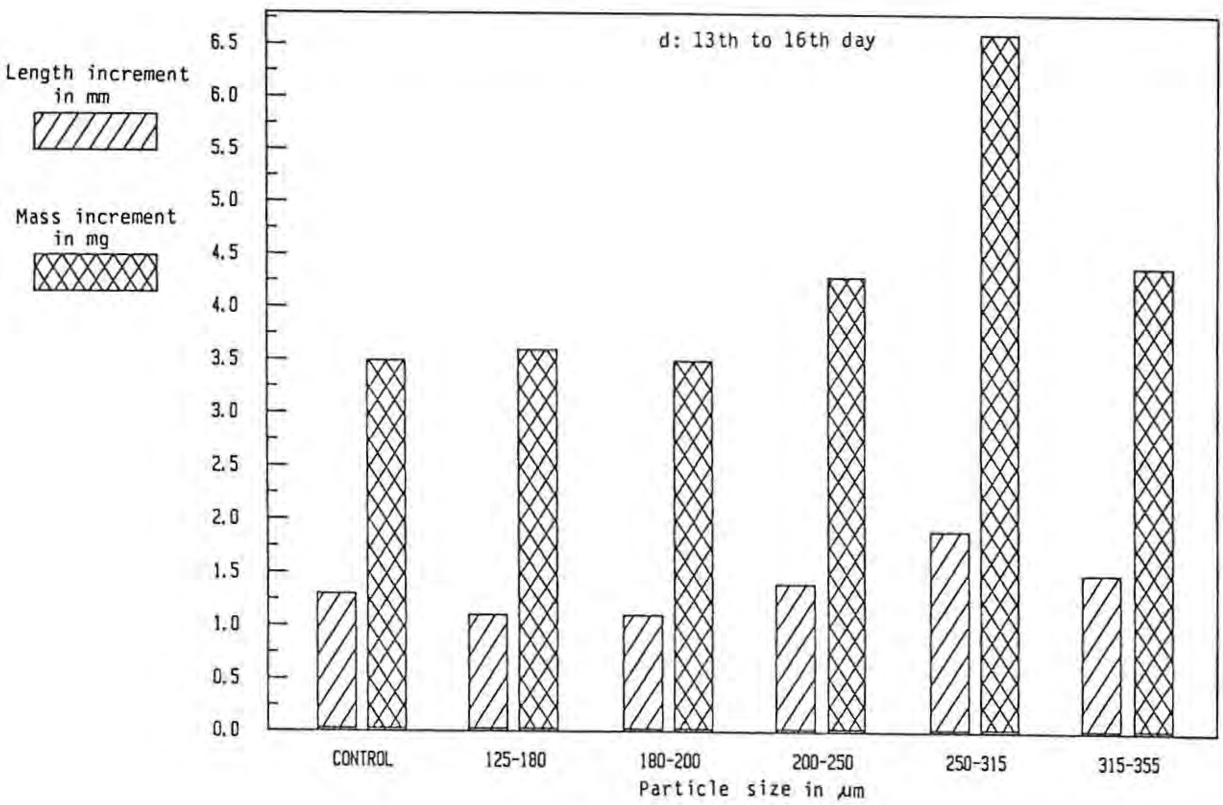


FIGURE 7c & d. INCREMENTS IN LENGTH AND MASS OVER FOUR DAY PERIODS OF C. GARIEPINUS LARVAE FED ON DIFFERENT FEED PARTICLE SIZES IN EXPERIMENT 2

EXPERIMENT 3. MINERAL SUPPLEMENTATION

INTRODUCTION

Very little is known about dietary mineral requirements of fishes but the minerals which have demonstrated biological functions, either in element form or incorporated into specific compounds, are the same as required by other animals (Lovell 1977a; Lall 1979; Piper et al. 1982). Seven major minerals are required in large amounts and constitute 60 to 80% of all the inorganic materials in the fish body. These include: calcium, phosphorus, sulphur, sodium, chlorine, potassium and magnesium (Lovell 1977a; Lall 1979; Piper 1982). The other essential minerals are trace elements, required only in small amounts. These include iron, copper, iodine, manganese, cobalt, zinc, molybdenum, selenium and fluorine (Piper et al. 1982).

Although, according to Lall (1979), the study of mineral requirements of fish has been neglected so far, it has been demonstrated that both dissolved and dietary minerals are important to the health and vigour of fish (Lall 1979; Piper 1982). Minerals are normally present in adequate levels in the usual ingredients of fish feeds. If not, minerals can easily be supplemented to the feed (Piper et al. 1982).

The object of this experiment was to determine whether Torula yeast had a sufficient mineral content. Two groups of larvae were fed on the established control diet (yeast & vitamins) and two other groups of larvae were fed on the same feed to which 4% dried kelp seaweed had been added as a mineral supplement. Dried Kelp Seaweed (a "Vita Force" health food product) contains high levels of all dietary minerals and trace elements.

METHODOLOGY

Parental fish were caught in Donkerpoort Dam. The larvae were hatched in recirculating, matured tap water. They were then divided into four groups and placed in four of the 25 litre aquaria (Type 2) as described in Chapter 3.

The physico-chemical conditions and stocking density were as follows:

Temperature : 24-25 °C
pH : : 7,9-8,3
Oxygen saturation : 95%
Flow rate : 5 litres/min.
Stocking density : 10 larvae/litre

Groups 1 and 2 were fed on the control diet (yeast & vitamins). Groups 3 and 4 were fed the same basic control diet plus 4% dried kelp at the cost of yeast. The larvae were fed three times daily in excess of 25% of body wt. per day.

RESULTS AND DISCUSSION

The results of Experiment 3 are shown in Table 12 and Figures 8 and 9. Mortalities were negligible throughout the experiment. Groups 1 and 2 grew slightly faster until the 10th day. From the 10th day onwards, growth rates were more or less equal so that on the 25th day, Group 2 was significantly larger in length ($p < 0.05$) than Groups 3 and 4. Group 1 had a low significant difference from Groups 3 and 4 ($p < 0.2$).

Since there was no benefit from the higher mineral content of the feed to which dried kelp had been added, the mineral content of Torula yeast (given below) seems to be adequate. The levels of the seven major minerals are shown as a percentage of total dry matter by weight (Crampton & Harris 1969):

Calcium : 0,57
Phosphorus : 1,68
Sulphur : unknown
Sodium : 0,01
Chlorine : unknown
Potassium : 1,88
Magnesium :12,80

Of the trace elements in Torula yeast, only the following values are available (Crampton & Harris 1969):

Iron	0,01% of dry matter by weight
Copper	13,4mg/kg
Manganese	12,8mg/kg
Zinc	99,2mg/kg

It is surmised that the lower growth rates of the two groups which had dried kelp added to their feed can be attributed to the 2% reduction of total protein in their diet.

TABLE 12.

GROWTH IN LENGTH AND MASS OF FOUR GROUPS OF <u>C. GARIEPINUS</u> LARVAE FED ON TWO DIFFERENT DIETS IN EXP.3

DAY 1:

GROUP	N	L	S	M	M+L	CF
All	10	8	0.78	3.4	11.4	1.00

DAY 5:

GROUP	N	L	S	M	M+L	CF
1	8	8.6	0.95	4.3	12.9	1.02
2	7	8.6	0.86	4.3	12.9	1.02
3	7	8.4	0.76	4.0	12.4	1.02
4	7	8.5	0.86	4.1	12.6	1.01

DAY 10:

GROUP	N	L	S	M	M+L	CF
1	8	10.0	0.52	6.5	16.5	1.00
2	7	10.0	0.45	6.8	16.8	1.05
3	8	9.2	0.49	4.9	14.1	0.96
4	9	9.8	0.57	5.9	15.7	0.97

DAY 25:

GROUP	N	L	S	M	M+L	CF
1	15	15.5	0.80	28.6	44.1	1.26
2	15	15.8	0.91	27.9	43.7	1.16
3	15	15.0	1.12	26.9	41.9	1.3
4	15	15.1	0.94	24.7	39.8	1.17

N = number of individuals in sample
L = mean total length in mm.
S = standard deviation of L
M = mean mass in mg.
CF= condition factor

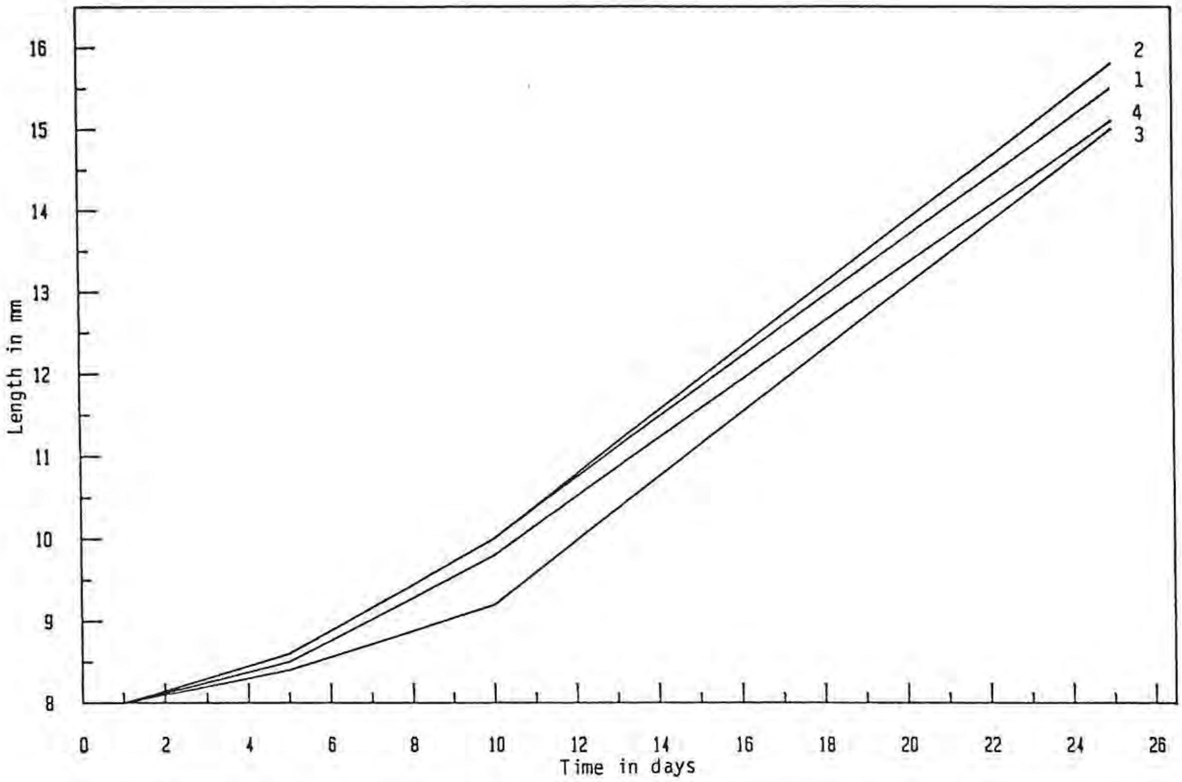


FIGURE 8. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 3

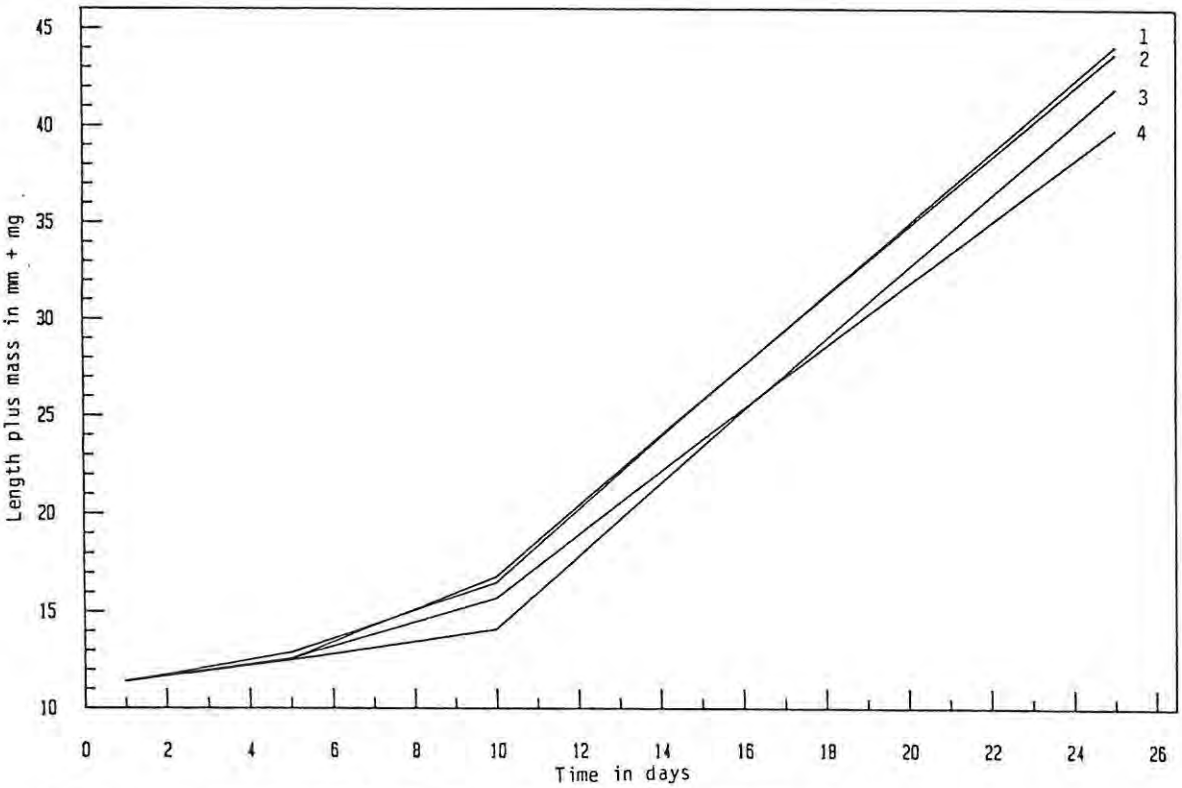


FIGURE 9. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 3

EXPERIMENT 4. DIETARY LIPID REQUIREMENT

INTRODUCTION

Dietary lipids provide a concentrated energy source, having at least 2,25 times more energy per unit weight than carbohydrates or proteins (Stickney 1977b; Piper et al. 1982). Apart from being an important energy source, lipids also serve several other functions such as reserve energy storage, insulation of the body, transport of fat soluble vitamins, formation of essential lipids and hormones for certain body processes and forming structural components of membranes (Piper et al. 1982). Suitable supplementation of oils to diets has produced increases in both energy and protein retention (Stickney 1977b; Viola & Rappaport 1979; Viola, Rappaport, Arielli & Mokady 1981; Millikin 1982).

High levels of poly-unsaturated fatty acids (PUFA), with carbon chains 18 to 20 units in length, are generally found in the tissues of fishes (Stickney 1977b). PUFA of the W3 and W6 families in particular seem to play an important role in fish nutrition (Castell 1979). (The "W" nomenclature of fatty acids is a designation where the "W" or "omega" -number identifies the position of the first double bond, counting from the methyl end. Linolenic acid, for instance, is written as 18:3W3. The first number indicates the number of carbons, the second number the number of double bonds and the last number the position of the double bonds).

Essential fatty acid requirements differ from species to species (Takeuchi & Watanabe 1982; Takeuchi, Satoh & Watanabe 1983), but generally, fish require PUFA and highly unsaturated fatty acids (HUFA) such as 18:3W3 and 20;5W3 (Kanazawa, Teshima & Sakamoto 1982). *Salmo gairdneri* also require high levels of W3 HUFA whereas *C. carpio*, *Anguilla japonica*, *Tilapia* and *Oncorhynchus tshawytscha* seem to require 18:2W6 fatty acids as well (Takeuchi & Watanabe 1982; Castell 1979).

Castell (1979) in a review of lipid requirements of finfish noted that warmwater species have high levels of W6 fatty acids in their tissues and that the W6/W3 ratio decreases with a decrease in temperature. If trends in fatty acid composition of the fish can be taken as clues to their esential fatty acid requirements, Castell (1979) predicted that fish raised in warmer water such as carp, channel catfish and tilapia, may do better with a mixture of W9, W6 and W3 PUFA in their diets.

Fish oils such as cod liver oil are high in W3 PUFA and low in W6 fatty acids. Practical diets for warmwater fish would, therefore, also require some plant oil which is high in W6 and W9 PUFA.

The requirement of fish for PUFA creates problems with respect to feed storage, as this type of fatty acid is very liable to oxidation (Castell 1979). The products of oxidation and rancid fats may be toxic. Care has to be taken to preserve these fatty acids by using antioxidant in the feed.

Total lipid levels in practical diets range from 3,5 to 12% of dry diet (Garling and Wilson 1977). As with the case of proteins, larval fish have higher lipid requirements than adult fish, eg. the total lipid requirement of salmonids is given as follows (Piper et al. 1982):

Fry : 15% of dry weight of diet
Fingerlings : 12% "
Older fish : 9% "

Excess body lipids due to high energy feeds are normally unwanted in fish production, but if fish are being reared in a hatchery for release into ponds, high body lipid composition may be beneficial as an energy source during the acclimation period (Millikin 1982).

The object of this experiment was to quantify the lipid requirement of the larvae. The Group 2 feed used in Experiment 1, was adopted as a control diet (yeast & vitamins). The first group of larvae were fed on the control diet while the other three groups had 6, 10 and 16% of oil supplement in their diets respectively.

METHODOLOGY

The Group 2 larvae of Experiment 1 were used for this experiment. They were maintained on the original diet (control diet in this experiment) up to their 15th day of feeding, whereafter four groups of 180 larvae each were placed into four of the 25 litre aquaria (Type 1) as described in Chapter 3. The physico-chemical conditions and stocking density were as follows:

Temperature	: 23-25 °C
pH :	: 7,9-8,3
Oxygen saturation	: 95%
Flow rate	: 5 litres/min.
Stocking density	: 7 larvae/litre

The larvae were fed three times daily and their ration was in excess of 25% of body weight per day. The oil supplement was an equal mixture of cod liver oil and soya bean oil. This made the supplement high in levels of W3 and W6 fatty acids, which were demonstrated to be essential for other fish species (Castell 1979).

The feed formulations were as follows:

Group 1. (Control) :	Torula yeast	95%
	"Nutri B"	0,4%
	Ascorbic acid	0,14%
	"Weider High Prot."	4,5%

Group 2. Control + 6% oil mixture added.

Group 3. Control + 10% oil mixture added

Group 4. Control + 16% oil mixture added

RESULTS AND DISCUSSION

The results of Experiment 2 are shown in Table 13 and Figures 10 and 11. It is evident from the results of the experiment that the addition of oil to the diet adversely affected the larvae. On the 15th day, the larvae fed on the control diet were highly significantly larger in length than the other three groups ($p<0.001$). The mean mass of the Group 1 larvae was more than double that of Group 4. Low condition factors and high mortalities also reflected the detrimental effect of oil in these diets.

The following explanation for the poor results is offered: The experimental feeds were prepared one day before the start of the feeding trials and then stored in clear glass containers at room temperature. Castell (1979) reported that PUFA of the W3 series are particularly subject to rancidity, but since the experiment was only run for 15 days, it was thought not to be neccessary to preserve the feed in any other way than by drying. The results of the experiment, however, indicate that deterioration of the nutrients did occur. Products of lipid oxidation may react with other nutrients such as proteins and vitamins and reduce their available dietary levels, or the oxidation products may be toxic (Castell 1979).

TABLE 13.

GROWTH IN LENGTH AND MASS OF FOUR GROUPS OF *C. GARIEPINUS* LARVAE FED ON DIETS WITH VARYING LEVELS OF LIPID SUPPLEMENTATION IN EXP. 4.

GROUP	N	L	S	M	M+L	CF	ACCUMULATED TOTAL MORTALITY
		DAY1					
All	10	12.0	0.52	11.8	23.8	1.08	
		DAY7					
1	10	17.1	0.89	37.4	54.5	1.24	
2	11	16.0	1.51	32.2	48.2	1.29	
3	9	15.6	1.80	30.6	46.2	1.32	
4	9	16.3	1.20	31.9	48.2	1.21	
		DAY 15					
1	16	22.0	1.23	72.0	94.0	1.16	5%
2	16	19.1	1.31	46.6	65.7	1.12	50%
3	16	18.0	1.56	40.0	58.0	1.14	50%
4	15	18.0	1.52	34.6	52.6	0.99	40%

N = number of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

Group 1: Control
Group 2: Control + 6% oil
Group 3: Control +10% oil
Group 4: Control +16% oil

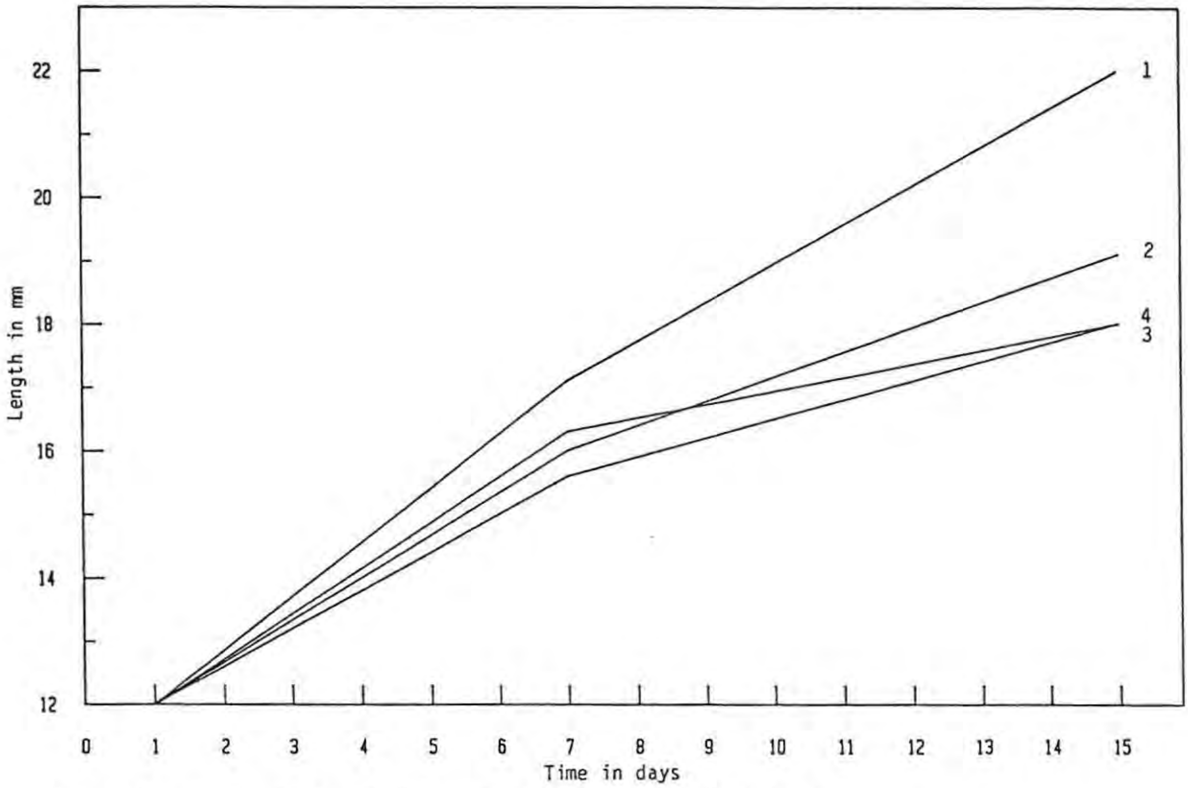


FIGURE 10. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 4

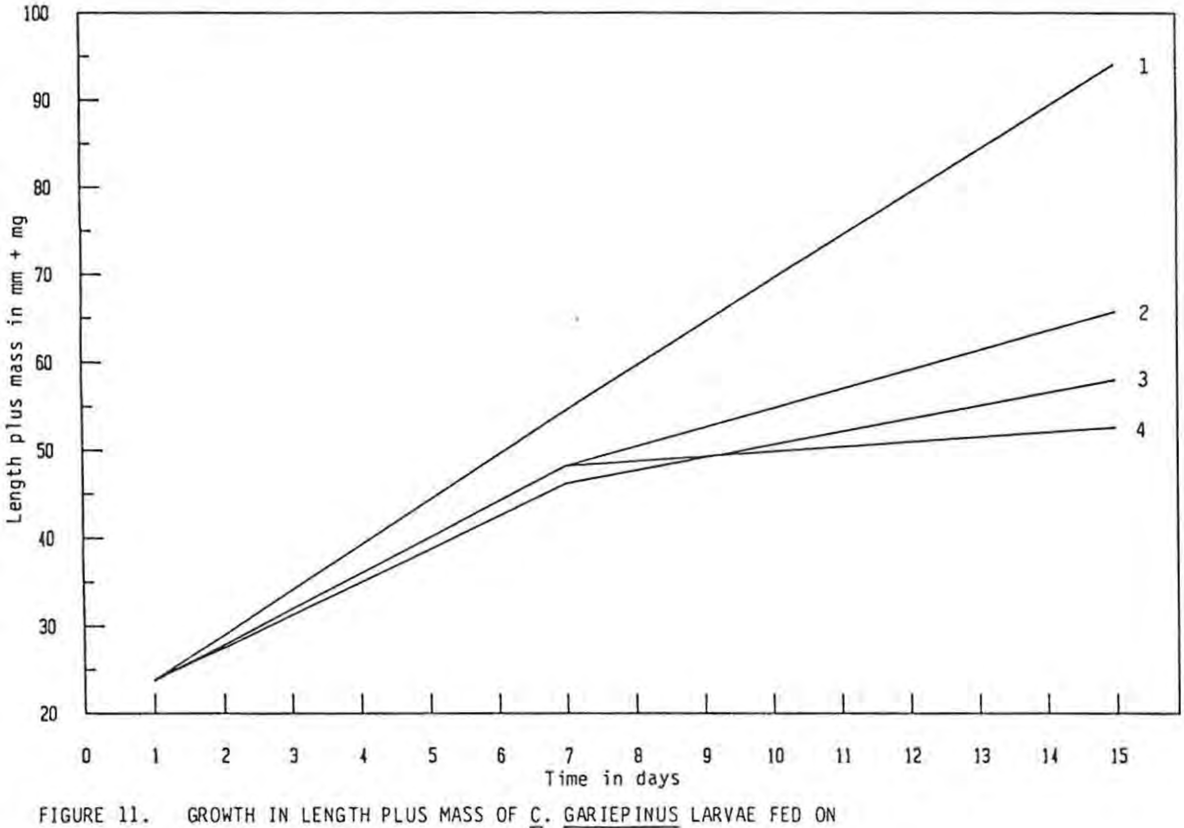


FIGURE 11. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 4

EXPERIMENT 5. PRESERVATION OF LIPID SUPPLEMENT AND APPROXIMATION OF REQUIRED LEVEL OF SUPPLEMENTATION

INTRODUCTION

In Experiment 4, it was noted that oil supplementation to the feeds adversely effected the larvae. It was suspected that this was an indirect effect, caused by the rapid rancidization of the highly unsaturated fatty acids in the dietary oil mixture. Castell (1979) warned that feeds containing these fatty acids should be stored in airtight containers, at reduced temperature and with minimum exposure to UV radiaton. These measures were applied to the feeds used in this experiment and compared with feed that was freshly prepared every day. Quantitative requirement for dietary lipids was also investigated.

METHODOLOGY

Parental material was also obtained from Donkerpoort Dam. At the onset of the experiment the larvae were already 13 days old and they had been fed on the established control diet(yeast + vitamins) up to that stage. The larvae were placed in seven of the 75 litre aquaria (Type 1) as described in Chapter 3. The physico- chemical conditions were as follows:

Temperature	: 24-26 °C
pH :	: 8,0-8,2
Oxygen saturation	: 95%
Flow rate	: 5 litres/min.
Stocking density	: 2,7 larvae/litre

The established feed formulation (yeast & vitamins) was used with different levels of oil supplementation. Feed ration was in excess of 25% of body weight per day. Feeding frequency was twice daily.

The following feed mixtures and different treatments were used: (An equal mixture of cod liver oil and soya bean oil was added as a percentage of total weight).

GROUP 1: Control feed plus 5% oil mixture, kept in clear glass bottle at room temperature (same treatment of feed as used in Experiment 4).

GROUP 2: Control feed plus 5% oil mixture, kept in dark glass bottle in refrigerator at 3-5°C.

GROUP 3: Control feed plus 5% oil mixture, freshly prepared every day.

GROUP 4: Control feed plus 2% oil mixture, kept in dark glass bottle in refrigerator.

GROUP 5: Control feed plus 8% oil mixture, kept in dark glass bottle in refrigerator.

GROUP 6: Control feed plus 12% oil mixture, kept in dark glass bottle in refrigerator.

GROUP 7: Control feed, no oil added.

RESULTS AND DISCUSSION

The results are shown in Table 14 and Figures 12 and 13. The results conclusively show that Torula yeast is in fact deficient in lipids. The results further indicate that the feed requires chemical stabilization after the oil mixture has been added.

Except for Group 1, which received unpreserved feed, all the groups showed a better response than the control group (Group 7), which received no oil supplement. The difference in length between Group 7 (control) and Group 3 (5% oil, refrigerated) was highly significant by the 24th day ($p<0.001$).

Group 5, which had 5% oil added to their feed which was kept in a transparent container at room temperature, fared the worst. All the other groups which had oil supplements, either prepared freshly or refrigerated in dark containers, were significantly larger in length than the Group 5 larvae by the 24th day ($p<0.05$ for Group 4 ; $p<0.02$

for Group 6 ; $p<0.01$ for Group 2 and $p<0.001$ for Groups 5 and 6). Group 5 also had the highest mortality rate and, except for the control diet, it had the lowest condition factor.

It can also be deducted from the results that refrigeration and prevention of irradiation of the feed did not prevent degradation of food quality, since the Group 3 larvae (5% oil, fresh) were significantly larger in length ($p<0.05$) than Group 2 (5% oil, refrigerated).

As far as the quantitative requirement for oil is concerned, Groups 2 and 5 which had 5 and 8% oil supplements, showed the best results, with no significant length difference between these two groups ($p<0.2$). The only significant differences that emerged were between Groups 5 and 6 (8% and 12% oil) and between Groups 5 and 4 (8% and 2% oil).

At this stage it could be concluded that 5-8% supplementation (7-10% total lipid content) is the optimum level. This was, however, re-examined in subsequent experiments because of the older larvae that were used in this experiment.

TABLE 14.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 5

DAY 1:

GROUP	N	L	S	M	M+L	CF
All	10	12.0	0.75	12.3	24.3	1.13

DAY 8:

GROUP	N	L	S	M	M+L	CF
1	8	14.9	1.2	21.8	36.7	1.07
2	7	14.7	0.8	25.4	40.3	1.25
3	8	14.6	0.9	27.3	41.9	1.42
4	7	14.2	0.9	27.0	41.2	1.53
5	8	14.0	1.3	25.4	39.4	1.49
6	9	15.2	1.0	27.5	42.7	1.28
7	6	14.2	1.3	21.3	35.5	1.20

DAY 24:

GROUP	N	L	S	M	M+L	CF	ACCUMULATED TOTAL MORTALITY
1	12	17.5	2.1	40.2	57.7	1.25	40%
2	8	19.9	1.3	59.2	79.1	1.27	5%
3	14	21.4	2.1	71.1	92.5	1.24	5%
4	17	19.1	2.1	54.2	73.3	1.31	5%
5	12	20.6	1.9	66.7	87.3	1.30	5%
6	13	19.3	1.1	55.0	74.3	1.29	10%
7	15	18.6	1.2	43.7	62.3	1.14	10%

N = numbers of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

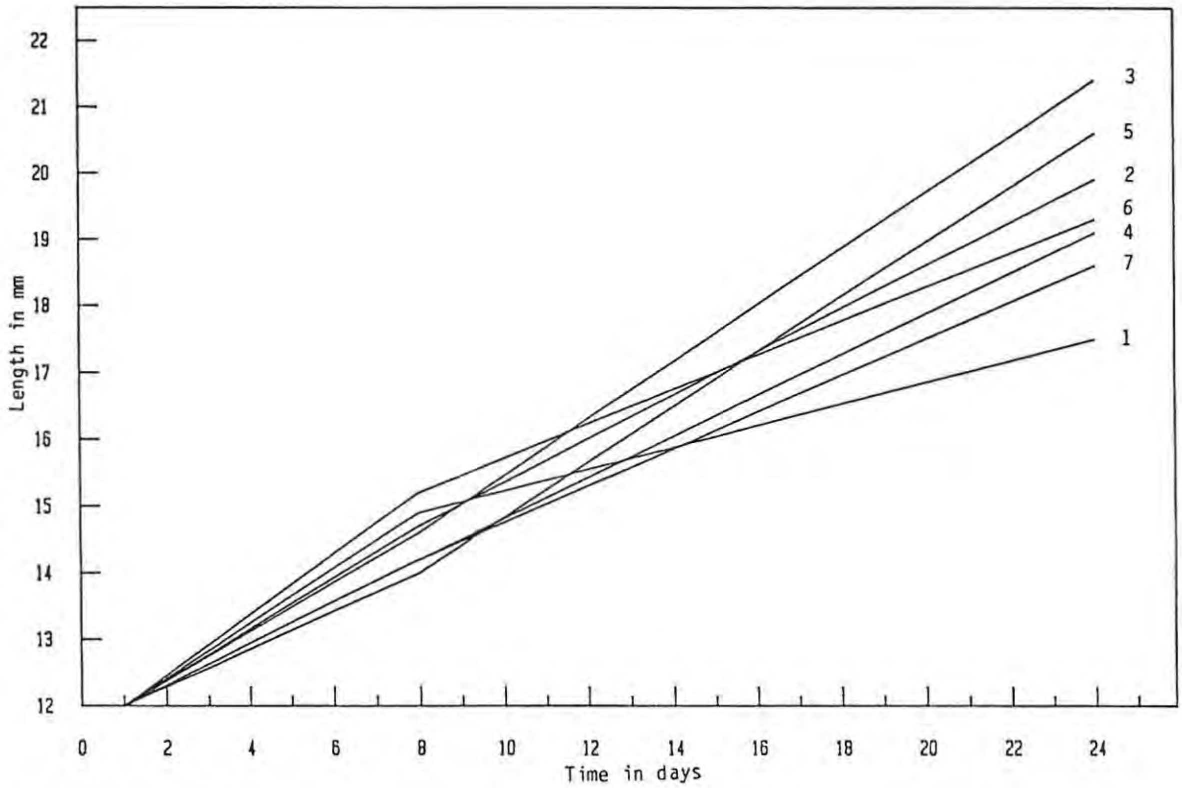


FIGURE 12. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 5

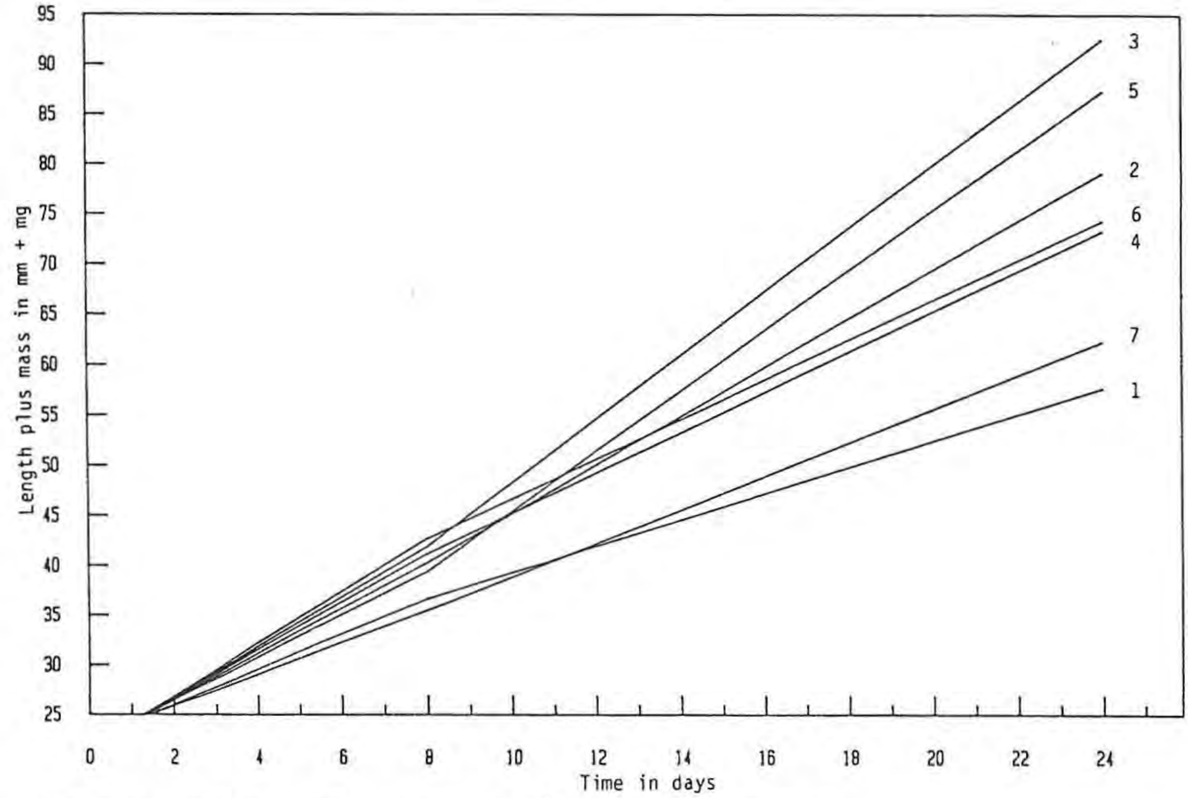


FIGURE 13. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 5

EXPERIMENT 6. OPTIMUM LIPID SUPPLEMENTATION LEVEL, USE OF ANTIOXIDANTS, VITAMIN PREMIX AND METHIONINE SUPPLEMENTATION

INTRODUCTON

Piper et al. (1982) recommended that oxidation of unsaturated fatty acids should be prevented by the addition of a commercial antioxidant to fishfeeds. The use of a commercial antioxidant, "Endox", to prevent rancidity of oils and deterioration of food quality was investigated. "Endox" is a formulation of antioxidants, stabilizers and emulsifiers for compound feeds, concentrates and mineral supplements, manufactured by Kemin Europa NV. Piper et al. (1982) listed antioxidants which are effective in limiting breakdown of oils and other nutrients in fishfeeds. These are butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), ethoxyquin and vitamin E. Although much research, by various authors and institutions has been done on the preservation effects of "Endox" on nutrients(sales brochure, Kemin Europa NV), a search of the literature did not reveal anything on the use of "Endox" in fishfeeds.

The object of this experiment was also to obtain more accurate information on the quantitative total lipid requirement of the larvae. Whereas before 2, 5, 8 and 12% oil supplementation levels were tested, it was now possible to narrow the levels down to 6, 8 and 10%.

Because it was suspected that methionine is a limiting EAA in Torula yeast, a commercially available methionine supplement was included in one of the test diets to determine whether it might enhance growth. The effect of using a more complete vitamin supplement was also investigated.

METHODOLOGY

This was the first experiment carried out at Rhodes University. Broodstock were obtained from Amalinda Warmwater Fish Station. The parents were induced to spawn at Amalinda and the fertilized eggs were transported to Rhodes University where they were incubated in part of the recirculating system.

Sixteen of the 50 litre tanks in the recirculating system, as described in Chapter 3, were stocked with 500 larvae each. Water quality and conditions remained constant throughout, as described in Chapter 3, with the exception of temperature. Temperature could not be maintained at the usual 24°C because of a cold spell. On days 8, 9, 10, 11 and 12, the temperatures were 21, 19, 20, 21 and 23°C respectively. For the remaining duration of the experiment the temperature was constant at 24 ± 0,5°C.

The larvae were fed 2 - 3 times daily in excess of 25% of body weight per day. As a sufficient number of larvae were produced, there were two groups of larvae for each test diet. Tables 15 and 16 show the formulations and compositions of the eight test diets that were used in Experiment 6.

It should be noted that the methionine supplement ("Naturmade" Inositol & Choline) contains 25mg of methionine per gram as well as 50mg inositol and 50mg choline per gram. Inositol and choline are not known to incur hypervitaminoses effects in fishes (Piper et al. 1982).

TABLE 15.

FORMULATIONS OF EXPERIMENTAL FEEDS USED IN EXPERIMENT 6. values represent percentage of total weight

INGRED.	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7	GROUP 8
Torula	95	95	95	95	95	95	89	95
Nutri B	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
21 plus	--	--	--	--	--	--	--	
Vit. C	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Weider.	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Methionine	--	--	--	--	--	--	--	--
Endox	--	--	125ppm	250ppm	--	--	--	--
Oil mix	--	+6%	+6%	+6%	+8%	+10%	+6%	+6%

RESULTS AND DISCUSSION

The results are shown in Table 17 and Figures 14 and 15. As far as the quantitative oil requirement is concerned, the following is evident: Group 2 with 6% oil mixture added to their feed, although statistical significance is not high ($p<0.1$), grew faster in length than the control group (Group 1) which received no oil supplement. Groups 5 and 6 which had 8 and 10% oil added to their feeds respectively, fared the worst, the larvae being significantly smaller in length on the 14th day than Group 2 ($p<0.01$) and the control group ($p<0.1$ for Group 5 and $p<0.05$ for Group 6). The different levels of oil supplementation did not have a strong influence on growth rates and condition factors. It was felt that the results were not conclusive enough and that further experimentation concerning optimization of lipid supplementation was required.

The use of "Endox" as an antioxidant certainly proved beneficial: The feeds for Groups 3 and 4 which had 125 and 250ppm antioxidant added respectively, were prepared once only at the onset of the experiment. All the other feeds were prepared at the same time but the oil supplements were only added just prior to feeding. Because the feeds for Groups 3 and 4 gave better results than the feed for Group 2 ($p<0.05$ and $p<0.01$ respectively), it could be concluded that the antioxidant not only preserved the lipids but prevented deterioration of other essential nutrients. It was decided to use 250 ppm "Endox" in all subsequent experimental feeds. This is the dosage recommended by the manufacturers.

The methionine supplement in the feed for Group 7 proved beneficial. The larvae had a highly significant difference in length ($p<0.001$) from those fed on Feed 2, which had essentially the same nutrient composition, except for the methionine supplement. The inositol and choline levels were also higher in the feed for Group 7, but these two vitamins were already excessive in the feed for Group 2. As can be seen in Table 16, the methionine supplement in the feed for Group 7, raised the level of pure methionine from 1,5 to 2,5% of dietary protein. This is considerably closer to the average value required by other fish of 3,1% of dietary protein (Millikin 1982).

The more complete vitamin premix supplemented to the feed for Group 8 (see Table 16) also showed better results than its control group (Group 2, $p<0.001$). It was decided to use "21 Plus" instead of "Nutri B" as a premix in combination with "Weider High Protein" in subsequent experiments. As can be seen in Table 16, this vitamin supplement increases the theoretical content of vitamins in the feed to levels well above the values recommended for warmwater fish (Piper _et al._ 1982).

Duplication of the test groups in this experiment demonstrated the accuracy of the experimental procedures as no significant differences for $p=0.2$ emerged between any of the duplicates by the 13th day.

Mortalities were uniformly high for the test groups, presumably because of the temperature fluctuation that was experienced.

TABLE 17.

GROWTH IN LENGTH AND MASS OF <u>C. GARIEPINUS</u> LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 6

DAY 1:

GROUP	N	L	S	M	M+L	CF
All	10	7.68	0.56	2.86	10.54	0.94

DAY 6:

GROUP	N	L	S	M	M+L	CF	MEAN L	MEAN M+L
1a	13	8.92	0.70	4.89	13.81	1.05		
1b	12	8.96	0.58	4.97	13.93	1.05	8.94	13.87
2a	11	9.10	0.62	4.97	14.07	1.01		
2b	12	9.17	0.58	4.68	13.85	0.93	9.14	13.96
3a	12	9.20	0.68	5.25	14.45	1.03		
3b	12	9.42	0.81	5.32	14.74	0.98	9.31	14.60
4a	10	9.40	0.78	5.18	14.58	0.96		
4b	13	9.35	0.56	5.12	14.47	0.96	9.38	14.53
5a	13	8.60	0.52	4.60	13.20	1.10		
5b	11	8.75	0.59	4.85	13.60	1.10	8.68	13.40
6a	12	8.77	0.53	4.71	13.48	1.06		
6b	12	8.83	0.50	4.85	13.68	1.07	8.80	13.58
7a	10	9.30	0.85	5.96	15.26	1.13		
7b	12	9.32	0.41	6.05	15.37	1.14	9.31	15.32
8a	10	9.20	0.89	5.80	15.00	1.14		
8b	12	9.28	0.72	5.55	14.83	1.06	9.24	14.92

DAY 13:

GROUP	N	L	S	M	M+L	CF	MEAN L	MEAN M+L	ACCUMULATED TOTAL MORTALITY
1a	12	10.12	0.52	6.95	17.07	1.04			24%
1b	11	10.38	0.60	7.12	17.50	0.99	10.25	17.29	22%
2a	14	10.67	0.72	7.75	18.42	0.99			38%
2b	15	10.68	0.68	7.69	18.37	0.98	10.68	18.40	26%
3a	14	11.02	0.55	8.82	19.84	1.03			35%
3b	17	11.27	0.60	8.96	20.23	0.98	11.15	20.04	30%
4a	14	11.45	0.59	9.52	20.97	1.00			37%
4b	15	11.68	0.52	9.26	20.94	0.92	11.57	20.96	39%
5a	14	9.68	0.67	5.31	14.99	0.90			26%
5b	10	9.89	0.59	5.40	15.29	0.86	9.79	15.14	30%
6a	11	9.60	0.71	5.29	14.89	0.92			35%
6b	15	9.79	0.65	5.67	15.46	0.93	9.70	15.18	40%
7a	13	12.26	0.65	11.88	24.14	1.02			39%
7b	14	12.19	0.68	11.53	23.72	1.01	12.23	23.93	37%
8a	12	11.72	0.53	11.37	23.09	1.11			41%
8b	15	11.87	0.57	10.68	22.55	1.01	11.80	22.82	45%

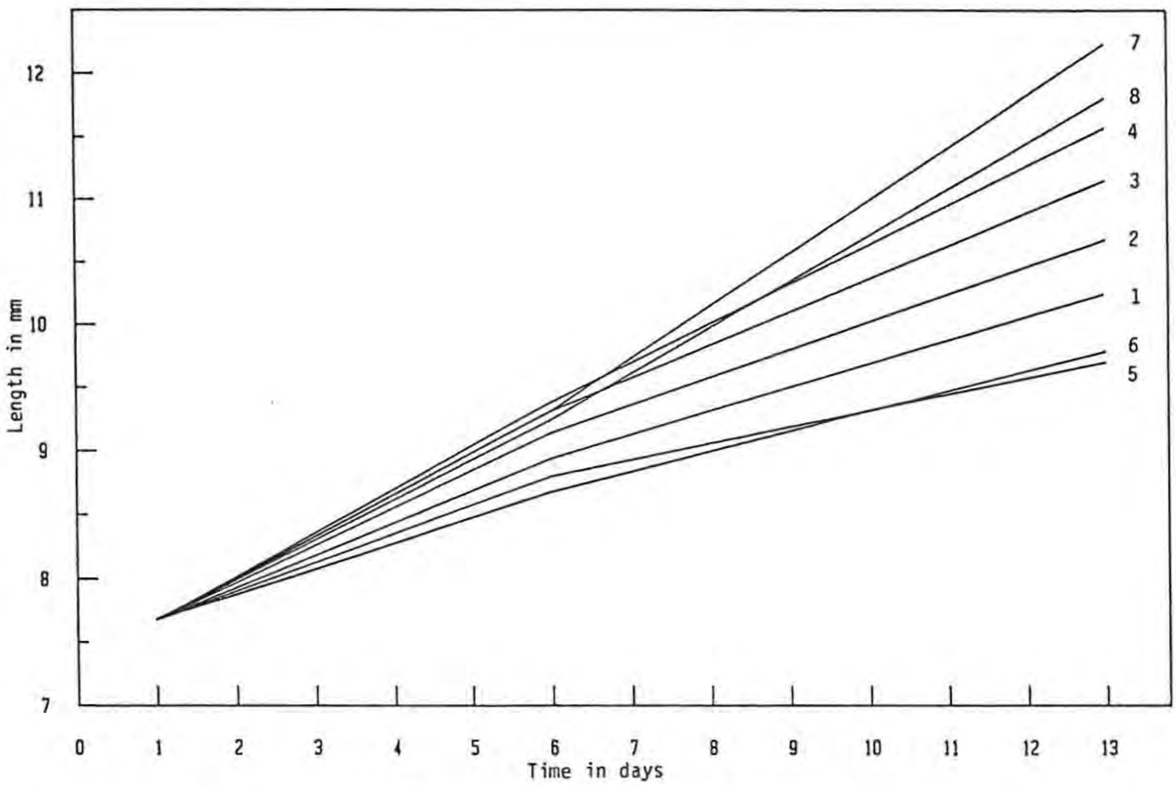


FIGURE 14. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 6

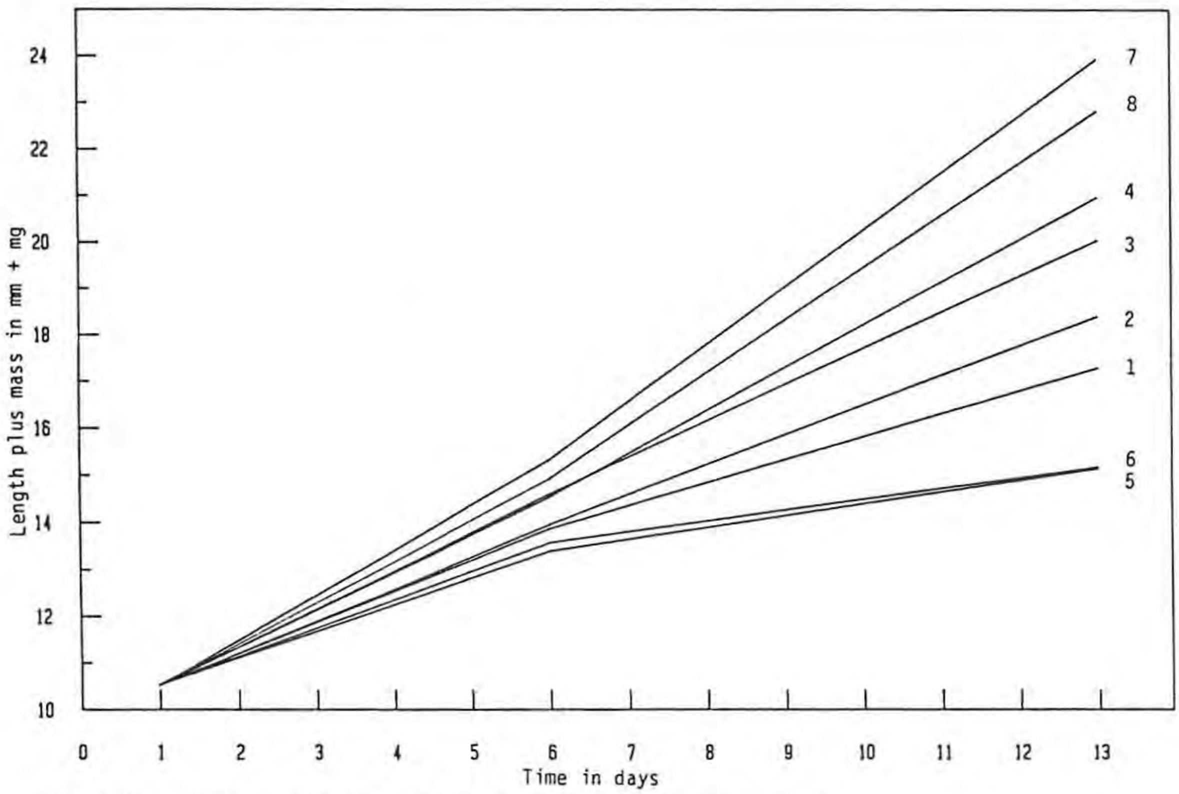


FIGURE 15. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 6

EXPERIMENT 7. QUANTITATIVE REQUIREMENTS FOR METHIONINE AND DETERMINATION OF THE EFFECTS OF A HIGHER LEVEL OF VITAMIN PREMIX SUPPLEMENTATION

INTRODUCTION

In the previous experiment it was shown that the methionine and additional vitamin supplement enhanced growth and survival. These two changes to the test diet were used to formulate an improved control diet which was used in Experiments 7, 8 and 9. In this experiment, an attempt was made to quantify the larvae's requirement for dietary methionine and to determine the optimum level of the vitamin premix in the feed.

METHODOLOGY

Fish were spawned at the Amalinda Fish Station and fertilized eggs were transported to the recirculating system at Rhodes University, four to six hours after fertilization. Experiments 7 to 9 were run concurrently, using a total of 15 groups of larvae. These groups consisted of 500 four-day old larvae each, stocked into 15 of the 50 litre tanks in the recirculating system as described in Chapter 3. The physico- chemical properties of the water remained constant as described in Chapter 3. Stocking density was 10 larvae per litre.

High mortalities occurred in all tanks on the eighth and ninth days as a result of a bacterial infection. Symptoms included loss of appetite, lethargy, unco-ordinated swimming and ascites (belly dropsy). Each tank was treated with 4ppm "Furanace" (Dainippon Pharmaceutical Co., Osaka, Japan) for 20 minutes every day. The larvae that were visibly affected or already dead were removed from the tanks. By the 10th day, conditions returned to normal. All the surviving larvae had regained their appetite and vigour and there was no sign of ascites.

This experiment was concerned with six of the 15 groups of larvae: The six feed formulations and reasons for each formulation were as follows:

GROUP 1: 95% Torula yeast ; 0.4% "Nutri B" ; 0.14% ascorbic acid ; 4.5% "Weider High Protein" ; 250ppm "Endox".
(Previous experiment's control diet + "Endox", for comparison with Group 2, to serve as a replicate for the "Nutri B" vs. "21Plus" test in Exp.6).

GROUP 2: 95% Torula yeast ; 0.4% "21 Plus" ; 0.14% ascorbic acid ; 4.5% "Weider High Protein" ; 250ppm "Endox".
(For comparison with Group 1, to recheck the effect of "21 Plus" against "Nutri B").

GROUP 3: (CONTROL) 95% Torula yeast ; 0.4% "21 Plus" ; 0.14% ascorbic acid ; 4.5% "Weider High protein" ; 150mg/kg Methionine ; 250ppm "Endox" +8% oil supplement added lastly.
(Basic formulation for this experiment).

GROUP 4: Basic formulation, but vitamin supplement is doubled.
(to determine whether vitamin supplementation in basic formulation is adequate).

GROUP 5: Basic formulation, but methionine supplement is doubled.
(To determine whether methionine supplementation in basic formulation is adequate).

GROUP 6: Basic formulation + 8% oil mixture, but methionine supplement is halved.
(To determine whether methionine supplement in basic formulation was perhaps excessive).

As in previous experiments, the larvae were fed in excess of 25% of body weight per day. Feeding frequency was three times daily.

RESULTS AND DISCUSSION

The results are given in Table 18 and Figures 16 and 17. The different methionine supplements had very little effect on growth rates. The growth rates of groups 3, 5 and 6 were very similar. There was no significant difference between the lengths of these groups by the 14th day. However, in conjunction with the pattern shown in the mass curves for these groups, the results seem to indicate that methionine supplementation of 150mg/kg is the optimum level. This effects a final methionine level in the feed of 2,5% of dietary protein. This requirement corresponds well with I. punctatus which requires methionine at 2,3% of dietary protein (Millikin 1982). The mean methionine requirement of the four fish species listed in Table 2 is 3,6% of dietary protein. It should be noted that the feed for Group 3 also had cystine at 0,3% of dietary protein. Cystine can replace 50 to 60% of the methionine requirement of fishes (Harding In: Millikin 1982).

The results of the vitamin premix trials firstly indicate that the "21Plus" premix (Group 2) is indeed superior to the "Nutri B" premix (Group 1 $p<0,05$). This substantiates the results concerning these two premixes in Experiment 6. Furthermore, by doubling the "21Plus" premix (Group 4), an even better growth response was elicited. The Group 4 larvae were significantly larger in length than the control group (Group 3) by the 14th day ($p<0,05$). As this was the highest level of vitamin supplementation ever used, it was thought neccessary to investigate the effects of even higher vitamin supplementation levels. This was done in Experiment 10, in which the optimum level of vitamin premix supplementation was also established.

TABLE 18.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 7

DAY1:

GROUP	N	L	S	M	M+L	CF
All	12	7.24	0.45	2.40	9.64	0.94

DAY 10:

GROUP	N	L	S	M	M+L	CF
1	15	10.56	0.56	7.35	17.91	0.97
2	15	10.50	0.59	7.90	18.40	1.06
3	12	11.04	0.62	8.27	19.31	0.96
4	15	10.97	0.61	7.96	18.93	0.94
5	15	10.69	0.68	7.59	18.28	0.97
6	15	10.67	0.48	7.81	18.48	1.00

DAY 14:

GROUP	N	L	S	M	M+L	CF	TOTAL ACCUMULATED MORTALITY
1	15	11.48	0.62	9.43	20.91	0.98	10%
2	15	11.97	0.58	10.83	22.80	1.00	10%
3	15	12.34	0.72	11.98	24.32	1.01	15%
4	16	12.89	0.57	12.84	25.73	0.96	15%
5	15	12.10	0.61	11.01	23.11	0.98	10%
6	15	12.34	0.58	11.54	23.88	0.98	15%

N = number of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

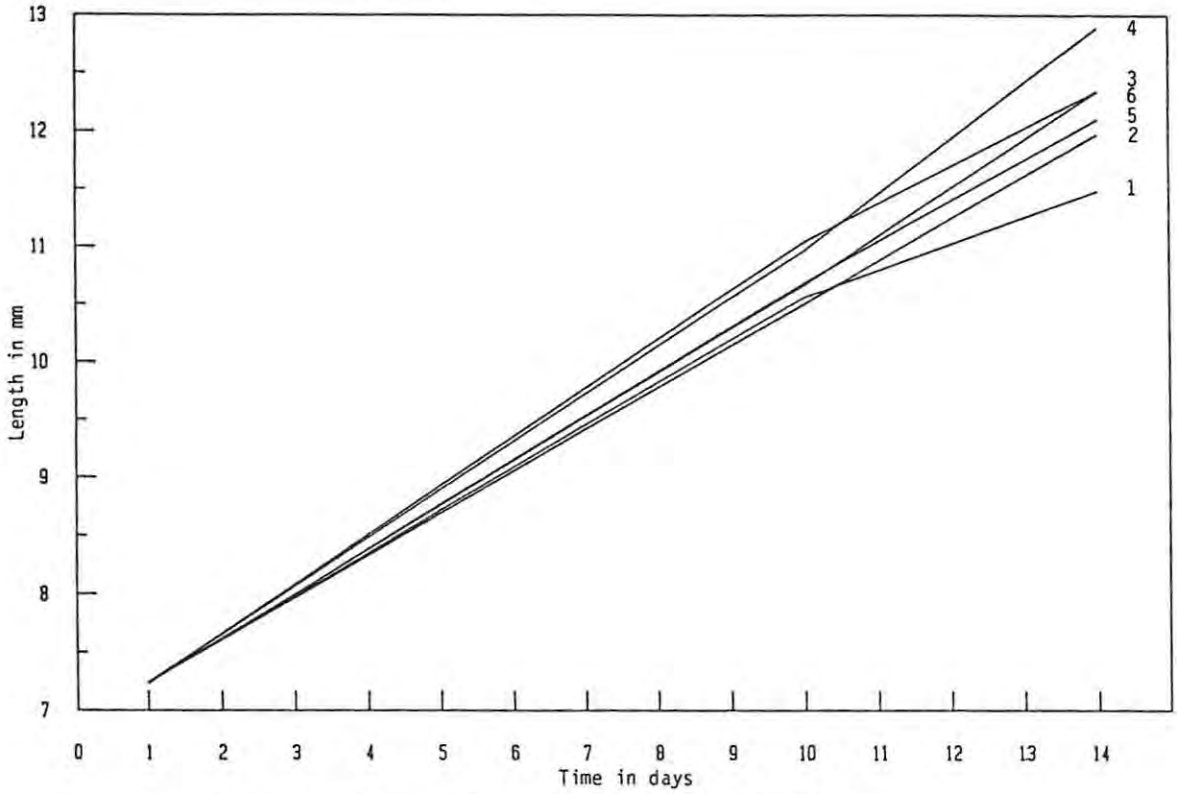


FIGURE 16. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 7

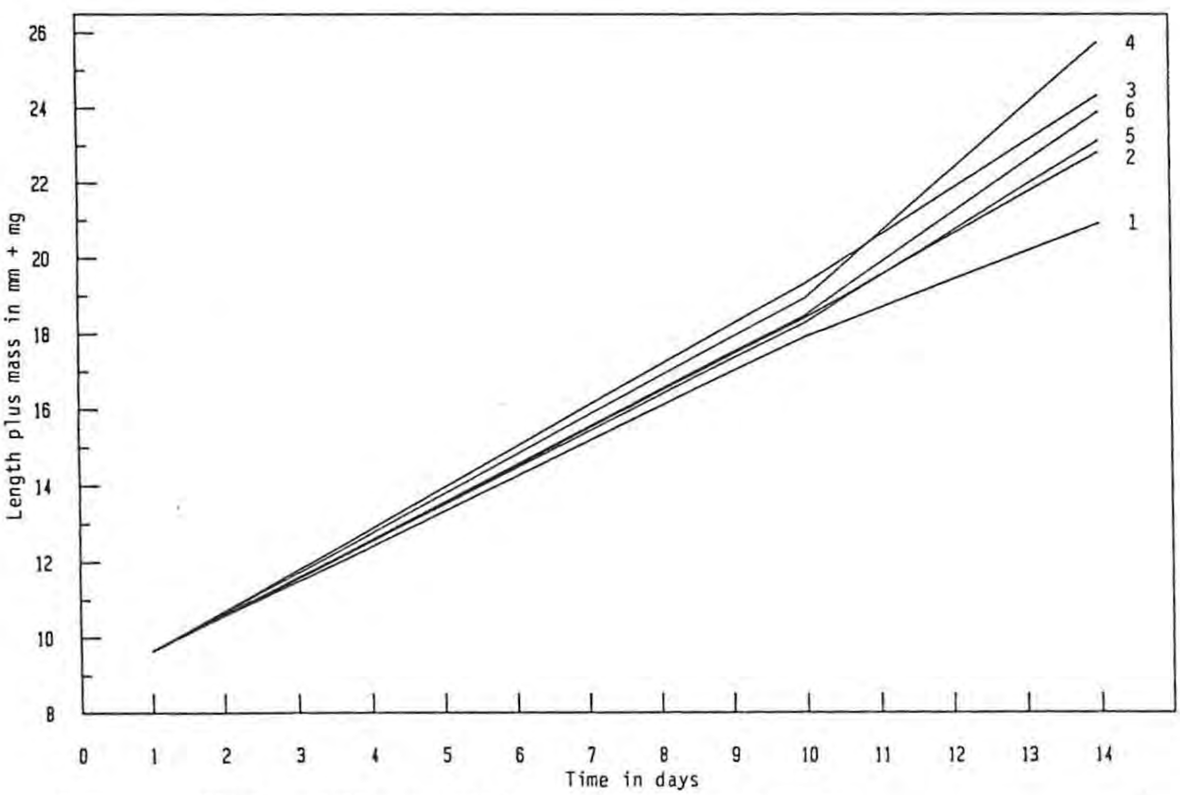


FIGURE 17. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 7

EXPERIMENT 8. FURTHER DIETARY LIPID TESTS

INTRODUCTION

As mentioned in the discussion of Experiment 6, it was felt that the results concerning lipid supplementation were not yet conclusive and that further experimentation was required. In this experiment, three feeds with varying levels of oil supplementation were again tested against the control feed which had no oil supplement. Secondly, the effect of "Endox" on growth rate and survival of the larvae was again investigated to replicate the antioxidant test in Experiment 6. Furthermore, because oil has such a marked effect on leaching (Chapter 2), it was thought that the enhancement of growth rate by adding oil to the diets might have been due largely to this fact and not because of the direct nutritional value of the oil. To investigate this, a control diet was washed with water to remove the leachable content from the feed. It was then dried and split into two portions. Eight percent oil supplement was added to one portion and the other portion was used without oil. By doing leaching tests on samples of these two diets, it was found that their rates of leaching when placed into the water, were, as could be expected, minimal. The only difference between these two diets, therefore, was their lipid content.

METHODOLOGY

As mentioned before, this experiment was run concurrently and in the same rearing system as Experiments 7 and 9. The larvae, water systems and conditions were the same as described in Experiment 7.

This experiment was concerned with eight of the 15 groups of larvae. The respective feed formulations were as follows:

GROUP 1: 95% Torula yeast ; 0.4% "21Plus" ; 0.14% ascorbic acid ;
4.5% "Weider High Protein" ; 150mg/kg methionine ;
250ppm "Endox".
(Basic formulation for this experiment)

GROUP 2: Basic formulation + 6% equal mixture of cod liver- and soya bean oil added at the end.

GROUP 3: Basic formulation + 8% oil mixture.

GROUP 4: Basic formulation + 10% oil mixture.

GROUP 5: Basic formulation + 8% oil mixture without "Endox".
(To recheck effect of "Endox")

GROUP 6: Basic formulation + 8% oil mixture with only 125ppm "Endox".
(Half strength "Endox").

GROUP 7: Basic formulation, washed.
(All leachable content removed to compare with Feed 8).

GROUP 8: Basic formulation, washed + 6% oil mixture.
(To find direct nutritional effect of oil on larvae).

The eight groups of larvae received their respective feeds three times daily in excess of 25% per body weight per day.

RESULTS AND DISCUSSION

The results are shown in Table 19 and Figures 18 and 19. Firstly, the results show conclusively that the use of an antioxidant when oil is supplemented to the feed, is of vital importance. Group 5, which had no "Endox" in their feed, showed a decrease in mean mass between the 10th and 14th day. This group showed the worst growth rate and the highest accumulated mortality. The use of 250ppm "Endox" is therefore recommended.

Secondly, the results substantiated the indication in Experiment 6 that the 6% level of oil mixture supplementation is optimal. Group 2 (6% oil) fared the best, being significantly larger in length than Group 3 (8% oil) ($p<0.05$) as well as Group 1 (basic formulation) ($p<0.01$). Supplementing 10% of oil to the formulation (Group 4) had a detrimental effect, as the Group 4 larvae were significantly smaller in length at the end of the experiment ($p<0.01$) than the Group 1 larvae (basic formulation). A higher accumulated mortality (20%) was also encountered in Group 4. Castell (1979) reported that excessive levels of W3 and W6 fatty acids in fishfeeds may be detrimental because they compete with W9 fatty acids for active enzyme sites. Piper et al. (1982) reported detrimental effects caused by excessive dietary lipids in S. gairdneri.

Lastly, in comparing the growth rates of Groups 7 and 8, it can be seen that the beneficial effect of oil was not only due to its restricting effect on leaching, but also due to its nutritional value. On the 14th day the Group 8 larvae were significantly larger in length than those of Group 7 ($p<0.02$).

TABLE 19.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 8

DAY1:

GROUP	N	L	S	M	M+L	CF
All	12	7.24	0.45	2.40	9.64	0.94

DAY 10:

GROUP	N	L	S	M	M+L	CF
1	13	10.77	0.60	7.96	18.73	0.99
2	14	10.93	0.55	8.50	19.43	1.02
3	12	11.04	0.62	8.27	19.31	0.96
4	13	10.62	0.62	7.64	18.26	0.99
5	14	10.73	0.54	7.49	18.22	0.95
6	15	10.77	0.42	7.40	18.17	0.92
7	14	10.10	0.46	6.45	16.55	0.97
8	12	10.30	0.54	6.93	17.23	0.98

DAY 14:

GROUP	N	L	S	M	M+L	CF	TOTAL ACCUMULATED MORTALITY
1	14	12.09	0.58	11.13	23.22	1.00	10%
2	13	12.85	0.62	12.96	25.81	0.98	10%
3	15	12.34	0.72	11.98	24.32	1.01	15%
4	12	11.32	0.72	9.51	20.83	1.03	15%
5	17	11.03	0.52	7.28	18.31	0.85	10%
6	15	11.41	0.61	9.51	20.92	1.01	15%
7	13	10.50	0.72	7.10	17.60	0.95	15%
8	12	11.21	0.74	8.24	19.45	0.92	15%

N = number of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

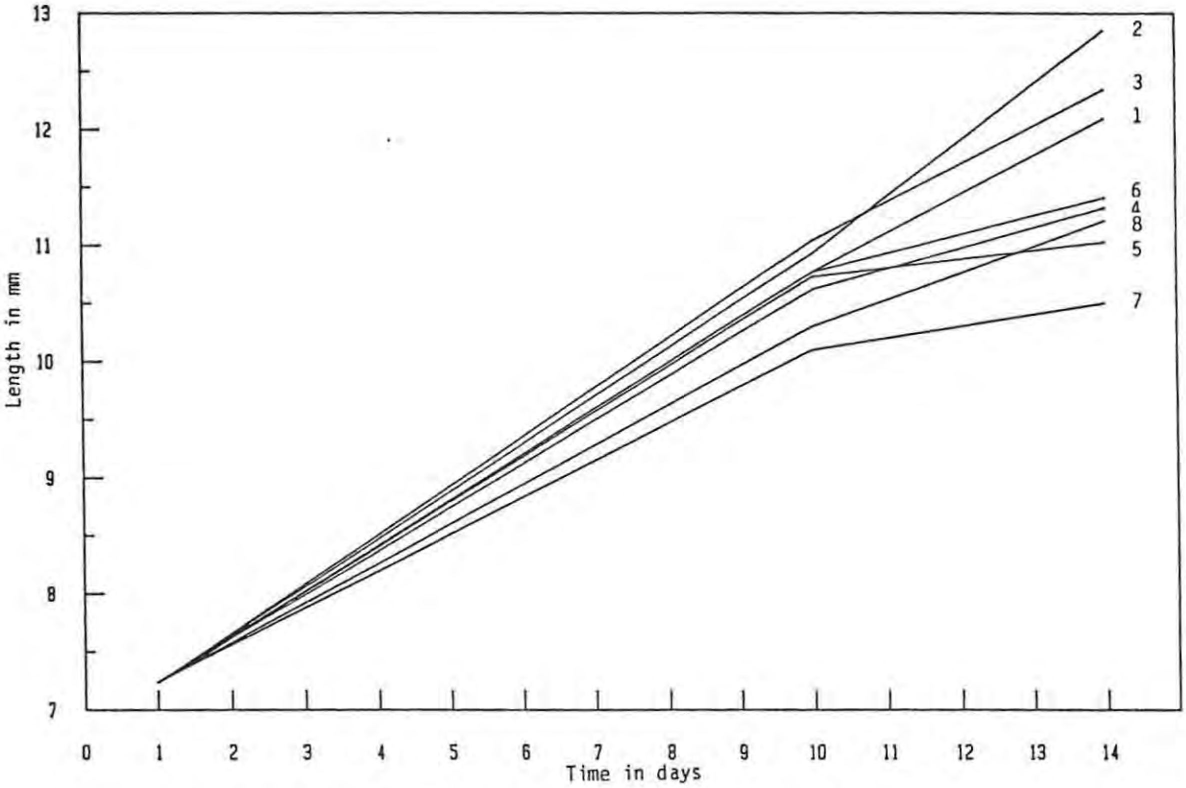


FIGURE 18. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 8

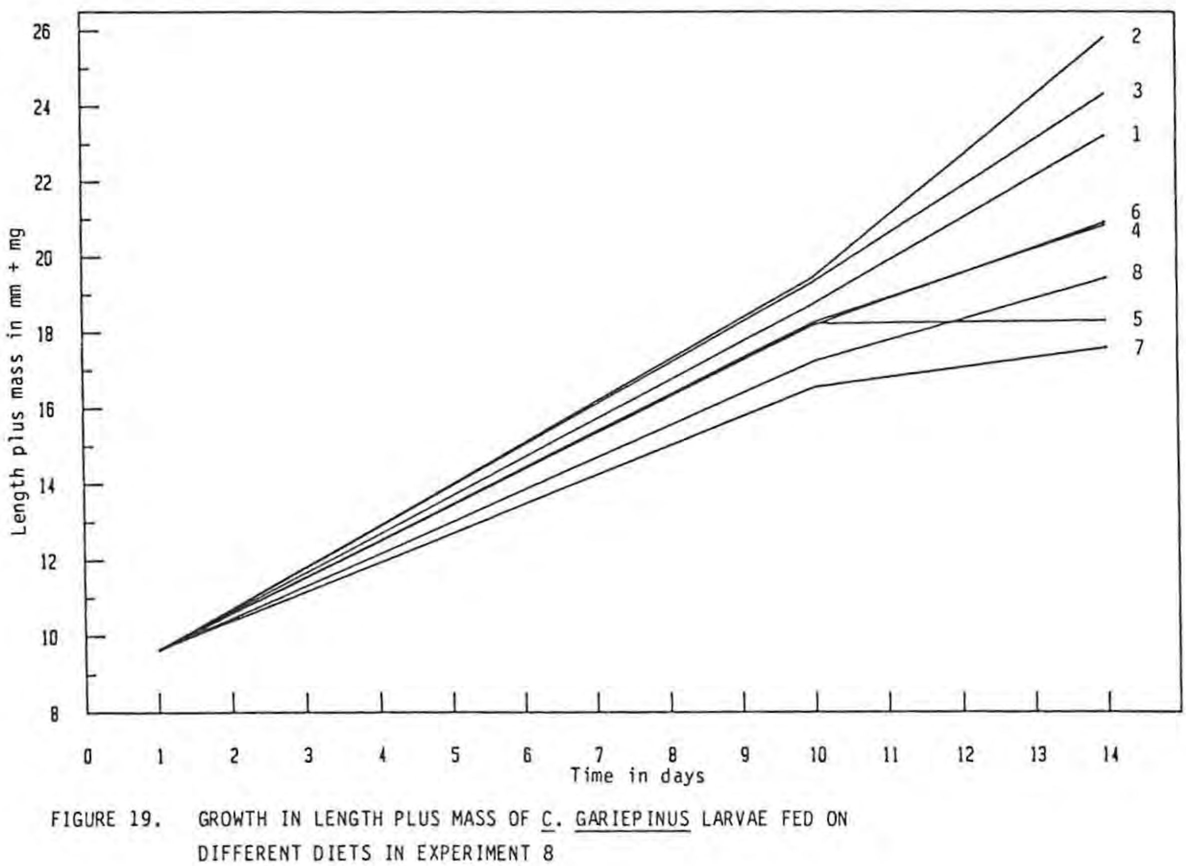


FIGURE 19. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 8

EXPERIMENT 9. DIETARY STARCH TESTS

INTRODUCTION

Only limited information is available on the role of carbohydrates in fish nutrition. Piper et al. (1982) mentioned that the only known function of carbohydrates in fish food is supplying energy and it seems that carbohydrates do not supply any essential nutrients that cannot be obtained from other nutrients in the feed. No specific quantitative carbohydrate requirements have been determined for any fish species (Wilson 1977a; Piper et al. 1982).

Pieper & Pfeffer (1979) have shown that trout can utilize dietary carbohydrates, especially in the form of gelatinized maize starch and sucrose as a source of energy. This also had a protein sparing effect. High levels of carbohydrates in fishfeeds have been known to produce liver abnormalities, poor growth and high mortalities in salmonids (Wilson 1977a; Piper et al. 1982) but this effect has not been found in I. punctatus. Although the necessary enzymes for digestion and utilization of carbohydrates have been found in fish, the role of dietary carbohydrates and the contribution of glucose (final product in carbohydrate digestion) to the total energy requirement of fishes remain unclear (Piper et al. 1982).

I. punctatus have been found to utilize polysaccharides (starch, dextrin) more readily than disaccharides or simple sugars (Wilson 1977). It was, therefore, decided to investigate the use of pure soluble starch as a carbohydrate supplement in the experimental feed.

METHODOLOGY

This experiment was run concurrently with Experiments 7 and 8. The origin of the larvae, the water systems and conditions were the same as described in Experiment 7. This experiment is concerned with three of the 15 groups used in this set of experiments. The feed formulations were as follows:

GROUP 1: 95% Torula yeast ; 0.4% "21 Plus" ; 0.14% ascorbic acid ; 4.5% "Weider High protein" ; 150mg/kg Methionine ; 250ppm "Endox" ; 8% equal mixture of cod liver- and soya bean oil added lastly.
(Control formulation).

GROUP 2: Basic formulation + 6% starch.

GROUP 3: Basic formulation + 8% oil mixture + 10% starch.

The three groups of larvae received their respective feeds three times daily in excess of 25% per body weight per day.

RESULTS AND DISCUSSION

The results are shown in Table 20 and Figures 20 and 21. The results conclusively show that by supplementing starch to the feed at 6 and 10% of total weight, the growth rates of the larvae were adversely affected. By the 10th day, the Group 1 larvae (control, no starch added), were significantly larger in length ($p<0,01$), than those of Groups 2 and 3 (6 and 10% starch added respectively). By the 14th day the difference was increased ($p<0,001$). Furthermore the condition factors of Groups 2 and 3 were considerably lower than that of Group 1 (0,90 vs. 1,01).

The carbohydrate content of the feed given to the control group (Group 1) was 21% of dry weight, most of this being the carbohydrate content of the yeast in the feed. By supplementing starch to the feed at 6 and 10% of total weight, the carbohydrate content of the feeds for Groups 2 and 3 was increased to 25,5 and 28% respectively. As stated before, no information is available on quantitative dietary requirements for carbohydrates. However, Cowey & Sargent (1972) suggested a maximum digestible carbohydrate level of 25% for most fish species. Jauncy & Ross (1982) also suggested that carbohydrate levels in fishfeeds should be restricted. Furthermore, the fact remains that lipids and proteins supply more energy per gram than carbohydrates (Wilson 1977b; Jauncy & Ross 1982). The question therefore arises whether the carbohydrate level of 21% in the control diet is not perhaps excessive. For practical purposes, this was, however, not tested as it would have necessitated extracting carbohydrate from Torula yeast, without affecting other nutrients.

TABLE 20.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 9

DAY1:

GROUP	N	L	S	M	M+L	CF
All	12	7.24	0.45	2.40	9.64	0.94

DAY 10:

GROUP	N	L	S	M	M+L	CF
1	13	10.77	0.60	7.96	18.73	0.99
2	15	10.08	0.73	6.56	16.64	0.99
3	15	10.19	0.47	6.10	16.29	0.89

DAY 14:

GROUP	N	L	S	M	M+L	CF	TOTAL ACCUMULATED MORTALITY
1	14	12.09	0.58	11.13	23.22	1.00	10%
2	15	10.70	0.62	7.08	17.78	0.90	15%
3	15	10.62	0.52	6.95	17.57	0.90	15%

N = number of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

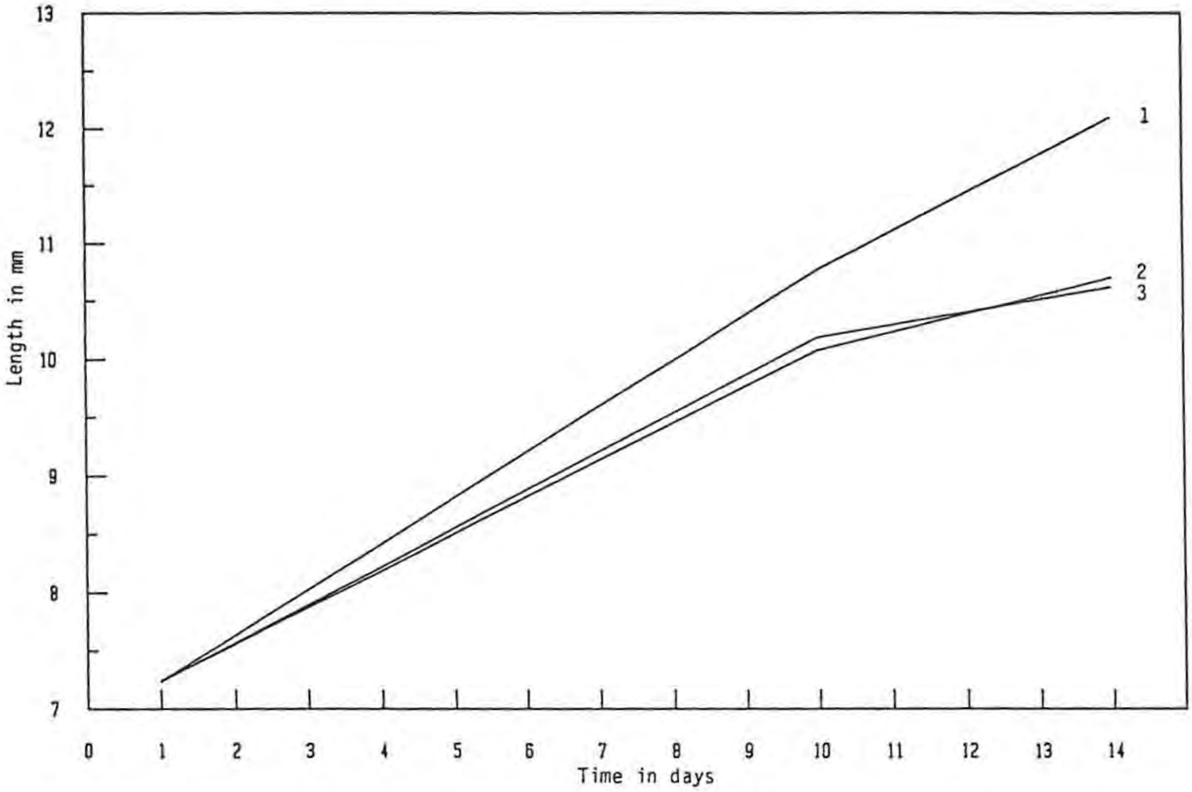


FIGURE 20. GROWTH IN LENGTH OF *C. GARIEPINUS* LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 9

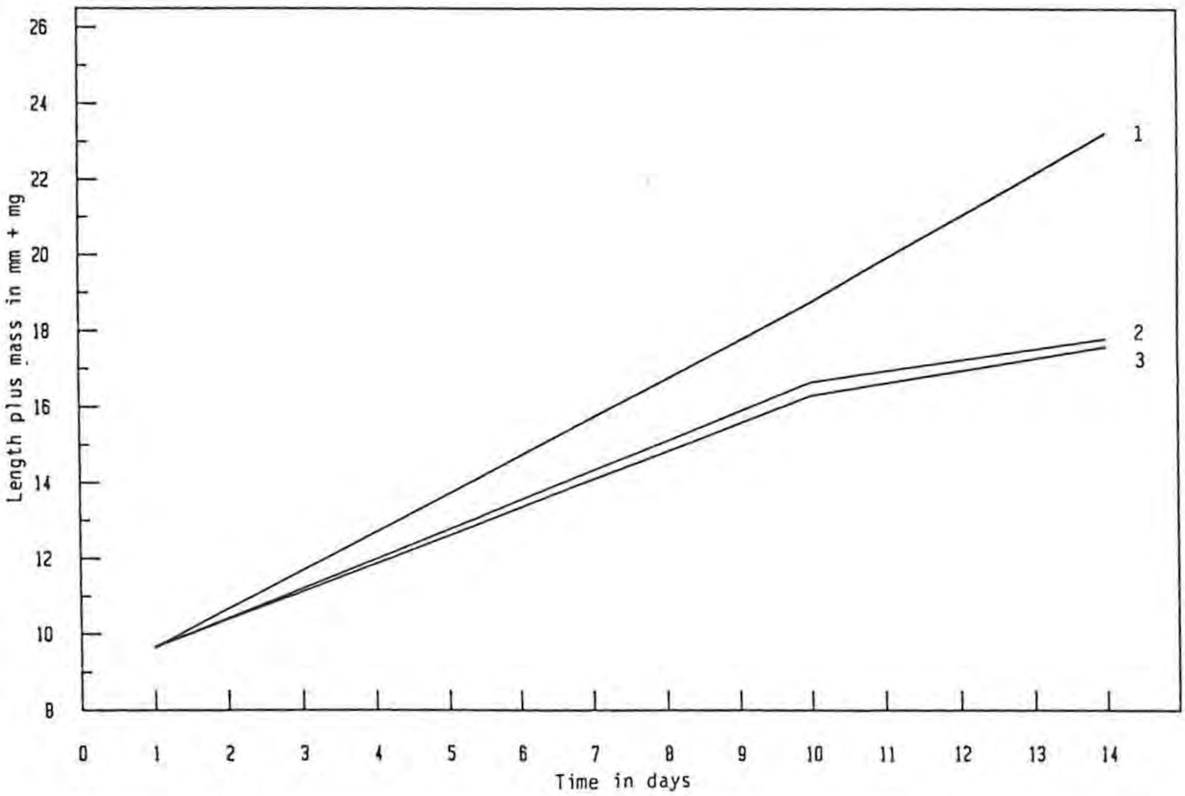


FIGURE 21. GROWTH IN LENGTH PLUS MASS OF *C. GARIEPINUS* LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 9

EXPERIMENT 10. FINAL FEED FORMULATION

INTRODUCTION

This was the last feeding trial in which different feed formulations were tested in order to finalize an optimal feed formulation. The following four issues had to be investigated:

1. Because of the favourable results obtained with the higher levels of vitamin supplementation in Experiment 7 (0,8% "21Plus"), further optimization was attempted by testing four different levels of "21Plus" supplementation (up to as high as 1,2 % of total dry weight). These levels of vitamin supplementation far exceeded those recommended for warmwater fishes by Jauncy & Ross (1982), Millikin *et al.* and Piper *et al.* (1982), but the fact remains that vitamin loss through leaching is higher from larval feed particles than from larger feed pellets.

2. To re-test the optimum lipid supplementation level. As was shown in Experiment 8, the results concerning lipid supplementation, were not conclusive.

3. To investigate the value of fishmeal as an additional protein source to possibly improve amino acid ratios. Fishmeal is generally used as a main constituent of fishfeeds (Spinelli, Mahnken & Steinberg 1979) and in most cases, it was found to be superior to other protein sources (Spinelli *et al.* 1979; Ohmae, Suzuki & Shimna 1979).

4. To replicate part of the methionine supplementation trial in Experiment 7 by investigating the effect of doubling the methionine supplement.

In addition, because of the disease that was encountered in the previous set of experiments, three mg/kg "Furanace" was included in the feeds that were used in this experiment. "Furanace", or nifurpirinol, possesses a wide antibacterial spectrum and acts to control both gram negative and gram positive infections. With a minimum inhibitory concentration below 1ppm, its antibacterial activity is effective against important fish pathogens such as Aeromonas, Vibrio and Chondrococcus, being 10-30 times as potent as other commonly used bactericides such as furazolidone and tetracycline. It was considered safe to administer "Furanace" at the recommended dosage of 4ppm in the feed for *C. gariepinus* larvae, since levels as high as 200ppm in the feeds for goldfish and 300ppm in the feeds for guppies were demonstrated not to be toxic to the fish (brochure, Dainippon Pharmaceutical Co., Osaka, Japan).

METHODOLOGY

Parental material was one female and one male catfish of approximately eight kg each, which had been collected from the P.K. le Roux Dam in 1981. These large fish were difficult to handle but 900ml of eggs were produced (±400 000). Hatching success was low at only 15%, but even so this meant that more than 60 000 larvae were produced. The large percentage of dead eggs in the funnels caused heavy fungal infection despite the malachite green treatment. It was known from previous experience that larvae which are kept in water heavily infected with fungus soon become susceptible to bacterial infection characterized by ascites. The larvae were, therefore, bathed in a 4ppm "Furanace" solution before being stocked into the sixteen tanks which were used in this experiment.

Twelve tanks in the recirculating system as described in Chapter 3 were stocked with 1000, four day old larvae each, i.e. 20 larvae per litre. Physico-chemical conditions remained stable at the standard levels for the system. Due to a shortage of available tanks, four 5 litre glass jars with aerated tap water were also stocked with 100 larvae each (Groups 13-16). The water was changed daily and the conditions in the glass jars were as follows:

Temperature : 22 - 24°C
pH : 7,5 - 7,7
Oxygen saturation : 90%

The basic formulation used in this experiment was as follows:

Torula yeast : 93%
"21Plus" : 0,4%

"Ascorbic acid : 0,14%
Methionine supplement : 2,7% (i.e. 150mg methionine/kg)
"Weider High Protein" : 3,8%
"Endox" : 250ppm
"Furanace" : 4ppm

The following variations on the basic formulation were applied to produce the experimental feeds:

In the recirculating system:
GROUP 1: Basic formulation without oil
GROUP 2: Basic formulation plus 2% oil
GROUP 3: Basic formulation plus 4% oil
GROUP 4: Basic formulation plus 6% oil
GROUP 5: Basic formulation plus 8% oil
GROUP 6: Basic formulation plus 6% oil and 300mg/kg methionine
GROUP 7: Basic formulation plus 6% oil with 0,6% "21Plus"
GROUP 8: Basic formulation plus 6% oil with 0,8% "21Plus"
GROUP 9: Basic formulation plus 6% oil with 1,2% "21Plus"
GROUP10: Basic formulation plus 6% oil with 1,2% "21Plus", but without "Weider High Protein"
GROUP11: Basic formulation plus 6% oil but substituting 25% of yeast with fishmeal
GROUP12: Basic formulation plus 6% oil but substituting 50% of yeast with fishmeal

In the glass jars:
GROUP13: Basic formulation plus 6% oil (no fishmeal)
GROUP14: Basic formulation,plus 6% oil but substituting 20% of yeast with fishmeal
GROUP15: Basic formulation,plus 6% oil but substituting 30% of yeast with fishmeal
GROUP16: Basic formulation,plus 6% oil but substituting 40% of yeast with fishmeal

Feeding rations were in excess of 25% of body weight per day and the larvae were fed three times daily. The established feed particle size ranges were used. The experiment was run for 10 days and only one set of samples was taken at the end of this period.

RESULTS AND DISCUSSION

The results are shown in Table 21 and Figures 22 and 23. Doubling the vitamin supplement (Group 8) seemed to be beneficial, but no significant difference in length emerged for $p<0,05$ when compared to Group 4. Mean mass of the sample was also higher. As there was no indication of hypervitaminoses or for that matter any detrimental effect and because of the good results it produced in the previous experiment, it was decided that this high level of vitamin supplementation is preferable. Group 10, which also had double the vitamin supplement but without "Weider High Protein", showed no significant difference in lengths as compared to Groups 4 or 8. This was to be expected as, theoretically, the "21Plus" supplement especially when doubled, supplies all the neccessary vitamins, and the "Weider High Protein" Supplement becomes obsolete. (The "Weider High Protein" supplement initially played a more important role as part of the vitamin supplement and was not, as the name wrongly indicates, an important part of the protein source in the feed).

As far as the different levels of oil supplementation are concerned (Groups 1 - 5), no significant differences in length emerged by the 10th day of feeding. It would seem, however, that the 6% supplementation (Group 4) gave the best results, as mean mass, mean length and condition factor are the highest for this group. Also considering the results of previous experiments, it was concluded that the 6% level of oil supplementation is the optimum. This effects a total lipid level in the feed of 9% of dry weight. Dupree (1969) found I. punctatus fingerlings to utilize dietary lipids most effectively when included in the feed at levels between 8 and 16% of dry weight. Jauncy & Ross (1982) recommended dietary lipid levels of 10% for first feed fry of tilapia. Generally, dietary lipid requirements of warmwater fish are between 3,5 and 12% of dry weight of the feed (Piper et al. 1982).

Replacing 25% of the yeast in the basic formulation with fishmeal (Group 11) proved to be beneficial as a significant difference in length ($p<0.05$) emerged between Group 11 and Group 4. A higher condition factor was also obtained. However, replacing more than 25% of the yeast with fishmeal (Group 12) had a detrimental effect. This out-come was also reflected in Groups 13 - 16 in the glass jars (Figure 23). The larvae fed on feed which had more than 25% of the yeast replaced with fishmeal were significantly smaller in length than the other groups ($p<0.02$). It was, therefore, concluded that the protein basis of the dry feed should consist of 75% Torula yeast and 25% high quality, brown fishmeal.

The higher level of methionine supplementation (Group 6) did not prove to be beneficial when compared to the control group, (Group 4). This substantiated the findings in Experiment 7 and it was therefore decided that the methionine supplementation level of 150mg/kg is sufficient.

All the groups of larvae were characterised by having unusually high condition factors. A possible explanation could be different phenotypic morphometrics of the P.K. le Roux Dam catfish that were used as broodstock.

On completion of the preceding experiments, a conclusion was reached as to the best formulation which could be achieved in this project. This formulation is given in Table 22 and the nutrient composition is given in Table 23.

TABLE 21.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 10

GROUP	N	L	S	M	M+L	CF
All	15	7.52	0.60	2.97	10.49	1.04

DAY 10:

GROUP	N	L	S	M	M+L	CF
1	16	10.50	0.52	8.03	18.53	1.08
2	11	10.64	0.60	8.86	19.50	1.15
3	17	10.76	0.50	9.03	19.79	1.13
4	12	10.81	0.75	9.53	20.34	1.18
5	15	10.50	0.60	8.02	18.52	1.08
6	18	10.69	0.60	9.43	20.12	1.20
7	18	10.53	0.58	8.28	18.81	1.10
8	15	11.10	0.73	10.33	21.43	1.18
9	18	10.67	0.45	9.37	20.04	1.20
10	19	11.19	0.52	10.88	22.07	1.22
11	20	11.47	0.55	11.45	22.92	1.19
12	15	10.59	0.58	9.01	19.60	1.18
13	20	10.90	0.48	8.90	19.80	1.07
14	16	10.98	0.46	9.15	20.13	1.08
15	15	10.45	0.33	7.82	18.27	1.07
16	18	10.03	0.40	7.6	17.63	1.16

N = number of individuals in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

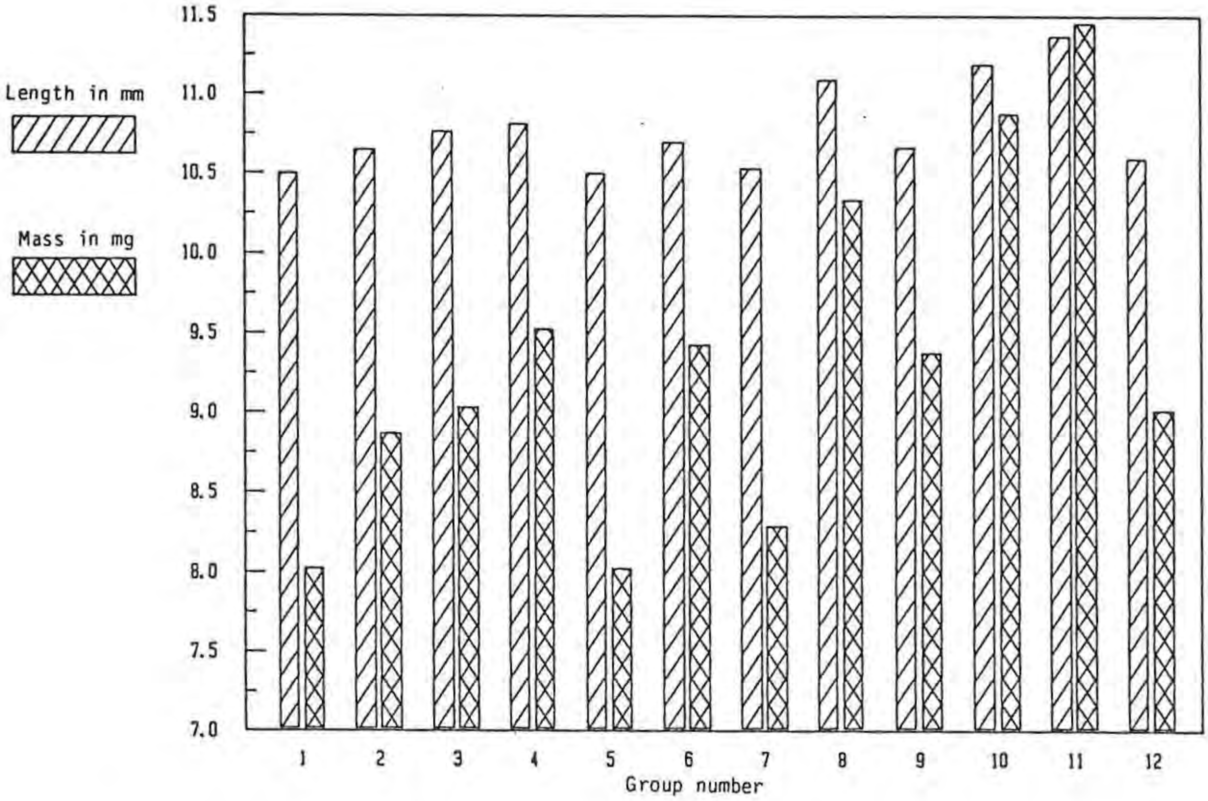


FIGURE 22. LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 10 (GROUPS 1 TO 12)

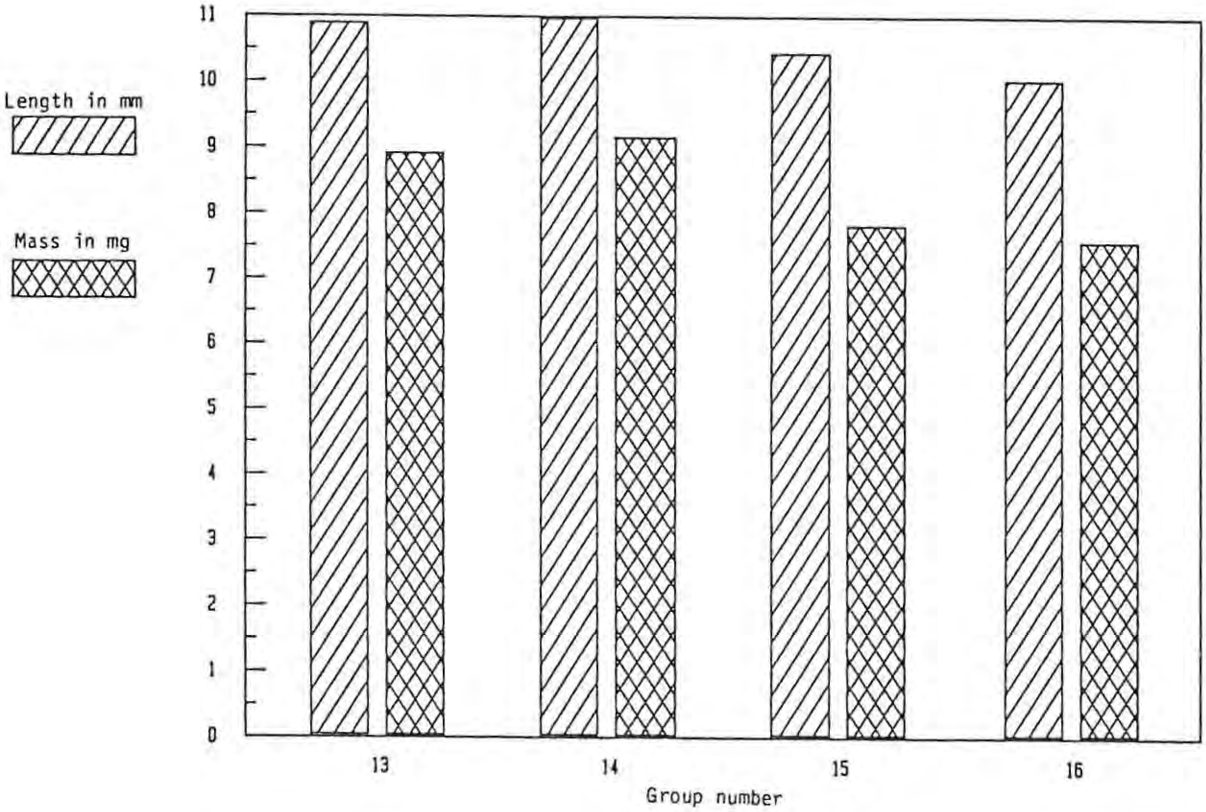


FIGURE 23. LENGTH AND MASS OF C. GARIEPINUS LARVAE FED ON DIFFERENT DIETS IN EXPERIMENT 10 (GROUPS 13 TO 16)

TABLE 22.

FORMULATION OF THE ESTABLISHED OPTIMAL DRY FEED FOR *C. GARIEPINUS* LARVAE

FISHMEAL:	23.3%
TORULA YEAST:	69.8%
"VITA FORCE 21PLUS"	0.8%
ASCORBIC ACID:	0.1%
METHIONINE SUPPLEMENT	6.0% (150 mg/kg pure)
"FURANACE"	4ppm.
"ENDOX"	250ppm.

PLUS 6% OIL MIXTURE (Equal mixture of cod liver and soya bean oil).

TABLE 23

NUTRIENT COMPOSITION OF THE ESTABLISHED OPTIMAL DRY FEED FOR C. GARIEPINUS LARVAE

PROXIMATE ANALYSIS (% dry weigth)

		REQUIRED VALUES FOR OTHER FISHES (Piper et al. 1982)
Protein	55.4	35-55
Lipids	9.11	3.5-12
Carbohydr	21.2	?
Minerals	13.3	?

AMINO ACIDS (% of dietary protein)

		MEAN REQUIREMENT (From Table 1.) (Millikin 1982)
Arginine	3.9	4.33
Histidine	2.6	1.88
Isoleucin	5.5	2.25
Leucine	6.5	4.03
Lysine	8.4	5.37
Methionine	1.7	3.10
Phenylalanine	3.3	5.80
Threonine	3.8	2.80
Tryptophan	6.6	1.10
Valine	4.4	3.40
Cystine	0.3	?
Tyrosine	1.4	?

VITAMINS (mg/kg unless indicated otherwise)

		REQUIRED VALUES FOR OTHER FISHES (Piper et al. 1982)
A (IU/kg)	65000	5500
D (IU/kg)	12000	1000
E (IU/kg)	943	50
K (IU/kg)	100	?
Thiamine	35.7	20
Riboflavine	70.7	20
Pyridoxine	18.8	20
Pantothenic.	445	50
Biotin	10.7	0.09
Choline	8500	551
B12	1.9	0.02
Niacin	590	100
Ascorbic acid	1500	100
Folic acid	13.2	5
Inositol	2860	100

EXPERIMENT 11. RATION, RETENTION TIME AND FEED UTILIZATION

INTRODUCTION

Optimum growth and feed efficiency depends not only upon those aspects of nutrition mentioned so far, but also on the amount of food and the frequency at which it is given to the fish. Overfeeding causes water pollution as well as poor food conversion, while underfeeding decreases production (Nose 1979; Webber & Huegenin 1979; Bryant & Matty 1980; Piper et al. 1982).

Feed ration is expressed in terms of percentage of bodyweight per day (Jauncy & Ross 1982). This daily ration is usually divided into several portions which are given at certain intervals throughout the day (feeding frequency).

It appears that there is an optimum feeding frequency above which additional feedings have no advantage (Kono & Nose 1971; Andrews & Page 1975). This frequency is related among other parameters, to stomach size (Kono & Nose 1971) and gastric evacuation time (Nose 1979). Gastric evacuation rate, in turn, is dependant on temperature (Brett & Higgs 1970; Jobling, Gwyther & Grove 1977), the size of a single meal and the physical and chemical properties of the food (Elliot 1972).

Feed utilization can be expressed as food conversion ratio in terms of grams of dry food fed per gram live weight gained (Jauncy & Ross 1982; Piper et al. 1982). The food conversion ratio does not take into account the protein level of the feed. This is done by the calculation of the protein efficiency ratio which is the weight of fish produced per unit weight of dietary protein fed (Jauncy & Ross 1982).

The amount of dry feed that catfish larvae can consume at a time was investigated to serve as a guideline for ration size when feeding catfish larvae under intensive culture conditions. The food retention time of the larvae was monitored to serve as a guideline for feeding frequency. The larvae's food conversion ratio was determined by monitoring amounts of food consumed vs. mass gained to give a further indication of the suitability of the established dry feed. The protein efficiency ratio was also determined.

METHODOLOGY

One male and one female catfish (±8kg each) from the P.K. le Roux Dam and one male and one female (±1kg each) from Amalinda Fish Station were spawned. The eggs from the two females were of equal size, but the newly hatched larvae from the large female were on average 0,44mm longer than the larvae from the small female ($p<0,01$). The eggs from the large female had been split into two portions; one being fertilised by the small male and the other by the 8kg male. There was no significant difference in size of the larvae that hatched from these two batches for $p<0.05$. Only the larvae from the large female was used in this experiment.

Two tanks in the recirculating system as described in Chapter 3 were stocked with 500 four-day old larvae. The Group 1 larvae were fed three times daily on the established optimal dry feed formulation. Each day, 10 minutes after the midday feeding, five larvae were removed from Tank 1. These larvae were examined under a stereo microscope and the number of newly ingested feed particles present in their stomachs was determined. Since the feed particles had a known mean dry weight, the mean weight of dry feed consumed could be calculated. The amounts of feed consumed per day was taken to be three times the weight of feed consumed per meal. The mean weight of the larval sample ($n=5$) was then determined and the food conversion ratio was calculated for each day.

The Group 2 larvae were fed on the optimal dry feed which had been stained either red or green with commercial food dyes. The progress of the stained food through the gastro-intestinal tracts could therefore be monitored. The effect of different feeding frequencies and starvation on food retention time was determined.

Gross energy of the dry feed was determined by burning five 1g samples in an adiabatic bomb calorimeter. It was also attempted to quantitively collect faeces from the larvae to determine gross energy so as to determine a value for the metabolizable energy (Jauncy & Ross 1982). It was, however, found to be impractical and a more unconventional technique was followed. The larvae's gross energy value per unit body weight was determined by firing eight samples of freeze dried larvae in the calorimeter with no feed in their stomachs. The value for gross energy per unit body weight was applied to the daily mass gain. This was done to determine the amount of gross energy built into the larvae's tissues per given amount of feed consumed. The value for this conversion ratio discounts the utilized energy which is expended on metabolism and locomotion and which is not otherwise fixed into the body through chemical processes.

RESULTS AND DISCUSSION

The daily mean mass gain vs. daily food consumption of the Group 1 larvae is shown in Table 24. The feed conversion ratio i.e. the mass of dry food required (in gram) to produce one gram of live fish varied between 1,5 and 0,95. The total feed conversion ratio over the 11 day period was 1,1. This figure compares favourably with figures given for I. punctatus which vary from 1,29 (balanced protein diet) to 2,45 (poorly balanced diet) (Andrews 1977). Trout fed on commercial "Ewos" trout feed had a food conversion ratio of 1,29 (Anon 1980). Taken that 55% of the dry feed was crude protein, the protein efficiency ratio over this 11 day period was 0,6 gram protein fed per gram wet mass gain. This is slightly less efficient than figures obtained for I. punctatus (0,37 - 0,46 gram protein fed per gram wet mass gain).

The gross energy of the dry diet was found to be 18,2 ±0,18 kJ/g (mean of five samples ±SD). The gross energy of larvae (empty stomach) was found to be 20,8 ± 0,26 kJ/g dry wt (mean of eight samples of 30 larvae ±SD). The energy retention ratio over the 11 day period was 9,77 kJ of energy consumed per kJ gained in the tissues of the larvae.

Food retention times on the 6th day of feeding were as follows: Where the larvae were fed once and then starved, gastric evacuation time was six hours and the total retention time was nine hours. Where larvae were fed every hour, gastric evacuation time was 3 hours 30 min. and total retention time was six hours. This suggests that in practise, feeding frequencies of 3-4 hours can be recommended. The periods after which 75% of the larvae had empty stomachs and then empty intestines were taken to be the gastric evacuation or -retention times respectively.

The size of a single meal varied from 4,5 to 10,3% of body weight. From this, the daily feed ration was calculated (see Table 24). It can be seen that although the daily amount of feed consumed rose steadily from the first to 11th day of the experiment the ration (in % of body wt./day) reached a peak on the 7th day (31%) and then started declining. There is an inverse relationship between fish size and food intake on a % of body wt./day basis (Jauncy & Ross 1982). Macintosh & De Silva (In: Jauncy & Ross 1982) recommended a feeding ration of 36% of body wt./day for the first feeding of *Oreochromis mossambicus* and *Sarotherodon niloticus* larvae and that this should be decreased gradually to 12% of body wt./day on the 20th day. Hogendoorn (1981) found that a ration of 10% of body wt./day gave optimal results with *C. lazera* fingerlings of 0,5 to 10g. Continuous feeding during part or all of the day gave better growth than feeding two or four definite meals per day.

Piper *et al.* (1982) proposed another method of determining feed ration:

$$\text{The percent of body weight to feed daily} = \frac{\text{conversion} \times 3 \times \Delta L \times 100}{L}$$

where ΔL equals the daily increase in length and L the fishes' length at the time of determination. When this formula was applied to the values in Table 24, the results corresponded very closely with the values for ration as determined by calculating the larvae's consumption as a % of body wt./day (see ration(b) Table 24).

TABLE 24

DAILY MASS AND LENGTH GAIN, FEED CONSUMPTION, CONVERSION RATIO AND RATION OF *C. GARIEPINUS* LARVAE FED ON THE ESTABLISHED OPTIMAL DRY FEED IN EXPERIMENT 10

DAY	MEAN MASS (mg)	MASS GAIN (mg)	MEAN LENGTH (mm)	FEED CONSUMED (mg)	CONVER-SION RATIO	RATION (a) (%body wt/day)	RATION (b) (%body wt/day)
1	2.2	---	6.8	0.30	---	13	---
2	2.4	0.2	7.0	0.51	1.50	21	13
3	2.7	0.3	7.3	0.60	1.70	22	21
4	3.2	0.5	7.7	0.78	1.20	24	19
5	3.8	0.6	8.1	1.06	1.30	28	19
6	4.9	1.1	8.9	1.23	0.96	25	26
7	5.2	1.3	9.3	1.60	0.95	31	13
8	7.7	1.5	10.4	1.86	1.07	24	34
9	9.5	1.8	11.2	2.07	1.03	22	22
10	11.4	1.9	12.0	2.39	1.09	21	22
11	13.5	2.1	12.8	(2.68)	1.14	20	21
	TOTAL:	11.3		12.4	→ 1.10		

CONVERSION RATIO = FEED CONSUMED / MASS GAINED
RATION(a) = 100 x FEED CONSUMED / MEAN MASS
RATION(b) = (CONVERSION RATIO x 300 x LENGTH GAIN) / LENGTH

EXPERIMENT 12. FEEDING FREQUENCY AND THE EVALUATION OF THE OPTIMAL DRY FEED

INTRODUCTION

In the preceding experiment, the results concerning gastric evacuation time, indicated that the larvae should be fed at intervals of 3-4 hours. In this experiment the effect of different feeding frequencies on growth rate and survival of C. gariepinus larvae was investigated. As a final evaluation of the established optimal dry feed it was tested against natural food organisms. Under natural conditions, zooplankton and especially planktonic crustaceans seem to be the most important food items of C. gariepinus which are less than 20mm in total length (Greenwood 1966; Holl 1968; Pham 1976; Bruton 1979a). As mentioned before, most researchers working on C. gariepinus larvae , found zooplankton to be superior to artificial feeds (Van der Waal 1972; Hogendoorn 1980; Msiska 1981).

METHODOLOGY

This experiment was run concurrently with Experiment 11 and the means of obtaining the larvae are described under that experiment.

Six tanks in the recirculating system as described in Chapter 3 were stocked with 500 four-day old larvae each. The seventh tank was stocked with 2000 larvae to determine the effect of higher stocking density on the growth and survival of the larvae.

A variety of live food organisms was collected with a plankton net from the conditioning reservoir of the Grahamstown Sewerage Works. A rich culture of these organisms was established in an open tank, outside the hatchery. High numbers of rotifers (6/ml) and high concentrations of unidentified protozoans were present. Cladocerans were also plentiful and ranged in size from 200 to 1000 µm in length. Ostracods were less plentiful (10/litre) and small free swimming copepods were also present (±100/litre). Insect larvae numbered ± 5 per litre. The water was tinged light-green with unicellular algae. Spirogyra and associated organisms made up 1,2g per litre of water.

Organic particulate matter was 0,6g/litre. This culture provided a wide range of food organisms with adequate numbers of organisms, small enough (<200μm) to be available as a first feed for the catfish larvae.

Larval rearing tanks 1 and 3 were supplied with five litres of the plankton culture three times daily. This ensured high levels of food organisms for all daylight hours as well as most of the night.

The established dry feed formulation as shown in Table 24 was used for comparison. Ration was in excess of 25 % of body wt./day. The feeding regimes were as follows:

GROUP 1: Plankton diet; 6am, 12noon, 6pm
GROUP 2: Dry feed; every two hours for 12hours per day
GROUP 3: Plankton diet; 6am, 12noon, 6pm
GROUP 4: Dry feed; every four hours for 24hours per day.
GROUP 5: Dry feed; 6am, 12noon, 6pm
GROUP 6: Dry feed and plankton mixture; 6am, 12noon. 6pm
GROUP 7: Dry feed; 6am, 12noon, 6pm; High stocking density (40/litre)

RESULTS AND DISCUSSION

The results are shown in Table 25 and Figures 24 and 25. The growth curves of all the groups are characterised by their exponentiality, as would be expected from healthy, growing larvae. Mortalities were negligible in all groups. The larvae in Groups 1 and 3, which received only plankton, both showed a decline in their growth rates between days 6 and 8, but recovered to show growth rates equal to that of the best group (Group 6) between the 8th and 11th days of feeding. The reason for this is unknown as no distinct transition in preferred type of food organism was noticed. Throughout the experiment, the larvae showed no preference for any specific food organism. The larvae's stomach contents could easily be monitored as their ventral sides are transparent. During the first three days, organic detritus, rotifers and plant material made up, on estimation, at least 50% of their stomach contents. This was interspersed with usually two to four large food organisms such as cladocerans, ostracods or duckweed spores (300-500 μm in diameter) per individual larva. From the 4th day onwards, less detritus and more whole organisms were detected in the larvae's gastro-intestinal tracts. Plant material, especially duckweed spores and bits of Spirogyra seemed to be indigestible as the plant cells were still intact on reaching the rectums of all the larvae that were examined. The crustaceans were however wholly digested and not even bits of carapace were found in the distal parts of the intestinal tracts. Small chironomid larvae were ingested from the 6th day onwards. The cladocerans and ostracods were the most consistent food items found in the catfish larvae. The larvae were observed to predate effectively upon even the quickest organisms such as ostracods and free swimming copepods.

The group of larvae that received the dry feed three times daily (Group 5) fared significantly better than either of the two groups which were fed on the plankton diet three times daily (Groups 1 and 3). It should be noted at this stage that although the plankton was supplied only three times daily, the levels of food organisms in the tanks were high throughout the day. Moreover, unlike with the dry feed, the larvae were observed to feed continuously on the plankton. Even so, the larvae of Group 5 were significantly longer than those of Groups 1 and 3 on the 11th day ($p<0,01$). Groups 1 and 3 had the lowest condition factors throughout the experiment.

The larvae which were fed on the plankton and dry feed mixture (Group 6), fared the best and showed a consistently higher growth rate throughout the experiment. On the 11th day this group was significantly larger in length than any of the other groups ($p<0,05$ and less) except for Group 7 where $p<0,1$. The larvae fed actively and exclusively on the dry feed at meal times, but resumed their less active but continuous feeding on the live food organisms soon afterwards.

As far as feeding frequency is concerned, the best results were obtained by feeding the larvae every four hours for 24 hours of the day. The Group 4 larvae were significantly larger in length by the 11th day, than Groups 5 and 2 ($p<0,02$). This substantiated the findings in Experiment 11.

Group 7, which was stocked at a higher density (40/litre) grew faster than Group 5 (10/litre) which had the same feeding regime. By the 11th day the Group 7 larvae were significantly longer than those of Group 5 ($p<0,01$). It can, therefore, be assumed that higher stocking densities elicit a more intensive and longer feeding response. Stocking density is an aspect of larval rearing which does not fall within the realms of this project but it certainly requires investigation in future research.

TABLE 25.

GROWTH IN LENGTH AND MASS OF C. GARIEPINUS LARVAE FED AT DIFFERENT FEEDING FREQUENCIES AND ON LIVE AND ARTIFICIAL FEED IN EXPERIMENT 12

DAY 1:

GROUP	N	L	S	M	M+L	CF
All	15	7.84	0.34	2.89	10.73	0.90

DAY 6:

GROUP	N	L	S	M	M+L	CF
1	34	9.71	0.51	5.96	15.67	1.00
2	39	10.24	0.50	6.39	16.63	0.92
3	33	10.00	0.60	5.79	15.79	0.90
4	35	10.34	0.50	7.90	18.24	1.11
5	32	10.12	0.53	6.48	16.60	0.97
6	38	10.31	0.58	7.57	17.88	1.07
7	33	10.30	0.60	7.92	18.22	1.12

DAY 8:

GROUP	N	L	S	M	M+L	CF
1	15	10.21	0.62	6.91	17.12	1.01
2	14	11.03	0.68	8.21	19.24	0.96
3	12	10.13	0.65	6.70	16.83	1.00
4	17	11.34	0.58	10.16	21.50	1.09
5	20	11.16	0.59	8.81	19.97	0.99
6	15	11.41	0.60	10.38	21.79	1.10
7	16	11.18	0.60	10.05	21.23	1.13

DAY 11:

GROUP	N	L	S	M	M+L	CF
1	25	12.42	0.60	10.93	23.35	0.91
2	28	12.97	0.65	13.74	26.71	1.01
3	30	12.67	0.68	11.41	24.08	0.89
4	30	13.51	0.65	15.77	29.28	1.03
5	31	13.12	0.62	14.83	27.95	1.05
6	32	13.95	0.67	16.23	30.18	0.97
7	31	13.61	0.68	15.97	29.58	1.02

N = number of larvae in sample
L = mean total length in mm
S = standard deviation of L
M = mean mass in mg
CF= condition factor

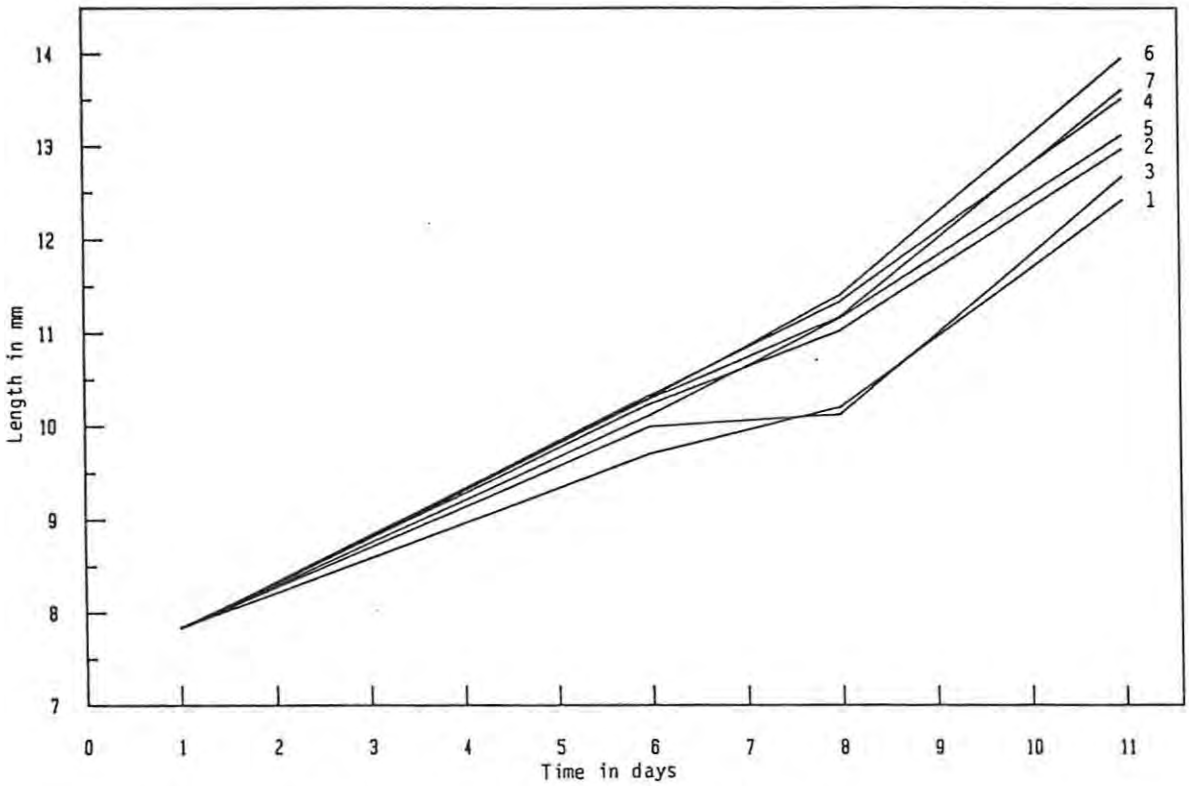


FIGURE 24. GROWTH IN LENGTH OF C. GARIEPINUS LARVAE FED AT DIFFERENT FEEDING FREQUENCIES AND ON DIFFERENT DIETS IN EXPERIMENT 12

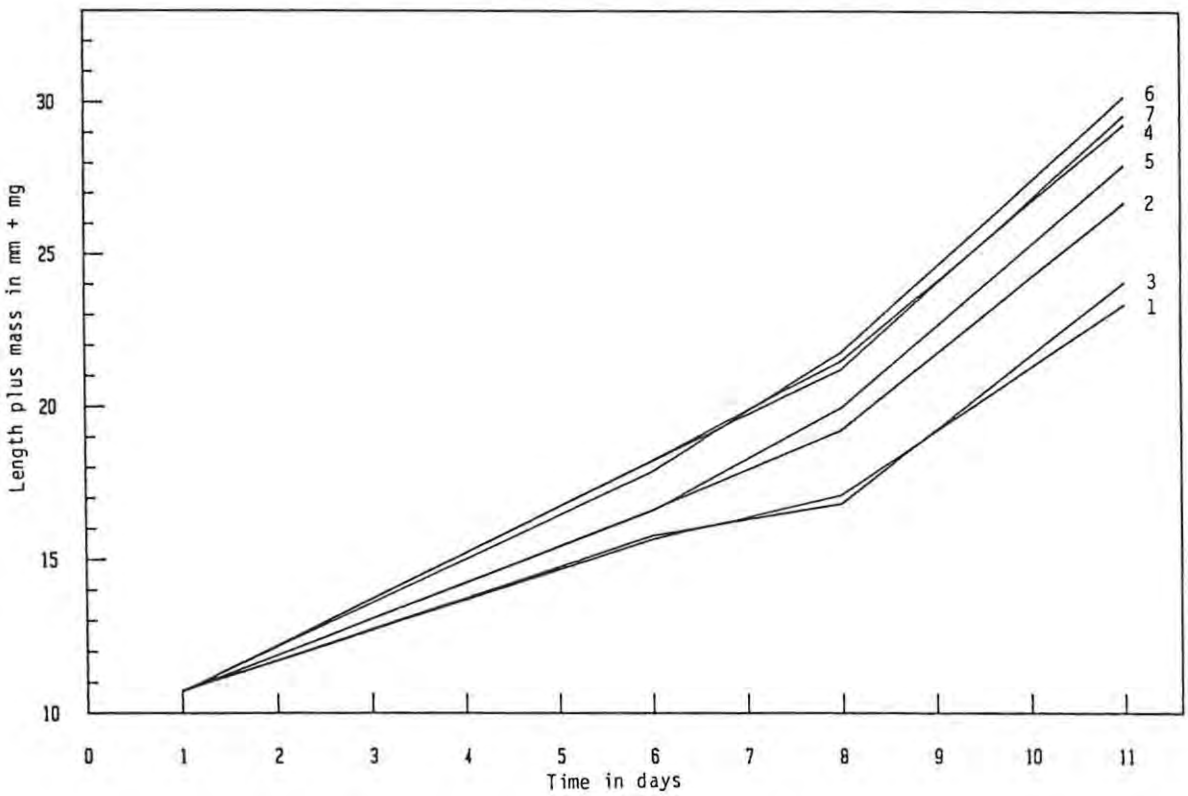


FIGURE 25. GROWTH IN LENGTH PLUS MASS OF C. GARIEPINUS LARVAE FED AT DIFFERENT FEEDING FREQUENCIES AND ON DIFFERENT DIETS IN EXPERIMENT 12

CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

The following conclusions and recommendations are based on the discussions of the preceding experiments. Aspects of feed manufacture, nutritional requirements of the larvae and feeding techniques are discussed. Finally, the evaluation of the feed is discussed.

FEED MANUFACTURE AND PHYSICAL REQUIREMENTS

One of the objectives of this project was to establish manufacturing techniques that produce a feed which meets the physical requirements of larval fishfeed as discussed in Chapter 2. Meyers (1979a) stated that there are essentially two main techniques for the manufacture of artificial larval feeds. The first is the preparation of a water stable matrix of dry ingredients followed by suitable grinding and sieving to required particle size. The alternative is the incorporation of dietary components into a properly sized microcapsule. The first of these two techniques was adopted as a basis from which to establish detailed techniques for the manufacture of dry larval feed for C. gariepinus larvae. The manufacturing techniques as described in Chapter 2, were found to be most suitable to achieve the required physical (or non-nutritional) properties of the feed.

Firstly, the techniques allow the feed particles to be graded into different particle sizes, an essential property of larval feed (Meyers 1979a; Van Limborgh 1979; Webber & Huguenin 1979; Jauncy & Ross 1982).

A second requirement of larval feed is that the larvae must be able to identify it as food (Appelbaum 1978, 1979b, 1980b). The Torula yeast based feeds were all readily accepted by the larvae from the first feeding at 90 - 100 hours after hatching. It was found that freeze dried, compacted and granulated C. gariepinus eggs were not accepted by the larvae and neither was C. gariepinus muscle which had been treated in the same manner. Torula yeast-based feeds induced an intensive feeding response for approximately two minutes after each feeding. This is fortunate, since the percentage of leachable nutrients that do leach from the feed particles is only

10% by the end of the first minute and 23% by the end of the second minute after immersion. Minimal leaching is another required property of larval fishfeeds (Randall-Robinette 1977; Csávás et al. 1979; Ghittino 1979; Goldblatt et al. 1979; Luquet & Rumsley 1979; Meyers 1979a; Van Limborgh 1979). The long physical stability of the feed in water limits water pollution. It was, however, found neccessary to remove uneaten feed from the rearing container at least once daily.

C. gariepinus larvae were found to feed willingly from the surface and in midwater but the majority of feed was consumed on the bottom of the rearing tanks. No adjustment of the specific gravity of the feed was, therefore, required (Appelbaum 1979b; Lovell 1977b; Meyers 1979a).

The low moisture content of the feed promotes stability during storage (Randall Robinette 1977; Csavas et al. 1979; Van Limborgh 1979). The manufacturing techniques as described do not necessitate exposure of the feed to high temperatures. This is an important factor in preserving heat labile nutrients (Meyers 1979a). Lastly, the cost and time involved in manufacturing the feed, using the established ingredients and techniques, are reasonable.

NUTRITIONAL REQUIREMENTS

Proteins

From what has come to light in this project, the nutritional requirements of C. gariepinus larvae do not seem to differ much from those of other warmwater fish in general (compare the nutrient composition of the most successful dry feed that could be established in this project, with the requirements of warmwater fishes (Table 24)). The crude protein content of the established feed (55,4%) is at the top of the range of protein requirements of other warmwater fishes (35 - 55%) (Piper et al. 1982). Good protein conversion rates for adult I. punctatus are considered to be 0,37 to 0,44 (Andrews 1977), but larval fish have higher dietary protein requirements and consequently poorer protein conversion rates (Andrews 1977; Cowey

1979a; Millikin 1982). Therefore, the demonstrated protein conversion rate of 0,6 gram protein fed per gram live fish gained, for the 11 day period in Experiment 10 seems to indicate that the amino acid ratios in the established feed are well balanced.

It was shown that C. gariepinus larvae require methionine at ±2,5% of their total dietary protein at a cystine level of 0,3%. This corresponds closely with I. punctatus which requires methionine at 2,3% of total dietary protein in the absence of cystine (Millikin 1982).

The high content of Torula yeast in the established feed formulation confirms the findings of Hecht (1981,1982) that Torula yeast is an excellent source of nutrients for C. gariepinus larvae.

The protein to energy ratio of the established feed is 30,4 mg protein per kJ. This is somewhat higher than the optimum protein to energy ratio demonsrated for I. punctatus fingerlings of 21 mg protein per kJ (Wilson 1977b).

Although it seems that the amino acids in the established dry feed are well balanced, research on optimum dietary amino acid ratios for C. gariepinus larvae is strongly recommended. Optimization of these ratios will increase protein utilization efficiency.

Lipids

The total dietary lipid requirement of C. gariepinus larvae was demonstrated to be approximately 9% of the total weight of the dry diet. In combination with a crude protein level of 54%, best growth and survival responses were obtained. This compares well with available data on the lipid requirements of I. punctatus fingerlings (Dupree 1969; Page & Andrews 1973; Murray, Andrews & Deloach 1977; Dupree, Gauglitz, Hall & Houle 1979). These authors found that I. punctatus fingerlings require a dietary lipid level of approximately 12% of the dry weight of the feed.

An equal mixture of cod liver oil and soya bean oil was used as a lipid supplement to the experimental feeds. This mixture contains 23%

W3, and 28% W6 fatty acids (Jauncy & Ross 1982). This lipid supplement was formulated on the assumption that fish require high levels of W3 and W6 fatty acids in their diets (Castell 1979, Jauncy and Ross 1982; Millikin 1982; Piper et al. 1982). Castell (1979) reported that there is probably a unique balance of W3, W6 and W9 series fatty acids that is specific for a fish species under the particular set of environmental conditions in which it lives. It is, therefore, recommended that research be done on qualitative dietary lipid requirements of C. gariepinus larvae.

Apart from having nutritional value, the oil supplement had a limiting effect on nutrient leaching. Significantly increased growth responses were recorded for larvae fed on diets supplemented with 6% of the oil mixture (total lipids = 9%), provided the feed had been suitably preserved.

Vitamins

Specific vitamin requirements of C. gariepinus larvae have not been established, but the levels of vitamins in the established dry feed were shown to be sufficient, as further vitamin premix supplementation did not result in increased growth of the larvae (Experiment 8). The vitamin levels in the established feed are uniformly high in comparison with requirements of warmwater fish in general (Table 23). It must be noted, however, that the vitamin levels of the established feed shown in Table 23 are the levels present before the feed particle manufacturing process takes place. Even though no severe heat treatment is involved in the manufacturing process, high vitamin losses due to oxidation and heat denaturation can still be expected (Goldblatt et al. 1979). High loss of water soluble vitamins due to leaching is also possible. Research on minimum, specific vitamin requirements of C. gariepinus larvae and the effect of processing, storage and leaching on dietary vitamins is also recommended, as the vitamin supplement to the established feed makes up 24% of the cost of the ingredients. Formulating a vitamin premix specifically for C. gariepinus larvae would greatly decrease the cost of the feed. Furthermore, the 11 water soluble vitamins and the four fat soluble vitamins which were considered in this project and which are generally considered essential in fish diets, may not be the only essential vitamin-like substances. Halver (1979) suggested that several unknown growth promoting components may yet be discovered.

Minerals

Minerals are normally present in adequate amounts in the usual ingredients of fishfeeds (Piper et al. 1982). The mineral content of Torula yeast was shown to adequately provide for the requirements of C. gariepinus larvae, since supplementation of minerals to the test diet in Experiment 3 showed no increased growth response.

Carbohydrates

Carbohydrates make up 21% of the nutrient composition of the established feed. This seems to be sufficient as supplementing starch to the test diets had only detrimental effects on the larvae (Experiment 9).

Since the only known function of carbohydrates in fish nutrition is supplying energy (Piper et al. 1982) and since lipids and proteins are more efficient energy sources in fishfeeds (Wilson 1977; Jauncy & Ross 1982), it is felt that research on dietary lipids should have priority in future. This, however, does not mean that research on carbohydrates should be neglected altogether. The role of carbohydrates in the larvae's nutrition and its contribution to the total energy requirements would be of considerable interest.

As no quantitative dietary carbohydrate requirements have been demonstrated for other fish species (Wilson 1977; Piper et al. 1982), the only comparison that can be made, is with the carbohydrate content of natural food organisms. The carbohydrate content of the zooplankters Daphnia, Cyclops, Diaptomus, Gammarus, Chaeborus, and chironomid larvae vary from 4 to 8% (Yurkowski & Tabacheck 1979).

Additives

Apart from incorporating raw materials that satisfy the nutritional requirements of the larvae, it was found neccessary to add two other supplements to the feed. Firstly, bacterial infection of the larvae led to the inclusion of "Furanace", a wide spectrum bacteriacide and fungicide in the feed. "Furanace" was shown to be effective against bacterial infection and including small amounts of it (4ppm) in the feed, is preferable to administering it directly into the hatchery water system. Large amounts of the bactericide would be required if it were to be present in sufficient concentrations in the water. Where a recirculating system is employed, it will have a detrimental effect on the bacterial populations that are responsible for maintaining water quality.

Secondly, it was found to be necessary to use an antioxidant ("Endox") in the larval feed. It was found to be effective, included at a concentration of 250ppm in the feed (Experiment 6). Antioxidants are common additives in practical fish feeds (Castell 1979; Jauncy & Ross 1982, Piper et al. 1982).

Feeding techniques

There are essentially three different methods by which feeds are dispensed. These are feeding by hand, automatic feed dispensers and demand feeders (Webber & Huguenin 1979; Jauncy & Ross 1982; Piper et al. 1982). Hand feeding was found to be preferable to automatic feed dispensing, since more rigid control could be exercised over the experiments. Hand feeding had the important advantage that feeding behaviour and demands of the fish could be noted. Mechanical demand feeders are impractical due to the small size of the larvae.

Optimum feed particle sizes were established for *C. gariepinus* larvae. The feed particle diameter should be 2,2% of the mean total length of the larvae. This confirms the recommendations by Van Limborgh (1979) that feed particles for non-salmonids that hatch from eggs approximately 1mm in diameter (as in the case with *C. gariepinus*), should not exceed 100-200 μm.

Furthermore, it was shown that a daily feed ration of 25% of the larvae's mean body weight should be maintained for the first two weeks of feeding. The optimum feeding frequency was found to be every four hours for 24 hours per day. This corresponds closely with recommendations for O. mossambicus and S. nilotica larvae. Macintosh & De Silva (In: Jauncy & Ross 1982) advocated that these larvae initially be fed a minimum of four times per day, using a ration of 36% of body wt /day.

EVALUATION OF THE FEED

Piper et al.(1982) stipulate five measurements to evaluate feeds for fish production under hatchery conditions. These are: (1) Fish growth, (2) Feed conversion, (3) Cost to rear a unit weight of fish, (4) Proteins and energy required to rear a unit weight of fish, (5) Mortality and dietary deficiency symptoms.

Evaluating the established feed by these measurements, the following conclusions could be reached: Firstly, the growth rate obtained with the established dry feed was superior to the results obtained with natural food organisms. Secondly, a feed conversion ratio of 1.1 grams of feed to produce 1 gram of larvae compares favourably with instances in the literature. I. punctatus fed on a well-balanced diet gave feed conversion ratios of 1,29 (Andrews 1977). Trout larvae fed on commercial "Ewos" trout starter feed had a food conversion ratio of 1,16 (Anon 1980). As far as cost is concerned, roughly 1 kg of feed is required to produce 500g of larvae for the first 14 days of feeding (33 000 larvae). The current retail prices of the ingredients of 1kg of the feed add up to R5.50 . By comparison, the cost of the imported commercial dry feed for C. carpio larvae "Ewos C10 Larvstart" was R17.10 per kg in 1981 (Hecht and Viljoen 1982). Roughly 2kg of protein and 18 200 kJ are required to rear 500g of 14 day old C. gariepinus larvae. Mortalities were negligible and dietary deficiency symptoms were not observed for the established feed.

It can be stated with reasonable confidence that the established dry feed as formulated in Table 22 and manufactured according to the methods described in Chapter 2, is suitable as a sole food source for C. gariepinus larvae during their first two weeks of feeding.

The preparation techniques described in Chapter 2 have to be closely followed when manufacturing the feed as these techiniques best produce the characteristics which are required for an artificial larval feed.

The established feed is suitable for use in commercial hatcheries. It can also be used as a standardized and proven food source for further research on the rearing of C. gariepinus larvae.

The objectives of this project have been met. Certain dietary requirements of C. gariepinus larvae were established and a formulation and manufacturing process to produce a feed which is superior to natural food was established.

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