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**Optimization of feeding and growth performance of African
catfish (*Clarias gariepinus* Burchell, 1822) fingerlings**

**Thesis submitted for the degree of
Doctor of Philosophy**

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Dedicated

to

***My parents, my wife Farjana
and
daughter Sabrina***

In the name of Allah, the most compassionate and the merciful

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DECLARATION

I declare that I carried out the work for and was principal contributor to the intellectual content of all papers published or in press in relation to this thesis (see Chapters for detail).

ABSTRACT

The present studies were undertaken because feeding remains the single most important determinant of the economic viability of fish culture. The research identified the factors pertinent to feeding strategies and growth performance of African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings. Existing literature relating to the feeding and growth of African catfish is reviewed and the key factors highlighted.

A preliminary experiment investigated the effect of the three most important factors - density, light and shelter - on the growth and survival of *C. gariepinus*. Low density, low light intensity and shelter enhanced growth rates, although not the rates of survival of *C. gariepinus* fingerlings. The second preliminary experiment was conducted in order to establish an appropriate methodology for measuring feed intake and gastric evacuation. The X-ray method using radio opaque Ballotinis proved successful for accurate estimation of feed intake and gastric evacuation of *C. gariepinus*. These two studies provided information on environmental parameters in catfish rearing and the appropriate techniques for monitoring feed consumption and evacuation rate.

Using feed marker and X-ray technology, based on gastric evacuation and return of appetite, maximum daily feed intake was estimated and a feeding schedule for fingerlings of this species proposed. The effects of particle size and energy level of food on gastric evacuation are evaluated and optimum feed particle sizes and energy levels were determined. Fingerling *C. gariepinus* grow best on diets of intermediate pellet size (1.5 and 2 mm) and intermediate dietary energy level (22.84 kJ g⁻¹), resulting in high feed intake and feed utilization and low food conversion.

Although this species is believed to have a nocturnal feeding habit, to date no research has established a diel rhythm. Using infrared video technology and continuous recording of feeding activities a precise diel rhythm was identified. Predominantly a nocturnal feeder, *C. gariepinus* shows two distinct feeding peaks given access to feed for 24 h - one immediately after the onset of dark phase and the second just prior to the onset of the light phase.

In order to maximize growth performance and feed intake, fish were fed with diets of intermediate pellet size and energy level in three different modes - following their feeding rhythm, only in light phase and in light and dark phase continuously. Fish fed in response to their rhythmic feeding peak had highest weight gain, feed intake and feed utilization and lowest feed conversion. On this basis, a comprehensive feeding guide for fingerling *C. gariepinus* was established.

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Chapter 1

GENERAL INTRODUCTION

... and ... which ... (1997) ...

1.1 *CLARIAS* CULTURE

Catfish of the genus *Clarias* are commercially very important in many countries, especially in Asia and Africa. In 1995, the world production of Clariid catfish was more than 0.2 million MT which was the second most important group of farmed catfish in the world (FAO, 1997). The principal cultured species in this group are *C. gariepinus*, *C. batrachus*, *C. macrocephalus* and *C. anguillaris*.

Clarias gariepinus (Burchell, 1822) was first cultured in the central part of Africa in 1970 (Hogendoorn, 1979). Problems such as stunted growth and overpopulation in tilapia culture systems promoted attempts in the early seventies to identify species more suitable for African aquaculture (Micha, 1971). Particularly, in the last quarter of this century, considerable interest has been shown in the potential of *C. gariepinus* culture (Haylor, 1992a). CTFT (1972) and Micha (1973) demonstrated its growth and production potential. It was found that African catfish is a highly suitable alternative to tilapia in subsistence fish farming in Africa and using low grade feed composed of some local agricultural by-products, the yields of catfish from ponds could be as much as 2.5 times higher than those of tilapia (Hogendoorn, 1983). At present it is cultured on a commercial and subsistence basis in at least twelve African countries, the most important of which in terms of tonnage produced, are Mali, Nigeria, Ethiopia and Ghana (FAO, 1997). Among Asian countries it is farmed mainly in Thailand, the Philippines, China, Israel, Malaysia and Indonesia. In Europe, it has been cultured in the Netherlands, Germany, Belgium, (Verreth *et al.*, 1993) and in Latin America in Brazil, (Hecht *et al.*, 1996). Recently countries such as Bangladesh (Mollah and Hossain, 1994), India (Tripathi, 1994) and the Czech Republic (Adamek and Sukop, 1995) have begun to farm the species on both extensive and intensive bases. Research activities,

experimental and commercial culture have been widely undertaken throughout Africa as well as in Asia (China, Israel, Thailand, India and Bangladesh) and Europe (the Netherlands and Scotland) (Haylor, 1992a).

Despite the considerable research effort and availability of a well developed technical knowledge in the different fields of African catfish culture systems, total production in 1995, (39,218 MT) (Table 1.1) was very low in terms of world freshwater fish production (18,145,100 MT). It accounts for less than one fifth of total Clariid catfish production (200,294 MT) (FAO, 1997).

A major bottleneck associated with the development of commercial culture of African catfish, as in most other cultured species, is the reliable supply of fish seed for stocking (Hogendoorn, 1979, 1980; Janseen, 1987; Uys and Hecht, 1985; Verreth and Bieman, 1987; Appelbaum and Van Damme, 1988). Therefore, the development of culture technology for the early stages in intensive hatchery production is an essential prerequisite to the development of African catfish culture.

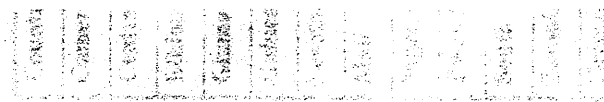


Table 1.1 World aquaculture production (tonnes) of the African catfish, *Clarias gariepinus* 1986-1995
(Data source: FAO, 1997) (F = FAO estimate)

Country	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Cameroon	6	7	9	8	6F	4F	2	2	0	0
Central African R.	3	3	2	2	5	5	1	1F	5F	10F
Ethiopia	-	-	-	-	-	-	-	-	-	643
Ghana	134	116	130	80	80	110	130	165	170	200
Guinea	1	1	1	1F	1F	1F	5F	5F	5F	4
Lesotho	3	2	2	2F	2F	2F	3F	5F	5F	4F
Mali	15251	13925	13969	17959	17637	17195	17134	16096	15752	33250
Nigeria	-	-	-	-	-	-	3850	3500	3600	4000
Rwanda	-	-	-	3	5	6	2	2F	5F	4F
South Africa	-	10	137	203	850	1150	450	50	50	10
Zambia	-	-	-	-	-	20	50	70	70	74
Greece	-	-	-	-	-	-	-	-	57	-
Netherlands	300	300	400	475	500	500	500	900	1000	1019
TOTAL	15698	14364	14650	18733F	19086F	18993F	22127F	20796F	20719	39218

1.2 CULTURE POTENTIAL OF *CLARIAS GARIEPINUS*

African catfish have all the criteria stated by Huet (1972) as desirable in species suitable for culture:

- adapted to the climate
- high growth rate
- able to mature and reproduce in captivity
- accept and thrive on cheap feeds
- acceptable to the consumer
- support high population densities
- resistant against disease.

Haylor (1993a) summarized the attributes of *C. gariepinus* for aquaculture:

Wide natural distribution

The African catfish is a eurytopic species, widely distributed throughout the Pan-African Region from Asia Minor to South Africa (from the Middle east in the North to the Orange river in South Africa in the South) (Clay, 1977; Bruton, *et al.*, 1981; Teugels, 1984). It has the widest natural latitudinal range (about 70°) of any freshwater species in the world (Hecht *et al.*, 1988). Within this range it lives in a wide variety of habitats from temperate to tropical streams, rivers, pans, swamps, underground sinkholes, shallow or deep lakes (Uys, 1989), ponds, submerged rice fields and impoundments.

Ability to air-breathe

In addition to gills African catfish have accessory air-breathing organs occupying the upper part of each branchial cavity. This organ, having an arborescent shape, enables

the fish to breathe atmospheric air, thus tolerating very low dissolved oxygen levels. This attribute contributes to their market value under private market conditions where fish are sold alive with little or no water, so that if not sold one day the live fish can be taken back to market the next day.

High acceptability to the consumers

C. gariepinus is a delicious fish and highly esteemed. Many kinds of dishes are prepared from its meat when smoked, fried and curried. It is favoured by African consumers for its taste and high fat content (Mann, 1964). Balon (1972) observed African catfish as one of the four most highly sought after fish in the Lake Kariba (Africa) region. There is a higher demand for African catfish than tilapia in Nigeria, Cameroon and Gabon (de Kimpe and Micha, 1974).

Culture and reproduction in captivity

The African catfish is well suited to all types of freshwater and brackish waters. It easily breeds in captivity. It is a seasonal spawner and the stimulus to spawn is associated with heavy rainfall (Clay, 1979; Bruton, 1979a). Outside the spawning season a general regression of the gonads takes place (Bruton, 1979a, Van Oordt and Goos, 1987) and consequently, natural spawning terminates in a culture systems. *C. gariepinus* can be artificially induced to reproduce using hormone treatments (Hogendoorn, 1979; Richter, *et al.*, 1987). The species is highly fecund (Gaigher, 1977; Bruton, 1979a; Hogendoorn, 1979).

Invaluable nutritional potential

The African catfish is an opportunistic omnivorous predator (Clay, 1979; Bruton, 1979b) and, therefore, ideal for aquaculture. It can consume a wide range and size of plant and animal feed items (ranging from small aquatic weeds to detritus and larger plants) and from zooplankton to relatively large fish, crustaceans, chironomid larvae. (Bruton, 1978; Spartaru *et al.*, 1987). It is mainly a nocturnal feeder and feeding does not depend on eyesight (Hecht and Appelbaum, 1988). Spartaru *et al.* (1987) observed intermittent feeding and an apparent ability to utilize infrequent, large meals. In addition, in culture systems, this fish can easily adapt to a variety of supplementary and formulated complete feeds.

Food conversion ratios

African catfish fingerlings are highly efficient feed convertors showing very good Feed Conversion Ratios (FCRs) in culture systems (1:1) (Hogendoorn, 1981, 1983, Uys, 1989) when fed on commercial pelleted food, but also grow very well when fed low cost feeds manufactured from agricultural by-products (Bok and Jongbloed, 1984; Michiels, 1987). A bioenergetic study found that about 70% of feed was metabolised and the utilization of metabolized feed energy for weight gain above maintenance was 80% efficient (Hogendoorn, 1983). In addition, a high ratio of feed energy is available for production as compared with that required for maintenance.

Fast growth rate

Rapid growth rate is one of the most favourable aspects of the biology of African catfish culture in terms of its aquaculture potential (Haylor, 1992a). Under optimal management conditions, they grow to over 10 g at an age of two months and more than 200 g in 5

months in tanks. Food Conversion Ratios (FCRs) can be < 1.0 and in small experimental ponds the fish can grow up to 300 g in 5½ months and reach marketable size (0.8 - 1.0 kg) within a year (Huisman and Richter, 1987). Trials indicate that the specific growth rate (SGR) of small fish (0.3 – 3 g) was 11% per day whereas for large (95 – 200 g) fish SGR is around 2% (Hogendoorn, 1983).

Environmental tolerance

C. gariepinus is a very hardy fish and can tolerate a wide range of environmental factors and survive rough handling and low levels of management (Clay, 1979). Moreover, it can survive in a wide range of temperatures (Quick and Bruton, 1984), including conditions with large diurnal fluctuations (13.5-27.5 °C, adults, Donnelly, 1973). According to Babikar (1984) the temperature tolerance range for this species is 6-50 °C. The species also survives salinities of up to 15 ppt (Clay, 1977).

Resistance against disease

C. gariepinus is tolerant of parasitic infection and no major outbreak of other types of diseases has been reported under culture condition (Huisman and Richter, 1987).

Suitable for high density culture

The African catfish are highly suitable for high density intensive aquaculture because of its rapid growth and efficient feed utilization (Hogendoorn, 1983). It can be easily cultured at high stocking density in a flowing water culture system (250-300 larvae L⁻¹, and flow rate = 200 L h⁻¹) (Hecht, 1982; Huisman and Richter, 1987).

It is not surprising therefore, that the species has long been regarded as one of the most suitable species for culture in Africa (El Bolock and Koura, 1960; Micha, 1971, 1975; Richter, 1976; Hogendoorn, 1979; Hecht, 1985). Hogendoorn (1983) summarized the attributes of the species for culture:

- it matures and easily reproduces in captivity.
- it grows fast and efficiently,
- it tolerates high densities,
- it is hardy, and
- it survives in adverse water quality conditions.

From both biological and socio-economic points of view, the African catfish is highly suitable for aquaculture, with good prospects for both developing and developed countries.

1.3 OBJECTIVES OF THE PRESENT WORK

Once a promising candidate for fish culture is selected, the possibilities and constraints in various phases of its culture must be elaborated to provide the basis for a production programme. In fish culture, the production cycle starts with young, immature fish capable of rapid and efficient growth. Therefore, good quality fish seed for a selected species must be available in large numbers.

Several methods of larval rearing proposed by authors showed highly varying success (10-90%) (Huisman, 1985; Hecht *et al.*, 1988). The successful large-scale rearing of larvae has remained a major constraint mainly as a result of inadequate nutrition during the larval and postlarval period coupled with poor hatchery management (Hecht and Appelbaum, 1987). Therefore, the development of culture technology to produce large

numbers of fry and fingerlings using appropriate feed with well-developed primary nursing technology in intensive hatchery system may solve the problem.

The three most important factors which can limit the growth of a fish are ration, body size and temperature (Stauffer, 1973; Elliot, 1975); ration as the driving force, temperature as the major rate-controlling force and fish weight as a scaling factor which adjust these rates to the size of a growing individual (Stauffer, 1973). Among the three factors, body size and temperature can be favourably manipulated easily and inexpensively. Particularly, in tropical countries, temperature is not a problem in culture systems. Since feeds are the major cost in any culture system (Shang, 1981), as with other cultured species, it is of prime importance to define feeding strategies for this species which give the best growth performance, optimum food utilisation and food conversion ratio and the least amount of waste produced from the culture systems.

Thus, the questions are: what type of feed to give the fish, how much, when and with what frequency. These questions are related to feed preference, feed intake, satiation, digestion, absorption, assimilation, excretion and the corresponding metabolic losses; and then determining whether body weight will be gained or lost. These factors are governed by internal and external, biotic and abiotic factors, such as water quality - (temperature, light regime, O₂, NH₃, CO₂, pH); stocking density, individual body weight, feeding rhythms, maturity.

In order to derive a suitable feeding strategy for *C. gariepinus* research will be conducted on three main areas - a) Optimisation of daily feed intake, b) Feed particle size and dietary energy level and c) Feeding rhythms.

a) The most important factors that impact directly on the maximum daily feed intake of fishes include the duration of feeding (satiation time), individual meal size (“stomach capacity”), the time between meals (feeding interval) and interaction among factors. If gastric evacuation is closely related to return of appetite (Ware, 1972) the daily feed intake can be favourably adjusted by manipulating the size of ration and timing of its presentation.

b) The types of feed and feed particle sizes are among the two most important factors that have significant effect on feed intake of fish (Fänge and Grove, 1979; Durbin and Durbin, 1980; Jobling, 1987). Knowledge of influence of the factors is a pre-requisite for optimizing production of a fish species because of their role in determining food acceptance, growth and feed efficiency (Jobling *et al.*, 1993).

c) *C. gariepinus* is said to feed at night (Bruton, 1979a; Hogendoorn, 1981; Viveen *et al.*, 1985; Britz and Pienaar, 1992). Although a number of authors have studied the diel rhythms of feeding activity in fish such as brown bullhead, (Eriksson and Van Veen, 1980), Asian stinging catfish (Sundararaj *et al.*, 1982), thick lipped mullet (Wright and Eastcott, 1982), South American armoured catfish (Boujard *et al.*, 1990), rainbow trout (Boujard and Leatherland, 1992a), Atlantic salmon (Kadri *et al.*, 1991; Fraser *et al.*, 1993), sea bass (Sanchez-Vazquez *et al.*, 1994), European catfish (Anthouard *et al.*, 1987; Boujard, 1995) to the best of my knowledge only two papers has described the diel rhythm of African catfish, *Clarias gariepinus* (Bruton, 1979b in field; Britz and Pienaar, 1992 in laboratory). However, in culture systems, this species is still fed during day time and such a feeding practice may have negative effects on the

growth performance and feed utilisation and obviously increase the amount of uneaten feed and consequently the source of pollution.

The overall aim of the present project is to present the result of the experiments conducted on these three areas. Specific objectives include

- (i) to evaluate the daily feed intake of African catfish fingerlings,
- (ii) to evaluate the effect of feed quality and particle size on gastric evacuation and growth and
- (iii) to evaluate the feeding rhythms under conditions of constant feed access and photoperiod (Light : Dark 12 h : 12 h) and to assess a suitable feeding schedule for this species.

In order to achieve these goals four key experiments were identified. However, before starting the main experiments two preliminary studies were carried out. The first evaluated the effects of three most important abiotic factors – density, photoperiod and shelter on growth and survival of *C. gariepinus* (Chapter 4). The second preliminary experiment was carried out to elucidate a suitable methodology for the gastric evacuation experiment (Chapter 5). The first key trial was to carry out a quantitative estimation of maximum daily feed intake of *C. gariepinus* (Chapter 6). In Chapter 7 and 8, the effects of quality and particle size of feed on gastric evacuation and growth are investigated. An evaluation of diel rhythm of feeding activity is summarized in Chapter 9. Finally, in a follow-up experiment (Chapter 10), growth, survival and food conversion ratio of *C. gariepinus* applying results from other experiments were investigated.

APPENDIX

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... aquaculture conditions. There are
... which contain some species which are of

Chapter 2

A REVIEW OF SOME ASPECTS OF THE BIOLOGY AND FEEDING PRACTICES OF *C. GARIEPINUS* AND SOME RELATED WORKS

2.1 TAXONOMY AND IDENTIFYING CHARACTERISTICS OF *C. GARIEPINUS*

Catfishes belong to the Order Siluriformes and there are some 2,211 species worldwide, representing 8 % of the total number of fish species (Nelson, 1984). Most African catfishes are either too small or too difficult to culture or encounter too much consumer resistance to be successful aquaculture candidates. There are only three African Siluroidea Families which contain some species which could be considered suitable for food fish culture: the Claroteidea (formerly Bagridae), the Schilbeidea and the Clariidae.

The following anatomical features characterize the fishes of the Family Clariidae:

- a single rayed dorsal fin, which may be short or long,
- presence of adipose fin in some species,
- strong and sharply pointed spines in the pectoral,
- a long anal fin
- whisker-like sensory barbels around the mouth,
- a large broad head,
- small eyes,
- swimbladders,
- Weberian apparatus and
- a suprabranchial organ for airbreathing

Recent revisions of the systematics of African catfish have resulted in several widespread species being synonymised under the name *Clarias gariepinus* (Ozouf-Costaz *et al.*, 1990). These include *C. capensis* of Southern Africa, *C. mossambicus* of

Central Africa and *C. lazera* of the West and North Africa and Asia Minor. *C. gariepinus* has been placed in the subgenus *Clarias* (*Clarias*) together with the west African species *C. anguillaris*, *C. senegalensis* and others (Teugels, 1986).

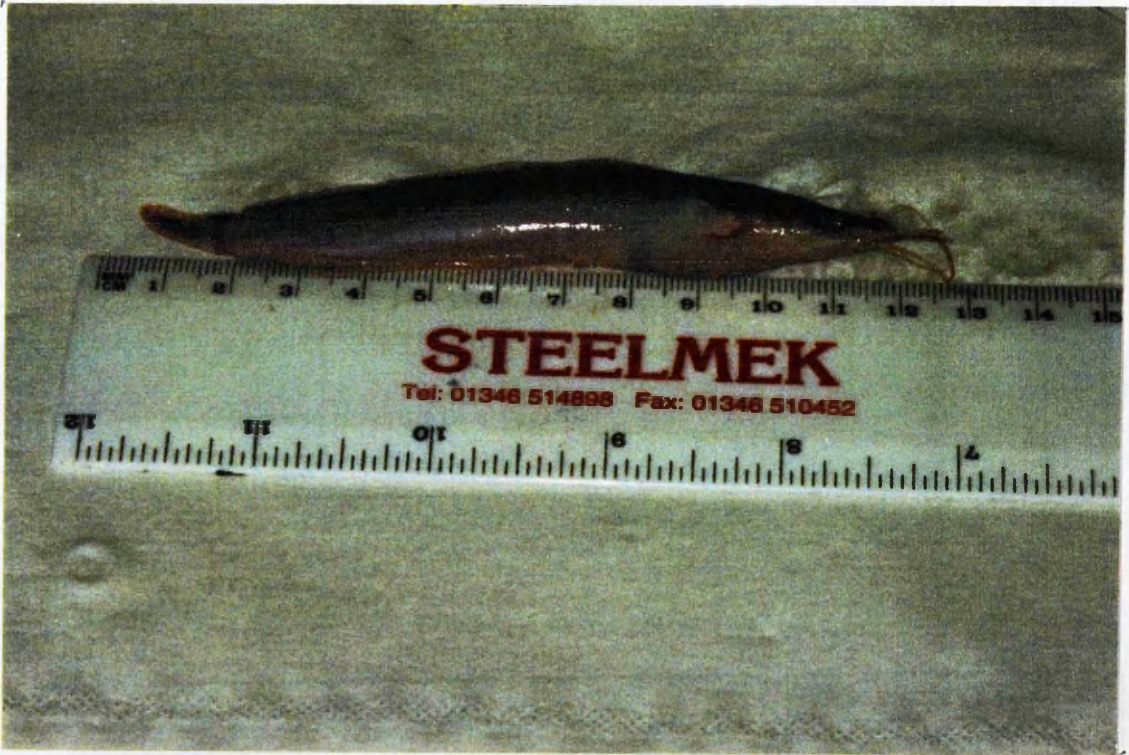
The distinguishing characteristics of *C. gariepinus* are:

- large and bony head with small eyes,
- dorsal and anal fins long,
- no adipose fin,
- pectoral fins with stout serrated spine, used for defence or walking on land,
- large and terminal mouth,
- four pairs of barbels,
- colour varies from sandy-yellow through grey to olive with dark greenish-brown marking, belly white, and
- well-developed suprabranchial organ.

(Teugels, 1984, 1986, 1996)

2.2 BIOLOGY

Clarias gariepinus is an elongated freshwater teleost with a dorso-ventrally flattened head and laterally flattened body (Figure 2.1). It has a scaleless slimy skin with dark pigmentation on dorsal and lateral parts of the body. The mouth is relatively wide by comparison with other fish rendering catfish able to feed on a variety of food items ranging from minute zooplankton to fish. The species is also able to suck benthos from the bottom and can tear pieces off cadavers with small jaw teeth and can swallow prey such as fish whole. The mouth circumference of this gape-limited predator, which is



a) *Fingerling*



b) *Adult*

Figure 2.1 *African catfish, Clarias gariepinus (Burchell, 1822)*

about ¼ of its total length, determines the maximum size of its prey. A 30-cm (approximately 200 g) catfish having a mouth circumference of about 7.5 cm can encompass the body circumference of small tilapia of up to 8-10 cm. (Viveen *et al.*, 1985).

The different life stages of *C. gariepinus* were defined by Haylor (1992b) and Viveen *et al.* (1985) expressed these in terms of size range (Table 2.1)

Table 2.1 *The different life stages of Clarias gariepinus*

	Definition (Haylor, 1992)	Size range (Viveen <i>et al.</i>, 1985)
Eggs	Pelagic	1-1.6 mm
Larvae	Young fish starts exogenous feeding but still lacks accessory breathing organs	5.0-7.0 mm
Fry	Airbreathing fish up to 1 g	8.0-30.0 mm
Fingerling	Immature airbreathing fish between 1 g and 5 g	3.0-10.0 cm
Grower	Immature airbreathing fish more than 5 g	
Adult fish		32.0-140.0 cm

2.3 FACTORS AFFECTING GROWTH OF *C. GARIEPINUS*

2.3.1 Temperature

Like other poikilothermic animals, the growth process in fish is highly susceptible to and dependent upon changes in temperature. Among water quality parameters, temperature is the most important determinant of growth and metabolism of fish. It acts as a controlling factor to pace the metabolic requirements for food and to govern the rate processes involved in food processing (Brett, 1979).

C. gariepinus is a very temperature tolerant species and can survive in a wide range of temperatures (Quick and Bruton, 1984). The thermal zone of normal activity is 18-45 °C and the zone of feeding is 15-50 °C (Babiker, 1984). Between 25 °C and 30 °C, the scope for growth in *C. gariepinus* increases with increasing temperature (Verreth and Bieman, 1987). Although Clay (1979) stated a temperature preferendum of 32.7 ± 1.5 °C for *C. gariepinus*, Viveen *et al.*, (1985) subsequently reported this as 27°C; however, according to Hogendoorn (1983) the maximum feeding of *C. gariepinus* is reached at 30°C for the size range of 0.3-70 g. This statement is also supported by Britz and Hecht (1987), as they found the temperature for fastest growth rate and the temperature preferendum of both larval and post-larval African catfish corresponds to 30 °C. Other water quality requirements are summarized in Table 2.2.

2.3.2 Stocking density

The stocking densities which are commercially most appropriate for fish rearing depend upon a number of both biological and economic factors (Haylor, 1991). The economic factors are mainly site- and situation- specific and can be determined for a given situation by a feasibility study. The biologically most appropriate stocking density is the highest which still allows the optimum growth and highest survival rate in any given situation.

Like many other fish species, territoriality, intraspecific aggression and sibling cannibalism are recognized in *C. gariepinus* (Hecht and Appelbaum, 1987, 1988; Haylor, 1991; Kaiser *et al.*, 1995). In most studies involving heavy mortalities of *C. gariepinus*, the causes were believed to be intraspecific agonistic behaviour and

Table 2.2 Water quality requirements for African catfish (Viveen et. al., 1985)

Water quality parameters	Limit
O₂	> 3 ppm
pH	6.5 – 8
N₂	> 102 % saturation
CO₂	< 15 ppm
NH₃	< 0.05 ppm
NH₄⁺	< 8.80 ppm (pH 7)
NO₂⁻	< 0.25 ppm
NO₃⁻	< 250 ppm
Cu	< 0.03 ppm
Zn	< 0.1 ppm
Cd	< 0.0006 ppm
Salinity	< 15000 ppm

cannibalism (Van der Waal, 1978; Britz, 1986; Smith and Reay, 1991; Hecht and Pienaar, 1993).

Stocking density has been found to be one of the principal factors regulating agonistic behaviour of this species (Kaiser *et al.*, 1995) and therefore survival and growth as well. In experimental culture systems, young *C. gariepinus* have been cultured at a range of stocking densities between 5 and 300 fish L⁻¹ (Hecht, 1982; Hecht and Appelbaum, 1987; Appelbaum & Van Damme, 1988; Haylor, 1991). In an experiment with the fry of *C. gariepinus* kept at different stocking densities (50 L⁻¹, 100 L⁻¹ and 150 L⁻¹), Haylor (1991) found that fish increased rapidly in weight, with significant ($P > 0.05$) increases in weight for each successive 5-day period measured between day 15 and day 35. At 50 fry L⁻¹ the fish gained significantly more weight over each 5-day period than at the higher stocking densities, there being no significant ($P < 0.05$) differences in weight gain between fish at 100 L⁻¹ and 150 L⁻¹. Although survival rates increased with the increasing stocking densities there were no significant differences in survival rate among the three different stocking densities. However, above 100 fry L⁻¹ cannibalism was the principle cause of death, whereas at lower stocking densities aggressive encounters were more commonly observed and at 50 fry L⁻¹ non-cannibalistic death accounted for nearly 79% of fry mortality (Haylor, 1991).

Under experimental culture conditions, *C. gariepinus* starts air breathing when it attains a length of ~ 2 cm, 14 days after first feeding at 30 °C (Haylor, 1991). Fry are not constrained by dissolved oxygen level and they can survive without dissolved O₂ for a long period of time if their respiratory apparatus remains moist; hence they can be cultured at high stocking densities (Hogendoorn, 1983).

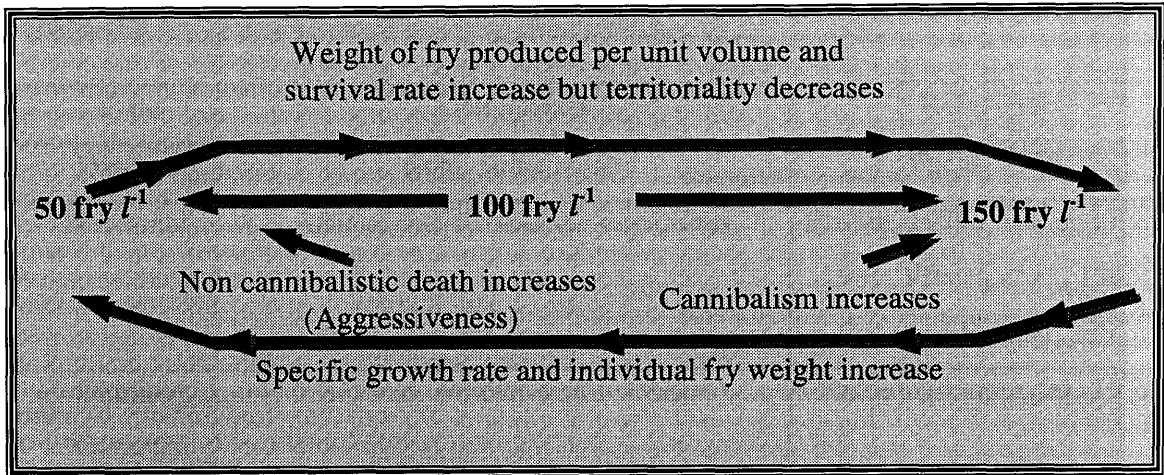


Figure 2.2 The effect of different stocking on growth and survival of *C. gariepinus* fry (after Haylor, 1991)

It is observed from the published literature that most stocking density experiments have been carried out with first feeding larvae or fry of *C. gariepinus* (Hecht, 1982; Hecht and Appelbaum, 1987; Appelbaum and Van Damme, 1988; Haylor, 1991). The growth and survival of the fingerling stages of this species, however, have not been the subject of detailed investigation to determine the optimum stocking density.

2.3.3 Light and photoperiod

Light is known to act as a powerful directive factor synchronizing the endogenous cycles of metabolism and activity in fish and other organisms (Britz and Piennar, 1992). It stimulates brain-pituitary responses which radiate through the endocrine and sympathetic systems (Brett, 1979) and synchronize the physiology and activity rhythms of fish (Thorpe, 1978). Most fish do not feed constantly but follow cyclical rhythmic feeding patterns which have been widely studied in a number of fish species (Boujard, 1995) (Table 2.3). The rhythmic activity of fish is known to be synchronized by daily fluctuation in environmental cues, and light is generally regarded as the main factor (Manteifel *et al.*, 1978; Tomiyama *et al.*, 1985). Although temperature, dissolved

Table 2.3 Feeding rhythms in different fish species

Type	Fish species	Reference
Diurnal	Sole, <i>Solea solea</i>	Fuchs, 1978
Nocturnal	African catfish, <i>Clarias gariepinus</i>	Bruton, 1979a; Hogendoorn, 1981; Viveen <i>et al.</i> , 1985; Britz and Pienaar, 1992
Diurnal/ Nocturnal	Seabass, <i>Dicentrarchus labrax</i>	Barahona-Fernandez, 1979
Diurnal/ Nocturnal	Brown Bullhead, <i>Ictalurus nebulosus</i>	Eriksson and Van Veen, 1980
Nocturnal	Catfish, <i>Ictalurus sp.</i> Eel, <i>Anguilla anguilla</i>	Meske, 1981
Nocturnal	Stinging catfish, <i>Heteropneus fossilis</i>	Sundararaj <i>et al.</i> , 1982
Nocturnal	Brown hakeling, <i>Physiculus maximowiezi</i> Bermuda catfish, <i>Promethichthys prometheus</i> Japanese conger, <i>Conger myriaster</i>	Arimoto <i>et al.</i> , 1983
Nocturnal	Sea catfish, <i>Arius felis</i>	Stelle, 1985
Nocturnal	European catfish, <i>Silurus glanis</i>	Anthouard <i>et al.</i> , 1987 Boujard, 1995
Nocturnal	Driftwood catfish, <i>Entomocorus gameroi</i>	Rodriguez <i>et al.</i> , 1990
Nocturnal	Armoured catfish, <i>Hoplosternum littorale</i>	Boujard <i>et al.</i> , 1990 Boujard <i>et al.</i> , 1992
Nocturnal	African catfish, <i>Heterobranchus longifilis</i>	Kerdchuen and Legendre, 1991
Diurnal Nocturnal	Atlantic salmon, <i>Salmo salar</i>	Kadri <i>et al.</i> , 1991 Fraser <i>et al.</i> , 1993
Diurnal	Rainbow trout, <i>Oncorhynchus mykiss</i>	Boujard and Leatherland, 1993
Nocturnal	Walking catfish, <i>Clarias batrachus</i>	Singh and Srivastava, 1993
Diurnal	Baramundi, <i>Lates calcarifer</i>	Barlow <i>et al.</i> , 1995
Nocturnal	European catfish, <i>Silurus glanis</i>	Boujard, 1995

oxygen and carbon dioxide are examples of other factors influencing the pattern of feeding activity (Randolph and Clemens, 1976), the main daily environmental rhythmic¹ Zeitgeber, however, is the periodicity of light/dark alteration (Boujard and Leatherland, 1992a).

2.3.3.1 Nocturnal adaptation of *C. gariepinus*

According to Schwassmann (1971), most fish can be conveniently classified into two categories - diurnal, relying predominantly on vision, and nocturnal, which rely more on tactile, chemical or electrical senses. Having a poor acuity of vision *C. gariepinus* does not rely on visual stimuli for food detection (Hecht and Appelbaum, 1988). It recognizes its prey mainly by touch and smell (Viveen *et al.*, 1985) primarily through an array of circum-oral barbels. The dependence upon tactile and chemosensory prey detection is an adaptation for nocturnal and turbid water feeding (Viveen *op cit.*), in common with many other silurids (Lowe-McConnell, 1975). Another adaptation to nocturnal feeding habit was reported by Lissman and Machin (1963), who discovered an ability of *Clarias spp.* to detect minute electric fields (0.75 Vcm^{-1}) which they believe plays a role in prey location by enabling the animal to fix upon muscular electrical activity and/or prey location by water movement in the Earth's magnetic field. The same adaptation in Japanese catfish *Parasilurus asotus* has also been reported with the catfish was apparently able to locate nearby prey by means of its electric sense (Asano and Hanyu, 1986).

¹ The diel activity patterns of fish are the expressions of endogenous circadian rhythms synchronized by environmental factors (such as light) called 'Zeitgebers' (Schwassmann, 1980).

2.3.3.2 Feeding rhythms

Since most marine and freshwater fishes show a cyclical daily activity pattern (Schwassmann, 1971), the understanding of rhythmicity can be of prime importance to maximizing the growth and survival of a fish population in a culture system. In culture systems, the timing of meals has a prominent effect on locomotor and air breathing activity and food utilization by fish (Boujard *et al.*, 1990), as well as their growth rate, food conversion efficiency and body composition (Noeske *et al.* 1981; Sundararaj *et al.*, 1982; Noeske and Spieler, 1984; Ottaway, 1978). Parker (1984) recommended taking diel cycles into account because of their possible influence on the metabolic utilization of food. Synchronization of rearing activities with biological rhythms may improve the efficiency of production and the quality of the farmed product.

In an experiment with Atlantic salmon, *Salmo salar*, Kadri *et al.*, (1991) found that this species showed a marked feeding rhythm, being highest in early morning and lowest in early afternoon. Boujard *et al.*, (1990) reported that feed demands of south American armoured catfish, *Hoplosternum littorale* started at dusk and increased throughout the night with a peak between 02.00 and 05.00 with a marked peak of air-breathing and locomotor activities in dusk. Boujard (1995) found European catfish, *Silurus glanis* to be strongly nocturnal. After training them to adopt diurnal feeding rhythms, they not only reduced voluntary feed intake but resumed their nocturnal behaviour in less than 24 h when they had again free access to feed.

The development of ecologically acceptable fish culture must be able to realize improved growth performance of fish and minimization of effluent production. The economy of a fish farm is greatly dependent on the efficiency with which fish utilize the

food supply. In many farms food wastage is high, leading to high production costs and poor economy (Alänarä, 1992). The feeding efficiency of fish can be improved markedly if feed delivery is tailored to daily rhythms in appetite (Kadri *et al.*, 1991). Handy and Poxton (1993) reported that the most effective way of reducing water pollution from fish culture is to minimize feed loss and feed wastage, which can be reduced by presenting food when the fish are most motivated to feed. Moreover, feed is the major production cost in fish culture (Boujard, 1995), so minimizing feed loss not only reduces water pollution but also lowers production costs. In culture systems, most of the species, however, are still fed during daytime and feeding rhythms are not considered when designing feeding schedules. Such feeding practices may have negative effects on the growth performance and survival and feed utilization and may increase the amount of food wastage and consequently the source of pollution and cost of fish culture as well.

2.3.4 Shelter

The shelter seeking behaviour of a number of fish species has long been documented (Huet, 1972; Britz and Pienaar, 1992, Table 2.4). Fish need protection from predators, especially when they are small and vulnerable, so they can hunt for food whilst avoiding predators (Burke, 1991). The provision of shelter ensures a refuge for non-schooling fish, facilitates feeding and protects from visual predators thus improving survivorship. Potts and Hulbert (1994) carried out field studies and found that in conditions of decreasing availability of shelter, pelagic baitfish abundance decreased while predator abundance increased. Increasing availability of shelter decreases the efficiency of many predatory species (Northern pike, *Esox lucius*, Savino and Stein,

Table 2.4 Different type of shelters used by fish and the purpose

Species	Type of shelter	Purpose	References
Piranha, <i>Serrasalmus spilopleura</i>	Water hyacinth roots	Refuge from predators and feeding	Sazima and Zamprogno, 1985
Driftwood catfish, <i>Entomocorus gameri</i>	Benthic and floating substrata	Avoid predators	Rodriguez <i>et al.</i> , 1990
Atlantic cod, <i>Gadus morhua</i>	Seagrass, rock reef etc.	Protection from predator	Tupper and Boutilier, 1995
Multi species	Well vegetated littoral areas	Protection from excess sunlight and predators	Sumer <i>et al.</i> , 1995
Atlantic salmon, <i>Salmo salar</i>	Shallow and deep lakes : stones and macrophytic vegetation	Mainly spawning	Halvorsen and Joergensen, 1996

1989; Largemouth bass, *Micropterus salmonoides*, Miranda and Hubbard, 1994; Atlantic cod, *Gadus morhua*, Tupper and Boutilier, 1995).

Providing shelter decreased the intra-specific aggressive interaction among European eels, *Anguilla anguilla* and improved growth performance (Kushnirov and Degani, 1991). In an experiment with African catfish, *Clarias gariepinus* in captivity, Britz and Pienaar (1992) found very obvious refuge-seeking behaviour. The authors recommended shelter principally for the larvae which are not very strong swimmers and have poor visual acuity. They, therefore, are able to seek refuge in shelter and forage more widely for food and in this way can avoid visual detection by predators, yet feed efficiently. It has also been suggested that shelters may suppress mortality due to cannibalism during culture (Britz and Pienaar 1992).

2.3.5 Feeding

2.3.5.1 Feeding level

Rapid growth is one of the favourable aspects of the biology of *Clarias gariepinus* in terms of aquaculture potential. As a consequence, however, the conventional approach to the assessment of feed requirements based on periodic weighing can not be easily achieved (Haylor, 1992a).

Specific growth rate (SGR) remains somewhat constant over short culture intervals and consequently feeding level (expressed as % of bw d⁻¹) can be kept constant over these intervals and the resulting growth performance may be compared by the SGR (% bw d⁻¹). However, in younger fish this rule is no longer tenable. Although this period is not very long, during this time fish weight increases twenty to fifty fold, dry matter content

changes considerably and the specific growth rate decreases continuously and rapidly (Verreth and den Biemen, 1987). Thus Hogendoorn (1980) reported a rapid decrease in SGR of *Clarias gariepinus* from 85% d⁻¹ to below 20% d⁻¹ of the body weight in the first 28 days of feeding. For *Clarias gariepinus*, therefore, fixing the feeding level as a percentage of body weight based on periodic weighing, is only a poor approximation of feed requirements (Haylor, 1992a).

2.3.5.2 Feeding frequencies

To date no clear picture has emerged from experiments (Hogendoorn, 1980; Uys and Hecht, 1985; Hecht and Applebaum, 1987; Verreth and den Bieman, 1987; Appelbaum and Van Damme, 1988; Verreth and Van Tongeren, 1989) specifically designed to investigate feeding frequencies and no consensus exists as to how much and at what frequency feed should be offered (Haylor, 1993b). Hogendoorn (1981) investigated the effect of the number of meals on growth, survival and feed conversion of *Clarias gariepinus* fingerlings (0.5-10 g). Fish fed continuously for 24 h per day gave the fastest growth and highest average final weights. Fish which received feed 12 h per night grew almost as rapidly but food conversion ratio was improved. The remaining fish which received feed as 2 or 4 meals or 12 h continuously per day grew more slowly and showed less efficient conversion of feed. All experimental fish received 10% of their body weight daily. The same has also been reported in another African catfish *Heterobranchus longifilis* (Kerdchuen and Legendre, 1991), where all the fish received 3% of their body weight daily.

Uys and Hecht (1985) recommended feeding every 4 h which resulted in faster growth than feeding every 2 h for 12 h per day or every 6 h for 18 h per day for *Clarias*

gariiepinus. The results indicate that the feed conversion and growth rate are significantly affected by feeding frequency as has been reported with carp (Huisman, 1974).

The subject of maximizing daily feed intake with optimum number of meals for *Clarias gariiepinus* in order to achieve a maximum growth rate clearly still remains to be addressed. However, it has long been considered that feeding frequency can be scheduled according to the rate of gastric evacuation (Brett and Higgs, 1970; Eggers, 1977; Elliott and Persson, 1978; Jobling, 1981) (detail in chapter 2.4)

2.4 GASTRIC EVACUATION

In fish farming, it is of prime importance to define feeding strategies which provide the best growth performance and the optimum feed conversion ratio. The match between feed intake and the amount of feed presented determines the amount of non-ingested feed, which is a source of pollution and lost revenue to the fish farmer.

Estimation of the rates of food consumption by fish (*i.e.*, feed intake) have wide spread use in ecological, fisheries and aquaculture research (Rice and Cochran, 1984; Jobling *et al.*, 1995). In the field of ecology and fisheries, food consumption estimates have been made in order to quantify population mortality due to predation and the production of the fish population. In aquaculture, however, the same information is needed to quantify the daily ration of fish (Jobling *et al.*, 1995). Accurate and precise techniques for determining rates of gastric evacuation (GER) in fishes are essential (Olson and Mullen, 1986), in order to accurately model daily ration and food consumption (Figure 2.3) in fish (Eggers, 1977; Elliott & Persson, 1978; Jobling, 1981).

Food is usually broken down in the fish stomach through a combination of muscular contractions of the gastric wall and enzymatic reaction in an acid medium. The resulting products are expelled from the stomach through the pyloric sphincter into the small intestine through a process called gastric evacuation (Bromley, 1994), the gastric evacuation rate being defined as the rate at which food passes through the stomach. Bajkov (1935) was among the first to estimate daily food consumption of fish using rates of gastric evacuation. However, it was recognized by Ricker (1946) as having an important bearing on fish production in terms of estimating the 'daily ration' which he defined as the size of the daily meal expressed as a percentage of body weight. Since then the model of Bajkov (1935) has been widely applied either in its original form or with slight modification (Darnell and Meierotto, 1962; Backiel, 1971; Noble, 1973). Models in common usage today are based on the assumption that gastric evacuation is an exponential process over time as proposed by Elliott and Persson (1978) (Huebner and Langton, 1982; Macdonald *et al.*, 1982; Elliott, 1991; Haylor, 1993b). As enzyme reactions are essentially exponential processes (Fábián *et al.*, 1963; Jennings, 1965), it is likely that gastric evacuation proceeds at an exponential rate (Elliott and Persson, 1978).

Factors found to be important in assessment of gastric evacuation rates include water temperature, food composition (physical and chemical properties), dietary energy content, meal size and food particle size (Windell 1978; Jobling 1981; Durbin *et al.*, 1983; Smith 1989; Bromley 1994). He and Wurtsbaugh (1993) investigated the effects of water temperature, fish size and meal size on gastric evacuation rates and after analyzing results from 121 published paper (22 different fish species) concluded that

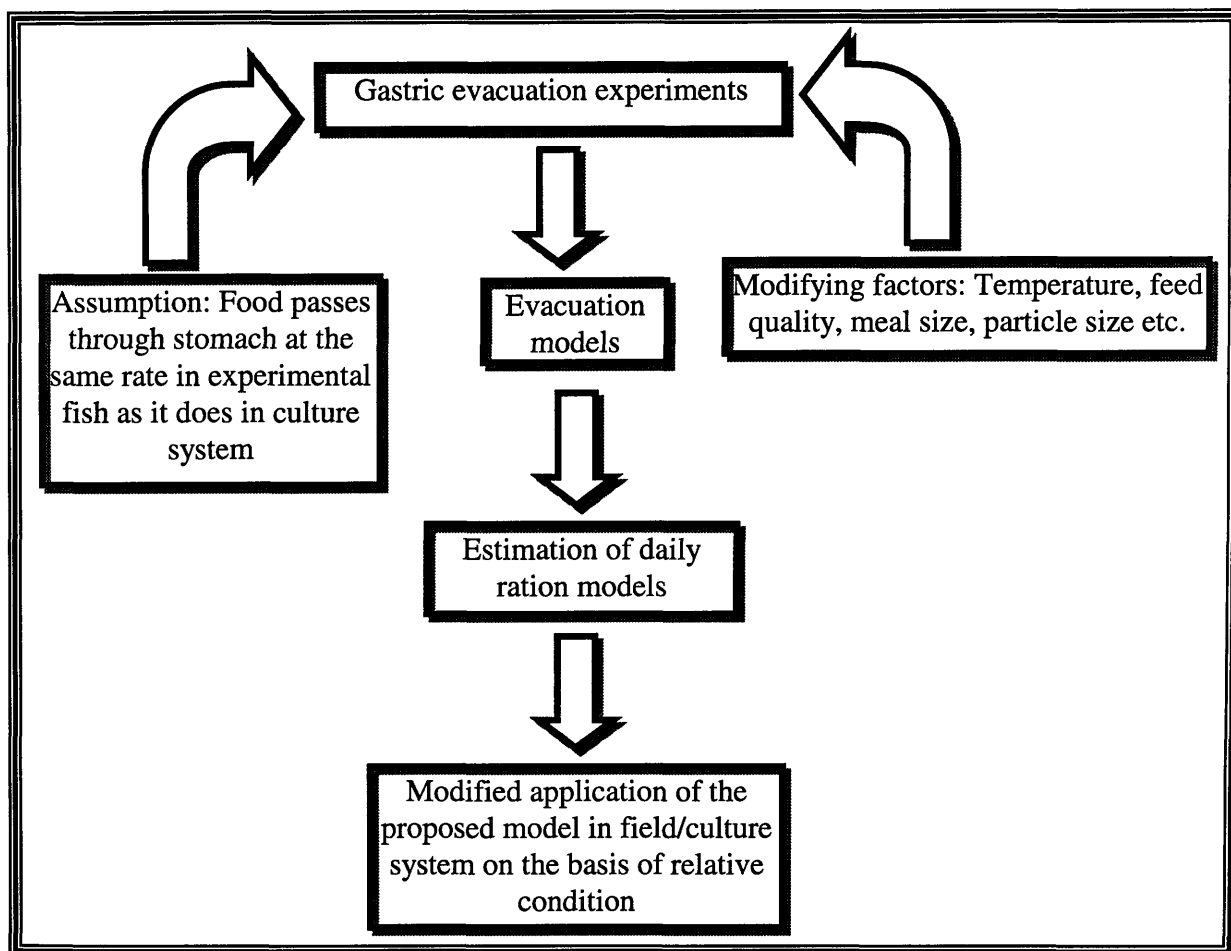


Figure 2.3 *Flow chart of the procedures of estimating daily ration based on gastric evacuation*

both temperature and meal size had a significant effect but fish size did not. Jobling (1980) found that different sizes of fish belonging to a single species and fed a particular feed will take the same time to empty their stomachs. Although not thoroughly studied, the evidence indicates that season does not influence gastric emptying rates either (Windell, 1978). However, force feeding (Windell, 1966; Swenson and Smith, 1973) and starvation (Goddard, 1974; Sarokon, 1975) have a pronounced effect on gastric evacuation rate (GER).

2.4.1 Water temperature

The successive steps in the transformation of fish feed to fish tissue are influenced by numerous physical, chemical and biological factors, but none is more important than water temperature (Windell, 1978). Temperature significantly affects the rate at which food is processed in the stomach (Fänge and Grove, 1979; Buckel and Conover, 1996). The rate tends to increase with rising temperature, reaching a maximum near the upper temperature tolerance limit for the species (Smit, 1967; Shrable *et al.*, 1969; Brett and Higgs, 1970). Beyond the maximum, food-processing rate drops precipitously (Tyler, 1970), the fish ultimately losing appetite, ceasing feeding and becoming extremely lethargic.

In a recent study with age-0 bluefish, *Pomatomus saltatrix*, fed with bay anchovy, Buckel and Conover (1996) found increasing evacuation rate with temperature (temperature - 21, 24, 27 and 30 °C; evacuation rate- 0.157, 0.199, 0.273 and 0.376 respectively) using the exponential model of Elliott and Persson (1978). The time taken for total gut evacuation and 50% evacuation at different temperatures for a range of fish species is presented in Tables 2.5 and 2.6.

Table 2.5 Emptying time for 50% stomach evacuation of fish at different temperature (after Windell, 1978)

Species	Temperature °C	Time to 50 % empty (h)	Reference
<i>Lepomis macrochirus</i>	5	31	Kitchell, 1970
	10	11.5	
	15	7.5	
	20	5	
	25	4.5	
<i>Gadus morhua</i>	2	13	Tyler, 1970
	5	11	
	10	5	
	15	4	
	19	5	
<i>Ictalurus munctatus</i>	10	15.5	Shrable <i>et al.</i> , 1969
	15.5	13.5	
	21.1	9	
	23.9	6	
	26.6	4	
	29.4	7	
<i>Oncorhynchus nerka</i>	3.1	25.6	Brett and Higgs, 1970
	5.5	12	
	9.9	6	
	14.9	3.4	
	20.1	2.7	
	23	2.6	
<i>Salmo gairdneri</i>	5	25	Windell <i>et al.</i> , 1976
	10	15.1	
	15	9.2	
	20	5.6	
<i>Salmo trutta</i>	5.2	9.9	Elliott, 1972
	9.8	5.9	
	15	3.3	

Table 2.6 Emptying time for 100% stomach evacuation of fish at different temperature (after Fänge and Grove, 1979)

Species	Temperature °C	Time to 100 % empty (h)	Reference
<i>Salmo trutta</i>	0	35	Otto, 1976
	2-4	12-18	
	6-8	10	
	12-15	3	
<i>Salmo gairdnerii</i>	8	27	Grove <i>et al.</i> , 1976
	11	24	
	15	22	
	8.5	26.5	Grove <i>et al.</i> , 1978
	13.5	18.2	
18	15		
<i>Oncorhynchus nerka</i>	3.1	147	Brett and Higgs, 1970
	5.5	79	
	9.9	38	
	14.9	23	
	20.1	18	
<i>Ptychocheilus oregonensis</i>	6	111	Steigenberger and Larkin, 1974
	10	38	
	15	14	
	20	10	
	24	8	
<i>Silurus glanis</i>	5	206	Fábián <i>et al.</i> , 1963
	10	87	
	15	49	
	20	28	
	25	20	
<i>Ictalurus punctatus</i>	10	24	Shrable <i>et al.</i> , 1969
	16	24	
	22	7-10	
	27	3-4	
<i>Gadus morhua</i>	2	72	Tyler, 1970
	5	58	
	10	25	
	15	20	
	19	20	
<i>Pleuronectus platessa</i>	1	36	Edwards, 1971
	5	25	
	9	16	
	14	12	
	20	10	
<i>Fundulus heteroclitus</i>	6	27	Nichols, 1931
	10	12	
	15	9	
	20	7	
	25	5	
	30	3	

<i>Perca fluviatilis</i>	5	115	Fábián et al.,1963
	10	63	
	15	49	
	20	27	
	25	21	
<i>Stizostedion lucioperca</i>	5	257	Fábián et al.1963
	10	157	
	15	83	
	20	45	
	25	28	
<i>Gobius minutus</i>	5	18-20	Healey, 1971
	10	16-18	
	15	14	
<i>Mullus barbatus</i>	15	25	Lipskaya, 1959
	20	14	
	25	8	
<i>Channa punctatus</i>	20	48	Gerald, 1973
	28	24	
	33	20	
<i>Micropterus salmoides</i>	5	110	Molnár and Tölg, 1962
	10	50	
	15	37	
	20	24	
	25	19	
<i>Lepomis Microchirus</i>	5	69	Kitchell, 1970
	10	37	
	15	27	
	20	15	
	25	13	
<i>Pleronectes platessa</i>	5	67.3	Jobling and Davies, 1979
	10	36.5	
	15.5	31.3	
	21	20.2	

2.4.2 Fish size

With increasing fish size, GER has been observed to decrease (Hunt, 1960; Smith *et al.*, 1989; Hayward and Bushman, 1994), increase (Swenson and Smith, 1973; Cochran and Alderman, 1982) or be unaffected (Brett and Higgs, 1970; Elliott, 1972; Jobling, 1980; Brodeur, 1984; Lambert, 1985; dos Santos and Jobling, 1991). Boisclair and Leggett (1991) and Bromley (1994) pointed out that these contradictory results are most likely due to differences in interpretation of data and method of estimation.

For example, relative GER values expressed as $\text{g food remaining g}^{-1} \text{ food initial h}^{-1}$, in an experiment involving both small and large bluefish at 21 °C were similar. However, the absolute GER values (g food h^{-1}) for small and large bluefish were very different - 0.030 and 0.167 respectively (Buckel and Conover, 1996). dos Santos and Jobling (1991) noted that when Atlantic cod, *Gadus morhua* are fed meals of the same relative size ($100 \cdot \text{g prey} \cdot \text{g}^{-1} \text{ predator}$), gastric evacuation time was independent of body size. Juanes and Conover (1994) also found no difference in GER between small, medium, and large bluefish when fed fish prey.

2.4.3 Type of food

The type of food ingested by fish has significant effects on gastric evacuation rates (Elliott, 1972 (Table 2.7); Fänge and Grove, 1979; Durbin and Durbin, 1980; Jobling, 1986; see Bromley, 1994 for review).

Table 2.7 Emptying time for different food types at fixed temperature by *Salmo trutta* and *S. gairdneri* (after Elliott, 1972)

Fish	Type of food	Emptying time h
<i>Salmo trutta</i>	<i>Oligochaetes</i>	22 (90%)
	<i>Protonemura sp.</i>	26
	<i>Hydropsyche sp.</i>	30
	<i>Tenebrio sp.</i>	49.5
<i>Salmo gairdnerii</i>	<i>Helodrilus sp.</i>	12
	<i>Gammarus sp.</i>	13
	<i>Arctopsyche sp.</i>	16

Workers who have detected decreased evacuation rates with less digestible food stuffs include Pandian (1967) (*Megalops* fed *Gambusia* or *Metapenaeus*), Western (1971) (*Cottus*, *Enophrys* fed on *Tubifex*, *Calliphora* or semifluid meals), and Kionka and Windell (1972) (*Salmo* fed various diets). The digestibility of the feed not only affects the emptying rate from the stomach, but may also determine the time after ingestion before weight decrease of the meal occurs (Jones, 1974). He found that *Merlangius* or *Melanogrammus* start to digest shell-less *Mytilus* almost immediately but that meals consisting of *Ophiopholis*, large crustacea or *Centronotus* require up to 10, 20 and 25 h, respectively, before weight loss begins.

2.4.3.1 Lipid level of feed

Fat concentrations in excess of 15% of dry weight probably have an inhibitory effect on gastric motility. Windell (1967) suggested that the presence of fat in the food may delay gastric emptying, possibly by stimulating the secretion from the intestinal wall of a

hormone similar to enterogastrone which in mammals inhibits gastric motility (Hunt and Knox, 1968). Diets with increased fat levels clearly decrease gastric evacuation rate in rainbow trout (Windell *et al.*, 1969). However, pelleted diets adjusted to show marked differences in lipid level of 6.5, 10.5 and 14.5% moved through the stomachs of rainbow trout at the same rate (Windell *et al.*, 1972).

2.4.3.2 Digestibility of food

Little attention has been given to the potential differential movement through the stomach of separate food fractions such as digestible organic matter and indigestible chitin, debris, pebbles, and plant material (Windell, 1978). Several workers observed a lingering of indigestible chitinous exoskeletons in the guts of fish (Mann, 1978; Gerking, 1952; Pandian, 1967). Significant amounts of chitin from aquatic invertebrates were observed in the stomach of bluegill sunfish, *Lepomis macrochirus* (Windell, 1978) and black bullhead, *Ictalurus melas* (Darnell and Meierotto, 1962) well after the digestible material had been evacuated. Total gastric evacuation time was affected by the presence of chitin in the food fed to brook trout, *Salvelinus fontinalis* (Hess and Rainwater, 1939) and megalop, *Megalops cyprinoides* (Pandian, 1967)

2.4.4 Energy content

Increases in the dietary energy content of food have been reported as reducing gastric emptying rates in fish (Windell, 1966; Elliott, 1972). Jobling (1988) found that minced herring diet with higher energy content enriched by the addition of fish meal and oil led to increases in the gastric emptying time of cod, *Gadus morhua*, which is in agreement with results of the experiments conducted with rainbow trout and marine flatfish (Windell *et al.*, 1969; Grove *et al.*, 1978; Flowerdew and Grove, 1979; Jobling, 1980).

For example in plaice, *Pleuronectes platessa*, an increase in dietary energy content from approximately 5 to 11 kJ ml⁻¹ resulted in doubling of gastric emptying time (Jobling, 1980), and, in rainbow trout, GET was reduced from 15 to 10 h when the energy content of food was reduced by 50% by dilution with kaolin (Grove *et al.*, 1978). Following a series of experiments with plaice, *Pleuronectes platessa*, Jobling (1981) reported that total energy content has more influence on gastric evacuation than either available (digestible) energy or specific nutrient content.

2.4.5 Meal size

Meal size and rate of gastric emptying have long received considerable attention from scientists (Hunt, 1960; Windell, 1966; Kitchell and Windell, 1968; Magnuson, 1969; Windel *et al.*, 1969; Brett and Higgs, 1970; Tyler, 1970; Beamish, 1971; Elliott, 1972; Swenson and Smith, 1973; Steigenberger and Larkin, 1974; Jobling *et al.*, 1977; Jobling, 1986). Although most studies show a positive correlation between meal size and evacuation rate (Windell, 1967; Kitchell and Windell, 1968; Bagge, 1977; Jobling and Davies, 1979; Brodeur, 1984; dos Santos and Jobling, 1991), a number of studies have found the relationship to be negative (Ruggerone, 1986) or that there is no relationship (Bromley, 1988).

Jobling (1981) summarized data on gastric emptying time for a variety of species and concluded that when expressed in the form of $GET = a(\text{meal size})^b$, the value of the exponent 'b' ranged from 0.35-0.83 (mean value 0.57 ± 0.15 SD), indicating that on average, the time taken to evacuate a meal increased with meal size. Elliott (1991) refers to evacuation rate as the slope of a regression line of the logarithm of stomach content plotted against time after feeding, *ie.*, an exponential model; and evacuation rate

varies only if the slope of the regression varies. Since the model is exponential, the food weight evacuated per unit time depends on stomach fullness and therefore, the greater the amount of food present in the stomach, the faster the absolute rate (unit weight per unit time) of evacuation. With increasing and decreasing meal size absolute rate may increase or decrease but the slope of the regression will remain constant. In conclusion, depending on the definition of rate, evacuation rate increases with meal size, and evacuation rate is constant with meal size; in other words, both arguments can be correct (Bromley, 1994).

According to Brett (1979), one of the most important factors which bears directly on the maximum food intake of fish is satiation feeding. Therefore, studies on formulating daily ration models have been carried out in relation to satiation feeding (Haylor, 1993b). In experiments with turbot, *Scophthalmus maximus*, Grove *et al.* (1985) and Bromley (1987) found close agreement between evacuation rate and satiation feeding of fish.

2.4.6 Particle size

Although closely related to the effect of meal size on digestion rate, few data are available on the effect of food particle size (Swenson and Smith 1973; Jobling 1986, 1987, 1988). Jobling (1987), however, suggested that food particle size was the most important factor governing gastric evacuation in fish. Tyler (1970) argued that the disintegration of a food particle probably begins at the outer surface and proposed models for estimating digestion rate based on particle surface area and particle weight (volume). It is most likely that both volume and surface effects influence the rate of stomach emptying and that digestion probably begins at the surface of a particle.

However, food volume probably influences peristalsis, which thereby facilitates mechanical and physical breakdown (Windell, 1978).

Large food particles have a lower surface-to-volume ratio than small particles and present a relatively smaller surface area open for reaction by gastric acid and enzymes (He and Wurtsbaugh, 1993) so the rates of digestion and fragmentation (consequently the GER) of large food items would be expected to be slower than those of same volume of food composed of a higher number of smaller particles (Jobling, 1987). This supports the findings of Swenson and Smith (1973), who reported that the evacuation rate of walleye, *Stizostedion viterum viterum* was higher when fed meals comprised of smaller prey (*Pimephales promelus*) comparing the meals of the same size comprised of larger prey.

Moreover, the observation that food particles must be broken down to a small size before they are passed from the stomach, through the pylorus and into the intestine has important consequences for predictions concerning the pattern of emptying to be expected when large food items are consumed (Jobling, 1986). When fish consume food items such as other fish, crustaceans and other animals and plants which are relatively large in comparison to their own body size, the time required to break down the majority of the food into fragments of suitable size for passage through the pylorus may be relatively long. Consequently, there may be a 'time lag' or initial emptying delay before there is any substantial diminution in the quantity of food remaining in the stomach (Jones, 1974; MacDonald *et al.*, 1982).

2.4.7 Force feeding and starvation

In conducting research with gastric evacuation, a number of workers resorted to placing food items directly into the stomach of fish (Hess and Rainwater, 1939; Hunt, 1960; Mölnár and Tölg, 1962; Windell, 1966, Shrable *et al.*, 1969; Edwards, 1971; Swenson and Smith, 1973; Steigenberger and Larkin, 1974). However, Windell (1966), Swenson and Smith (1973) and Persson (1986) provide convincing evidence that force feeding may cause physiological disturbance which in turn strongly affects certain physiological body processes. The latter authors reported an approximate two-fold difference in evacuation rate when comparing voluntary with force-feeding fish.

Fasting assumes considerable experimental and ecological significance for studies related to evacuation, digestibility, absorption, efficiency and growth. Windell (1966) found that fasting periods of 7, 14 and 25 days substantially decreased rate of gastric evacuation in bluegill sunfish, a 7-day starvation decreasing gastric evacuation by as much as 22% while a 25-day starvation period reduced gastric evacuation rate by 51% compared with normal evacuation rates. Rainbow trout, *Oncorhynchus mykiss* fasted for three and six days had significantly lower evacuation rates than fish which had fasted for 18 h when compared after 24 h of digestion (Sarokon, 1975). Among other workers, Tyler (1970), Brett (1971), and Jones (1974) reported that fish which have been deprived of food for a time prior to feeding show a slower gastric emptying rate than fish tested under continuous feeding condition.

2.4.8 Gastric evacuation model

The postulate that 'what goes up must come down' has been transmuted in fish feeding studies into 'what enters in must come out'. Using evacuation experiments to predict

feeding assumes that the amount of food expelled from the stomach mirrors the amount of food eaten (Bromley, 1994). The idea of intake = expulsion (Tyler, 1970; Talbot, 1985; Bromley, 1987) is based on the principle that, averaged over time, the amount of food evacuated from the stomach equals the amount consumed. The change in stomach content is a function of both feeding rate (+) and evacuation rate (-), and there have been attempts to exploit this approach.

In many studies the amount of food leaving the stomach has been found to be constant throughout the evacuation period; hence, the model is linear and stomach contents decreased linearly with time (Hunt, 1960; Swenson and Smith, 1973; Jones, 1974). Others described this relationship by a square root function which implies that the evacuation rate is dependent on the amount of food present in the stomach (Jobling and Davies, 1979; Jobling, 1981; Talbot *et al.*, 1984). However, the most common models used by authors are exponential where stomach contents were depleted at a constant rate and the relationship is expressed either in exponential or logarithmic equations (Brett and Higgs, 1970; Tyler, 1970; El-Shamy, 1976; Elliott and Persson, 1978; Grove and Crawford, 1980; Andersson, 1984; Persson, 1986; Jobling 1986, 1987; Macpherson *et al.*, 1989; Haylor, 1993b). A number of workers have also used square root models to express the GER (Windell, 1966; Swenson and Smith, 1973; Jobling, 1980, 1981) (Table 2.8). However, the accuracy of the exponential method has been tested under laboratory conditions and has been shown to give excellent results for a number of fish: brown trout, *Salmo trutta*, roach, *Rutilus rutilus* (Jobling, 1986) and a number of workers estimated daily ration for different fishes and shellfishes- largemouth bass, *Micropterus salmoides* (Cochran and Adelman, 1982), winter flounder,

Pseudopleuronectes americanus (Worobec, 1984), cephalopods (Jobling, 1985), coho salmon, *Oncorhynchus kisutch*

Table 2.8 Equations used to describe gastric evacuation (after Bromley, 1994)

*Equation	Model
$S_t = S_0 - Rt$	Linear
$S_t = S_0 \cdot e^{-Rt}$	Exponential
$S_t = S_0 - 2\sqrt{S_0 \cdot Rt} + (Rt)^2$	Square root

* R is the rate of gastric evacuation, S_0 , weight of meals eaten and S_t , weight of stomach contents t hours after ingestion of S_0 and t, time in hours after feeding.

(Ruggerone, 1989), turbot, *Scophthalmus maximus* (Corcobado-Onate *et al.*, 1991), perch, *Perca flavescens* (Hayward *et al.*, 1991), crab, *Cancer polyodon* (Wolff and Cerda, 1992), Cape hake, *Merluccius capensis* (Pillar and Barange, 1995) using this method.

2.5 CONCLUSION

Biologically the African catfish, *C. gariepinus* is undoubtedly an ideal aquaculture species (Hecht *et al.*, 1996). However, despite its many and loudly acclaimed virtues and the potential of this species for aquaculture, the production figures presented in Table 1.1 tell a different tale. Overall the production of *C. gariepinus* over the last decade has been disappointing. Initially farmers found themselves in a situation in which the product could not be promoted owing to the lack of fish, therefore they

increased the production. Given the cost of feed at the time, all the fish produced was sold at a highly acceptable margin, whereupon the farmers increased production further. At the same time feed producers increased the price of feed, which increased disproportionately with the gate price of fish. This trend, coupled with the generally protracted nature of a marketing campaign has resulted in farmers leaving catfish farming or changing to other species (Hecht *op. cit.*).

While the technologies for the farming of this species have now been developed with varying degree of success, there is still a great need for research on feeding strategies. Research on quantitative estimation of feed intake for *C. gariepinus*, the effect of different factors on their feeding and growth, presenting food according to their diel rhythm (*ie.*, when they are most motivated to feed) can greatly optimize its feed utilization and growth performance and thus decrease the amount of feed wastage and ultimately the cost of culture. Once the cost of culture decreases and there is a ready market for any species, farmers will begin to farm it on a large scale.

built in the Tropical Aquarium of the University of
temperature inside the building is maintained
regulated as 12:12 h light to dark regime (08:30-20:30).

Figure 2.11 depicted 32 white plastic tanks placed in
rows – the tank dimensions were 47 cm diameter, 25 cm
height, and 20 cm depth. The tanks were arranged in 4 rows

and 8 tanks in each row. The tanks were placed in a
room with a temperature of 25°C and a relative humidity of
70%. The tanks were placed in a room with a temperature of
25°C and a relative humidity of 70%.

Chapter 3

SYSTEM DESIGN

The system design is the process of defining the system
requirements and the system architecture. The system design
process is a multi-step process that involves the following
steps: 1. Requirements analysis, 2. System architecture design,
3. System design, 4. System implementation, 5. System testing,
6. System deployment, 7. System maintenance.

3.1 EXPERIMENTAL SYSTEM

A system was built in the Tropical Aquarium of the Institute of Aquaculture, Stirling, Scotland. Air temperature inside the building is maintained above 25 °C and photoperiod is regulated as 12:12 h light to dark regime (0830-2030, light period).

The system (Figure 3.1) comprised 32 white plastic tanks placed on two identical metal supporting tables – the tank dimensions were 40 cm diameter, 25 cm deep, self-cleaning with lids. The tanks drained into six 100 L pre-conditioned biofilter tanks (filled with packing materials to increase biofiltration, made of non-toxic propylene 3.5.2 (Dryden Aquaculture Ltd, Edinburgh, Scotland) with a total biofilter medium surface area 120 m² from which water flowed by gravity to a 100 L sump tank.

An electric pump (0.55 kW, Beresford, England) raised water to a 400 L header tank. More than 50% of the water from the header tanks overflowed through a solid filter (Open cellfoam matting) filled with broken shell before returning to the sump tank. Identical solid filters were placed at the inflow to the sump tanks. The filtration tank with broken shell acted as both mechanical filter removing solids and a source of CO₃²⁻ and HCO₃¹⁻ ions to buffer the water against pH fluctuations. A 3 kW electric heater controlled by a Deem 10/1193 thermister which linked to an on/off controller set at 30 °C.

Water was pumped from the sump to the header tank via a pipe (1½"). Two outflow pipes (1¼") from the header tank were plumbed into two different ring mains (1¼") which fed inlet pipes (½") to each rearing tank. The ring main equalised the water pressure to each inlet. A manual valve controlled flow to each ring main whereas flow

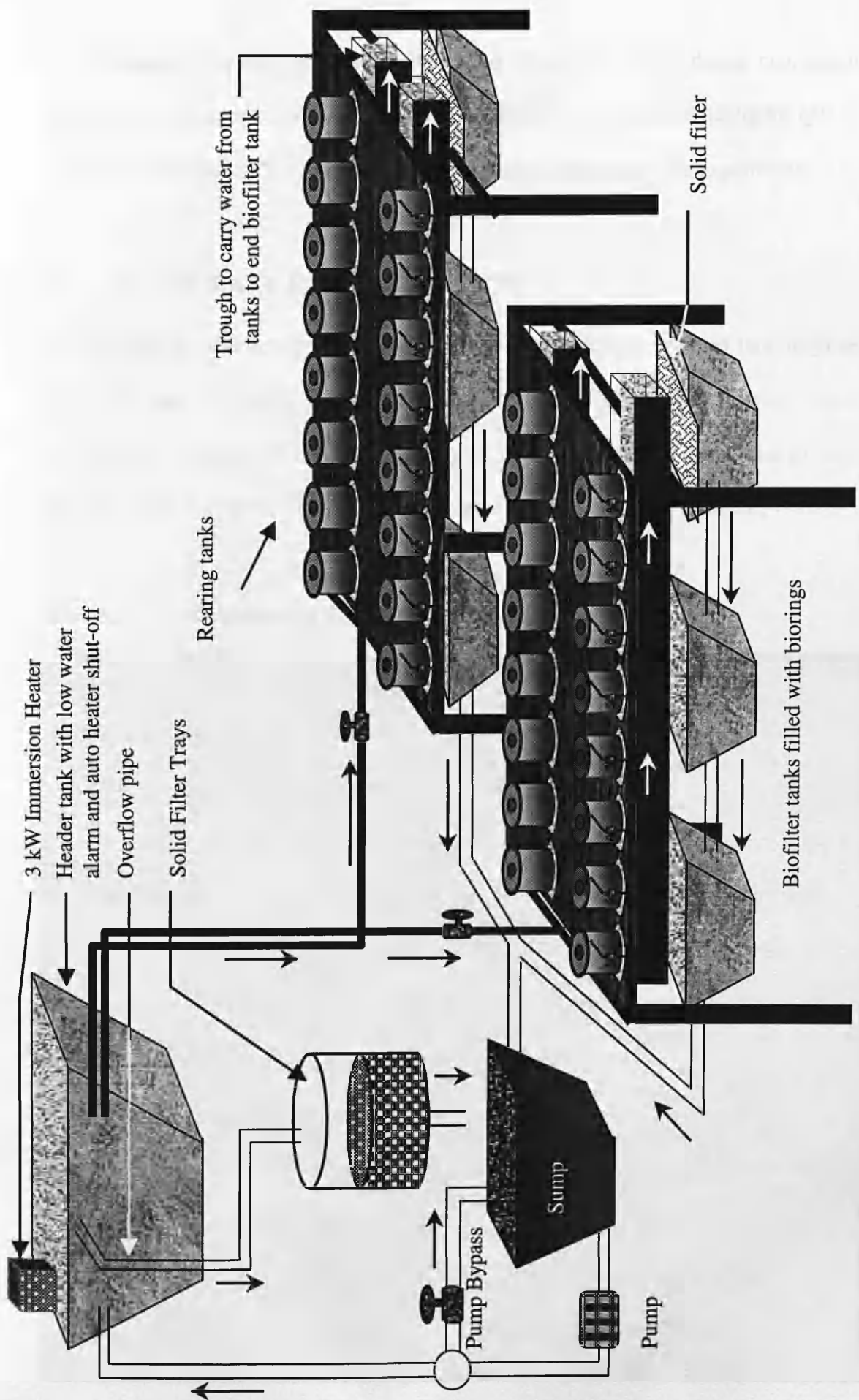


Figure 3.1 Three dimensional view of experimental system (See Plate 1 under Appendix 1)

in each rearing tank was controlled by individual valves. The system design maintained almost 100% O₂-saturation and nitrogenous metabolic levels remained negligible (pH = 7.8; NH₃ ≥ 0 ppm; NO₂ ≥ 0 ppm and NO₃ < 20 ppm) throughout the experiment.

3.2 FLOW RATE DETERMINATION

An appropriate flow rate for this type of fish is a compromise between tank hygiene (flushing) and fish energy expenditure (current velocity). Flow characteristics which facilitate the cleaning of solid wastes even at low flow rates are beneficial to tank hygiene, such as cylindrical tanks with a diameter to depth ratio of 10 (Haylor, 1992c).

Box 3.1 Calculation of flow rate based on oxygen requirements

Volume of each tank: 5 L Number of tanks: 32 Final fish weight: 10 g

Highest stocking density 10 fish L⁻¹

According to the following equation (Haylor, 1992c)

In a condition of 100% O₂ saturation

Relative O₂ consumption = $(649767 \times W^{-0.25}) / (1013 + 3.718T)$; W = Final fish weight
and T = temperature °C

$$= (649767 \times 10^{-0.25}) / \{1013 + (3.718 \times 30)\}, \text{ when } W = 10 \text{ g, } T = 30 \text{ }^\circ\text{C}$$

$$= 325 \text{ mg kg}^{-1} \text{ h}^{-1}$$

The lowest O₂ saturation level (at 30 °C) is 7.6 mg L⁻¹

Now, Water flow rate = $(\text{O}_2 \text{ consumption of fish mg kg}^{-1} \text{ h}^{-1}) / (\text{least O}_2 \text{ saturation level mg L}^{-1}) = 325 / 7.6 = 42.8 \text{ L kg}^{-1} \text{ h}^{-1}$

In the proposed stocking density 10 fish L⁻¹, final fish weight in a tank = 0.5 kg

Therefore, the flow rate for the proposed system = $0.36 \approx 0.4 \text{ L min}^{-1} \text{ tank}^{-1}$

The sedentary habit of catfish may contribute to the efficiency of its feed conversion. (Hogendoorn *et al.*, 1983). Therefore, an appropriate flow rate is adjusted to be the maximum flow rate that provides sufficient oxygen and at the same time allows the fish to maintain station without swimming (Haylor, 1992c).

Box 3.2 *Calculation of flow rate based on flows which do not elicit swimming*

According to Haylor (1992c), the maximum current velocity in which African catfish fry can maintain station without swimming -

$$C \text{ (cm s}^{-1}\text{)} = 0.1 \cdot \text{fish size mm} - 0.57 \dots\dots\dots(1)$$

In shallow tanks (diameter: depth ratio 10)

$$C_p = 1.33 \cdot F + 1.56 \dots\dots\dots(2)$$

$$\& C_c = 0.17 \cdot F + 0.69 \dots\dots\dots (3)$$

where C_p and C_c are peripheral and central current velocity in cm s^{-1} respectively and F is flow rate in L min^{-1} .

Now from equations 1 and 2, and 1 and 3 -

$$\text{Peripheral Current: Flow rate} = \{0.075 \times \text{fish length (mm)} - 1.6\} \text{ L min}^{-1} \dots\dots\dots(4)$$

$$\text{Central Current : Flow rate} = \{0.588 \times \text{fish length (mm)} - 7.41\} \text{ L min}^{-1} \dots\dots\dots(5)$$

Since the initial size of experimental fish is approximately < 40 mm, from equation 4 and 5, the maximum tolerable flow rate for this species 1.4 and 16.11 L min^{-1} on the basis of peripheral and central current respectively (the calculated flow rate on the basis of oxygen requirement is $0.4 \text{ L min}^{-1} \text{ tank}^{-1}$ only). Therefore, selected flow rate was $0.4 \text{ L min}^{-1} \text{ tank}^{-1}$.

3.3 WASTE REMOVAL

Box 3.3 *Estimating biofilter size based on ammonia production*

a) Feeding level = 10% bw

b) Daily ammonia production = {Fish kg x feed (% bw) × 0.03} g (Liao and Mayo, 1981)

c) Ammonia removal rate = 2 g ammonia (m² filter medium)⁻¹ d⁻¹

Proposed stocking density (highest) 10 fish L⁻¹, total fish weight in 32 tanks = 16 kg

Therefore, total daily ammonia production = (16 × 10 × 0.03) = 4.8 g

So the required biofilter = 4.8/2 = 2.4 m²

It must be stressed that this value is theoretical and as such does not include any safety margin. In addition these filters will also act as sedimentation tanks removing solid waste. To compensate for this it is normal to increase the theoretical value by 40-50 times. Therefore, a biofilter was selected of 96-120 m².

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Chapter 4

THE EFFECTS OF DENSITY, LIGHT AND SHELTER ON THE GROWTH AND SURVIVAL OF AFRICAN CATFISH, *C. GARIEPINUS* FINGERLINGS

Results concluded that under conditions of numerous...
...to the natural light regime...
...and growth enhanced. Small C. gariepinus...

4.1 INTRODUCTION

The feeding activities of fish are governed by a number of biotic and abiotic factors. The former includes the influence of body weight, maturity and sex, while among the latter, water quality, temperature, light regime, shelter, and stocking density are known to be important (Brett, 1979). These factors and their interactions determine scope for growth (Hogendoorn, 1983).

Growth and survival of African catfish (*Clarias gariepinus* Burchell, 1822) are known to be strongly influenced by stocking density (Hecht, 1982; Hecht and Appelbaum, 1988; Appelbaum and Van Damme, 1988; Haylor, 1991; 1992d), photoperiod and shelter (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992) in particular. Hecht and Appelbaum (1987) observed that lower stocking densities always gave the higher growth rate in an experiment with 25-day old *C. gariepinus* fingerlings (density range 5-20 fish L⁻¹). However, low stocking densities are also known to increase the rate of cannibalism, e.g. Haylor (1991) found that increasing stocking density from 50 fry L⁻¹ to 150 fry L⁻¹ did not increase the incidence of cannibalism significantly provided the fish were well-fed.

The species reportedly has nocturnal feeding habits (Bruton, 1979a; Hogendoorn, 1981; Viveen *et al.*, 1985). Britz and Pienaar (1992) working with 36 week-old *C. gariepinus* juveniles concluded that under conditions of continuous darkness or low light intensity, which approximated to the natural light regime, stress, aggression and cannibalism were reduced and growth enhanced. Small *C. gariepinus* are poor

swimmers and are ill-equipped to escape from a predator, hence the suggestion that shelter may also suppress cannibalism during culture (Britz and Pienaar, 1992).

In this experiment the effects of density, light and shelter on the growth and survival of *C. gariepinus* fingerlings were studied under controlled environmental conditions.

4.2 MATERIAL AND METHODS

4.2.1 Sources of fish

Male and female brood fish were reared in captivity to sexual maturity in the Tropical Aquarium, Institute of Aquaculture. Breeding was carried out using Ovaprim as an inducing agent, following procedures used for carp detailed by Nandeesh *et al.* (1990). Ovaprim (Glaxo India Limited) was injected into the female (1.5 kg) below one of the pectoral fins at a rate of 0.5 ml Kg⁻¹ (Total 0.75 ml) at 17.00 h. The female and a male of about same size were kept overnight in a separate 1-m diameter tank with secured lid supplied with recirculated water (30 ± 1 °C).

The following morning (09.00 h), the male was captured and killed. The testes were removed carefully and kept in a jar without any water. The female was then captured and ova were produced by gently stripping the animal and the eggs kept in a shallow uPVC plastic tray (without water). Milt obtained from the excised testes of the sacrificed male was mixed with the ova, by gentle swirling in the absence of water. A small amount of water at 30 °C was then added to the swirling

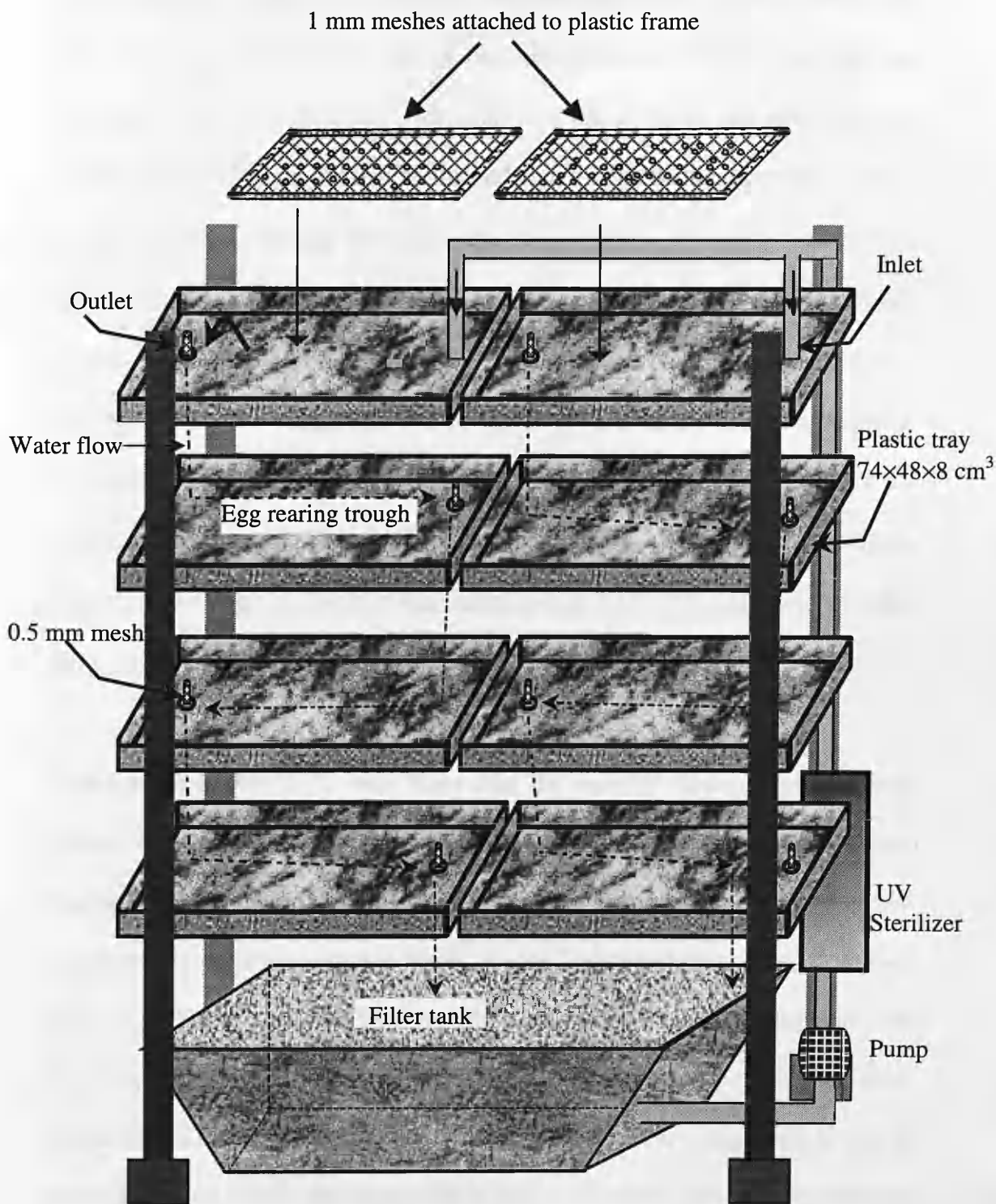


Figure 4.1 Incubation system used for hatching of *C. gariepinus* larvae (See Plate 2 under Appendix 1)

eggs to facilitate gentle movement and to activate amphimixis. After a few seconds more water was added to the side of the tray, resuspending the excess milt and washing it away. The fertilized eggs were then placed in an incubation/hatching system (Figure 4.1) in a single layer on horizontal 1 mm meshes attached to uPVC plastic pipe frames in egg rearing troughs ($740 \times 480 \times 80 \text{ mm}^3$). Continuously aerated water was recirculated over the eggs. An electric pump (Fluval 403 model, Animal House (UK) Ltd. Bristall, Batley, England) raised the water to the system. A 200 W thermostatic heater (Animal House (UK) Ltd. Bristall, Batley, England) controlled the temperature of the system. The water inflow was connected with a UV sterilizer (Model 30, 30 W and 240 V; Tropical marine Centre Ltd, Hertfordshire, England). The water temperature was maintained at $30 \pm 1 \text{ }^\circ\text{C}$. Light was excluded from the incubation system by covering the system with black polythene.

Larvae hatched after 24 h. Four hours after the onset of hatching the horizontal meshes were removed together with adhering egg shell and dead or unhatched eggs. Larvae were left undisturbed in their environment for a further 48 h when feed (unhatched, hydrated, decysted *Artemia*, Argent Chemical Laboratories, Redmond, USA) was offered. Thereafter feed was offered every two hours during day time. The following day, larvae were siphoned from the incubation troughs through 5 mm clear plastic tubing into a bucket and transferred to a 1m diameter rearing tank by gentle pouring from the bucket. The water temperature in the rearing tank was maintained at $30 \pm 1^\circ\text{C}$ and the photoperiod regulated, providing a 12 : 12 h light : dark regime (0830-2030, light period).

Table 4.1 *Composition of the supplemented diet, 2 mm trout pellets (BP Nutrition, Trouw UK Ltd) used. (This diet is made from cereal grains, fish products, oil seed products and by-products, land animal products oils and fats and minerals)*

Ingredient	Quantity	
	Manufacturer's analysis (%)	Independent Analysis (%)
Crude oil	7	7.66
Crude protein	40	42.64
Crude ash	10	8.86
Crude fiber	2.5	2.96
N-free extract (by subtracting)	-	28.86
Moisture	-	9.02
Vitamin A	10,000 iu kg ⁻¹	-
Vitamin D ₃	1000 iu kg ⁻¹	-
Vitamin E	100 iu kg ⁻¹	-
Total energy	-	22.7 kJ g ⁻¹

Table 4.2 *Feed application during weaning*

Day	Artemia %	Supplemented feed %
5	80	20
6	60	40
7	40	60
8	20	80
9	0	100

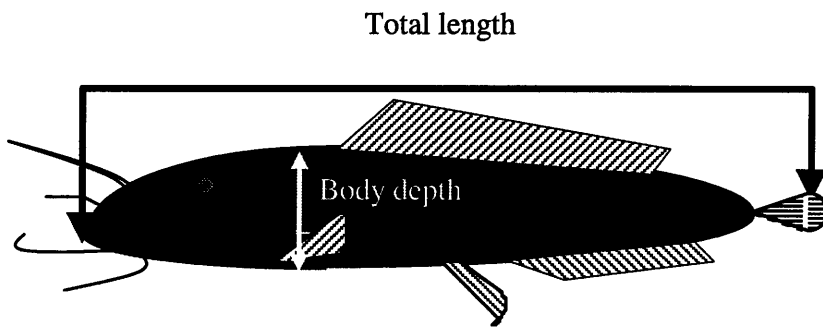


Figure 4.2a *Diagram showing the total length and body depth measurement of C. gariepinus*

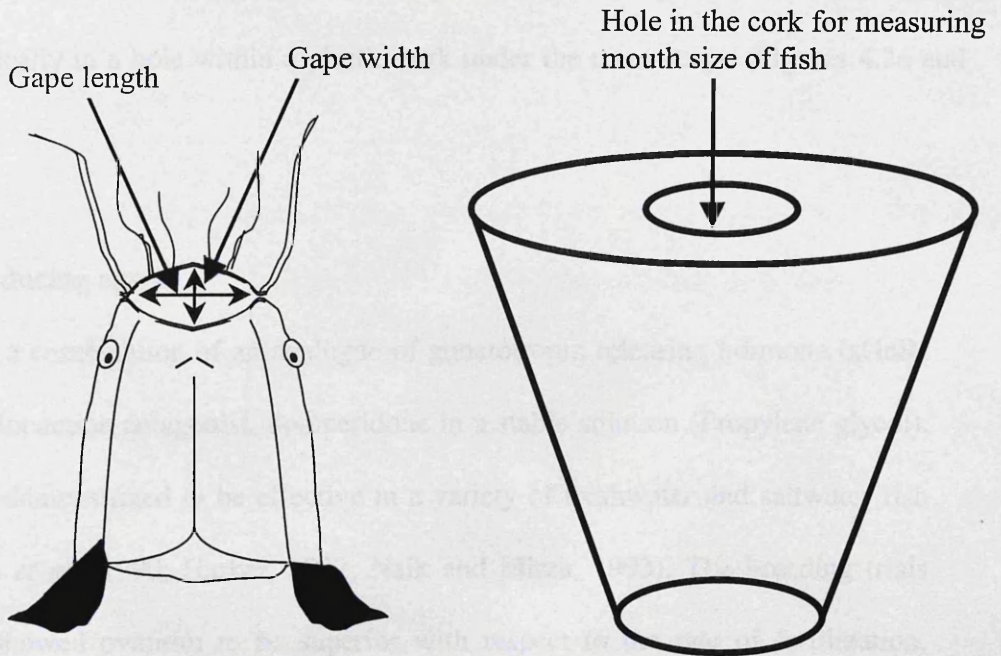
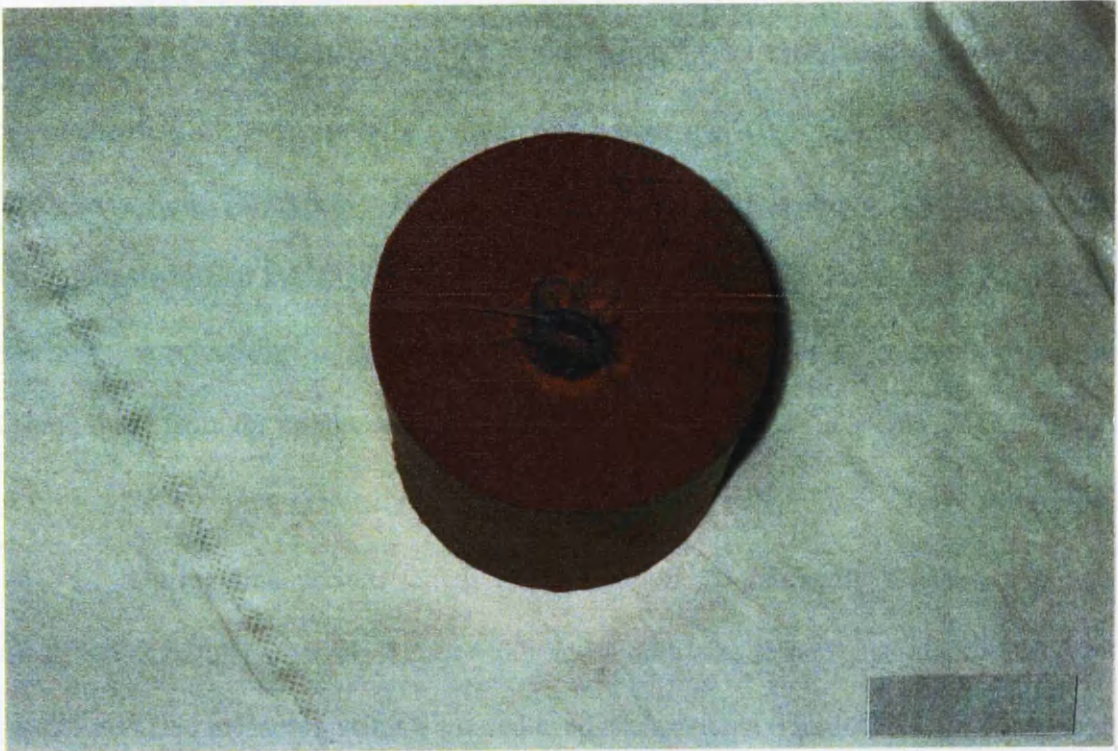


Figure 4.2b Photograph and diagram showing the gape of mouth measurement of *C. gariepinus*

Larvae were fed exclusively on *Artemia* (Argent Chemical Laboratories, Redmond, Wa, USA) for a period of 4 days from 48 h after hatching, and then weaned gradually by supplementing the *Artemia* with a commercial trout diet (B P Nutrition, Trouw (UK) Ltd, Shay Lane, Longridge, Preston) (Table 4.2). After weaning, larvae were fed continuously by belt feeder (Fiap Fish Technik, GMBH, D92277, Hohenburg, Papermill, Germany; supplied by Aquatic Service (International) Ltd., Hans, England) with feed crumb made from the commercial trout diet (at the beginning particle size range 250-500 μ for a week and then gradually 500 μ to 1500 μ for the remaining 9 days) for a further 16 days. During this period, length and weight of 20 randomly selected fish was measured at regular interval. Head width and mouth size (inner gape length and gape width) was also measured using a crossed eyepiece graticule (Graticules Ltd, Tonbridge, Kent, UK) attached to a binocular microscope. For measuring mouth size, fish were placed vertically in a hole within a plastic cork under the microscope (Figures 4.2a and 4.2b).

4.2.2 Inducing agent

Ovaprim is a combination of an analogue of gonatotropin releasing hormone (sGnR-Ha) and a dopamine antagonist, domperidone in a stable solution (Propylene glycol). It has been demonstrated to be effective in a variety of freshwater and saltwater fish (Nandeeshha *et al.*, 1990; Harker, 1992; Naik and Mirza, 1993). The breeding trials with carp showed ovaprim to be superior with respect to the rate of fertilization, hatching and the health of hatchlings as compared with pituitary extract, with no adverse effects noted on the brood fish or the offspring (Nandeeshha *et al.*, 1990).

4.2.3 Experimental procedure

Nine hundred 25-day old (mean weight 0.79 ± 0.01 g; mean total length 49.2 ± 0.91 mm) *C. gariiepinus* fingerlings were transferred at random (Figure 4.3) to twenty four cylindrical plastic tanks (40 cm diameter 25 cm deep, self-cleaning with lids) within a recirculation system. Water depth was maintained at 4 cm. A 12 h light:12 h dark regime (0830-2030, light period) was established and water temperature maintained at 30 ± 1 °C. Fish were stocked at a density of 10 fish L⁻¹ (50 fish per tank) in twelve tanks and 5 fish L⁻¹ (25 fish per tank) in the remaining twelve tanks. The assignment of tanks to treatments is detailed in Table 4.3. Tanks C, D, G and H were fully covered with black polythene to reduce light levels, while tanks E, F, G and H were provided with shelters made from inert plastic shade materials (Figure 4.4). The experiment was carried out over a 4-week period to investigate the effects of density, cover and shelter on growth.

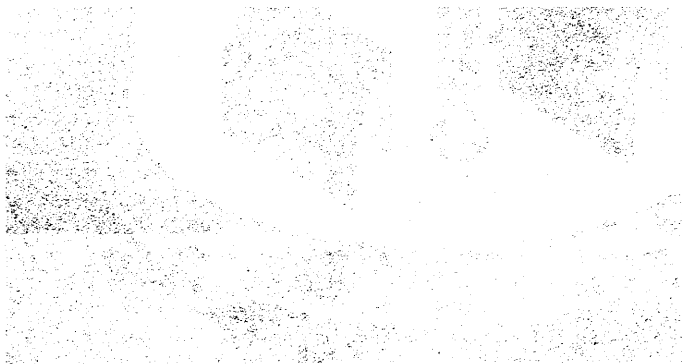
During the experimental period fingerlings were fed to satiation three times per day (0900, 1300 and 1700 h) on 2 mm trout pellets (BP Nutrition). During feeding, water flow was slowed down. Following first feeding in the morning, the debris was removed and the filter mats cleaned.

Fish were weighed every 7 days using a balance (Mettler PM6000; precision 0.01g, Leicester, Leich, UK). Water levels in the tanks were first lowered, then fish were caught by scoop net and placed on absorbent paper for 3-4 seconds in order to remove excess water. During weighing, tanks were emptied, and the tanks, shelter and outlet screen cleaned. After weighing fish were gently returned to the appropriate

Table 4.3

Assignment of tanks to individual treatments and combination of the treatments

Tanks	Treatment
A ₁ , A ₂ , A ₃	Density 5 fish L ⁻¹ ; Control
B ₁ , B ₂ , B ₃	Density 10 fish L ⁻¹ ; Control
C ₁ , C ₂ , C ₃	5 fish L ⁻¹ + Cover
D ₁ , D ₂ , D ₃	10 fish L ⁻¹ + Cover
E ₁ , E ₂ , E ₃	5 fish L ⁻¹ + Shelter
F ₁ , F ₂ , F ₃	10 fish L ⁻¹ + Shelter
G ₁ , G ₂ , G ₃	5 fish L ⁻¹ + Cover + Shelter
H ₁ , H ₂ , H ₃	10 fish L ⁻¹ + Cover + Shelter



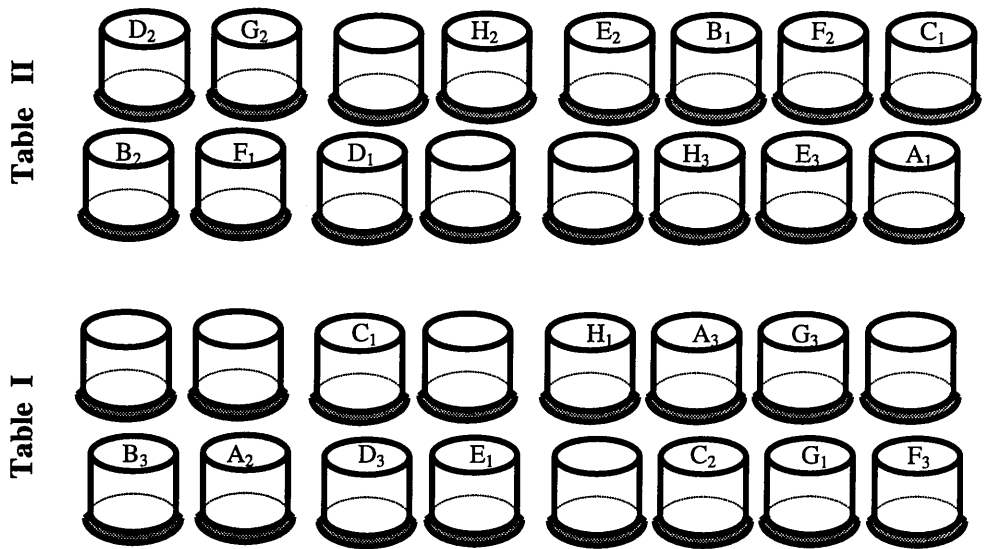


Figure 4.3 Random placing of rearing tanks in the system

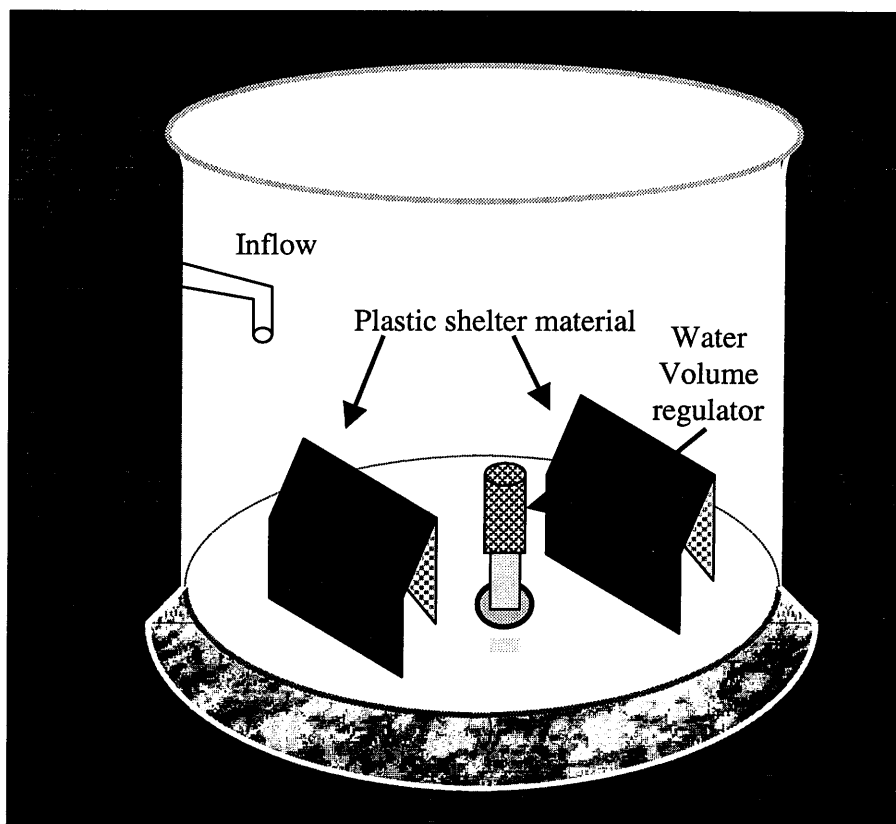


Figure 4.4 Shelter in rearing tank

tanks. It was observed, however, that fish did not resume feeding on the day of sampling. Dead fish were removed daily after feeding and the deaths noted. Each week, during weighing the number of fish in each tank was recorded.

4.2.4 Data analyses

Instantaneous growth rate (G_w) was determined as:

$$G_w = (\text{Ln } W_t - \text{Ln } W_0)/t$$

where Ln = natural logarithm; W_0 = Initial weight (g), W_t = Final weight (g).

Ninety-five percent confidence limits (CL) were calculated as:

$$\text{CL} = \bar{X} \pm t_{0.05 (n-1)} (S/\sqrt{n}), \text{ where } \bar{X} = \text{Mean weight, } t_{0.05 (n-1)} = \text{value from a}$$

Student's t-table where 0.05 is the proportion expressing confidence, $n-1$ is the degree of freedom and S = Standard deviation. The effects of density, cover and shelter on average weight and specific growth rate (G_w) were investigated using Duncan's Multiple Range Test (Zar, 1984). The mean number of mortalities on each day, expressed in terms of % surviving fish at the beginning of that day, was calculated as:

$$\bar{M} \% = \frac{\sum^a \frac{M_{t+1}}{N_t}}{a} \cdot 100$$

where $\bar{M} \%$ = mean % per capita mortality, a = number of replicates N_t = number of live fish on day t and M_{t+1} = number of dead fish.

In order to compare the total mortality for the period (day 25 - day 53), a single value representing mean % per capita mortality per day was calculated as

$$M \% \text{ day}^{-1} = \frac{\sum_{t=25}^{t=53} \frac{M_{t+1}}{N_t}}{\frac{29}{a}} \quad (\text{Haylor, 1991})$$

The effects of stocking density, light and shelter on mortality rate were explored by one way ANOVA with equal sample size.

4.3 RESULTS

From the day of hatching to 25th day after hatching mouth size of *C. gariepinus* increases some 5 times in inner gape length (from 1.02 ± 0.01 (CL) mm to 5.01 ± 0.34 mm) and 9 times in gape width (from 0.46 ± 0.04 mm to 4.18 ± 0.21 mm), while total length increases about 5.5 times (from 9.04 ± 0.14 mm to 49.22 ± 0.91 mm) (Appendix 1). Viveen *et al.* (1985) noted that in the field, *C. gariepinus* can encompass prey size almost $\frac{1}{4}$ of its own body size. However, it was observed that fish of total length between 30 - 50 mm did not ingest feed pellets greater than 2 mm in diameter in experimental conditions.

In all treatments fish increased rapidly in weight over the experimental period with significant ($P < 0.05$) increases in weight for each successive 7-day period measured between Day 25 and Day 53 (Figure 4.5). Prior to day 46, there was no significant difference in mean body weight between the treatments except for the fish in treatment G (5 fish L^{-1} , cover and shelter). During days 43-53, the mean weights of fish in treatments B (10 fish L^{-1}) and D (10 fish L^{-1} and cover) were lower than in the rest of the treatments. Greatest individual weight gains, over the

experimental period corresponded to Treatment G, where low stocking density, low light and shelter were provided. In this treatment fish gained significantly more weight over each 7-day period than in the other treatments ($P < 0.05$). Comparisons are presented between pairs of treatments, when either density or covering or shelter are varied (Table 4.4).

The weekly mean weights in Treatment G (low density, shelter, reduced light) were significantly higher than those in Treatment E (low density, shelter, ambient light) throughout the experimental period. By contrast, growth in the high density-treatments (Treatments B, D) and in treatments with high density and shelter (Treatments F, H) were unaffected by light levels. (Figure 4.5 and Table 4.4)

The outputs of the exponential growth model, applied to data for each treatment, are shown in Table 4.6. Instantaneous growth rate, G_w , was highest ($P < 0.05$) in Treatment G (5 fish L^{-1} , cover, shelter) and Treatment E (5 fish L^{-1} , shelter) followed by Treatment C (5 fish L^{-1} , cover). Lowest growth rates were observed in Treatments B (10 fish L^{-1} , control) and D (10 fish L^{-1} , cover) (Table 4.5).

Survival and mortality data are summarised in Table 4.6. Mean survival was in excess of 79 % in all treatments. Mean % mortality in treatment C (5 fish L^{-1} , covered tanks, no shelter) was significantly higher ($P < 0.05$) than in the other treatments.

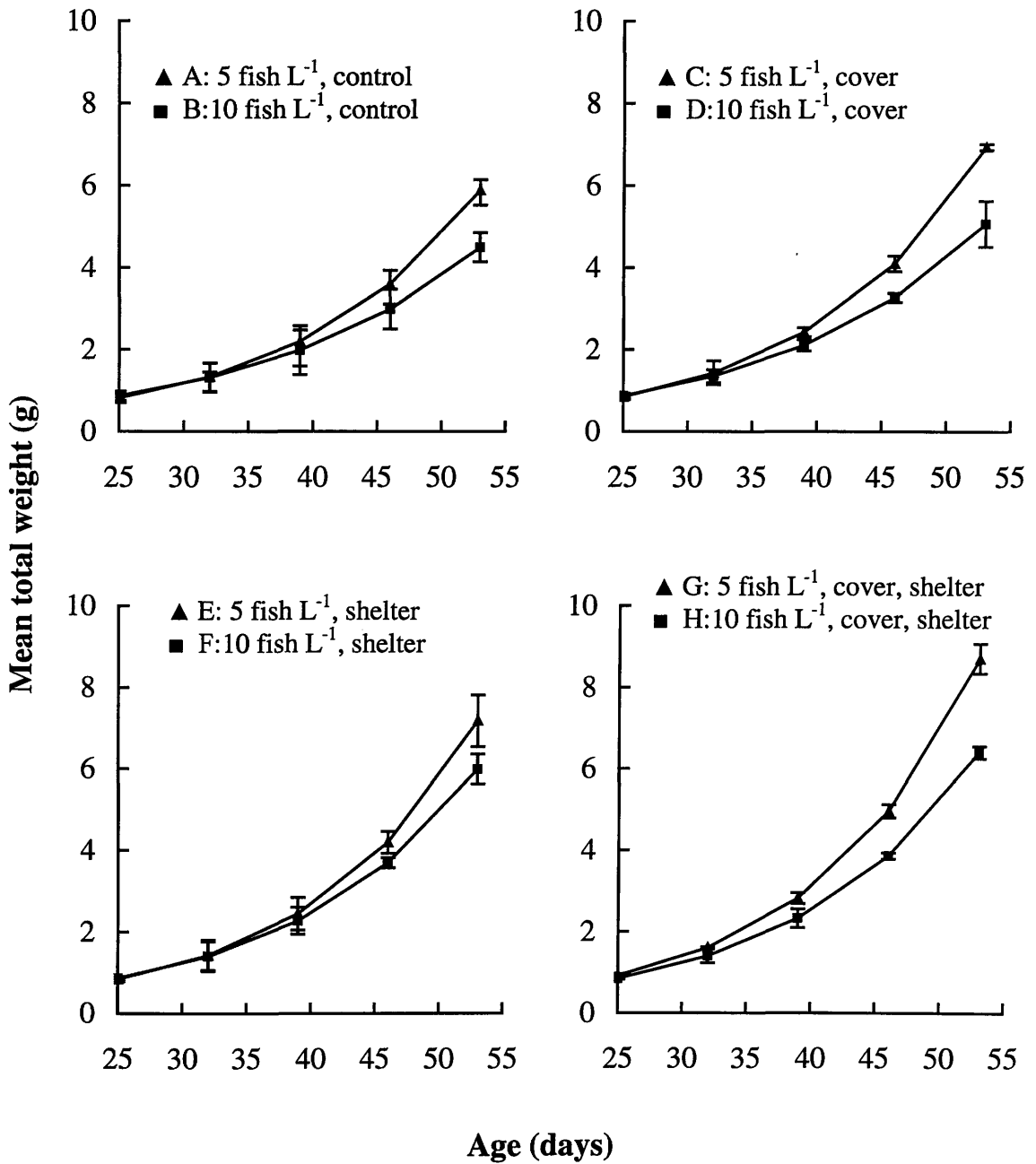


Figure 4.5 The weekly mean total weight (g) of *C. gariepinus* fingerlings in different treatments over the experimental period. Error bars are 95 % CL.

Table 4.4 Comparison between mean individual weights in each of two treatments where one criterion is variable. Only significant differences ($P < 0.05$) are indicated

Treatments/Weeks		Day 25 1 st	Day 32 2 nd	Day 39 3 rd	Day 46 4 th	Day 53 5 th
Density: 5 fish L ⁻¹ Density: 10 fish L ⁻¹						
A: Control	B: Control	-	-	-	-	A > B
C: Covered	D: Covered	-	-	C > D	C > D	C > D
E: Shelter	F: Shelter	-	-	-	E > F	E > F
G: Cover + Shelter	H: Cover + Shelter	-	G > H	G > H	G > H	G > H
A: 5 fish L ⁻¹	C: 5 fish L ⁻¹	-	-	C > A	C > A	C > A
No Cover Cover						
B: 10 fish L ⁻¹	D: 10 fish L ⁻¹	-	-	-	-	-
E: 5 fish L ⁻¹ + Shelter	G: 5 fish L ⁻¹ + Shelter	-	G > E	G > E	G > E	G > E
F: 10 fish L ⁻¹ + Shelter	H: 10 fish L ⁻¹ + Shelter	-	-	-	-	-
No shelter Shelter						
A: 5 fish L ⁻¹	E: 5 fish L ⁻¹	-	-	-	E > A	E > A
B: 10 fish L ⁻¹	F: 10 fish L ⁻¹	-	-	-	F > B	F > B
C: 5 fish L ⁻¹ + Cover	G: 5 fish L ⁻¹ + Cover	-	G > C	G > C	G > C	G > C
D: 10 fish L ⁻¹ + Cover	H: 10 fish L ⁻¹ + Cover	-	-	-	H > D	H > D
No shelter and cover Shelter and cover						
A: 5 fish L ⁻¹	G: 5 fish L ⁻¹	-	G > A	G > A	G > A	G > A
B: 10 fish L ⁻¹	H: 10 fish L ⁻¹	-	H > B	H > B	H > B	H > B

Table 4.5 *Exponential growth model in different treatments over a 4-week experimental period (Confidence limits are shown in parentheses). Instantaneous growth rates (G_w) with the same superscript are not significantly ($P < 0.05$) different.*

Treatment	Type	W_0 (CL) g	G_w (CL)	r^2	P
A	Density 5 fish L ⁻¹ Control	0.82 (0.05)	0.070 (0.003) ^b	0.99	<0.01
B	Density 10 fish L ⁻¹ Control	0.86 (0.09)	0.059 (0.004) ^a	0.95	<0.01
C	5 fish L ⁻¹ + Cover	0.84 (0.09)	0.075 (0.001) ^{cd}	0.98	<0.01
D	10 fish L ⁻¹ + Cover	0.87 (0.07)	0.063 (0.001) ^a	0.98	<0.01
E	5 fish L ⁻¹ + Shelter	0.83 (0.09)	0.079 (0.002) ^{ef}	0.98	<0.01
F	10 fish L ⁻¹ + Shelter	0.86 (0.05)	0.069 (0.001) ^b	0.97	<0.01
G	5 fish L ⁻¹ + Cover + Shelter	0.91 (0.03)	0.081 (0.001) ^f	0.98	<0.01
H	10 fish L ⁻¹ + Cover + Shelter	0.84 (0.05)	0.072 (0.002) ^{bc}	0.98	<0.01

Table 4.6 *A summary of the mean survival and mortality in the different treatments over the experimental period. Data with the same superscript are not significantly ($P < 0.05$) different.*

Treatment	Type	% survival	% mortality	CL
A	Density 5 fish L ⁻¹ Control	84.18 ^b	15.82 ^a	2.56
B	Density 10 fish L ⁻¹ Control	81.86 ^b	18.14 ^a	1.32
C	5 fish L ⁻¹ + Cover	79.61 ^a	20.39 ^b	0.66
D	10 fish L ⁻¹ + Cover	85.01 ^b	14.99 ^a	3.19
E	5 fish L ⁻¹ + Shelter	85.17 ^b	14.83 ^a	3.06
F	10 fish L ⁻¹ + Shelter	85.99 ^b	14.01 ^a	4.12
G	5 fish L ⁻¹ + Cover + Shelter	88.85 ^b	11.15 ^a	6.15
H	10 fish L ⁻¹ + Cover + Shelter	89.62 ^b	10.38 ^a	7.01

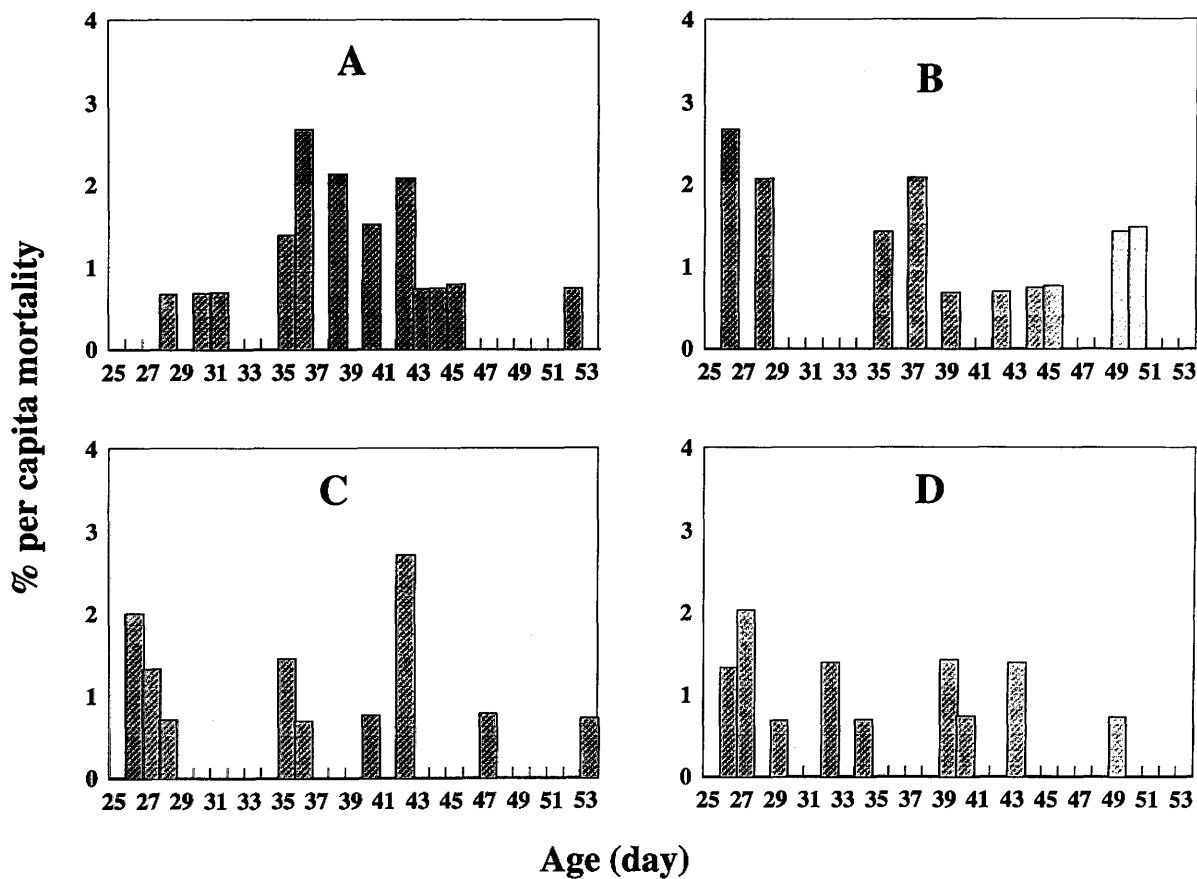


Figure 4.6a Mean % per capita mortality in relation to time in different treatments. A: 5 fish L^{-1} , control, B: 10 fish L^{-1} , control, C: 5 fish L^{-1} , cover and D: 10 fish L^{-1} , cover

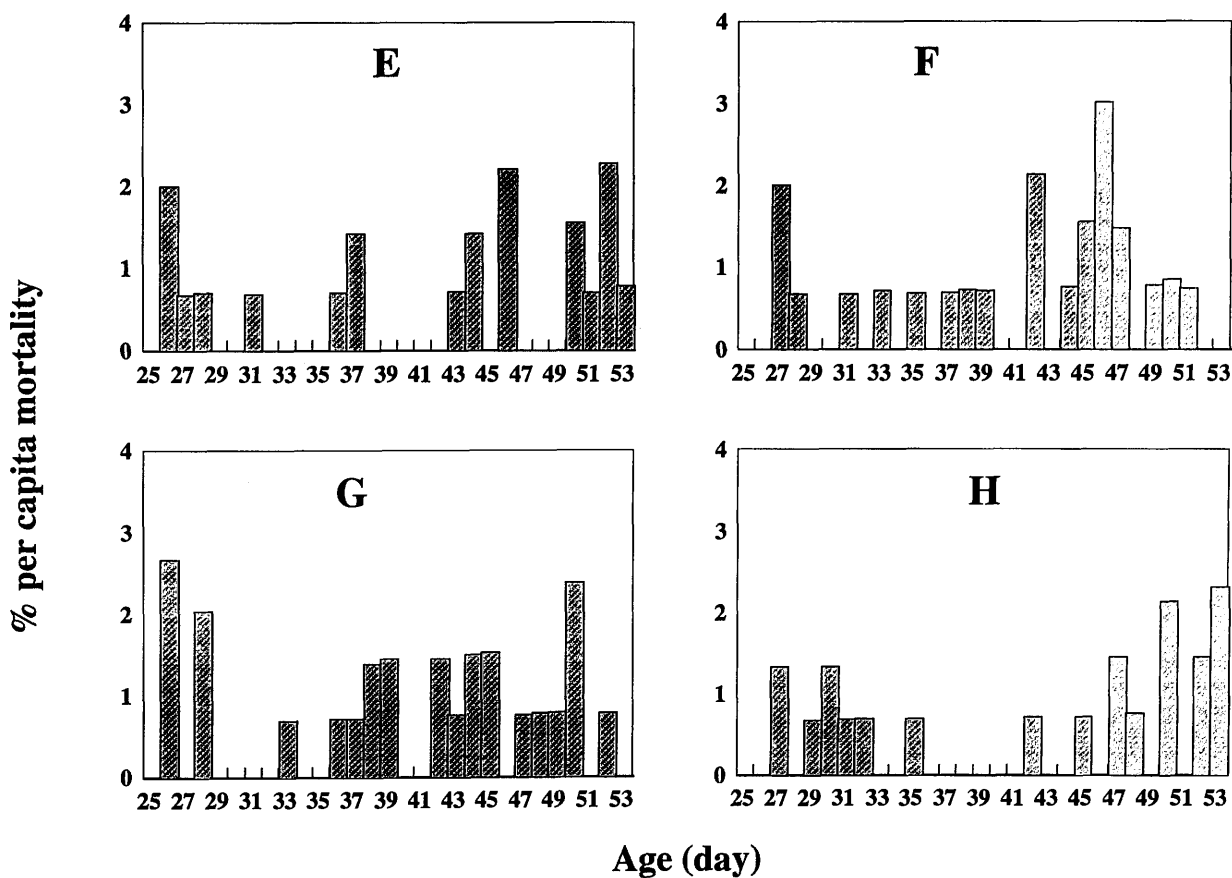


Figure 4.6b Mean % per capita mortality in relation to time in different treatments. E: 5 fish L^{-1} , shelter, F: 10 fish L^{-1} , shelter, G: 5 fish L^{-1} , cover, shelter and H: 10 fish L^{-1} , cover, shelter

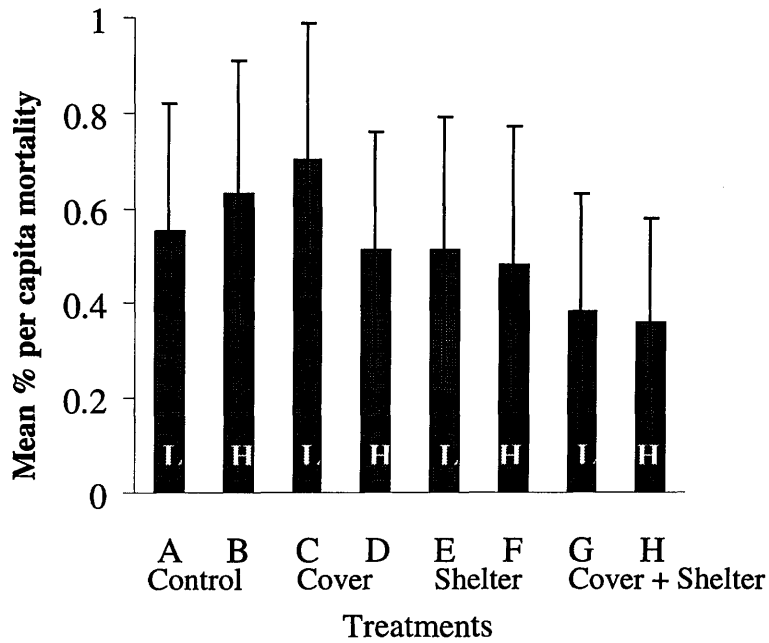


Figure 4.7 The mean % per capita mortality per day in different treatments (error bar represents 95 % CL). L: Low density (5 fish L⁻¹) and H: High density (10 fish L⁻¹)

Weekly sampling did not affect the mortality and there were no important fluctuations in physicochemical conditions. Figure 4.6a and 4.6b display the mean % per capita mortality values in different treatments while the mean daily % per capita mortality values in different treatments are summarised in Figure 4.7.

4.4 DISCUSSION

The growth of *C. gariepinus* fingerlings in this experiment was clearly density-dependent in common with findings in other studies (Hecht and Appelbaum, 1987; Haylor, 1991; Kaiser *et al.*, 1995). In all the treatments, the lower densities showed significantly higher ($P < 0.05$) specific growth rates (G_w). However, in some treatments, weekly mean individual weight did not appear to be density dependent during the earlier weeks. Mean individual weight in Treatment A (low density control) was only significantly higher than that in Treatment B (high density control) during the final week.

C. gariepinus is known to be a nocturnal feeder and believed to prefer low light conditions (Bruton, 1979a; Hogendoorn, 1981; Viveen *et al.*, 1985) and indeed Britz and Pienaar (1992) recorded the highest growth rate for groups of fish reared under a 24 h dark:0 h light regime (continual darkness). However, in the present experiment the effects of light on growth were only significant at low densities.

Except for the fastest growing fish at low density and reduced light the effect of shelter on growth only became apparent during the last two weeks. It is clear however that the provision of shelter and reduced light improved growth rates in both the low and high density treatments used. In a 50-day experiment with *C. gariepinus*,

Hecht and Appelbaum (1988) showed presence of shelter increased the amount of time spent resting and this may have influenced the growth of *C. gariepinus* in the present experiment.

The high survival rates of fingerlings in all the treatments in this experiment suggest a ready adaptation to intensive culture practices without any marked physiological or disease problem related to handling or other associated activities. In addition very few incidents of cannibalism were observed and these were not affected by the treatments. The principle cause of death in this experiment was aggressive encounters which are known to be common in *C. gariepinus* (Haylor, 1991). Aggressive behaviour has been found to increase with decreasing stocking density and the cause of mortality can be significantly reduced by the provision of shelter (Hecht and Appelbaum, 1988) and by increased stocking density (Haylor, 1991). In this experiment the total mortality (%) in only one treatment (C, low density, reduced light and no shelter) was significantly higher than in any other treatment. Fish under those conditions were more aggressive due to low density and the absence of shelter and were more active in reduced light level.

In conclusion, low density, low light intensity and shelter enhance growth rates, although not the rates of survival of *C. gariepinus* fingerlings. The provision of shelters and low light in hatcheries will be likely to benefit fry/fingerlings rearing facilities for *C. gariepinus*. The stocking density selected by operators, however, must take account of the conflicting effects upon aggressive behaviour (reduced by increased density) and growth rate (which is reduced by increased density). Guides to the change between fish weight gain and production per unit volume in relation to

stocking density are available for *C. gariepinus* fry and fingerlings (Haylor, 1991, 1992d; Haylor and Muir, 1998). For selecting a stocking density, a target weight for the end of the rearing period (based on economic and/or operational criteria) can be selected and the expected production per unit volume derived.

Chapter 5

The information contained in Chapter 5 is accepted (7th April 1998) and now in press in Aquaculture International – Hossain, Haylor and Beveridge 1998, 6 (1-7). Edited by Poxton M. G. and published by Chapman & Hall Ltd.

Chapter 5

AN EVALUATION OF RADIOGRAPHY IN STUDIES OF GASTRIC EVACUATION IN AFRICAN CATFISH FINGERLINGS

5.1 INTRODUCTION

Feeding strategies in fish farming should be aimed at optimising growth and food conversion, and at minimising waste. Development of successful strategies may be aided by knowledge about food consumption patterns. Meal size and time between meals are important factors affecting daily food intake (Brett, 1979; Talbot and Higgins, 1983), so accurate measures of gastric evacuation may assist in estimating consumption, and have value for the development of feeding strategies.

Much information on food consumption by individual fish and the movement of food through the stomach has come from analyses of stomach contents after gastrectomy (Brett and Higgs, 1970; Elliott, 1972; Thorpe, 1977) or from stomach pump experiments (Seaburg, 1957; Seaburg and Moyle, 1964; Strange and Kennedy, 1981). In the former method, sometimes it is very difficult to differentiate half-digested feed from blood, slime and other materials. Moreover, the method requires the sacrifice of fish and therefore, does not allow the study of intra-individual variability. The latter method is restricted to fish of relatively large size and also requires numerous feeding and considerable effort.

Worthwhile though gastric analysis methods are, the innate drawbacks have led to the development of other techniques. Mölnár and Tölg (1960) first described a method for determining gastric evacuation times in piscivorous fish by radiographic visualization of the disappearance of bony and other hard part of prey items from fish stomachs. Similar radiographic methods have been used by a number of workers to study gastric evacuation by following the passage through the gut of feed filled with radiopaque compound, barium sulphate (BaSO_4) as a contrast medium (Edwards,

1971, 1973; Goddard, 1970, 1974; Jobling *et al.*, 1977; Ross and Jauncey, 1981).

The X-ray method removes the need to sacrifice fish which can be used repeatedly.

Barium sulphate, however, is only adequately radiopaque at relative high concentration (25 % - Jobling *et al.*, 1977; fish meal:BaSO₄:water :: 1:1:4 - Ross and Jauncey, 1981) and may alter food composition. Therefore, it often requires force feeding which is only possible for large fish with a risk of injury and trauma. Furthermore, force feeding has an obvious effect on gastric evacuation. Swenson and Smith (1973) found that the evacuation rate of force fed walleye, *Stizostedion vitreum vitreum*, was approximately 50 % that for voluntary feeding fish.

Techniques which include marking food items with suitable radio-isotopes (such as ¹³¹I, ⁵¹Cr, ¹³⁷Cs) have been described (Kevern, 1966; Cowey and Sargent, 1972; Peters and Hoss, 1974; Storebakken *et al.*, 1981). Although these methods have been used to measure feed intake and gut evacuation of fish, their applications are restricted due to problems associated with safe formulation and disposal of radioactive feed. Isotopes used in fish feeding studies should be of low radiological hazard and should not lead to long term radioactive contamination. Furthermore, all the isotopes assimilated naturally, therefore the presence of isotope in the feed, during feed preparation and at the time of feeding may vary. However, in common with radiographic methods, using isotopes allows various measurements to be made without sacrificing fish and besides fish can be fed more naturally because the food composition remains unaltered.

Talbot and Higgins (1983) described a radiographic method for feeding studies on fish using radiopaque metallic iron powder as a feed marker. The method is applicable to both small and large fish, food preparation is easy, and there is little associated risk to fish welfare. Although the method has been validated in feeding studies of Atlantic salmon, *Salmo salar* (Talbot and Higgins, 1983; Thorpe *et al.*, 1990) and rainbow trout, *Oncorhynchus mykiss* (McCarthy *et al.*, 1993), a difference in the evacuation of marker and nutritional content of feed has been observed in Arctic charr, *Salvelinus alpinus* (Jørgensen and Jobling, 1988) and in Atlantic cod, *Gadus morhua* (dos Santos and Jobling, 1991). The method would thus appear to be species-specific to some extent and can only be used for evacuation studies when the marker moves through the gut at the same rate as digesta.

The present study sets out to evaluate radio-opaque Ballotini as a marker to estimate gastric evacuation in African catfish. Specifically, two important questions are posed: (i) are there significant differences between estimates of feed ingested from X-radiographs of stomach contents of fish given feed with Ballotini glass beads and from gastrectomy and (ii) are there any effects of markers on feed preference and gastric evacuation.

5.2 MATERIALS AND METHODS

5.2.1 Fish

Fish: *C. gariepinus* fingerlings of mean weight 0.95 ± 0.1 (SE) g, were obtained from broodstock maintained in the Institute of Aquaculture, University of Stirling following the procedure detailed in Chapter 4.2.1.

5.2.2 Selecting the size of Ballotini

Three samples each of 1 mg from four sizes of Ballotini glass beads (136-001, 0.23-0.32 mm; 136-002, 0.16-0.25 mm; 136-003, 0.11-0.19 mm; 136-004, 0.09-0.135 mm) were x-rayed in order to count the numbers present (Table 5.1)

Approximately 1 g (total length 4-5 cm) initial size of African catfish fingerlings were used for the main experiment. The mouths of fish at this particular period were measured as internal gape length 4.5 - 5 mm and gape width 4 - 4.15 mm. Although one should expect that they can intake feed pellet according to their mouth size (4 - 5 mm), from some preliminary trials it was observed that at this stage they do not ingest feed pellets greater than 2 mm in diameter.

Table 5.1 Number of different size of Ballotinis present in 1 mg

Ballotini size	No. of Ballotini per mg			
	0.23-0.32 mm	0.16-0.25 mm	0.11-0.19 mm	0.09-0.135 mm
I	26	49	202	380
II	24	52	189	369
III	24	50	221	377
Mean \pm SD	24.7 \pm 0.7	50.3 \pm 0.9	204 \pm 9.3	375.3 \pm 3.3

Considering the size of the feed pellet and the diameter of Ballotini and also the number present in 1 mg Ballotini; 0.23 - 0.32 mm size Ballotini appeared too big and 0.11 - 0.19 mm and 0.09 - 0.135 mm too small. In addition the latter two contain too many Ballotini in 1 mg. Therefore, 0.16-0.25 mm size Ballotini were selected.

5.2.3 Feed preparation

A commercial pelleted trout diet (same diet used in larval rearing) was ground to a fine powder in a hammer mill and Ballotini glass beads (136-002, 0.16-0.25 mm; Jencons Scientific, Leighton, Buzzard, Beds, UK) added at a concentration of 1% w/w. A little water was added to the mixture. After several hours mixing in a food mixer (Hobart A200) the feed was re-pelleted to a size of 2 mm (California Pellet Mill, Lab. Model CL2, West March, Daventry, Northants, UK), the pellets freeze-dried, and then stored in sealed containers at 5 °C until use. A control diet without Ballotini was prepared in the same way. Samples of marked feed (n = 28) of known weight (0.05 – 1.0 g) were X-rayed (Figure 5.1) to establish the relationship between pellet weight and the number of Ballotini present; $Y = 0.00419 + 0.00209X$; $r^2 = 0.99$; where Y = Weight of feed and X = number of Ballotini (Figure 5.2).

5.2.4 Experimental procedure

Seven hundred and fifty 26 day old *C. gariiepinus* fingerlings were transferred to thirty 40 cm diameter rearing tanks within a recirculation system. Stocking density was 25 fish per tank. Tanks were covered by thin black polythene to reduce light levels. Water depth was maintained at 4 cm and shelter was provided. A 12 h light : 12 h dark regime (0830-2030 h, light period) was established and water temperature maintained at 30 ± 1 °C. Between day 26 and day 40, the fish were fed to apparent satiation, three times per day (0900, 1300, 1700 h). Each meal lasted approximately 10-12 min. At specific times during days 41-43, fish in 10 tanks were fed marked feed (Treatment A), 10 tanks were provided with

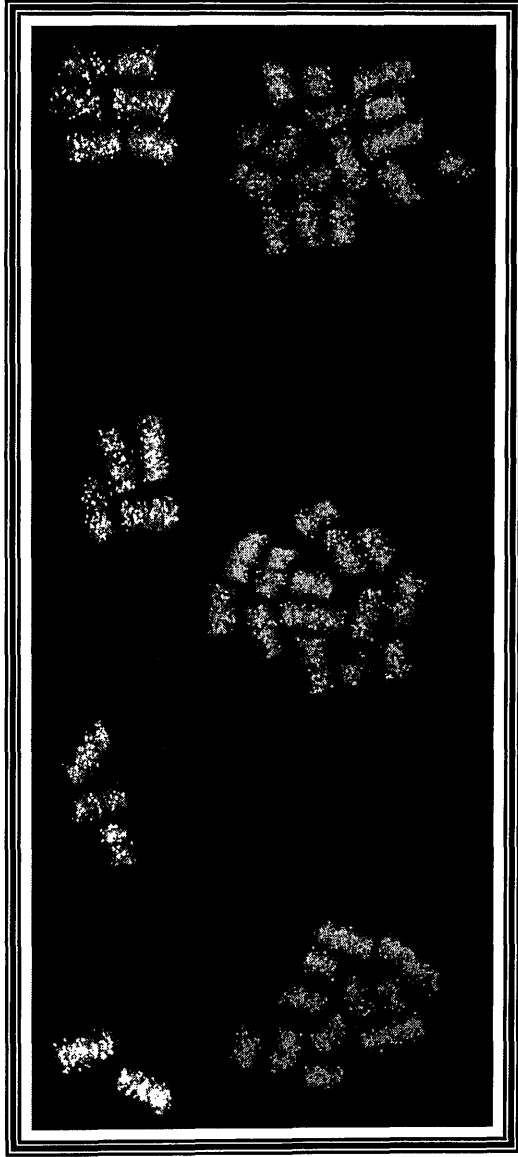


Figure 5.1 *X-ray photograph of feed pellet with Ballotini ($\times 2$)*

Table 5.2 *Feeding and sampling schedule on day 41- 43 for African catfish fingerlings fed three different diets. (A: marked feed, B: 50:50 mixture of marked and unmarked feed and C: unmarked feed)*

Tank No.	First Feeding			Deprivation Period (h)	Sampling time
	Day 41	Day 42	Day 43		
A ₁ , B ₁ & C ₁			09.00	0	09.00
A ₂ , B ₂ & C ₂			09.00	4	13.00
A ₃ , B ₃ & C ₃			09.00	8	17.00
A ₄ , B ₄ & C ₄			09.00	12	21.00
A ₅ , B ₅ & C ₅		17.00		16	09.00
A ₆ , B ₆ & C ₆		17.00		20	13.00
A ₇ , B ₇ & C ₇		17.00		24	17.00
A ₈ , B ₈ & C ₈		09.00		32	17.00
A ₉ , B ₉ & C ₉	17.00			40	09.00
A ₁₀ , B ₁₀ & C ₁₀	13.00			48	13.00

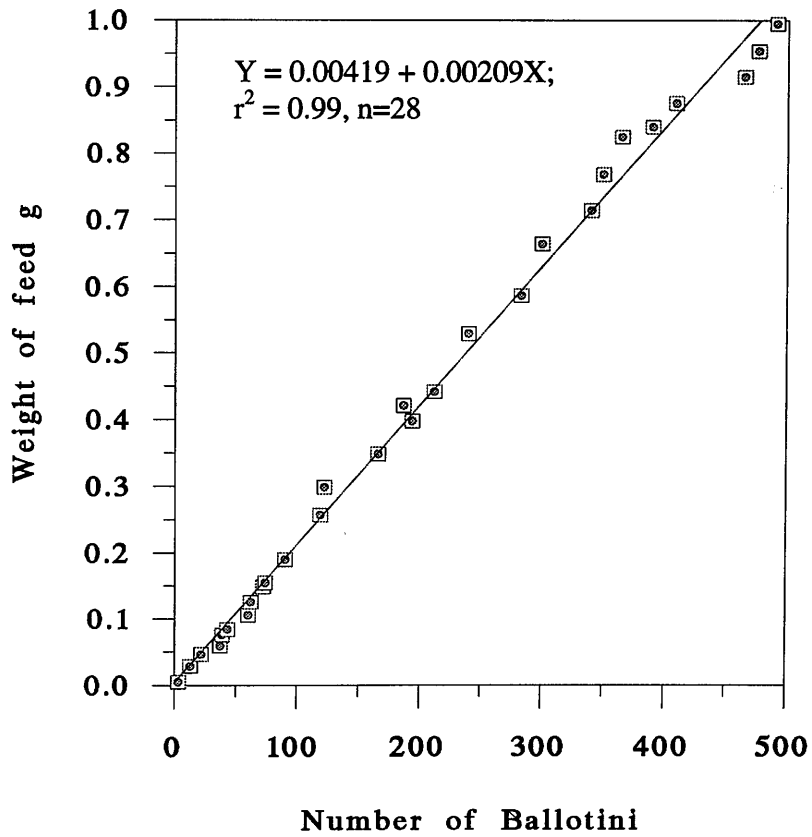


Figure 5.2 Regression line showing the relationship between weight and number of Ballotini

a 50:50 mixture of marked and unmarked feed (Treatment B) and fish in 10 tanks were fed unmarked feed (Treatment C). Fish were fed to apparent satiation and excess feed was removed after feeding ceased. Ten fish from each tank were then sampled at random at different time intervals following the termination of the meal (Table 5.2).

All procedures were performed on anaesthetised fish. The first samples of fish were anaesthetised using 100 ppm benzocaine solution, 5-10 min after they ceased feeding (handling or the application of anaesthetic 1-2 min after feeding was found to result in loss of ingested food). No losses of ingested feed were observed in any fish before or during X-raying. Fish were then weighed (Mettler PM6000 balance) and X-rayed. The stomach contents of dead fish were then carefully removed and dried at 40 °C overnight. The stomach contents were reweighed and calculated in terms of per cent body weight. Further samples were taken at intervals (Table 5.2) and the changes in the amount of marked feed present in the stomach with increasing time was used to estimate gastric evacuation rate (GER).

5.2.5 X-ray protocol

Both the marked feed pellets and fish were X-rayed using a Machlett Aeromax 2 X-ray apparatus (exposure time 2 s at 2 kV). Kodak Industrex film was used and the film developed using Kodak Industrex manual developer (4 min) and fixed by Kodak Industrex manual fixer (8 min) following washing (10 min) in cold, running tap water. The numbers of Ballotini were counted from X-ray plates observed under a

binocular microscope (x 40 magnification) and the amount of feed was estimated from the calibration curve.

5.2.6 Data analyses

Stomach content was expressed in terms of % body weight:

$$S = \frac{W_f}{W - W_f} \times 100$$

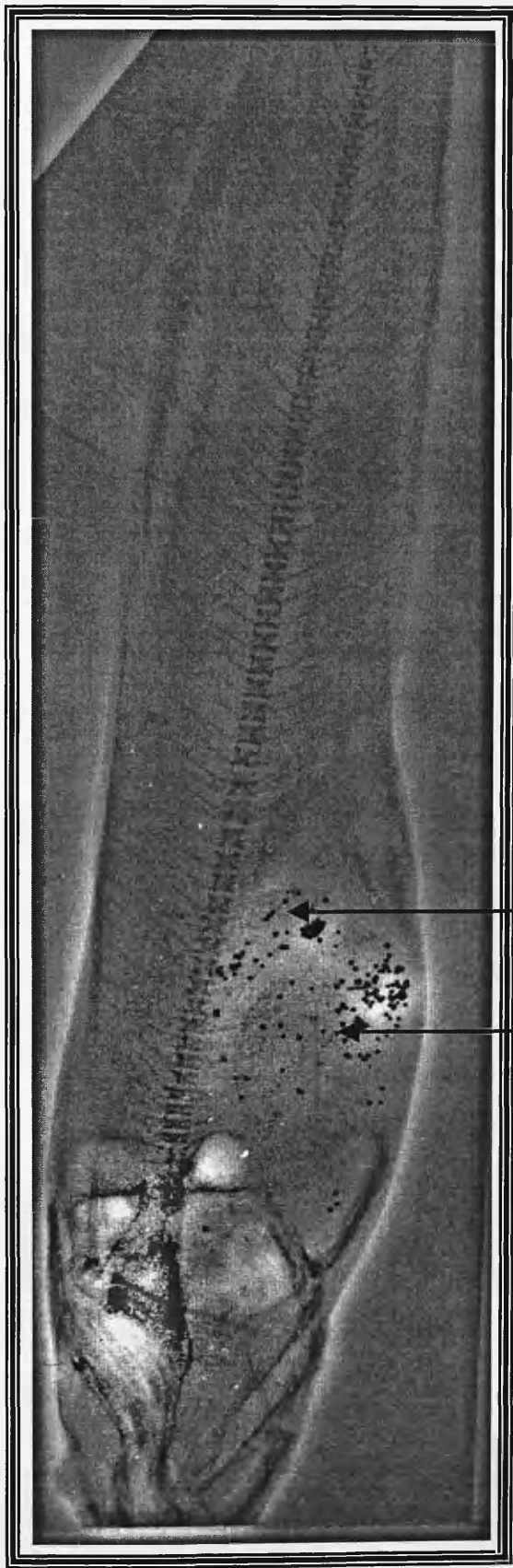
where W_f = Weight of feed in stomach (g), W = Weight of fish (g).

Stomach contents of fish fed the mixed diet were estimated by multiplying X-ray values by a factor of 2. Regression analysis was done using the absolute value (g) of stomach content obtained from two methods (stomach contents from fish fed unmarked feed were not included in regression) and significance test on regression coefficient was performed. Finally, the percent body weight data were arcsine transformed and then analysed by series of one way ANOVAs (Sokal and Rohlf, 1981)

5.3 RESULTS AND DISCUSSION

The stomach of fingerling African catfish was easily distinguished from other parts of the intestine from X-ray photographs of anaesthetised live fish (Figure 5.3). Stomach content was easily determine by counting the radio-opaque Ballotinis which showed up clearly on X-ray photographs. Ballotini present in other parts of the fish gut were not included in the estimation of stomach contents.

Highly significant correlations were found for the stomach content data obtained by both gastrectomy and X-ray method (Fig. 5.4). In addition, significance test on the



Ballotini present in stomach

Ballotini present in intestine

Figure 5.3 *Ballotini present in different parts of fish gut (× 8). Fish weight 5.25 g*

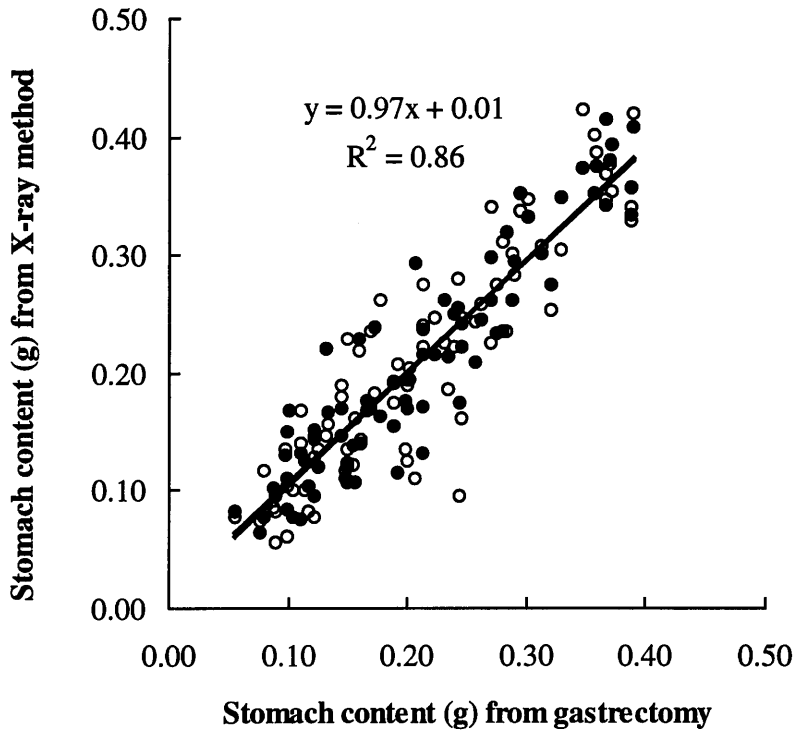


Figure 5.4 Relationships between stomach content (g) data obtained from gastrectomy and X-ray method. ● represents the data collected from fish fed 100% Ballotini marked feed and ○ represents the data collected from fish fed 50% marked and 50% unmarked feed. Since the stomach contents of fish fed unmarked feed were obtained by gastrectomy only, those data were not included in regression.

Table 5.3 *Stomach contents (% body weight) of fish in different treatments following different time intervals (Mean \pm 95% confidence limit values) (n = 10)*

Treatment	A Marked feed		B Mixed feed		C Unmarked feed
	Weight from gastreotomy	Estimated weight from Ballotini	Weight from gastreotomy	Estimated weight from Ballotini $\times 2$	Weight from gastreotomy
0	6.13 (0.16)	6.21 (0.22)	6.17 (0.18)	6.06 (0.38)	6.14 (0.25)
4	5.03 (0.17)	5.13 (0.38)	5.09 (0.33)	5.02 (0.38)	5.15 (0.20)
8	4.00 (0.27)	3.90 (0.11)	3.95 (0.39)	4.04 (0.42)	4.06 (0.31)
12	3.23 (0.33)	3.21 (0.52)	3.20 (0.41)	3.16 (0.54)	3.18 (0.39)
16	2.95 (0.41)	2.99 (0.32)	3.04 (0.37)	3.00 (0.47)	3.07 (0.36)
20	2.75 (0.43)	2.66 (0.28)	2.65 (0.47)	2.80 (0.41)	2.81 (0.24)
24	2.20 (0.33)	2.10 (0.18)	2.15 (0.27)	2.11 (0.22)	2.09 (0.26)
32	1.45 (0.18)	1.42 (0.14)	1.40 (0.12)	1.37 (0.19)	1.40 (0.17)

regression coefficient (slope) indicating a highly significant ($P < 0.05$) positive relationship between the two sets of data, and the slope (0.928) did not differ significantly ($P > 0.05$) from 1.

The stomach contents of fish immediately after feeding to satiation and after various deprivation periods are summarised in Table 5.3. The results show that average stomach contents at 0 h (feed consumption) ranged from 6.06 to 6.21 % body weight.

The ANOVAs performed on stomach content data obtained just after satiation prove that feed intake of fish fed three different diets are not significantly ($P < 0.05$) different. Therefore, the marker, Ballotini, has no effect on feed preference. Subsequent ANOVAs performed on data obtained at different time intervals also show that the differences are not significant, confirming that the marker has no effect on evacuation rate (Table 5.4).

Fig. 5.5 shows the evacuation of pelleted feed from the stomach after various deprivation periods. The data can be described by the equation:

$$S_t = S_0 e^{-Rt} \quad (\text{Elliott and Persson, 1978})$$

where S_0 = stomach contents after first feeding to satiation, S_t = stomach contents after time t , R is gastric evacuation rate and t is time (h). It was not possible to determine the exact point at which all fish stomachs were completely empty although this occurred after 32 h and before 40 h; hence, the last two points (40 and 48 h) were excluded from the regression. Gastric evacuation rates derived from five sets of data were not significantly different.

Table 5.4 One way ANOVA (Five groups with equal sample size)

H_0 : no difference in the quantity of marked feed (gastrectomy and X-ray method), unmarked feed (gastrectomy) or a 50:50 mixture (gastrectomy and X-ray method multiplied by 2) ingested after satiation (0 h) and different time intervals

Deprivation	Sources Of Variation	SS	Df	MS	F	$F_{(4,45) 0.05}$
0 h	Among Stomach Contents	0.12	4	0.03	0.18	2.58
	Within Treatments	7.45	45	0.17		
	Total	7.57	49			
4 h	Among Stomach Contents	0.14	4	0.03	0.14	
	Within Treatments	10.77	45	0.24		
	Total	10.91	49			
8 h	Among Stomach Contents	0.17	4	0.04	0.16	
	Within Treatments	11.92	45	0.26		
	Total	12.08	49			
12 h	Among Stomach Contents	0.02	4	0.01	0.01	
	Within Treatments	23.49	45	0.52		
	Total	23.51	49			
16 h	Among Stomach Contents	0.10	4	0.03	0.06	
	Within Treatments	17.84	45	0.40		
	Total	17.94	49			
20 h	Among Stomach Contents	0.24	4	0.06	0.16	
	Within Treatments	16.69	45	0.37		
	Total	16.93	49			
24 h	Among Stomach Contents	0.08	4	0.02	0.11	
	Within Treatments	7.87	45	0.17		
	Total	7.95	49			
32 h	Among Stomach Contents	0.03	4	0.01	0.13	
	Within Treatments	2.97	45	0.07		
	Total	3.01	49			

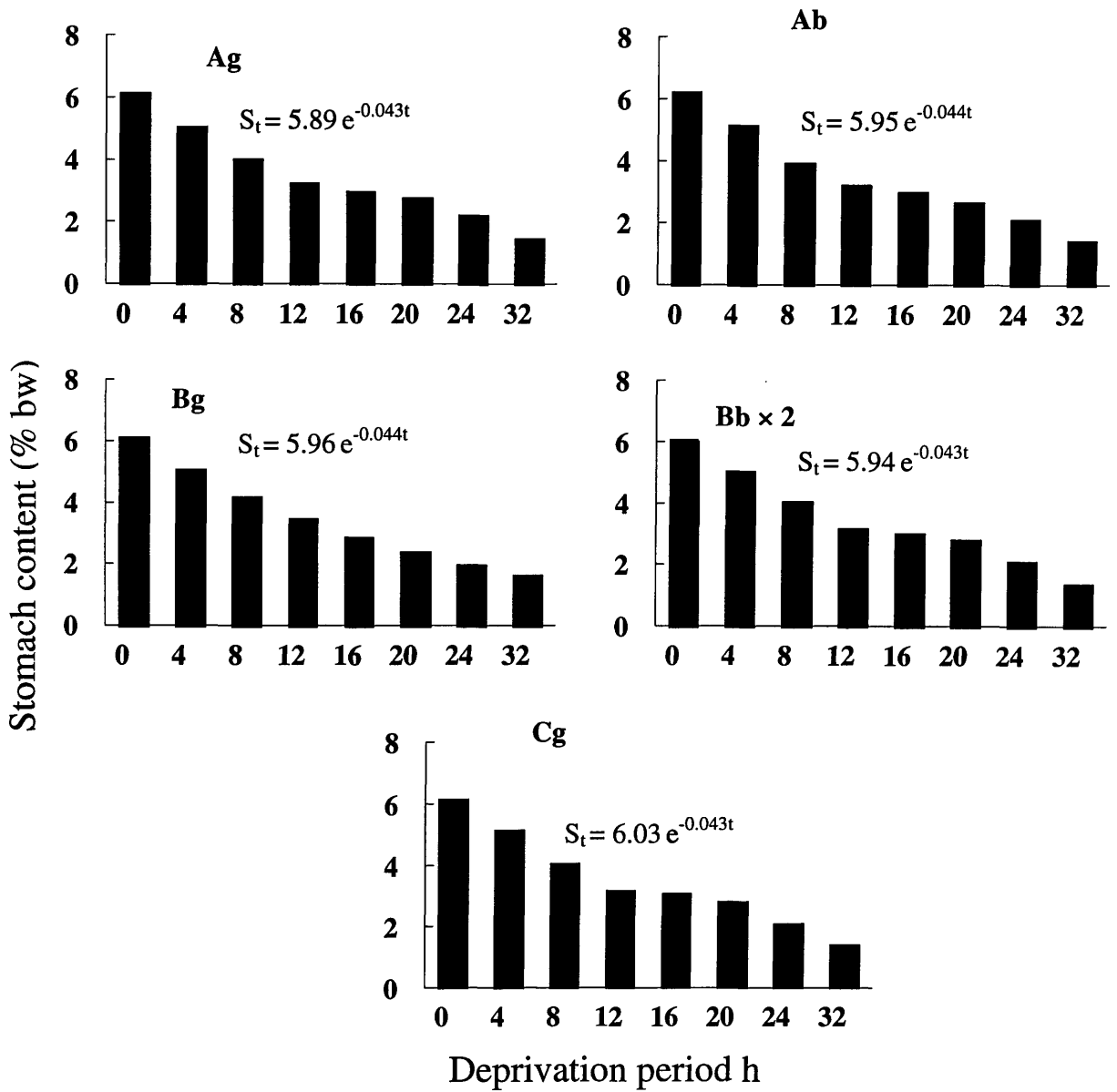


Figure 5.5 Gastric evacuation of African catfish fingerlings. After 40 h there were no feed in the stomachs. However, since it was not obvious at which exact point stomachs were completely evacuated, the last two points (40 and 48 h) were excluded from the evacuation rate calculation. Error bars are 95 % CL. A, B and C are the treatment no. and represent marked, mixed and unmarked feed respectively and g and b represent gastrectomy and Ballotini methods.

In conclusion, the results indicate that the inclusion of Ballotini in diets fed to *C. gariepinus* has no effect on feed ingestion or gastric evacuation rate. This is a useful and accurate method for estimating gastric evacuation and food intake in African catfish.

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Chapter 6

QUANTITATIVE ESTIMATION OF MAXIMUM DAILY FEED INTAKE OF AFRICAN CATFISH FINGERLINGS USING RADIOGRAPHY

Radiography is a non-invasive method of measuring the amount of feed intake of African catfish fingerlings (Clarias fahaka) under laboratory conditions. The method involves the use of a radioisotope as a tracer in the feed. The amount of feed intake is determined by measuring the radioactivity of the fish and the feed.

6.1 INTRODUCTION

A number of biotic and abiotic factors influence the growth of fish (Brett, 1979). Among them, the three most important factors - feeding level, body weight, and temperature interact with growth and feed conversion in a number of ways (Hogendoorn *et al.*, 1983). Feeding level or ration acts as a driving force, whereas temperature is a controlling force and body weight a scaling factor that adjusts these factors with respect to increasing fish size (Stauffer, 1973).

Appetite, feed intake, feeding frequency, digestibility, rate of feed movement through stomach and gut and, finally, absorption and conversion efficiency, are the major sequential steps in the transformation of fish feed into fish tissue. As research into the relationship between fish and their feed progresses from the largely qualitative to a more quantitative stage, accurate methods are required to estimate the optimum daily rate of food consumption.

Estimation of the gastric evacuation rate is a prerequisite for modelling of daily ration and food consumption in fish (Eggers, 1977; Elliott and Persson, 1978; Jobling, 1981). Gastric evacuation rate is defined as the rate at which food passes through the stomach and digestion is considered complete when the stomach becomes empty of all measurable remains (Windell, 1978).

The use of X-radiography in monitoring gastric evacuation rate was first described by Mölnár & Tölg (1960). Early methods involved mixing radio-opaque barium sulphate (BaSO_4) with feed (Edwards, 1971, 1973; Goddard, 1970, 1974; Jobling *et al.*, 1977; Ross and Jauncey, 1981). However, BaSO_4 is only sufficiently radio-

opaque at relatively high concentrations and can alter palatability and gut passage time. In 1983, a method was developed involving the inclusion of a particulate, X-ray dense marker in feed, which enabled quantitative determination of stomach contents of fish without palatability problem (Talbot and Higgins, 1983). However, in some fish species the rate of passage of markers appears to differ from that of other food components (Jørgensen and Jobling, 1988). In a recent experiment, Hossain *et al.*, (1998) observed that inclusion of radio-opaque marker Ballotini in diets fed to *C. gariepinus* had no effect either on ingestion or on gastric evacuation rate and concluded that the technique was an accurate method for estimating gastric evacuation and food intake in this species.

According to Hogendoorn (1983), maximum feeding level occurs at 30 °C for *C. gariepinus* in the size range 0.3 - 70 g. The fastest growth rate and temperature preferendum of this size group is also at 30 °C (Hogendoorn, *op cit.*; Britz and Hecht, 1987). Maximum fingerling growth rate therefore can be obtained by maximizing feed intake at this optimum temperature. Since stomach evacuation in young catfish is closely related to return of appetite (Haylor, 1993b), quantification of gastric (=stomach) capacity and evacuation can be used to estimate feed intake in relation to feeding schedule and hence to maximize feed intake.

The present experiment is designed to quantify feed intake in *C. gariepinus* fingerlings in relation to feeding schedule on the basis of stomach capacity and return of appetite, as measured by the X-ray method of Talbot and Higgins (1983) as modified by Hossain *et al.* (1998).

6.2 MATERIALS AND METHODS

6.2.1 Fish

C. gariepinus fingerlings of mean (\pm SE) weight 0.95 ± 0.1 g, were obtained from broodstock maintained in the Institute of Aquaculture, University of Stirling following the procedure detailed in Chapter 4.2.1.

6.2.2 Feed preparation

Feed mixed with Ballotini and control diet was prepared following the procedure in Chapter 5.2.3

6.2.3 Experimental procedure

Seven hundred and fifty 25-day old fingerlings were randomly allocated to thirty, 40-cm diameter round plastic tanks with a diameter:depth ratio of 10, within the recirculation system described in chapter 3.1, (water flow rate 0.4 L m^{-1}) at a stocking density of 25 fish per tank (5 fish L^{-1}).

Fingerlings were fed to satiation three times daily at 0900, 1300 and 1700 h with Ballotini- marked feed. Following the first feed in the morning, satiation time (the time from the onset of feeding until all fish in the tank ceased to respond to continued addition of feed), stomach capacity (the amount of feed in the stomachs of fish immediately after feeding to satiation, identified by X-ray, ventro-lateral view) and weight (Mettler PM6000 balance; precision 0.01g) of 30 randomly selected fish were determined every 5 days. All procedures were performed on anaesthetised fish.

Table 6.1 Feeding schedule on day 41- 43 for *C. gariepinus* fingerlings

Treatment	First Feeding			Deprivation Period (h)	Second feeding
	Day 41	Day 42	Day 43		
A			09.00	0	09.00
B			09.00	4	13.00
C			09.00	8	17.00
D			09.00	12	21.00
E		17.00		16	09.00
F		17.00		20	13.00
G		17.00		24	17.00
H		09.00		32	17.00
I	17.00			40	09.00
J	13.00			48	13.00

Fish were anaesthetised using 100 ppm benzocaine solution, and X-rayed 5-10 min after they ceased feeding. No losses of ingested feed were observed in any fish before or during X-raying.

On day 41 or 42, the fish in all 30 tanks were fed to satiation with marked feed pellets as usual. After various deprivation periods between 0 and 48 h, fish were again fed to satiation (See Table 6.1 for detailed feeding schedule). The second meal was of unmarked pellets. Following each deprivation period the satiation time was recorded as before. Fifteen fish from each treatment were then selected at random, anaesthetised, weighed and X-rayed (Figure 6.1; Contact photographs were taken by ILFORD Multigrade Enlarger Head using X-ray film as negative and processed by ILFORD 2150RC Print Processor, ILFORD Ltd., England; photographs were then scanned by a scanner- GT9500, Epson and finally modified and background changed by computer programme Corel Photo-paint 7). . The stomach contents of dead fish were then carefully removed and dried at 40 °C overnight. The stomach contents (marked + unmarked feed) were reweighed and calculated in terms of per cent body weight. The changes in the amount of marked feed present in the stomach with increasing time was used to estimate gastric evacuation rate (GER) and the changes in the amount of unmarked feed consumed with increasing deprivation time was used to quantify return of appetite (RA).

6.2.4 Statistical analyses

Ninety-five percent confidence limits (CL) were calculated as, $CL = \bar{X} \pm t_{0.05 (n-1)} (S/\sqrt{n})$. A single classification ANOVA was carried out to investigate difference in stomach capacity at various deprivation periods between 0 and 48 h. The % body



Figure 6.1a X-ray view of African catfish fingerling showing Ballotini in the stomach just after satiation

Figure 6.1b X-ray view of African catfish fingerling showing Ballotini in the stomach 48h after satiation



Figure 6.1b *X-ray view of African catfish fingerling showing Ballotini in the stomach 4 h after satiation*



Figure 6.1c X-ray view of African catfish fingerling showing *Ballotini* in the stomach 8 h after satiation



Figure 6.1d X-ray view of African catfish fingerling showing Ballotini in the stomach 12 h after satiation

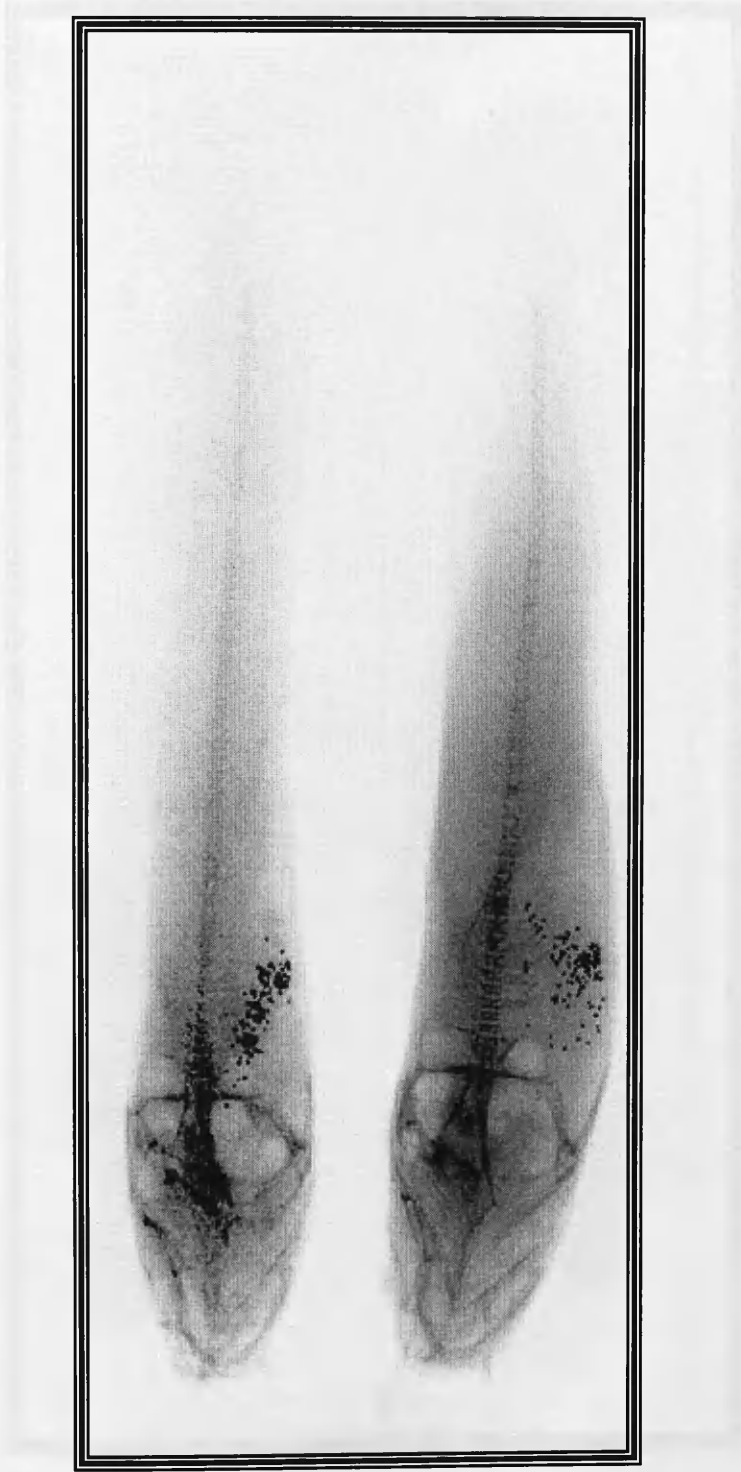


Figure 6.1e X-ray view of African catfish fingerling showing *Ballotini* in the stomach 16 h after satiation



Figure 6.1f X-ray view of African catfish fingerling showing Ballotini in the stomach 20 h after satiation



Figure 6.1g *X-ray view of African catfish fingerling showing Ballotini in the stomach 24 h after satiation*



Figure 6.1h *X-ray view of African catfish fingerling showing Ballotini in the stomach 32 h after satiation*

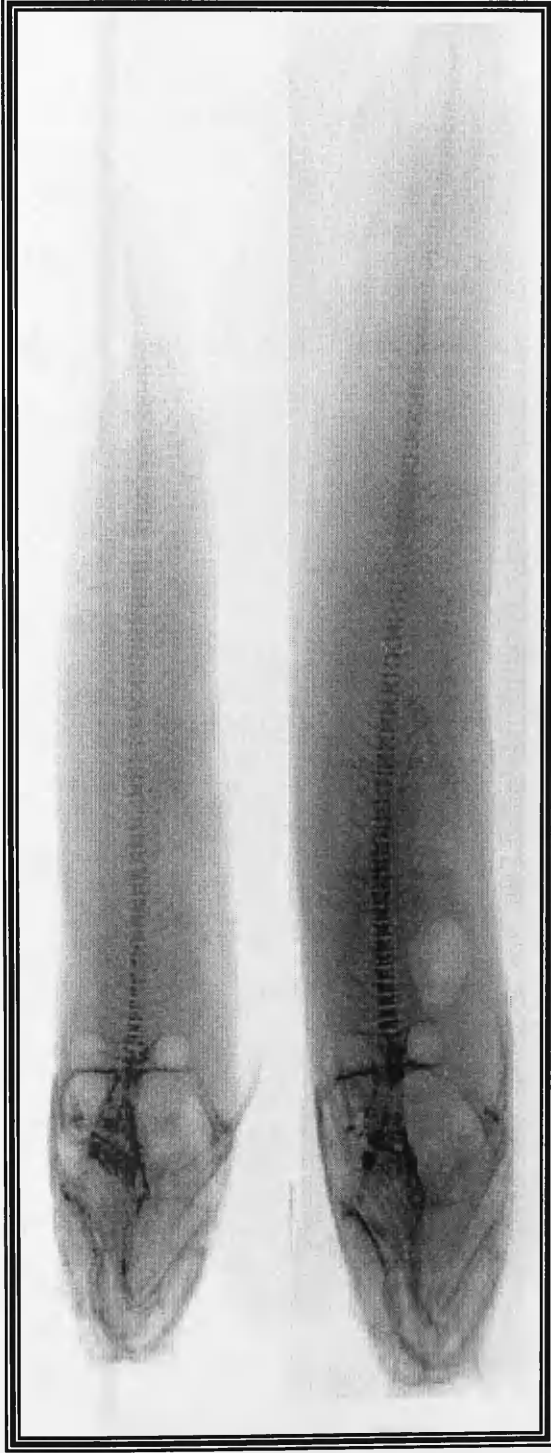


Figure 6.1i X-ray view of African catfish fingerling showing no Ballotini in the stomach 40 h after satiation

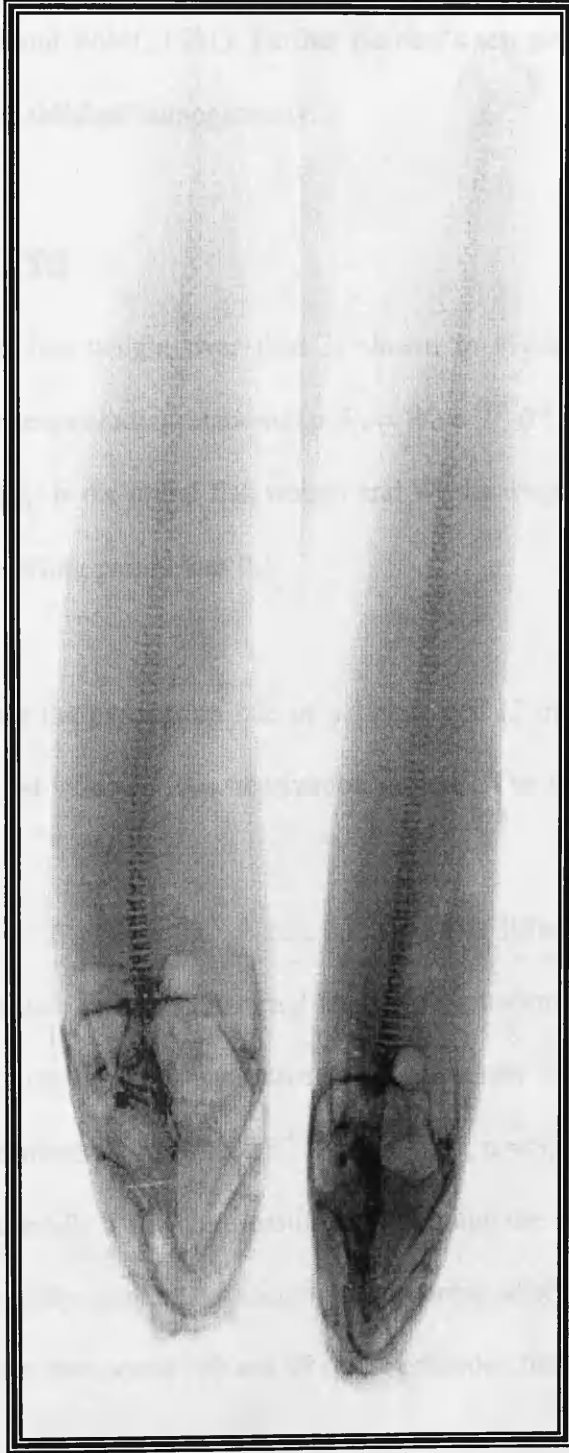


Figure 6.1j X-ray view of African catfish fingerling showing no Ballotini in the stomach 48 h after satiation (Photographs in this series are approximately 2.4 times enlarged)

weight data were arcsine transformed and a Bartlett's test revealed homogeneous variance (Sokal and Rohlf, 1981). Further Bartlett's test performed on the satiation time data also established homogeneity.

6.3 RESULTS

The increase in fish weight over time is shown in Figure 6.2. The data can be described by the exponential relationship $W_t = W_0 e^{Gwt}$ ($r^2 = 0.97$, $n = 5$, $P < 0.05$), where W_0 (0.95 g) is the initial fish weight and W_t the weight at time t . Growth rate (G_w) for the fingerling period was 0.1.

Figure 6.3 shows the evacuation rate of pelleted feed (2 mm) from the stomach of 41-43 day old fish after various deprivation periods. The data can be described by the equation

$$S_t = S_0 e^{-Rt} \dots\dots\dots(1) \text{ (Elliott and Persson, 1978)}$$

where S_0 = stomach contents after first feeding to satiation, S_t = stomach contents after time t , R is the rate constant, gastric evacuation rate and t is the time in hours.

A significant relationship ($S_t = 6.32 e^{-0.046t}$, $r^2 = 0.95$, $n = 8$, $P < 0.05$) was found for the data in Figure 6.3. It was not possible to determine the exact point at which fish stomachs were fully emptied although this occurred after 32 h and before 40 h. Therefore, the last two points (40 and 48 h) are excluded from the regression.

The return of appetite (amount of unmarked feed consumed) of the fingerlings is shown in Figure 6.4. The curve represents the level of consumption estimated from gastric evacuation parameters calculated from the data in Figure 6.3,

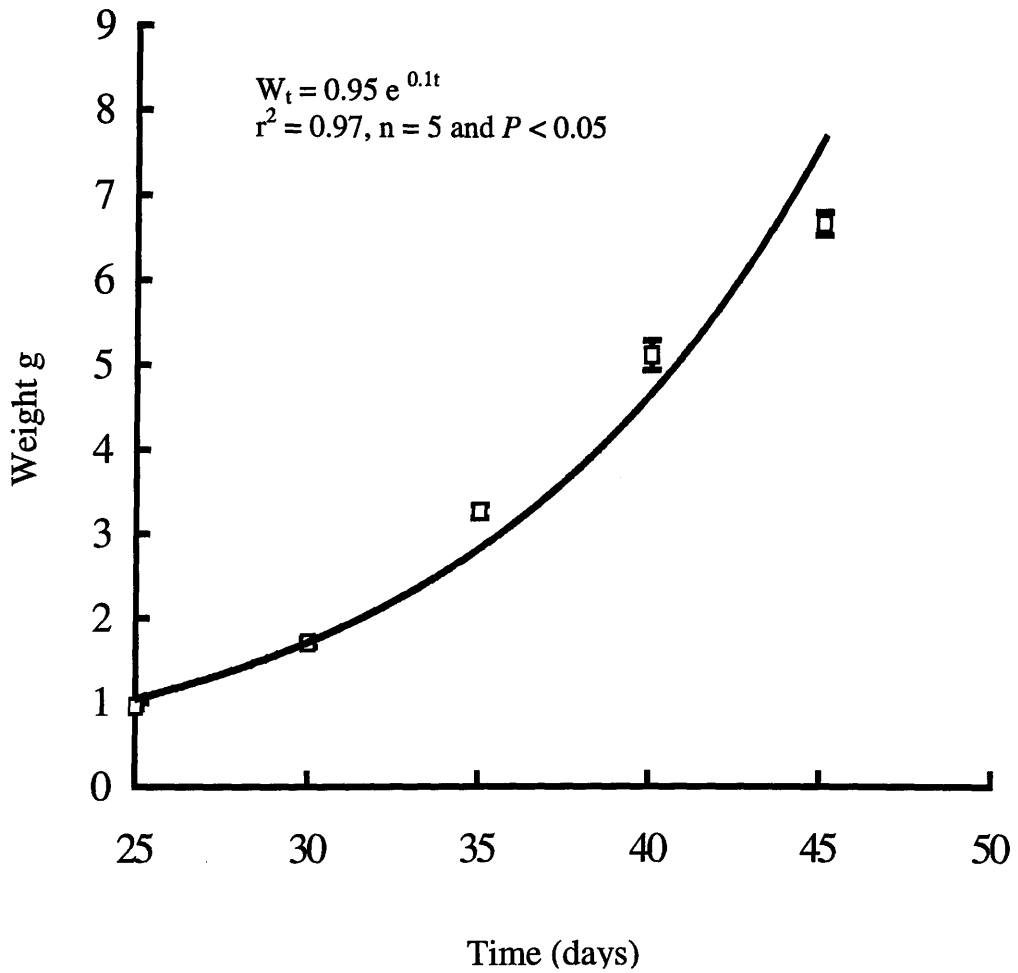


Figure 6.2 *Growth of African catfish fingerlings over the experimental period. Error bars represent 95% confidence limit*

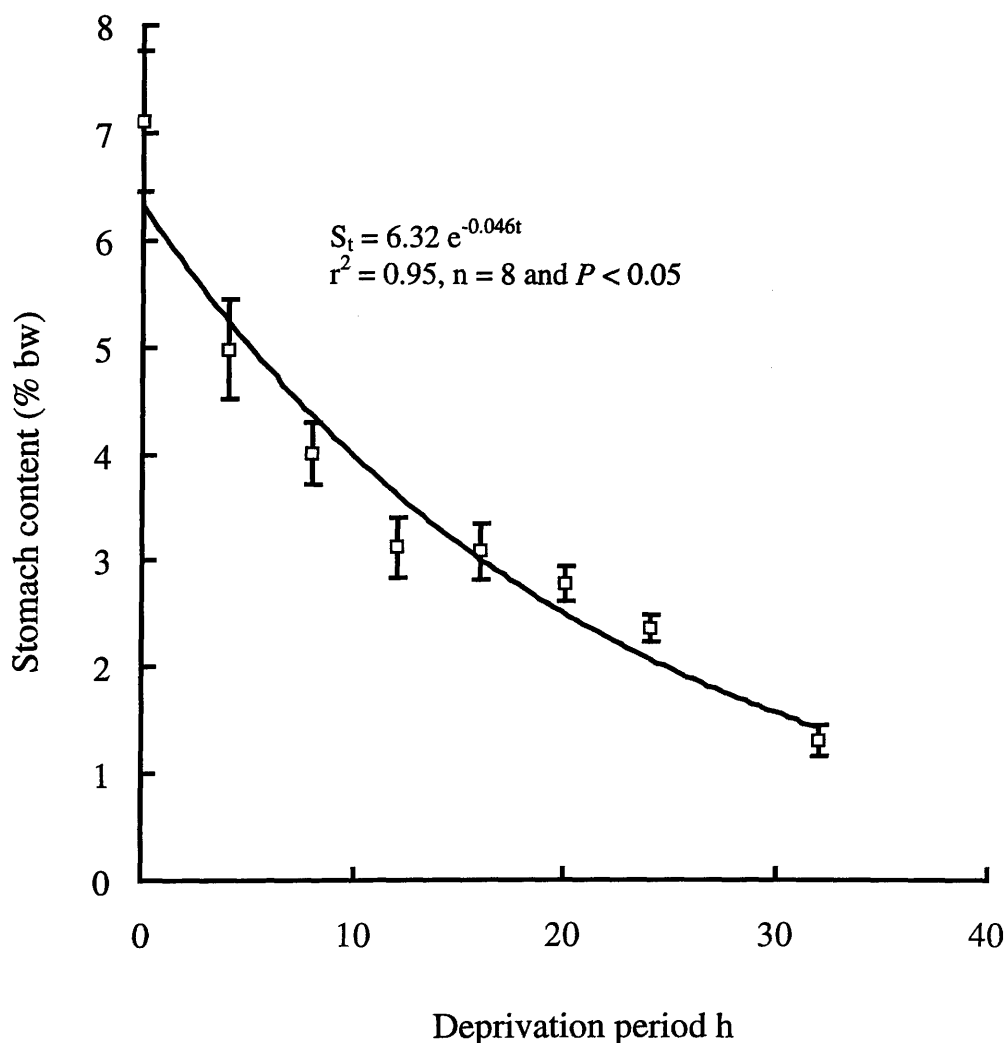


Figure 6.3 *Gastric evacuation of African catfish fingerlings. After 40 h there was no food in the stomachs. However, since it was not obvious at which exact point the stomachs were completely evacuated, the last two points (40 and 48 h), were excluded from the evacuation rate calculation. Error bars are 95 % CL. (15 out of 75 fish were randomly selected for each time point and mean fish weight at different time points were 6.09, 6.19, 6.33, 6.48, 5.92, 6.06, 6.22 and 6.32 g respectively).*

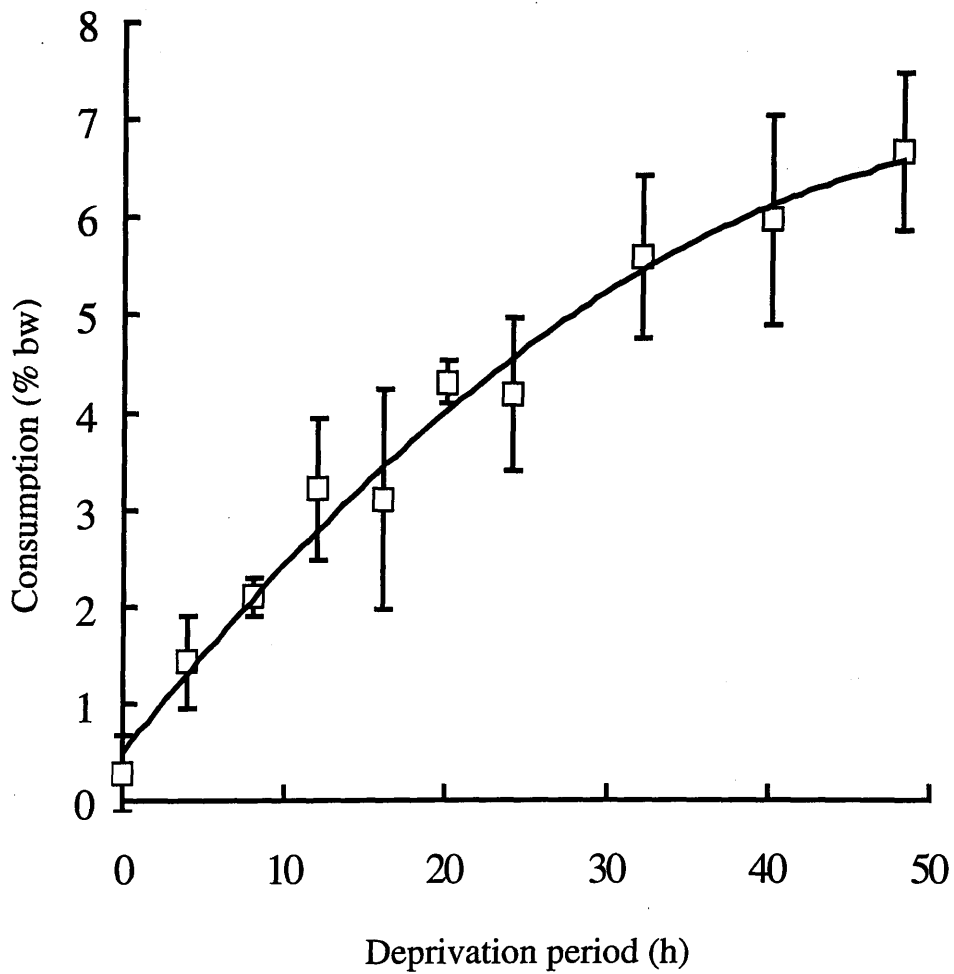


Figure 6.4 *The feed intake after different deprivation periods (Return of appetite) in C. gariepinus fingerlings at 30 °C. Error bars represent 95% CL.*

whereby consumption at time t , (C_t) can be determined from $C_t = S_0 (1 - e^{-Rt})$ (after Haylor, 1993b). Statistical analysis showed that there was no significant difference in consumption once the stomach was fully emptied, regardless of the deprivation period.

Figure 6.5 shows the time taken for fingerlings to reach satiation in relation to age. Over the experimental period satiation time remained constant ($F_{0.05(4, 145)} > F$; mean = 12 min 22 s \pm 35 s, 95% CL) (Table 6.2). On day 43, satiation times were recorded in relation to deprivation time. All approximated the mean satiation time except the satiation times recorded after the first three (0, 4 and 8 h) deprivation periods (Table 6.3).

Figure 6.6 shows the increasing stomach capacity in relation to fish weight (weight of feed measured after satiation meal), which can be expressed by linear relationship $S_0 \text{ g} = 0.0627 W \text{ g} + 0.03$ ($R^2 = 0.97$, $n = 5$, $P < 0.05$). If this relationship is expressed in % body weight terms then it becomes, $S_0 = (0.0627 + 0.03/W) \times 100$ (close to 6.27 %)

After each deprivation period, stomach capacity (marked feed remaining after first satiation meal + unmarked feed ingested in the second satiation meal after 0-48 h deprivation period) was measured by gastrectomy. Mean stomach capacity (6.30 \pm 0.29 %) was unaffected ($F_{0.05(9, 140)} > \text{calculated } F$) by deprivation time (Table 6.4).

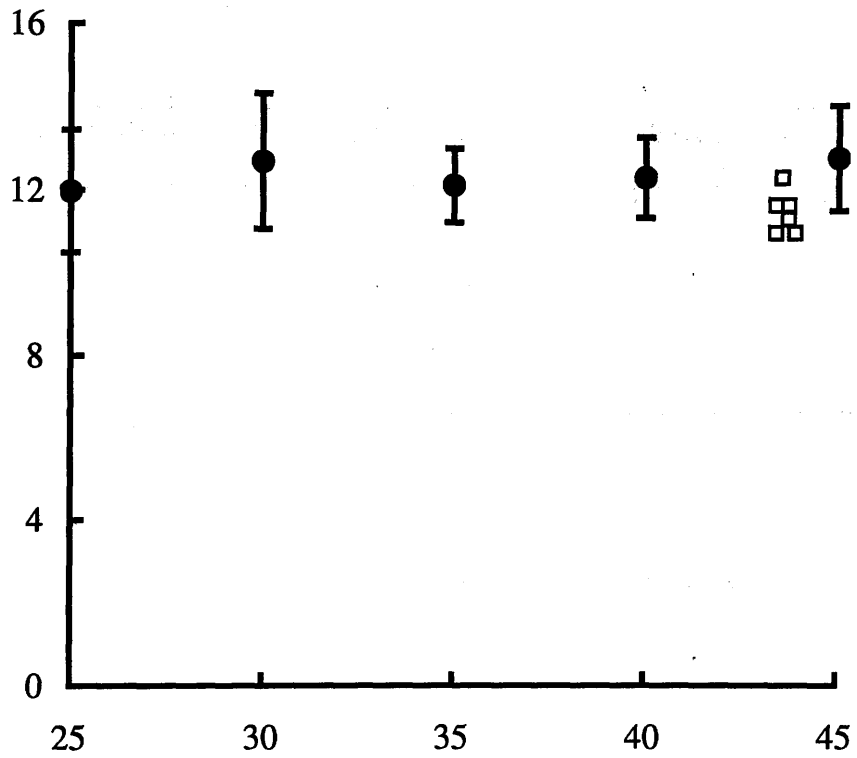


Figure 6.5 *Satiation time for C. gariepinus fingerlings over the experimental period. Error bars represents 95% CL. □ represents satiation time in relation to deprivation time on day 43 (first three sets of data from the satiation times on day 43 are excluded)*

Table 6.2 One way ANOVA (5 groups of equal sample size). The hypothesis (H_0) is that there is no difference between satiation time over the experimental period.

Source of variation	SS	Df	MS	F (calculated)	$F_{0.05 (4,145)}$
Between groups	112.27	4	28.07	2.19	2.43
Within groups	1854.57	145	12379		
Total	1966.84	149			

Table 6.3 Mean satiation time after various deprivation periods (0 - 48 h)

Deprivation period (h)	Mean satiation time (m)	95% CL
0	0.67	1.31
4	3.33	1.73
8	7.33	0.65
12	11.67	1.13
16	11.33	2.99
20	11.67	3.27
24	12.67	4.57
32	12.00	3.64
40	13.00	0.65
48	12.33	2.36

In this experiment, stomach capacity of fingerling was estimated by three methods - from feed intake over the experimental period (6.27 %) which comes from linear regression between increasing feed intake with fish body weight (Figure 6.6); from feed intake in relation to deprivation time (6.32 %) obtained from exponential regression between decreasing feed quantity with increasing deprivation time (Figure 6.3) and from the sum of the measured remains of the previous meal and feed ingested after subsequent satiation meal (6.30 ± 0.29 %), which was direct observation. All three gave approximately similar values.

6.4 DISCUSSION

The results of the preliminary experiments (Chapter 5) indicate that the inclusion of Ballotini in diets fed to *C. gariepinus* have no effect either on ingestion or on gastric evacuation rate and that this technique is a useful and accurate method for estimating gastric evacuation and food intake in African catfish.

Feed intake (stomach content) was unaffected by deprivation time (Table 6.4) indicating that regardless of deprivation time fish fed until the space available in their stomach was filled *i.e.*, that consumption (return of appetite) and gastric evacuation were inversely proportional. This relationship between gastric evacuation rate and food intake (return of appetite) is common to other studies with fish (Bajkov, 1935; Ricker, 1946; Magnuson, 1969; Brett, 1971; Elliott, 1975; Grove *et al.*, 1978; Ross and Jauncey, 1981; Charles *et al.*, 1984; Haylor, 1993b; Sims *et al.*, 1996).

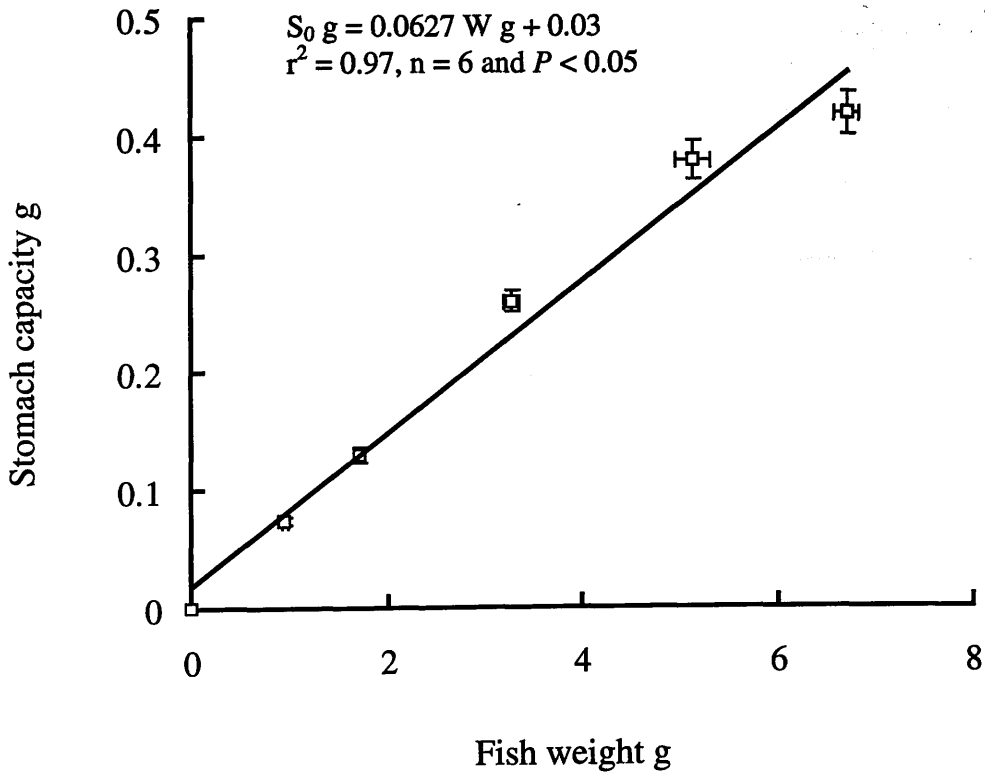


Figure 6.6 The changes of stomach capacity with increasing weight. Error bars are 95 % CL.

Table 6.4 Summary of analysis of variance (5 groups with equal sample size).
 The hypothesis (H_0) is that there is no difference between the summed quantity of marked + unmarked feed that remained in the stomach after various deprivation periods.

Source of variation	SS	Df	MS	F (calculated)	$F_{0.05} (9,140)$
Between groups	32.16	9	3.57	1.51	1.95
Within groups	331.54	140	2.37		
Total	363.70	149			

There is wide agreement that the exponential model of Elliott and Persson (1978) can be used to approximate the evacuation of small, easily digestible feed particles such as pellets in the stomach (Persson, 1986; Jobling, 1987; Macpherson *et al.*, 1989; Haylor, 1993b). Elliott and Persson (1978) observed that where no subsequent feeding occurs the quantity of food remaining in the stomach at time t is given by the Equation 1 above.

Since the rate of return of appetite is inversely proportional to gastric evacuation Haylor (1993b) expressed maximum consumption (C_t) at any time after satiation (t) as

$$\begin{aligned}
 C_t &= S_0 - S_t \\
 &= S_0 - S_0 e^{-Rt} \\
 &= S_0 (1 - e^{-Rt}) \dots\dots\dots (2)
 \end{aligned}$$

By expressing maximum stomach capacity (S_0) in terms of % body weight and gastric evacuation rate (R) in terms of % body weight over time Haylor (1993b) derived equations for estimating maximum daily consumption in relation to feeding schedules over 24 h and 12 h ($C_{24\text{ h}}$ & $C_{12\text{ h}}$ respectively)

$$C_{(24\text{ h})} = \frac{24}{t} S_0 (1 - e^{-Rt}) \dots\dots\dots (3)$$

$$\text{and } C_{(12\text{ h})} = S_0 (1 - e^{-12R}) + \frac{12}{t} S_0 (1 - e^{-Rt}) \dots\dots\dots (4)$$

Although temperature, meal size and quality of feed have an important effect on gastric evacuation rate, fish size does not. Fish of different sizes of a single species fed a standard weight of a particular feed will take the same length of time to empty their stomach (Jobling, 1980). He and Wurtsbaugh (1993) analyzed the effects of temperature, fish size and meal size on gastric evacuation rates of 22 fish species

from 121 published papers and found that while both temperature and meal size significantly affected GER fish size did not. In the present experiment, therefore, R can be considered as a constant throughout the fingerling period for estimating the daily consumption at 30 °C in fish fed to satiation.

The daily consumption of *C. gariepinus* fingerling can thus be estimated as

$$C_{(24\text{ h})} = \frac{24}{t} \times (0.0627 + 0.03/W) \times 100 \times (1 - e^{-Rt}) \dots \dots \dots (5)$$

$$C_{(12\text{ h})} = (0.0627 + 0.03/W) \times 100 \times (1 - e^{-12R})$$

$$+ \frac{12}{t} (0.0627 + 0.03/W) \times 100 \times (1 - e^{-Rt})$$

$$= (0.0627 + 0.03/W) \times 100 \times \{(1 - e^{-12R}) + \frac{12}{t} (1 - e^{-Rt})\} \dots \dots (6)$$

(from equations 3 and 4 and equation from Figure 6.6)

The estimated maximum daily feed intake for 1-8 g *C. gariepinus* fingerling fed over 24 h and 12 h each day and the % of the total ration for first and successive feeding are summarized in Tables 6.5 and 6.6. Interestingly, in an earlier experiment which investigated the effects of temperature, body weight and energy content on feed utilization, Hogendoorn (1983) recommended a feeding rate of 8.0 % bw day⁻¹ for 1 g and 5.6 % bw day⁻¹ for 5 g *C. gariepinus* fingerlings at 30°C in order to optimize growth. These feeding rates approximated those derived from the present experiment when the interval between two meals was 12 h, *i.e.*, feeding twice a day (Table 6.5).

In conclusion, it would appear that the gastric evacuation technique described here can be used to quantify daily feed requirements in other fish, although it must be borne in mind that requirements will change with culture conditions, fish species and feed type.

Table 6.5 *Estimated maximum feed intake (% body weight d^{-1}) for C. gariiepinus fingerlings (weight 1 - 8 g) fed 2 mm pelleted trout diet at 30 °C*

Feeding Schedule	Feeding Interval h	Weight of fish g							
		1	2	3	4	5	6	7	8
24 h daily	1	10.06	8.43	7.89	7.62	7.46	7.35	7.27	7.21
	2	9.83	8.24	7.71	7.45	7.29	7.18	7.10	7.05
	3	9.61	8.06	7.54	7.28	7.12	7.02	6.95	6.89
	4	9.40	7.88	7.37	7.12	6.96	6.86	6.79	6.74
	6	8.99	7.53	7.05	6.81	6.66	6.56	6.50	6.44
	8	8.60	7.21	6.75	6.52	6.38	6.28	6.22	6.17
	12	7.90	6.62	6.19	5.98	5.85	5.77	5.71	5.66
	24	6.22	5.21	4.88	4.71	4.61	4.54	4.49	4.46
12 h daily	1	8.96	7.51	7.03	6.79	6.64	6.55	6.48	6.42
	2	8.85	7.42	6.94	6.70	6.56	6.46	6.39	6.34
	3	8.74	7.32	6.85	6.62	6.48	6.38	6.31	6.26
	4	8.63	7.23	6.77	6.54	6.40	6.30	6.24	6.19
	6	8.43	7.06	6.61	6.38	6.25	6.15	6.09	6.04
	12	7.88	6.61	6.18	5.97	5.84	5.76	5.70	5.65

Table 6.6 *Percentage of daily rations to feed as first and subsequent ration (when feeding during daytime only).*

Feeding interval	First ration (% total)	No. of subsequent ration	Each of the subsequent ration (% total)
1	43.96	12	4.67
2	44.62	6	9.23
3	45.08	4	13.73
4	45.70	3	18.10
6	46.80	2	26.60
12	50.00	1	50.00

The information contained in Chapter 7 is submitted (18th March 1998) to Aquaculture Nutrition.

It has been suggested that particle size is an important factor in determining the rate of food intake in fish (Holtby, 1989). The rate of food intake is determined by the rate of food intake and the rate of food intake. Knowledge of the influence of particle size is a prerequisite to the selection of a fish species because of its role in determining food absorption (see review: Wankowski, 1977; Tabachnik, 1988; Subling et al., 1995).

Chapter 7

GASTRIC EMPTYING IN AFRICAN CATFISH: THE INFLUENCE OF FOOD PARTICLE SIZE

Food particle size (Holtby, 1989)

The rate of food intake is determined by the rate of food intake and the rate of food intake.

7.1 INTRODUCTION

Although it has been suggested that food particle size is an important factor governing gastric evacuation in fish (Jobling, 1987), few data are available on the effect of food particle size on feed intake (Swenson and Smith, 1973; Grove *et al.*, 1985, Jobling, 1986, 1987, 1988). Knowledge of the influence of particle size is a prerequisite to optimising production of a fish species because of its role in determining food acceptance, growth and food efficiency (Wańkowski, 1977; Tabachek, 1988; Jobling *et al.*, 1993).

Tyler (1970) pointed out that the disintegration of a food particle begins at the surface of the food item and proposed models for estimating digestion rate based on particle surface area and particle weight. It is likely that both volume and food particle surface area influence the rate of stomach emptying; digestion begins at the particle surface but food volume probably influences peristalsis and mechanical and physical breakdown (Windell, 1978).

Large food particles have a lower surface area-to-volume ratio than that of smaller particles and present a smaller surface area for the action of gastric acid and enzymes (He and Wurtsbaugh, 1993). Hence, the rate of fragmentation and digestion and consequently the gastric evacuation rate of large food items would be expected to be slower than that of the same volume of smaller particles (Jobling, 1987).

Food particles must be broken down to a small size before they are passed from the stomach through the pylorus and into the intestine. When fish consume food items such as fish,

crustaceans and other animals and plants which are large by comparison with their own body size, the time required to produce fragments of suitable size for passage through the pylorus may be relatively long (Jobling, 1986).

In recent years an extensive literature has appeared on gastric evacuation of fish (Elliott, 1975; Grove *et al.*, 1978; Fänge and Grove, 1979; Jobling, 1987; Haylor, 1993b; Bromley, 1994; Sims *et al.*, 1996), most for the purpose of determining daily ration and food consumption. The present study examines the effect of different particle size of a formulated diet on food intake, growth and gastric evacuation rate of *Clarias gariepinus* by the X-ray method (Hossain *et al.* 1998) (Chapter 5)

7.2 MATERIALS AND METHODS

7.2.1 Preparation of feed marked with Ballotinis

The Ballotini mixed commercial pelleted trout diet was re-pelleted in 4 different sizes - 1, 1.5, 2 and 3 mm following the procedure described in Chapter 5.2.3.

7.2.2 Experimental procedure

Three hundred 25-day old fingerlings (0.97 ± 0.7 g), were randomly allocated to twelve, 40-cm diameter round plastic tanks within the recirculation system described in Chapter 3.1 at a stocking density of 25 fish per tank (5 fish L^{-1}).

From day 26 (from the day fish started feeding), fish were fed the marked feed to apparent satiation three times each day (at 0900, 1300 and 1700 h). Every 5th day, following the

morning feed, the weights (precision 0.01g) of 15 fish taken from each treatment was determined.

On day 41 (at 0900 h) the fish in all twelve tanks were fed to satiation with marked pellet as usual. After various deprivation periods between 0 and 48 h (0, 4, 8, 16, 24, 32 and 48 h), ten fish from each treatment were selected at random, anaesthetized, weighed and X-rayed. All procedures were performed on fish anaesthetised using 100 mg ppm benzocaine solution. No losses of ingested feed were observed in any fish before or during the X-ray operation. The stomach contents were calculated in terms of per cent body weight following the relationship between feed weight and numbers of Ballotini. The changes in the amount of feed present in the stomach over time were used to estimate gastric evacuation rate (GER). Since no X-rayed fish was returned to the tanks (based on the assumption that the feeding and other behavioral pattern of fish have been changed for a certain time due to anesthesia and X-raying), on the last day of the experiment - day 45, the weights of five remaining fish were determined.

7.2.3 Statistical analyses

Ninety-five percent confidence limits (CL) were calculated as, $CL = \bar{X} \pm t_{0.05 (n-1)} (S/\sqrt{n})$; where \bar{X} = mean, $t_{0.05 (n-1)}$ = value from a t table where 0.05 is the proportion expressing confidence and n-1 is the degree of freedom and S = Standard deviation. The % body weights data were Arcsine transformed and a Bartlett's test used to confirm homogeneous variance (Sokal and Rohlf, 1981). A single classification ANOVA was carried out to

investigate difference in stomach capacity at various deprivation periods between 0 and 48 h.

7.3 RESULTS

The increase in fish weight over time is shown in Figure 7.1. Mean total weights (measured every 5 day) were not significantly different ($P < 0.05$) in fish fed 1.5 and 2 mm pellet but significantly higher than those of fish were fed 1 and 3 mm diets. The data in Figure 7.1 can be described by the exponential relationship $W_t = W_0 e^{G_w t}$, where W_0 is the initial fish weight and W_t the weight at time t and instantaneous growth rate is G_w .

1 mm;	$W_t = 1.04 \times e^{0.087t}$	$r^2 = 0.95$	$n = 6$	$P < 0.01$
1.5 mm;	$W_t = 1.04 \times e^{0.099t}$	$r^2 = 0.98$	$n = 6$	$P < 0.01$
2 mm;	$W_t = 1.12 \times e^{0.099t}$	$r^2 = 0.97$	$n = 6$	$P < 0.01$
3 mm;	$W_t = 0.95 \times e^{0.077t}$	$r^2 = 0.98$	$n = 6$	$P < 0.01$

Specific growth rate was calculated using the formula

$$SGR = (e^{G_w} - 1) \times 100$$

Figure 7.2 shows the specific growth rates over the experimental period and again there were significant ($P < 0.05$) differences in SGR values between the fish fed 1.5 and 2 mm pellets and those fed with 1 and 3 mm pellets. Fish fed 3 mm pellets performed lowest SGR.

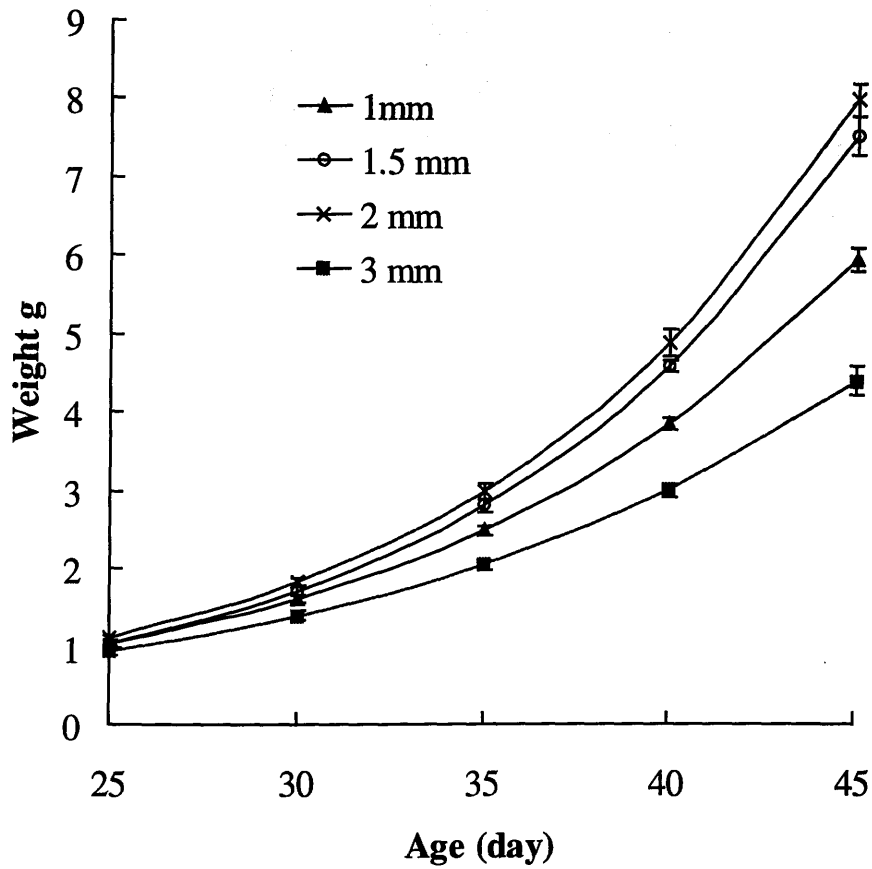


Figure 7.1 Mean weight of *C. gariepinus* fingerlings fed 4 pellet sizes of different diameter over the experimental period. Error bars represent 95% confidence limits

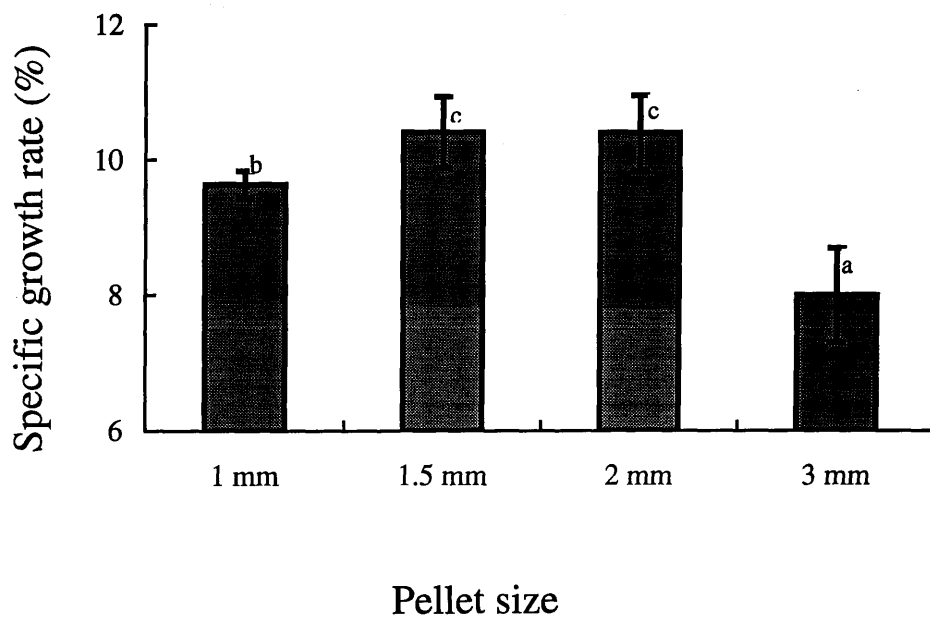


Figure 7.2 *Specific growth rate in fish fed pellet of 4 different sizes. Error bars represent 95% confidence limits. SGRs with same superscript are not significantly ($P < 0.05$) different*

Table 7.1 Mean stomach content \pm 95 % confidence limit (% body weight) of African catfish fingerlings fed four pellet sizes after various deprivation period. Stomach contents with the same superscript in a column are not significantly different ($P < 0.05$)

Deprivation period (h)	Pellet size			
	1mm	1.5 mm	2 mm	3 mm
0	4.67 \pm 0.42 ^a	6.47 \pm 0.57 ^a	6.54 \pm 0.26 ^a	3.89 \pm 0.23 ^a
4	3.44 \pm 0.62 ^b	5.21 \pm 0.67 ^b	5.44 \pm 0.53 ^b	3.47 \pm 0.12 ^b
8	2.53 \pm 0.25 ^c	4.19 \pm 0.31 ^c	4.53 \pm 0.30 ^c	3.09 \pm 0.34 ^{bc}
16	1.37 \pm 0.20 ^d	2.71 \pm 0.16 ^d	3.14 \pm 0.40 ^d	2.46 \pm 0.43 ^{cd}
24	0.74 \pm 0.20 ^e	1.76 \pm 0.22 ^e	2.18 \pm 0.29 ^e	1.96 \pm 0.25 ^d
32	0.40 \pm 0.09 ^f	1.14 \pm 0.18 ^f	1.51 \pm 0.20 ^f	1.56 \pm 0.14 ^e

Figure 7.1 shows the gastric evacuation rates in fish fed four different pellet sizes. The rate was highest in fish fed 1 mm pellet and lowest in fish fed 3 mm pellet. There was no significant difference between the fish fed 1.5 mm and 2 mm pellet.

Table 7.1 shows the stomach contents of pelleted feed of different pellet size from the stomach of 41-day old fish after various deprivation periods. The data can be described by the equation

$$S_t = S_0 e^{-Rt} \dots\dots\dots(1) \text{ (Elliott and Persson, 1978)}$$

where S_0 = stomach contents after first feeding to satiation, S_t = stomach contents after time t , R is the rate constant, gastric evacuation rate and t is the time in hours. Significant relationships were found for all four sets of data in Table 7.1. It was not possible to determine the exact point at which fish stomachs became fully empty although this always occurred after 32 h and before 48 h, hence, the last point (48 h) is excluded from the regression. The relationships for the four pellet sizes are:

1 mm;	$S_t = 4.67 \times e^{-0.077t}$	$r^2 = 0.96$	$n = 6$	$P < 0.01$
1.5 mm;	$S_t = 6.47 \times e^{-0.054t}$	$r^2 = 0.97$	$n = 6$	$P < 0.01$
2 mm;	$S_t = 6.54 \times e^{-0.046t}$	$r^2 = 0.97$	$n = 6$	$P < 0.01$
3 mm;	$S_t = 3.89 \times e^{-0.029t}$	$r^2 = 0.92$	$n = 6$	$P < 0.01$

Figure 7.3 shows the gastric evacuation rates in fish fed four different pellet sizes. While evacuation rate was highest in fish fed 1 mm pellet and lowest in fish fed 3 mm diet, there was no significant difference between the fish fed 1.5 and 2 mm pellet.

7.4 DISCUSSION

In the present experiment growth rate was found to be closely related to food particle size. The highest growth rate, apparent among fish fed 1.5 and 2 mm pellets, indicates that there is an optimum, intermediate particle size range and that feeding on both larger and smaller particle sizes adversely affects growth. The largest food items that fish can manipulate and engulf are not necessarily the most profitable (Wanzenboeck, 1995). Although large fish may be able to consume small particles, more energy may be required to capture an equivalent weight of small particles, adversely affecting net energy returns from foraging (Pandian and Vivekanandan, 1985). These findings and those of the present experiment are supported by studies on other species, including young Atlantic salmon (*Salmo salar*) (Wankowski, 1977), Arctic char (*Salvelinus alpinus*) (Tabachek, 1988) and common carp (Wang *et al.*, 1994).

There is wide agreement that the exponential model of Elliott and Persson (1978) can be used to approximate the evacuation rate of small easily digestible feed particles such as pellets from the stomach (Persson, 1986; Jobling, 1987; Macpherson *et al.*, 1989; Haylor, 1993b). In the present experiment, the smaller feed particles were evacuated more rapidly. In an experiment with cod, *Gadus morhua*, dos Santos and Jobling (1991) found whole herring, *Clupea harengus* were digested and evacuated from cod stomachs much more slowly than finely minced herring. Swenson and Smith (1973) reported that the stomach evacuation rate of walleye, *Stizostedion viterum viterum* was higher when fed meals composed of small prey (*Pimephales promelus*) than identical meals comprised of large prey. Mealworms were evacuated at a slower rate from the stomachs of pumpkinseed

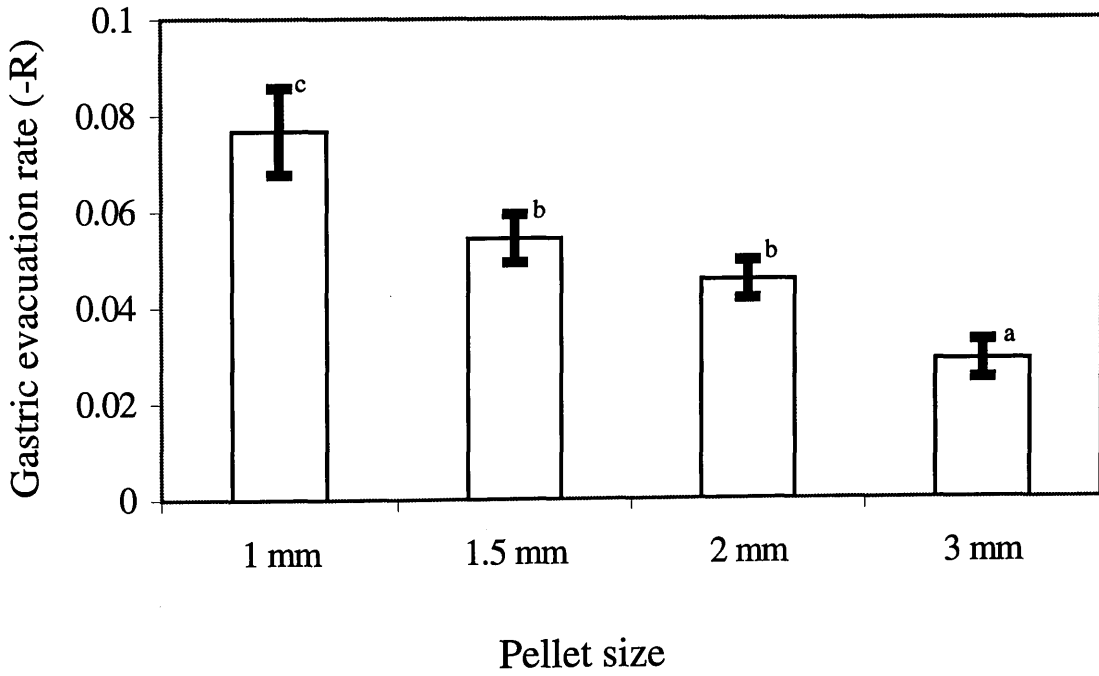


Figure 7.3 Gastric evacuation rate in *C. gariiepinus* fingerlings fed pellets of different diameter. Error bars represent 95% confidence limits. GERs with same superscript are not significantly ($P < 0.05$) different

sunfish, *Lepomis gibbosus* (Kitchell and Windell, 1968) and brown trout, *Salmo trutta* (Elliott, 1972) than other, smaller prey.

It is thus apparent from a range of studies that feeding fish small food particles results in faster stomach evacuation rates and that as a result, fish ingest more when frequent meals of smaller pellets are offered, even though, as in the present experiment, this results in poor growth. On the other hand, when catfish were fed larger particles in the present experiment, both the feed intake and growth rate were lower. Optimum feed efficiency and growth rates occurred when fish were fed intermediate pellet sizes.

of water in which the fish are kept. The
1970s. Some of the early work on
of determining the effect of food
of fish aimed at elucidating a general relationship
of food intake based on optimum particle size and energy level.
The amount of food ingested by fish have a significant effect on gastric evacuation

Chapter 8

THE INFLUENCE OF DIETARY ENERGY ON GASTRIC EMPTYING AND GROWTH RATES OF FINGERLING AFRICAN CATFISH

The influence of dietary energy on gastric emptying and growth rates of fingerling African catfish was investigated. The results showed that the rate of gastric emptying increased with increasing dietary energy levels. The growth rate of the fish also increased with increasing dietary energy levels. The results of this study are discussed in relation to the energy requirements of fingerling African catfish.

8.1 INTRODUCTION

In recent years a wide body of literature has appeared on gastric evacuation in fish (Elliott, 1975; Grove *et al.*, 1978; Haylor, 1993b; Sims *et al.*, 1996), most for the purpose of determining daily ration and food consumption. The present work is part of a larger study aimed at developing a general evacuation model and estimation of maximum feed intake based on optimum particle size and energy level.

The types of food ingested by fish have a significant effect on gastric evacuation rates (Fänge and Grove, 1979; Durbin and Durbin, 1980; Jobling, 1986; see Bromley, 1994 for review). Workers who have detected decreased evacuation rates with less digestible foodstuffs include Pandian (1967) (*Megalops* fed *Gambusia* or *Metapenaeus*), Western (1971) (*Cottus*, *Enophrys* fed on *Tubifex*, *Calliphora* or semifluid meals), and Kionka and Windell (1972) (*Salmo* fed various diets). The digestibility of the feed affects the emptying rate of the stomach and may also determine the time after ingestion before weight decrease of the meal in the stomach occurs. *Merlangius* or *Melanogrammus* start to digest shell-less *Mytilus* almost immediately but the meals of *Ophiopholis*, large crustacea or *Centronotus* required up to 10, 20 and 25 h respectively before weight loss began (Jones, 1974).

An increase in the dietary energy content of food has been reported to reduce gastric emptying rate in fish (Windell, 1966; Elliott, 1972). Jobling (1988) found that a high energy herring diet led to an increase in the gastric emptying time of cod, *Gadus morhua*, agreeing with results from experiments conducted with rainbow trout and marine flatfish (Windell, *et al.* 1969; Grove *et al.*, 1978; Flowerdew and Grove, 1979; Jobling, 1980). In plaice, *Pleuronectes platessa*, an increase in dietary energy content from approximately 5 to 11 kJ ml⁻¹ resulted in doubling of gastric emptying time (GET)

(Jobling, 1980), and, in rainbow trout, GET was increased from 10 to 15 h when the energy content of food was increased by 50 % (Grove *et al.*, 1978).

In this investigation the influence of dietary energy content on gastric evacuation and growth in the African catfish, *Clarias gariepinus* was studied using radio-opaque Ballotinis, following methods described by Hossain *et al.* (1998) (Chapter 5).

8.2 MATERIALS AND METHODS

8.2.1 Feed preparation

Four diets based on purified ingredients were prepared (Table 8.1) and Ballotini glass beads (136-002, 0.16-0.25 mm; Jencons Scientific) added at a concentration of 1% w/w. following the procedure described in Chapter 5.2.3. Four diets were formulated to cover a range of energy levels by varying lipid levels and α cellulose and maintaining approximately similar protein level. The prepared diets were analyzed for proximate composition following standard procedure AOAC (1990) and the results are presented in Table 8.1.

8.2.2 Experimental procedure

Three hundred fingerlings (0.99 ± 0.02 g in body weight) were randomly allocated to twelve, 40-cm diameter round plastic tanks within the recirculatory system described in Chapter 3.1. The fingerlings were fed three times daily at 0900, 1300 and 1700 h satiation with one of the four Ballotini marked diets. Weights and stomach capacities of 15 randomly selected fish from each treatment were measured following the procedure in Chapter 7.2.2.

Table 8.1 *Ingredients and proximate composition of experimental diets*

Ingredients	Diet I (%)	Diet II (%)	Diet III (%)	Diet IV (%)
Casein	36.00	36.00	36.00	36.00
Gelatin	8.50	8.50	8.50	8.50
Fish oil	6.75	9.00	11.25	13.50
Starch	30.00	30.00	30.00	30.00
CMC	2.00	2.00	2.00	2.00
Mineral premix	4.00	4.00	4.00	4.00
Vitamin premix	2.00	2.00	2.00	2.00
Cr ₂ O ₃	1.00	1.00	1.00	1.00
α Cellulose	9.75	7.50	5.25	3.00
Total	100.00	100.00	100.00	100.00
Nutrient composition (%)				
Moisture	6.01	6.35	6.59	6.77
Protein	41.56	41.84	42.05	42.21
Lipid	6.43	8.61	10.99	13.13
Ash	6.12	6.61	6.73	6.34
Energy kJ g ⁻¹	21.93	22.40	22.84	23.16

On day 41, the fish in all twelve tanks were fed to satiation with marked pellets as usual. After various deprivation periods between 0 and 48 h (0, 4, 8, 16, 24, 32 and 48 h), ten fish from each treatment were selected at random, anaesthetized, weighed and X-rayed. The changes in the amount of feed present in the stomach over time were used to estimate gastric evacuation rate (GER).

The energy digestibility (the proportion of dietary energy which is not excreted in the faeces and is assumed to be absorbed by the animal) of each of the test diets was determined by an indirect method, using chromic oxide as marker following the formulae:

Energy digestibility (%) =

$$100 - 100 \left(\frac{\% \text{ Cr}_2\text{O}_3 \text{ in food}}{\% \text{ Cr}_2\text{O}_3 \text{ in faeces}} \times \frac{\text{Energy in faeces}}{\text{Energy in food}} \right)$$

Faeces were collected twice daily using a tube and a filter and dried to constant weight at 60 °C. 50-100 mg triplicate samples of moisture free diet and faeces were then analysed for Cr₂O₃ content after the method of Furukawa and Tsukahara (1966) and energy content following standard procedure.

8.2.3 Data analyses

Stomach content was expressed in terms of % body weight:

$$S = \frac{W_f}{W - W_f} \times 100$$

where W_f = Weight of feed in stomach (g), W = Weight of fish (g). Ninety-five percent confidence limits (CL) were calculated as,

$$CL = \bar{X} \pm t_{0.05(n-1)} (S/\sqrt{n}),$$

where \bar{X} = mean, $t_{0.05 (n-1)}$ = t value from a two-tailed t table and S = Standard Deviation. A single classification ANOVA was carried out to investigate difference in stomach content at various deprivation periods between 0 and 48 h. The % body weight data were Arcsine transformed and a Bartlett's test revealed homogeneous variance (Sokal and Rohlf, 1981). Difference between regression coefficient was determined following the procedure of Fowler and Cohen (1990)

8.3 RESULTS

The increase in fish weight over time is shown in Table 8.2. Mean total weights (measured every 5 day) were not significantly ($P < 0.05$) different among the treatment groups except for last 5 days (Day 40 – 45) when the mean weights were significantly higher among fish fed diet III compared to fish fed other diets and fish fed diet I and IV performed the poorest weight gain. The data in Table 8.2 can be described by the exponential relationship, $W_t = W_0 e^{G_w t}$, where W_0 is initial fish weight, W_t the weight at time t and G_w the instantaneous growth rate.

Diet I;	$W_t = 1.09 \times e^{0.096t}$	$r^2 = 0.97$	$n = 5$	$P < 0.01$
Diet II;	$W_t = 1.10 \times e^{0.098t}$	$r^2 = 0.98$	$n = 5$	$P < 0.01$
Diet III;	$W_t = 1.05 \times e^{0.103t}$	$r^2 = 0.98$	$n = 5$	$P < 0.01$
Diet IV;	$W_t = 1.16 \times e^{0.084t}$	$r^2 = 0.96$	$n = 5$	$P < 0.01$

Specific growth rate was calculated using the formula

$$SGR = (e^{G_w} - 1) \times 100$$

Table 8.2 Mean weight (g) \pm 95 % confidence limit of African catfish fingerlings fed four diets over the experimental period. Mean weights with the same superscript are not significantly ($P < 0.05$) different between treatments

Day	Diet			
	I	II	III	IV
25	0.98 \pm 0.06	1.02 \pm 0.06	0.96 \pm 0.07	0.99 \pm 0.06
30	1.77 \pm 0.09	1.75 \pm 0.10	1.74 \pm 0.12	1.78 \pm 0.11
35	3.25 \pm 0.11	3.34 \pm 0.22	3.44 \pm 0.22	3.39 \pm 0.22
40	5.25 \pm 0.18 ^b	5.44 \pm 0.17 ^b	5.53 \pm 0.25 ^b	4.54 \pm 0.18 ^a
45	6.34 \pm 0.23 ^b	6.77 \pm 0.24 ^{bc}	7.14 \pm 0.20 ^c	5.10 \pm 0.54 ^a

Figure 8.1 shows the specific growth rates over the experimental period and again there was significant ($P < 0.05$) difference in SGR between the fish fed Diet IV and the other treatment groups.

Figure 8.2 shows the increasing stomach capacity in relation to fish weight (weight of feed measured after satiation meal) which can be expressed by linear relationship,

$S_t = S_0 \times W + c$; where S_t is stomach capacity (g) at time t , S_0 is initial stomach capacity (g) at beginning of the experiment, W is weight of fish in g and c is the constant;

Diet I;	$S_t = 0.076 \times W + 0.007$	$r^2 = 0.99$	$n = 5$	$P < 0.01$
Diet II;	$S_t = 0.075 \times W + 0.009$	$r^2 = 0.99$	$n = 5$	$P < 0.01$
Diet III;	$S_t = 0.064 \times W + 0.029$	$r^2 = 0.99$	$n = 5$	$P < 0.01$
Diet IV;	$S_t = 0.062 \times W + 0.022$	$r^2 = 0.99$	$n = 5$	$P < 0.01$

Stomach capacity in terms of percent body weight declined with increasing body weight.

Table 8.3 shows the stomach contents of experimental diets from the stomach of 41 day old fish after various deprivation periods. The data can be described by the equation

$$S_t = S_0 e^{-Rt} \quad (\text{Elliott and Persson, 1978})$$

where S_0 = stomach contents after first feeding to satiation, S_t = stomach contents after time t , R is the rate constant, gastric evacuation rate and t is the time in hours.

Significant relationships were found for all four sets of data in Table 8.3. The relationships for the four diets are:

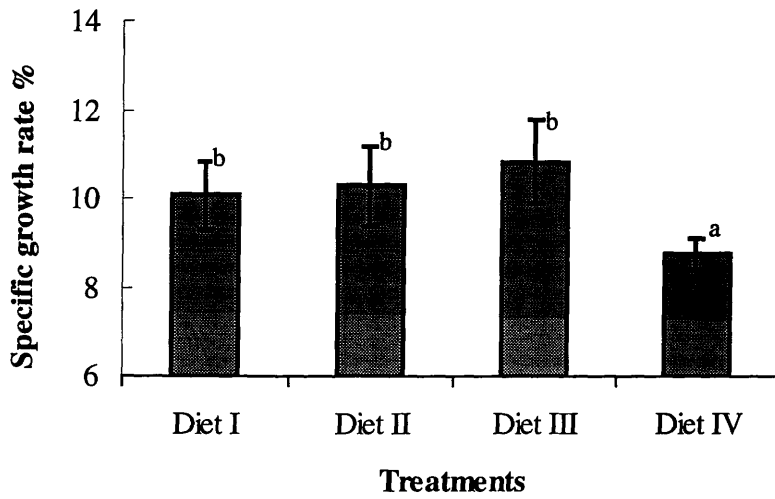


Figure 8.1 *Specific growth rate in fish fed four diets with different energy levels. Error bars represent 95% confidence limits. SGRs with same superscript are not significantly ($P < 0.05$) different*

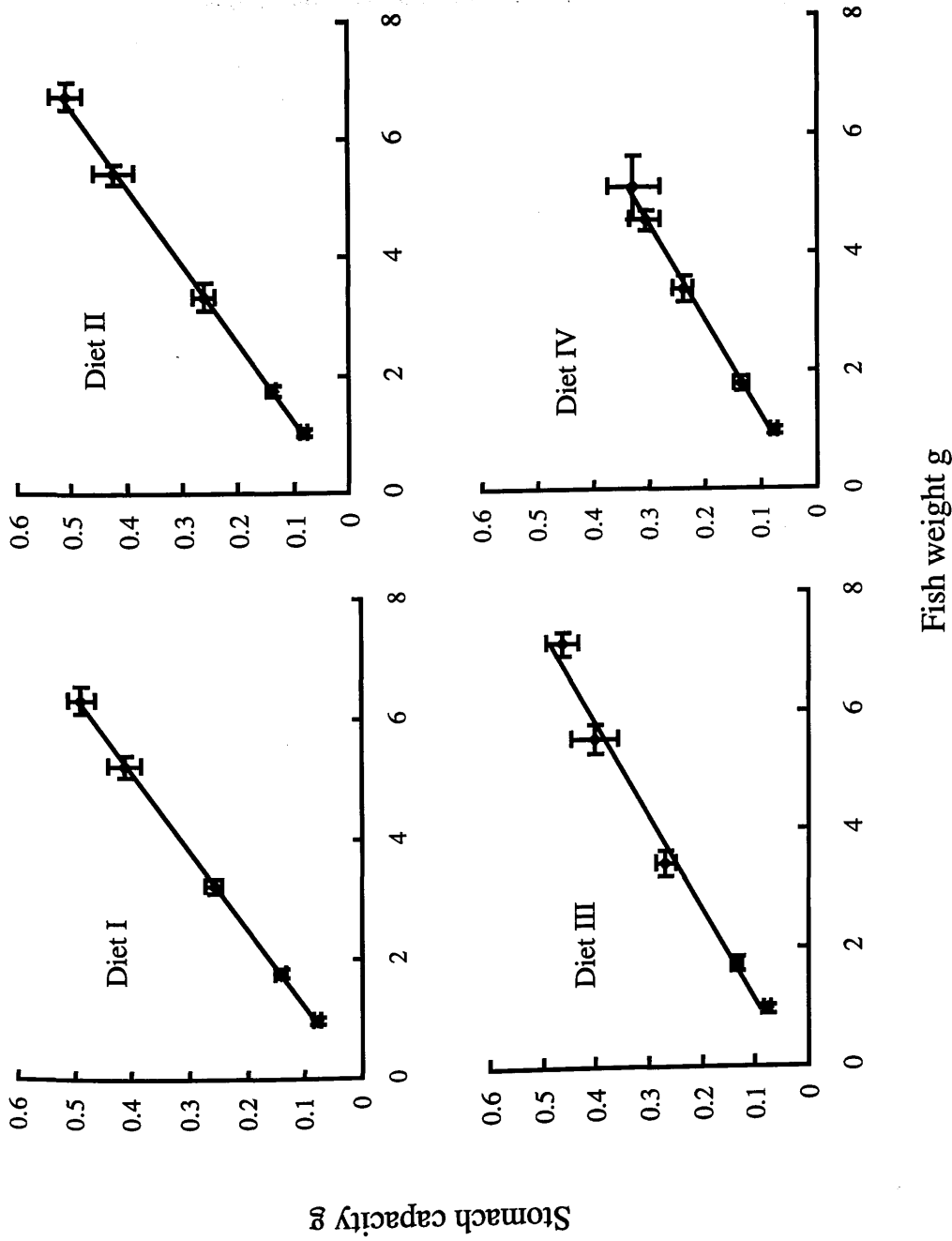


Figure 8.2 The changes of stomach capacity with increasing weight of fish fed four different diets. Error bars represent 95% confidence limit.

Table 8.3 Mean stomach content (% body weight) \pm 95 % confidence limit of African catfish fingerlings fed four diets after various deprivation periods. The stomach contents with same superscript are not significantly ($P < 0.05$) different after different deprivation period

Deprivation period (h)	Diet			
	I	II	III	IV
0	7.70 \pm 0.65 ^a	7.59 \pm 0.45 ^a	6.54 \pm 0.85 ^a	6.49 \pm 0.98 ^a
4	4.76 \pm 0.68 ^b	4.55 \pm 0.55 ^b	5.44 \pm 1.03 ^b	5.35 \pm 0.50 ^a
8	2.98 \pm 0.32 ^c	3.01 \pm 0.28 ^c	4.53 \pm 0.29 ^b	4.05 \pm 0.40 ^b
16	1.73 \pm 0.17 ^d	1.83 \pm 0.15 ^d	3.14 \pm 0.25 ^c	3.02 \pm 0.30 ^c
24	0.98 \pm 0.17 ^e	1.05 \pm 0.12 ^e	2.18 \pm 0.37 ^d	2.22 \pm 0.21 ^d
32	0.31 \pm 0.06 ^f	0.45 \pm 0.06 ^f	1.51 \pm 0.12 ^e	1.67 \pm 0.26 ^e

Diet I;	$S_t = 7.25 \times e^{-0.093t}$	$R^2 = -0.98$	$n = 6$	$P < 0.01$
Diet II;	$S_t = 6.73 \times e^{-0.083t}$	$R^2 = -0.99$	$n = 6$	$P < 0.01$
Diet III;	$S_t = 6.54 \times e^{-0.046t}$	$R^2 = -0.99$	$n = 6$	$P < 0.01$
Diet IV;	$S_t = 6.14 \times e^{-0.042t}$	$R^2 = -0.99$	$n = 6$	$P < 0.01$

Figure 8.3 shows the gastric evacuation rates in fish fed four different diets. While evacuation rate was highest in fish fed diet I and II and lowest in fish fed diet IV, there was no significant difference between the fish fed diets I and II.

The energy digestibility (%) is found to be significantly different between the four diets, highest in diet I and lowest in diet IV (Table 8.4).

The average weekly feed consumption of *C. gariepinus* fingerling over the experimental period is estimated using the formula detailed in Chapter 6.4 and presented in Table 8.5. Average weight gains are not significantly ($P < 0.05$) different in fish fed Diet I, II and III and fish fed diet IV showed poorer weight gain. Weekly feed consumption was highest in fish fed diet I and II and lowest in fish fed diet IV. While FCR was highest in fish fed diet I and lowest in fish fed diet III, there was no significant difference between the fish fed diet II and IV. The feed utilization efficiency (g gain per kJ energy) was highest in fish fed diet III and there was no significant difference between other treatment groups (Table 8.5).

8.4 DISCUSSION

There is wide agreement that the exponential model of Elliott & Persson (1978) can be used to approximate the evacuation of small easily digestible feed particles such as pellets in the stomach (Persson 1986; Macpherson *et al.* 1989; Haylor 1993b).

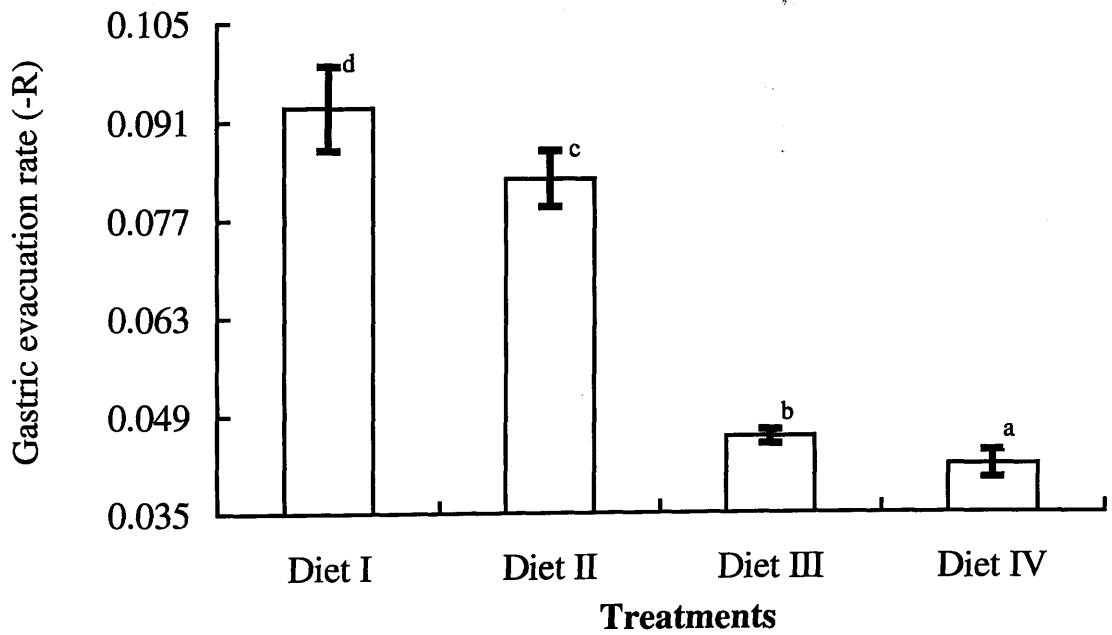


Figure 8.3 Gastric evacuation rates in *C. gariepinus* fingerlings fed diets with four different energy levels. Error bars represent 95% confidence limit. GERs with same superscript are not significantly ($P < 0.05$) different

Table 8.4 Energy digestibility of four dietary formulations fed to *C. gariepinus* fingerlings ($n = 3$)

Diet	DE \pm CL kJ g ⁻¹
I	91.33 \pm 0.19
II	90.79 \pm 0.28
III	89.78 \pm 0.14
IV	89.59 \pm 0.06

Table 8.5 Average weight per fish, feed consumption, feed conversion ratios and feed utilization efficiencies over the 20-day experimental period. Data with the same superscript are not significantly ($P < 0.05$) different

Diet	Average weight gain g fish ⁻¹ week ⁻¹	Average feed consumption g fish ⁻¹ week ⁻¹	¹ FCR	² FUE
I	1.34 ^b	1.77 ^b	1.40 ^c	0.045 ^a
II	1.44 ^b	1.65 ^b	1.18 ^b	0.049 ^a
III	1.55 ^b	1.00 ^a	0.64 ^a	0.079 ^b
IV	1.03 ^a	0.81 ^a	1.00 ^b	0.050 ^a

¹Feed conversion ratio (g consumption \cdot g gain⁻¹)

²Feed utilization efficiency (g gain \cdot kJ total energy⁻¹)

Haylor (1993b) derived an equation for estimating feed consumption in relation to feeding schedule over 12 h, (detailed in Chapter 6.4)

$$C = (S_0 \times W + c) \times \left\{ (1 - e^{-12R}) + \frac{12}{t} (1 - e^{-Rt}) \right\}$$

where C = consumption, W = weight of fish, c = constant, S₀ = stomach contents after first feeding to satiation, S_t = stomach contents after time t, R is the rate constant, gastric evacuation rate and t is the time in hours.

Estimation of feed consumption following the equation shows that fish fed the low energy diet (Diet I and II) consumed more feed. The lowest feed intake was apparent among the fish fed the highest energy level diet (diet IV) (Table 8.5). The average weight gain and specific growth rate, G_w (Fig. 8.1) did not differ significantly among fish fed diets I, II and III. Again the high energy diet IV showed the lowest weight gain and reduced G_w. This is in agreement with the results of an experiment conducted with three energy level diets with *C. gariepinus* by Machiels and Henken (1985).

Increasing the dietary energy content led to a reduction in gastric evacuation rate (Table 8.3 and Fig 8.3), as found in other studies (Flowerdew & Grove 1979; Jobling 1981,1988). While the feeding of high energy diets led to a decrease in gastric evacuation rate, the influence of digestible energy level on gastric evacuation appears to be of lesser importance than that of total energy. The present results show that the rates of gastric evacuation are more closely related to total energy than to digestible energy

Gastric evacuation rate (R) = -1.11 + 0.046 Total energy content

$$(n = 4, r^2 = 0.93 \text{ and } P < 0.05)$$

Gastric evacuation rate (R) = -1.66 + 0.079 Digestible energy content

$$(n = 4, r^2 = 0.85 \text{ and } P < 0.05)$$

In an experiment with plaice, *Pleuronectes platessa*, Jobling (1981) suggested that total energy was more important than digestible energy in determining rates of gastric evacuation and feed intake. From this experiment, it is also obvious that gastric evacuation is less dependent on digestible energy ($r^2 = 0.85$) than energy digestibility ($r^2 = 0.99$) (Fig. 8.4); which itself heavily depends on total energy ($r^2 = 0.99$; Fig. 8.5).

Feed consumption and feed energy intake differed markedly among groups, with food conversion ratios being lowest and food utilization efficiencies ($\text{g gain} \cdot \text{kJ total energy}^{-1}$) being highest among catfish fed the intermediate energy level diet (diet III, 22.84 kJ g^{-1}). It is thus apparent that fish ingest more when fed low energy diet with high associated evacuation rate, even though, as in the present experiment, this results in high FCR and low food utilization efficiency (FUE). On the other hand when catfish were fed on a high energy diet, although they ingested less and there was a low evacuation rate, the FCR remained high and the FUE low by comparison with results from fish fed the intermediate energy level diets. Therefore, it seems probable that the total energy of the diet limits the amount of digestible energy and this would have consequence for growth if the diet is of poor digestibility. This explains the lower growth from high energy diet in the present experiment.

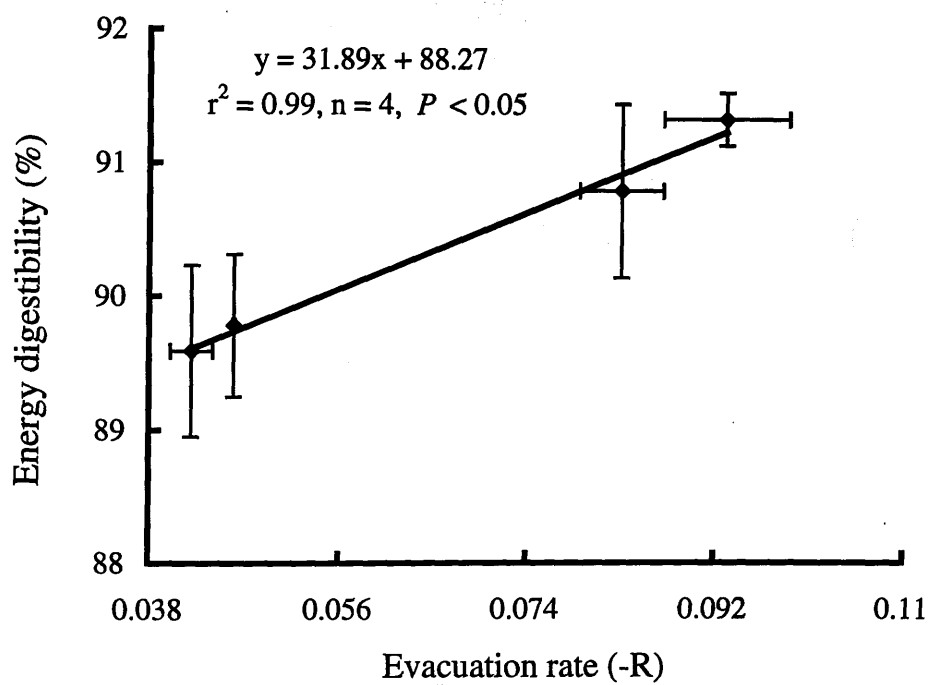


Figure 8.4 *The changes of evacuation rates with increasing digestibility. Error bars represent 95% confidence limit.*

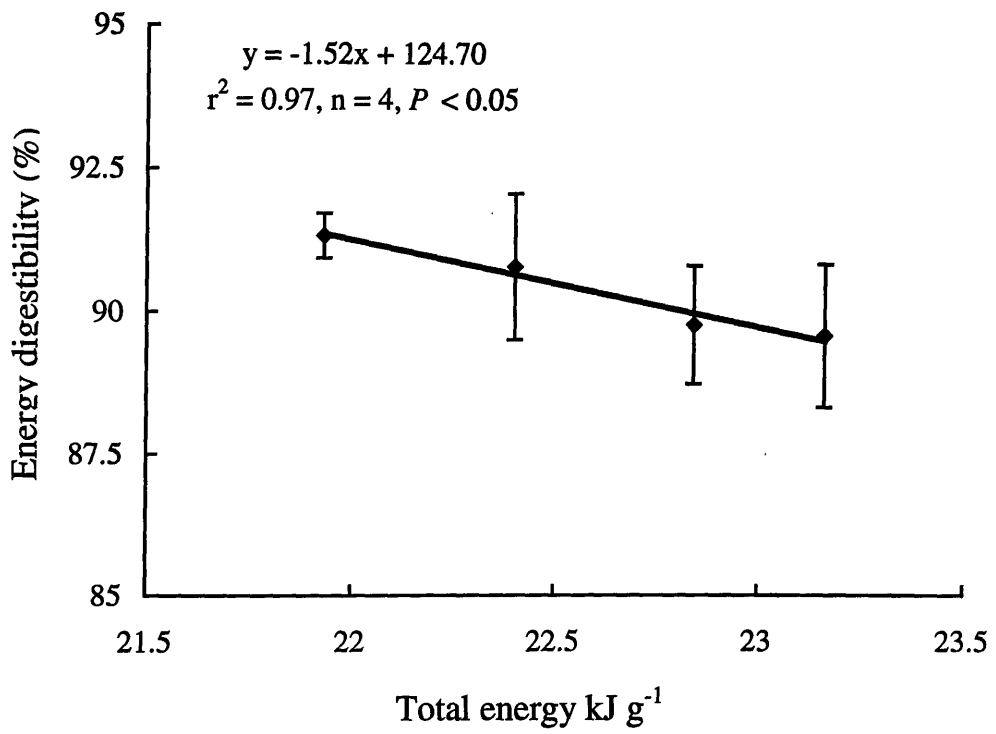


Figure 8.5 Relationship between total energy and energy digestibility. Error bars represent 95% confidence limit

The information contained in Chapter 9 was presented in the second COST 827 workshop on "The feeding behaviour of fish in culture" to be held in Umeå, Sweden, 20-22 August 1998.

Chapter 9

EVALUATION OF DIEL RHYTHMS OF FEEDING ACTIVITY IN AFRICAN CATFISH

9.1 INTRODUCTION

Although most feeding schedules for commercially and experimentally cultured fish assume that fish readily ingest food whenever given, there is no data to support this assumption. Most researchers who have studied the feeding activity of fish under experimental conditions with constant access to a source of food, or by means of self-feeders, have observed conspicuous diel feeding rhythms (Barahona-Fernandez, 1979; Eriksson and Van Veen, 1980; Sundararaj *et al.*, 1982; Steelle, 1985; Boujard *et al.*, 1990; Singh and Srivastava, 1993; Boujard, 1995; Kadri *et al.*, 1997), suggesting that control of feeding time is not necessarily regulated by natural variations in food availability. However, in a number of the studies undertaken to examine feeding rhythms, fish were fed during the normal working hours and were usually given a single meal per day; clearly this experimental protocol is an inappropriate design for studies of the animal's normal biology. Very few studies have focused on fish given free access to food for 24 hour a day (Boujard and Letherland, 1992a).

Although a number of researchers have stated that *C. gariepinus* is a nocturnal feeder (Viveen *et al.*, 1985; Hecht and Appelbaum, 1988; Britz and Pienaar, 1992), intrinsic feeding rhythms has not been evaluated under conditions of continuous feed supply. More detailed information is therefore required on daily pattern of appetite so that feeding schedules can be tailored to the feeding rhythm. The aim of this experiment is to investigate feeding rhythms of African catfish under conditions of constant feed access and photoperiod (LD 12:12), to establish peak feeding times (if any) and to determine the capacity of *C. gariepinus* to adapt to a feeding where access to food is restricted to the photophase only.

9.2 MATERIALS AND METHOD

9.2.1 Fish

Fingerling *C. gariepinus* (113.48 ± 1.87 mm total length) were obtained from broodstock maintained in the Institute of Aquaculture, University of Stirling following the procedure detailed in Chapter 3.1. After weaning, fish were fed continuously by a belt feeder on finely ground commercial trout diet for a further 16 days and then on 2 mm pellets until the end of the experiment in order to avoid inducing a feeding rhythm.

9.2.2 Experimental procedure

Trials were undertaken in a 1-m diameter self-cleaning fibreglass tank (water depth = 15 cm) with forty fingerlings within a recirculation system. Water temperature was maintained at 30 °C and photoperiod maintained as light:dark 12:12 h (Light level; 80 lx in light phase and 0 lx in dark phase), as measured by a light meter (Digital Lux Meter Model EP628, Eurisem Technics, Taiwan) installed in the tank within view of the camera. Between day 45 and day 59 (after hatching), 24 h video recordings were made following the procedure of Batty (1983). During the first phase of the recording (5 days) fish were fed for 24 h, then from the 6th day for the next 5 days fish had access to feed only during daytime. During an additional phase of 5 days, the fish again had continuous access to food (Table 9.1). Data were collected on the number of feeding responses (attacking or attempting to attack food pellets) and movements over the experimental period.

Table 9.1 Feeding schedule during the experimental period

Date Starts at 18.00 h	Day (Age)	Day (Expt)	Feeding time h	Feeding (% body weight)
15.01.98	45	1	24 h	6-7 (<i>ad libitum</i>)
16.01.98	46	2		
17.01.98	47	3		
18.01.98	48	4		
19.01.98	49	5		
20.01.98	50	6	12-h (During day only)	
21.01.98	51	7		
22.01.98	52	8		
23.01.98	53	9		
24.01.98	54	10		
25.01.98	55	11	24h	
26.01.98	56	12		
27.01.98	57	13		
28.01.98	56	14		
29.01.98	59	15		

9.2.3 Video observation

An infrared video observation system was set up in the Tropical Aquarium for recording the movement and feeding behaviour of the fish during the experiment (Figure 9.1). The fish were viewed by an infra-red sensitive video camera (Simrad Osprey OE1356) mounted approximately 1 m above the tank pointing downward and using four infrared light emitting diodes (Opto-Diode OD100) as the light source. The tank floor was covered with “Scotchlite ©” a retro-reflective material (3M company). A plastic infra-red transmitting filter (no. 177-143, Farnell, Leeds) covered the lens of the camera enabling feeding and movement in total darkness as well as in normal lighting conditions to be observed. The camera was connected by a waterproof cable to two video recorders and a monitor. The first video recorder was a time-lapse type (Panasonic AG 6124) which recorded feeding activity of fish for 24 h while the second one was conventional VHS video recorder programmed to record 20 minutes in every 3 h over a 24 h period. The video tapes were replayed on the time-lapse recorder for analysis at various speeds as well as frame-by-frame so that rapid feeding responses could be identified. Recording on the normal recorder allowed comparison with time-lapse recordings in which some temporal detail may have been lost.

The feeding activities were counted manually by playing the tape in slow motion and frame by frame where necessary while the movement of fish were counted using Mlogger a “spatial actograph” computer programme written by Dr M.T. Burrows and Mr S.G. Gontarek of Dunstaffnage Marine Laboratory, Oban, Scotland, UK. This software, which is described in more detail by Burrows (in prep.), compares captured images and records differences between them as movements within each cell of a 9 by 12 grid. The time lapse videos were replayed at their original recording speed into a

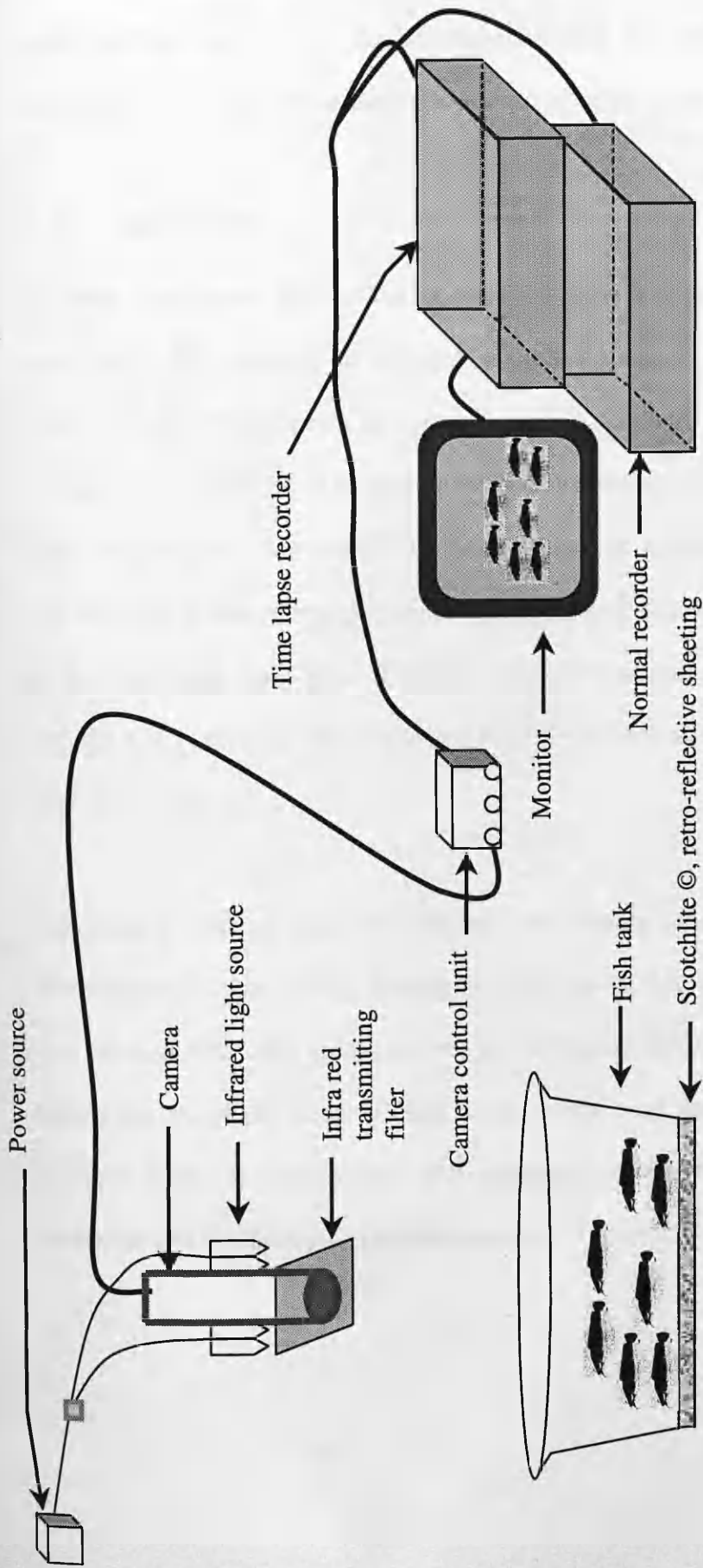


Figure 9.1 Video recording unit

computer fitted with a frame capture card and running this software. An index of movement per unit time over the experimental period was produced for each of 4 quadrants of the tank; each quadrant contained 12 of the activity cells.

9.3 RESULTS

A clear crepuscular rhythm was observed (Figure 9.2) in the number of feeding activities in first phase of the experiment (ANOVA based on hourly counting $F_{5,23} = 1.64$, $P < 0.05$). There was a very marked peak in the hours between 20.00 to 23.00 h, rising again at 06.00-08.00 h. When feeding was restricted to only the light phase there was a single peak at dawn and the feeding activity was higher in the first half of the day (08.00-13.00 h) than during the remaining day hours (14.00-19.00 h). In the final phase of the experiment, the pattern of feeding rhythm was almost identical to the first phase and the hourly feeding bites in the two phases were not significantly different ($F_{1,7} = 5.32$, $P < 0.05$).

Following a restricted phase of only day time feeding (2nd phase), fish were again given access to 24-h feeding, feeding activities on the first day were somewhat lower than on days with 24-h access to feed, the difference was not significant ($P < 0.05$). During the 2nd phase, when fish had access to feed only during day time, the feeding activities decreased by more than 30 % compared to the fish with 24-h access to feed during the first and final phase of the experiment (Figure 9.3).

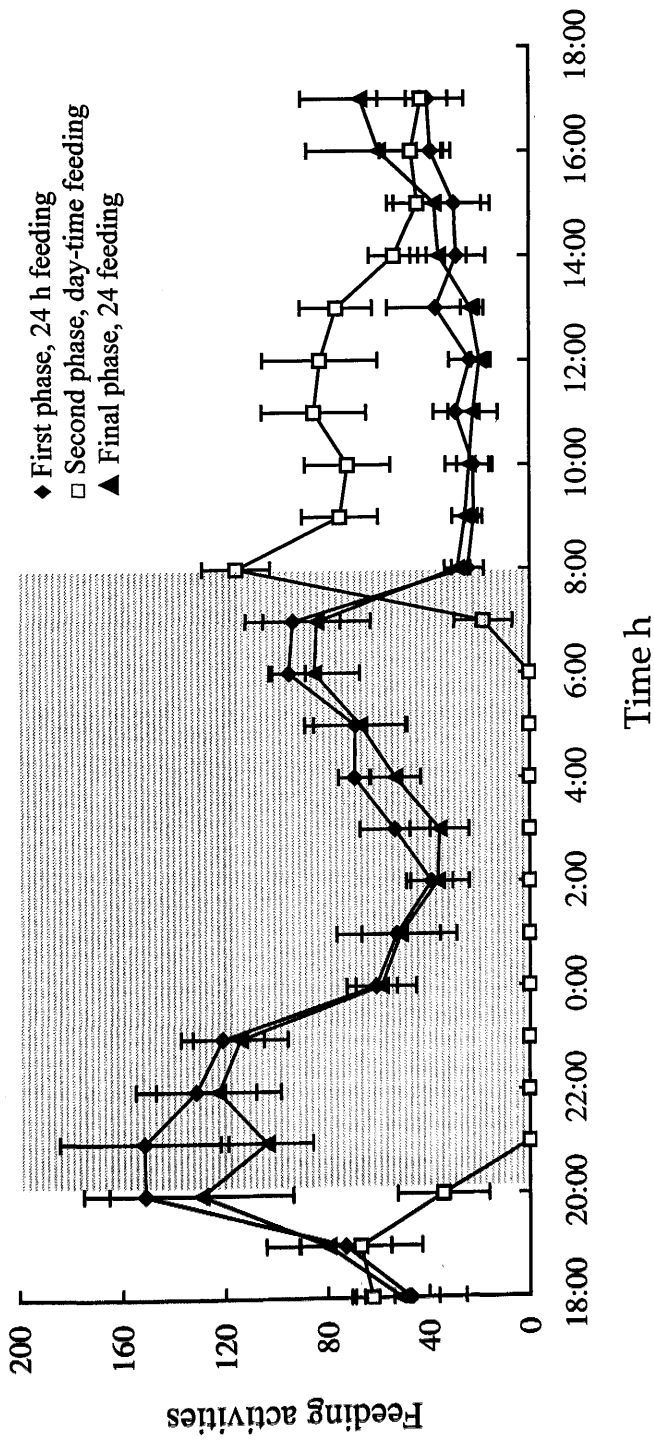


Figure 9.2 Mean feeding activities (counted as mean number of feeding responses in the whole tank in a particular hour from the feeding activities of 5 days) over the experimental period in three phases. Error bars represent 95% confidence limit. Shaded areas indicate dark phase.

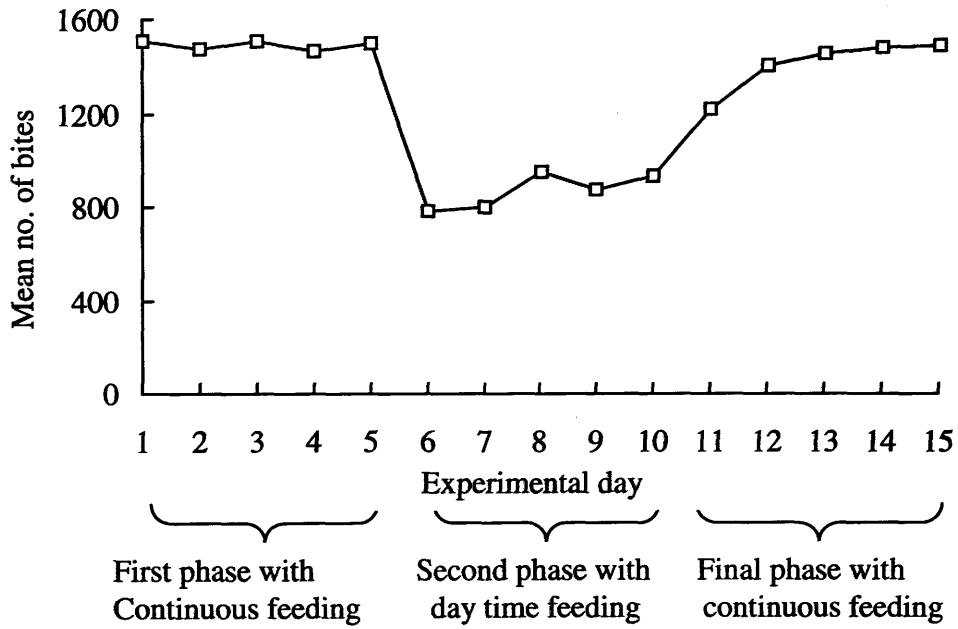


Figure 9.3 Mean no. of bites in a day over the experimental period

There was a clear diel rhythm in the movement of fish counted in the whole tank and below the feeder (Figures 9.4 and 9.5) and both matched with the feeding rhythm in all three phases. A series of regression analyses shows that the relationship between feeding activities and normal movement was closer during the first phase of the experiment and the weakest relationship was found when fish had access to feed only in daytime. However, the relationships between total movement in the tank and movement below the feeder were very close in all three phases of the experiment (Table 9.2).

Daily feed intake (%) during the first and final phases is presented in Figures 9.6a and 9.6b, which show that more than 70 % of the total feeding activities occurred during night time. In the second phase, when feed was restricted to only the light phase, more than 59 % of feeding activities were limited to the first half of the day (Figure 9.7).

Feeding activity (No. of bites · 24 h period⁻¹) decreased significantly during the second phase of the experiment and the mean number of bites was only 60 % of those compared when fish had access to feed for 24 h in the first and final phases. Although fish had access to feed restricted to day time only for 5 days during the 2nd phase, feeding activity in final phase was not significantly ($F_{1,8} = 5.32$) different with that during the first phase of the experiment (Figure 9.8).

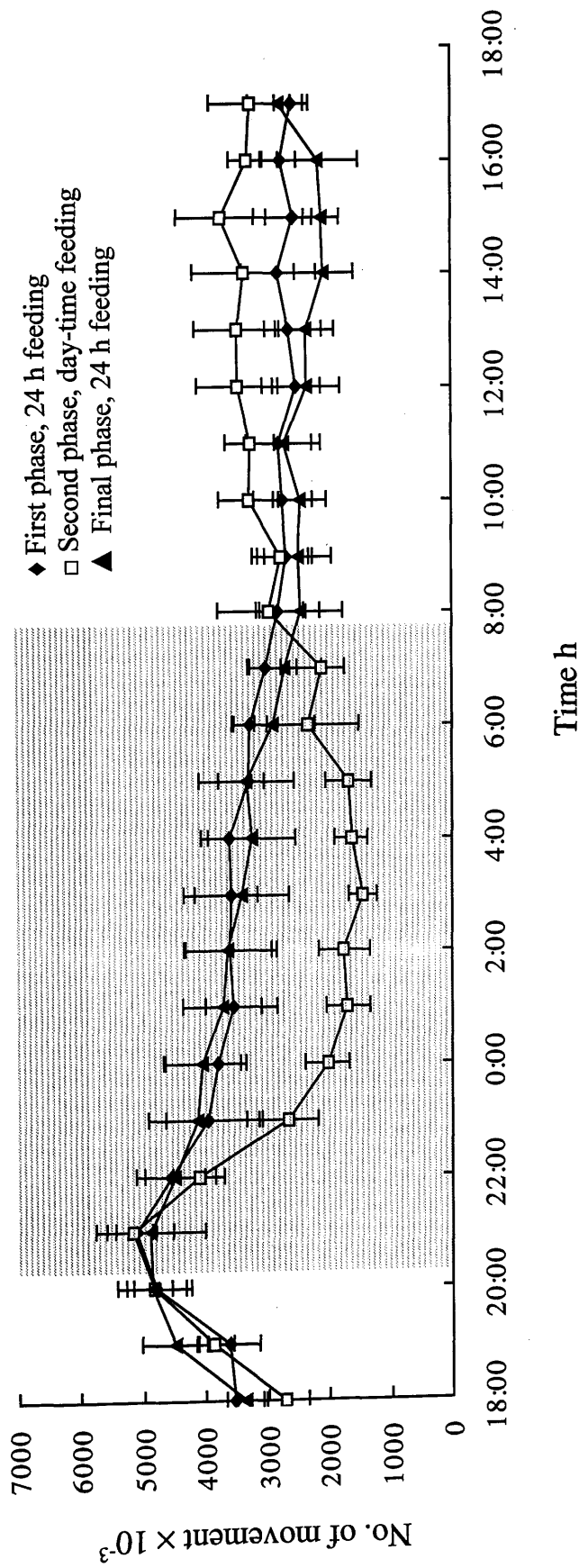


Figure 9.4 Mean number of movements in the whole tank over the experimental period. Error bars represent 95% confidence limit. Shaded areas indicate dark phase.

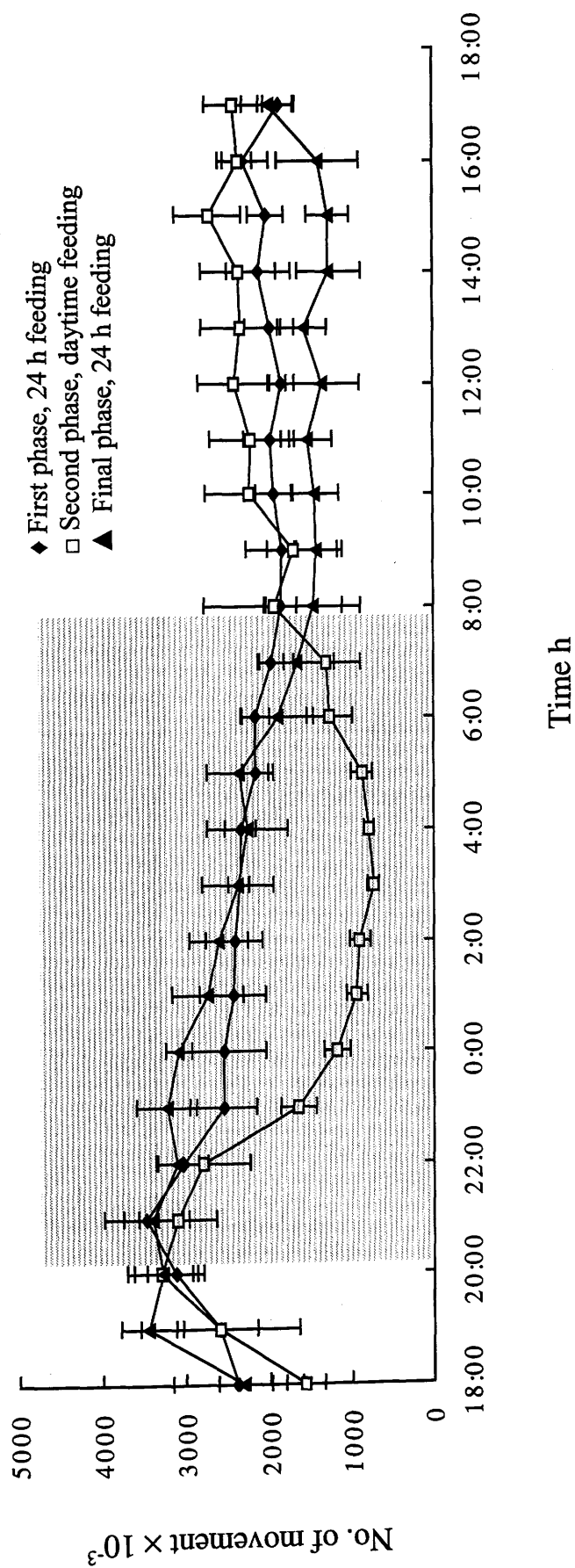


Figure 9.5 Mean number of movements below the feeder over the experimental period. Error bars represent 95% confidence limit. Shaded areas indicate dark phase.

Table 9.2 'r' values of the regression between feeding activity and normal movement of experimental fish in three phases

Experimental phase	Variables		
	Feeding Vs movement in whole tank	Feeding Vs movement below feeder	Movement in whole tank Vs below feeder
First phase (24-h feeding)	0.88	0.84	0.96
Second phase (day time feeding)	0.35	0.42	0.98
Final phase (24-h feeding)	0.75	0.73	0.98

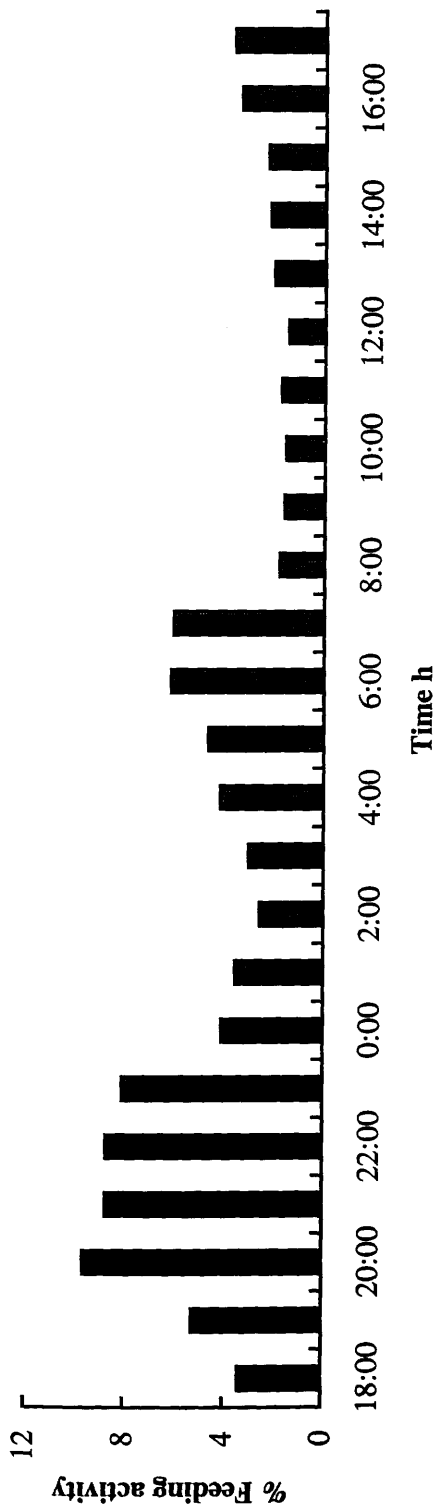


Figure 9.6a Mean % feeding activity in first and final phase of the experiment when fish had access to feed for 24 h

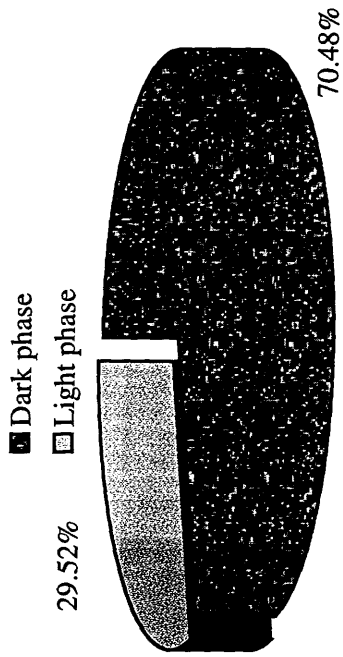


Figure 9.6b % feeding activities in first and final phase when fish had feed access for 24 h

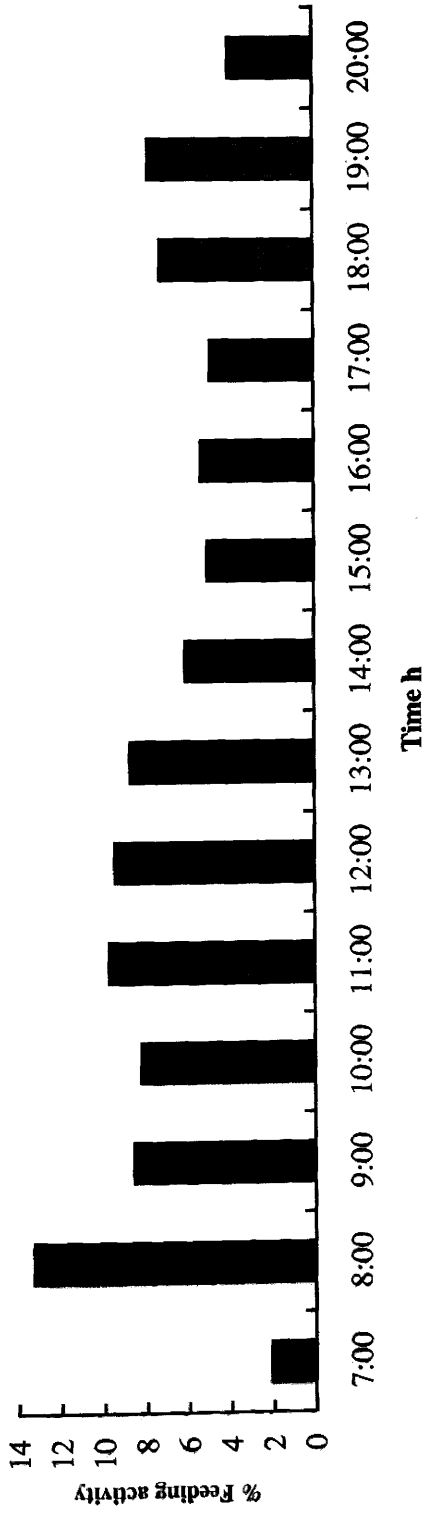


Figure 9.7 Mean % feeding activity in second phase of the experiment when fish had access to feed only in day time

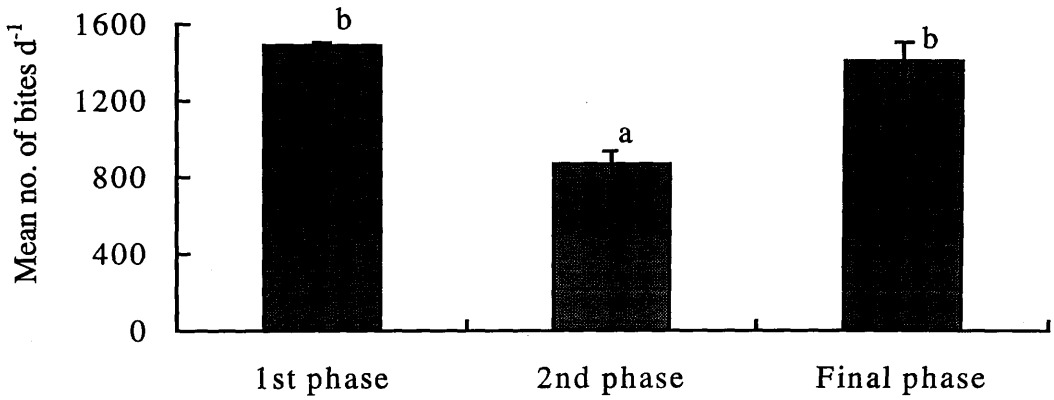


Figure 9.8 Mean number of bites d^{-1} in three phases. Error bars represent 95 % CL. Data with same superscripts are not significantly ($P < 0.05$) different

9.4 DISCUSSION

Activities recorded by two types of recorder were found to be in good agreement. Recordings done by the time-lapse recorder were vivid and sufficiently clear to quantify both movement and feeding activities. No mortality was recorded during the experiment. The feed waste was negligible (lower than 1%).

The diode used in this experiment had long wavelength (940 nm) and restricted bandwidth (15 nm) and emitted only 15 mW total radiant energy which was well beyond the spectral sensitivity of any fish since the cone pigments of most fish species have maximum absorption peaks around 455, 530 and 625 nm (Boujard *et al.*, 1992). The same author also observed that use of low intensity coloured light did not change the nocturnal feeding pattern of Armoured catfish, *Hoplosternum littorale* and that the darker phase was perceived as the scotophase by the fish regardless of the source of light used.

This experiment clearly shows that voluntary food intake in African catfish follows a diel cycle. Although food consumption during the light phase was erratic, the majority of food ingested occurred during the phase of darkness. The feeding activity began at the onset of the dark phase, with a very clear peak between 20.00 to 23.00 h and again increased although to a lesser degree, before the onset of the light phase (06.00 to 08.00). These findings are in agreement with Britz and Pienaar (1992), who observed African catfish as primarily a nocturnally active, tactile feeder, with a distinct crepuscular activity pattern.

In the present experiment *C. gariepinus* was found to be most active at night. This was conclusively demonstrated by Bruton (1979a) in Lake Sibaya, where *C. gariepinus*

hunts most actively at night and by the behavioral observations made under controlled condition by Britz and Pienaar (1992), which demonstrated that *Clarias* juveniles are negatively phototactic and display higher levels of swimming and browsing activity in darkness. Moreover, *C. gariepinus* is anatomically better adapted to seek prey and avoid being preyed upon under condition of low light and darkness. Acuity of vision is very poor and the fish relies primarily on the tactile, chemosensory and electrosensory functions of its four pairs of curcumoral barbels to detect food or prey and to explore its physical environment (Lissman and Machin, 1963; Bruton, 1979b; Hecht and Appelbaum, 1988). The relative importance of eyes and barbels in prey capture by juvenile *C. gariepinus* has been investigated by Hecht and Appelbaum (1988) who described the species as a tactile and possibly chemoreceptive rather than visual predators.

Although no clear peak was present (except a single and very narrow peak just after the onset of day light) in fish fed only during daytime, fish tended to take more food during first half of the day. The most likely explanation is that, fish were deprived of food throughout the night and when in the morning they had access to feed, there was an immediate rise in feeding activity. After this feeding remained almost constant throughout the first half of the day and then decreased during the second half.

It is evident, however, that *C. gariepinus* is not only active at night but it will opportunistically adopt a searching and feeding behaviour pattern if food or prey are available only during the light phase. This was shown experimentally by Bruton (1979b), in Lake Sibaya, and in laboratory conditions by Britz and Pienaar (1992). The present study has, however, demonstrated that when feeding is restricted to only the

light phase, fish displayed reduced browsing and swimming activity as well as feeding activity by comparison with when food was continuously available.

Fish activity in this experiment was clearly related to appetite when feed was given continuously. Kadri *et al.* (1991) also observed that feeding in Atlantic salmon, *Salmo salar* was closely related to swimming activity. *C. gariepinus* were more active at night when they had access to feed 24-h than fish those had access only in day time. However day light activity patterns did not differ significantly among treatment groups. From the regression between feeding activity and movement, it is clear that, feeding and movement of fish were more closely related in fish with 24-h access to food than among those fed only during day time.

In rainbow trout, *Oncorhynchus mykiss*, more than 98% of the feeding demand occurred during the photophase, regardless of the photoperiod, with a main peak at dawn and an occasional peak at dusk (Boujard and Letherland, 1992b). Boujard *et al.* (1990) observed a clear feeding rhythm in Atipa, (*Hoplosternum littorale*) a siluriform fish of the *Callichthyidae* family. Feed demand began at dusk and increased throughout the night with a peak at 0200 - 0500 hours, during which 3-h period the fish ate 40% of their total daily ration. In this experiment, *C. gariepinus* took more than two third of their total ration at night when they had constant access to food.

The effect of restricted feed access on feed intake suggests that under culture conditions, this fish species should be fed at night. In general, catfish do not refuse food during the day time. However, studies on the effect of meal time on feed intake and growth performance show poorer feed intake and growth performance fed by light

phase (Hogendoorn, 1981; Sundararaj *et al.*, 1982; Noeske *et al.*, 1985; Kerdchuen and Legendre, 1991)

9.5 CONCLUSION

C. gariepinus feeds by night. The observed diel rhythm suggests that the appetite of this fish may be under the control of an endogenous clock rather than the availability of feed. The effect of enforced diurnalism on growth performance needs further investigation. Indeed, it is of practical interest to determine if the observed reductions in feed intake also occurs under field conditions and what the effect is on growth performance, FCR and physiological condition of fish.

Chapter 10

THE OPTIMIZATION OF GROWTH, SURVIVAL AND PRODUCTION OF AFRICAN CATFISH

10.1 INTRODUCTION

One problem facing fish culturists is the need to obtain a balance between rapid fish growth and optimum use of the supplied feed. When fish are fed using self-feeders, growth and feed conversion are expected to be improved because the fish can regulate feed intake in relation to their energy needs (Kaushik and Médale, 1994) and their feeding rhythms (Boujard and Letherland, 1992a). In some species, such as the rainbow trout, *Oncorhynchus mykiss*, self-feeding can, however, lead to feed waste if the self feeding activity is too high (Boujard and Letherland, 1992b; Brännäs and Alanära, 1994). Nevertheless, a restriction of the time during which feed is made available may lead to reduced feed waste without any deterioration in growth performance, provided that the feeding periods are in phase with the feeding rhythms (Boujard *et al.*, 1996).

Time of feeding has been reported to affect feed intake or growth performance in goldfish, *Carassius auratus* (Noeske and Spieler, 1984), Indian catfish, *Heteropneustes fossilis* (Sundararaj *et al.*, 1982), channel catfish (Noeske *et al.*, 1985) and rainbow trout (Boujard *et al.*, 1995). In an experiment with African catfish, *Heterobranchus longifilis*, Kerdchuen and Legendre (1991) observed that fish fed during the night had higher growth rate than those fed during day time at the same feeding rate (3 % bw d⁻¹).

The effects of abiotic factors – density, shelter and photoperiod - and biotic factors (gastric evacuation, pellet size, energy levels) on growth and survival of *C. gariiepinus* and its diel rhythms were evaluated in earlier experiments. Here, findings from the experiments were combined together and growth, survival, feed utilization, and FCR of the *C. gariiepinus* fingerlings compared with control treatments.

10.2 MATERIALS AND METHODS

10.2.1 Fish

Three hundred and seventy five 25-day old fingerlings (0.98 ± 0.02 g) were randomly allocated to fifteen, 40-cm diameter round plastic tanks within the recirculation system described in Chapter 3.1 at a stocking density of 25 fish per tank (5 fish l^{-1}).

10.2.2 Feeding technique

Fish were fed on 2 mm trout pellet (22.7 kJ g^{-1} total energy) over the experimental period (25 day) following the feeding schedule detailed in Table 10.1. In treatments C and D, feed was administered by hand, while in the other treatments (A, B and E) feed was dispersed by belt feeders.

Before starting the experiment, different numbers of pellets were weighed (dry weight) and the weights plotted against pellet number to establish a relationship (Figure 10.1),

$$\text{Pellets weight} = 0.0127 \times \text{Number of Pellets} - 0.0002;$$

$$r^2 = 0.997; P < 0.01 \text{ and } n = 88.$$

Uneaten feed from the tanks was removed at 0800, 1400 and 2000 h every day, the numbers of pellets counted and their weight determined. The pellets remained intact during the time between feeding and collection of uneaten feed. Fish were weighed collectively every five days and mortalities recorded.

Table 10.1 Feeding schedule, mode of feeding, and timing of feed application in different treatments over the experimental period

Treatment	Feeding*	Mode	Timing	Feed/meal	Time/meal	No. of meals
A	24 h	Continuously	Continuously	100%	24 h, Belt	1
B	24 h	Continuously	18.00-02.00, 02.00-10.00, 10.00-18.00	51%, 31% and 18%	24 h (8 h), Belt	1(3)
C	During day only	4 h interval	08.30, 12.30, 16.30, 20.30	46%, 18%, 18% and 18%	5 min, Hand	4
D	During day only	4 h interval	09.00, 13.00, 17.00	34%, 33% and 33%	5 min, Hand	3
E	During night only	4 h interval	19.00-23.00, 03.00-07.00	60% and 40%	4 h, Belt	2

* First 5 days of the experiment fish were fed at 12 % bw day⁻¹ and then 10 % for 10 days and finally 8% for last 10 days

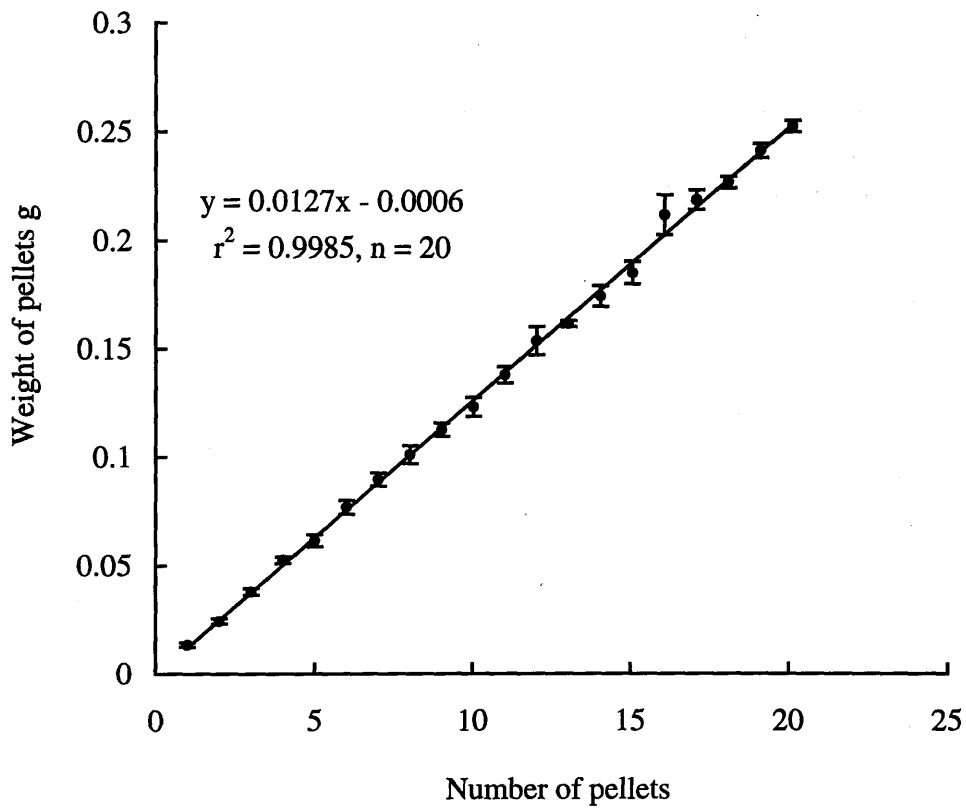


Figure 10.1 Regression between numbers and weights of pellets used in the experiment

10.3 RESULTS

The increase in fish weight over time is shown in Table 10.2. Mean total weights (measured every 5 days) were significantly ($P < 0.05$) different among the treatment groups and weights in the treatments B and E were significantly higher than those in the other treatments. The data in Table 10.2 can be described by the exponential relationship $W_t = W_0 e^{G_w t}$, where W_0 is initial fish weight, W_t the weight at time t and G_w the instantaneous growth rate. Exponential growth models for different treatments are presented in Table 10.3. Figure 10.2 shows the specific growth rates (calculated as $(e^{G_w} - 1) \times 100$) over the experimental period and again there was a significant difference ($P < 0.05$) in SGR between the fish in Treatments B and E and fish in the other treatments. Figure 10.3 shows feed intake and waste feed expressed as percentages of total feed given. The greatest amount of feed was wasted in Treatment D; food waste was least in Treatment B.

Food conversion ratios (FCRs) over the experimental period are presented in Table 10.4. FCRs were significantly different ($P < 0.05$) among the treatment groups throughout the experimental period. In all treatments, the FCRs were comparatively lower during the first ten days than during the last fifteen days. Figure 10.4 shows the mean FCR values for each treatment based on total weight gain and feed intake during the whole experimental period. Treatment E showed the best performance (lowest FCR), while the highest FCR was found for fish in Treatment A. Total energy intakes in the different treatments were calculated by multiplying feed intake (applied –wastage in g) with a factor of 22.7 (total energy in test diet 22.7 kJ g^{-1}) and feed utilization efficiencies ($\text{g gain kJ energy intake}^{-1}$) are presented in Figure 10.5.

Table 10.2 *Individual mean total weight (95 % confidence limit) g in different treatments over the experimental period. Weights with same superscript are not significantly ($P < 0.05$) different among the treatments*

	Treatments				
Day	A	B	C	D	E
25	0.98 (0.03)	0.95 (0.03)	0.98 (0.06)	0.99 (0.11)	0.97 (0.04)
30	2.45 (0.28) ^b	3.17 (0.10) ^c	1.93 (0.13) ^a	1.75 (0.06) ^a	3.19 (0.12) ^c
35	4.13 (0.22) ^c	5.52 (0.04) ^d	3.68 (0.11) ^b	3.38 (0.13) ^a	5.79 (0.32) ^d
40	5.74 (0.14) ^b	7.50 (0.20) ^c	5.29 (0.22) ^a	5.05 (0.06) ^a	7.77 (0.40) ^b
45	6.93 (0.18) ^b	8.84(0.30) ^c	6.59 (0.08) ^a	6.48 (0.25) ^a	9.28(0.15) ^c
50	8.03 (0.14) ^c	10.14 (0.19) ^d	7.38 (0.10) ^b	7.15 (0.09) ^a	10.12 (0.12) ^d

Table 10.3 Exponential growth model in different treatments over experimental period. 95 % confidence limits are shown in parentheses. G_w with same superscript are not significantly ($P < 0.05$) different among the treatments

Treatment	W_0 (CL)	G_w (CL)	R^2	P
A	1.41 (0.08)	0.080 (0.001) ^a	0.89	< 0.05
B	1.6 (0.04)	0.087 (0.001) ^b	0.84	< 0.05
C	1.27 (0.06)	0.081 (0.003) ^a	0.92	< 0.05
D	1.21 (0.08)	0.081 (0.002) ^a	0.94	< 0.05
E	1.64 (0.08)	0.087 (0.001) ^b	0.83	< 0.05

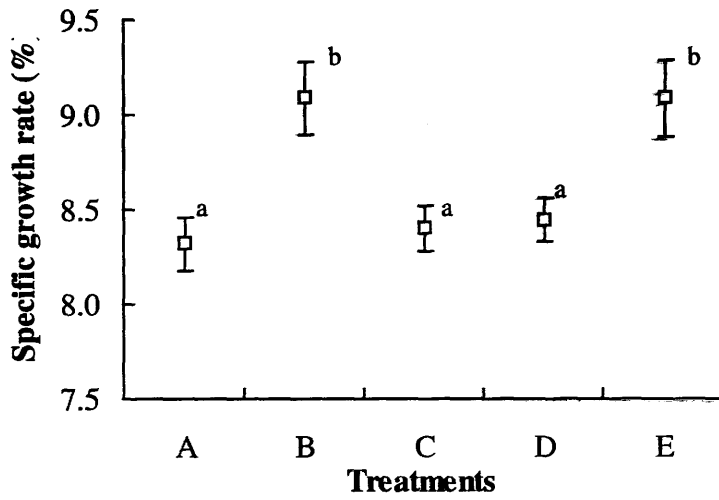


Figure 10.2 Specific growth rates of *C. gariepinus* fingerlings over the experimental period for the whole experimental period. Error bars represent 95% confidence limit. SGRs with same superscripts are not significantly ($P < 0.05$) different (See Table 10.1 for treatments' detail)

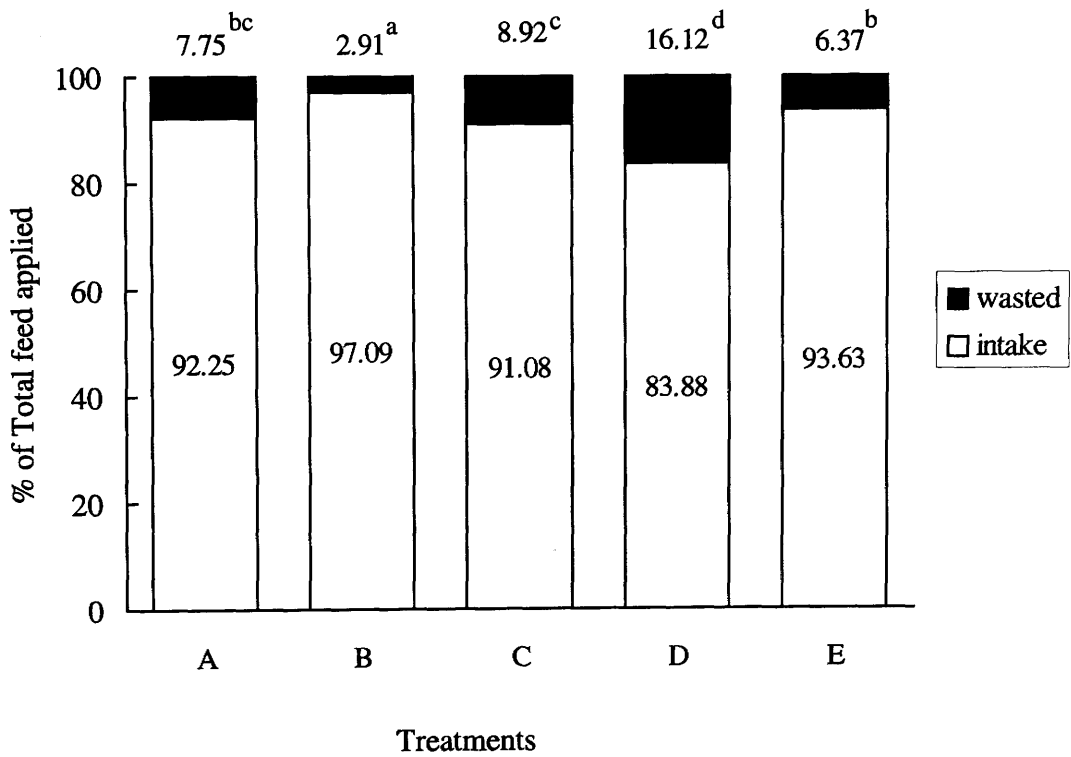


Figure 10.3 Mean % of feed intake and % feed wastage over the experimental period in different treatments. (See Table 10.1 for treatments' detail)

The fish in treatment E showed the highest FUE, while there was no significant difference among other treatments. Survival was very high in all the treatments and the differences were not significant ($P < 0.05$) among treatment groups (Figure 10.6).

10.4 DISCUSSION

Feeding schedule and mode of feeding were prepared according to the findings of previous experiments. Treatment D was considered as the control treatment and one third of total feed was applied to the experimental tanks three times a day during day time only. In Treatment A, feed was distributed evenly on the belt of feeder which was dispersed to the experimental tanks continuously for 24 h. Feeding mode for Treatment C was drawn up based on the findings of Chapter 6, 46% of total ration being given as the first meal in morning and the rest being given at 4 h intervals in 3 successive meals. In Treatments B and E, fish were fed over 24 h or only at night, respectively, following the findings of Chapter 9.

Using different feeding mode and feeding frequencies in the culture of *C. gariepinus* fingerlings, it was found that a body weight of about 10 g could be reached within 50 days after first feeding, taking into account that it took approximately one month to raise the fry to 1 g fingerlings. The rate of weight development compares favourably with the high values reported for channel catfish *Ictalurus punctatus* which increased in weight from 3 to 12.5 g in 4 weeks at about 28 °C (Stickney *et al.*, 1972).

The individual mean weights measured every 5 days and the specific growth rates in this experiment were found to be significantly higher when fish were fed according their feeding rhythm as evaluated in Chapter 9. This is in agreement with research on

several other species where workers found a marked effect of feeding time on growth performance and where feeding tailored to the feeding rhythm consistently gave the better results (Stinging catfish, *Heteriopneustes fossilis*, Sundararaj *et al.*, 1982; Gold fish, *Carassius auratus*, Noeske and Spieler, 1984; channel catfish, *Ictalurus punctatus*, Noeske *et al.*, 1985 catfish, *Heterobranchus longifilis*, Kerdchuen and Legendre, 1991, rainbow trout, *Oncorhynchus mykiss*, Reddy *et al.*, 1994; Boujard *et al.*, 1995). From this experiment, it is, however, obvious that, although *C. gariiepinus* can be fed continuously (Treatments A and B) or only at night time (Treatment E), feed should not be applied at the same rate over a feeding period, but following their feed demand. Feed demand can markedly fluctuate as fish behave according to their endogenous rhythm and not the availability of food.

Feeding following the diel rhythm of fish can greatly reduce wastage of feed. In this experiment feed wastage was significantly lower in Treatment B in which fish were fed continuously according to feed demand. By contrast, the highest feed wastage was observed in Treatment D with a feeding mode of three times a day with equal meal size.

Table 10.4 *Food conversion ratios (FCRs) in different treatments over the experimental period. 95 % CL are shown in parentheses. FCRs with same superscript are not significantly ($P < 0.05$) different among the treatments*

	Treatments				
Day	A	B	C	D	E
25-30	0.35 (0.09) ^b	0.24 (0.01) ^a	0.50 (0.09) ^{bc}	0.65 (0.12) ^{cd}	0.24 (0.01) ^a
30-35	0.67 (0.12) ^b	0.64 (0.06) ^b	0.48 (0.02) ^a	0.44 (0.09) ^a	0.56 (0.10) ^{ab}
35-40	1.19 (0.17) ^b	1.36 (0.14) ^b	1.06 (0.24) ^{ab}	0.85 (0.07) ^a	1.38 (0.22) ^b
40-45	1.84 (0.38) ^b	2.27 (0.56) ^b	1.57 (0.40) ^b	1.23 (0.24) ^a	1.75 (0.26) ^b
45-50	2.37(0.32) ^b	2.65(0.24) ^b	2.78 (0.58) ^b	2.74 (0.52) ^b	1.99(0.05) ^a

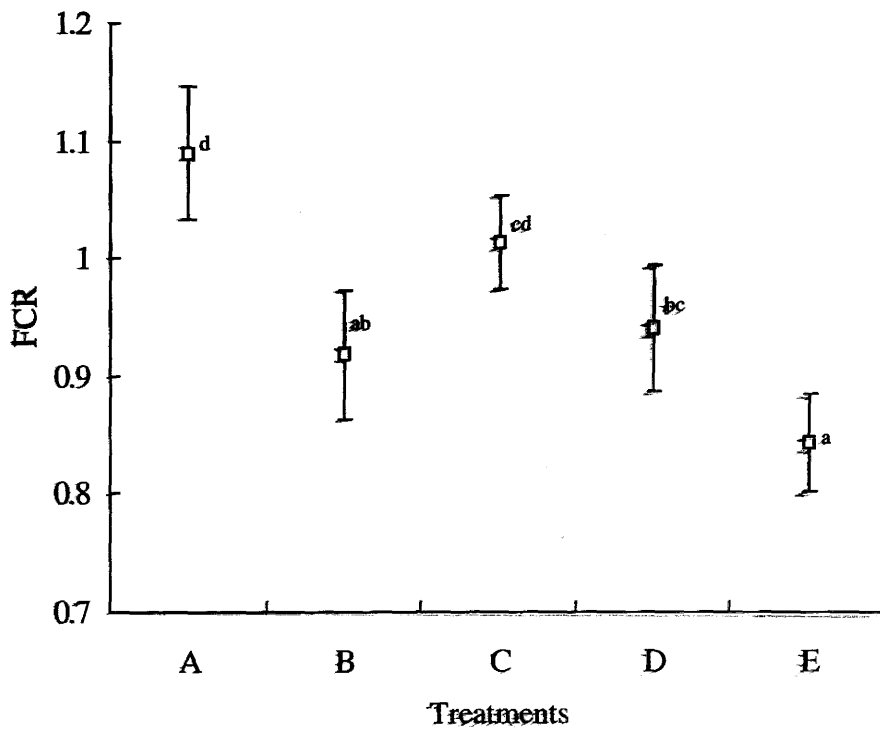


Figure 10.4 Food conversion ratio based on initial and final weight for total experimental period. Error bars represent 95% CL. FCRs with same superscript are not significantly ($P < 0.05$) different. (See Table 10.1 for treatments' detail)

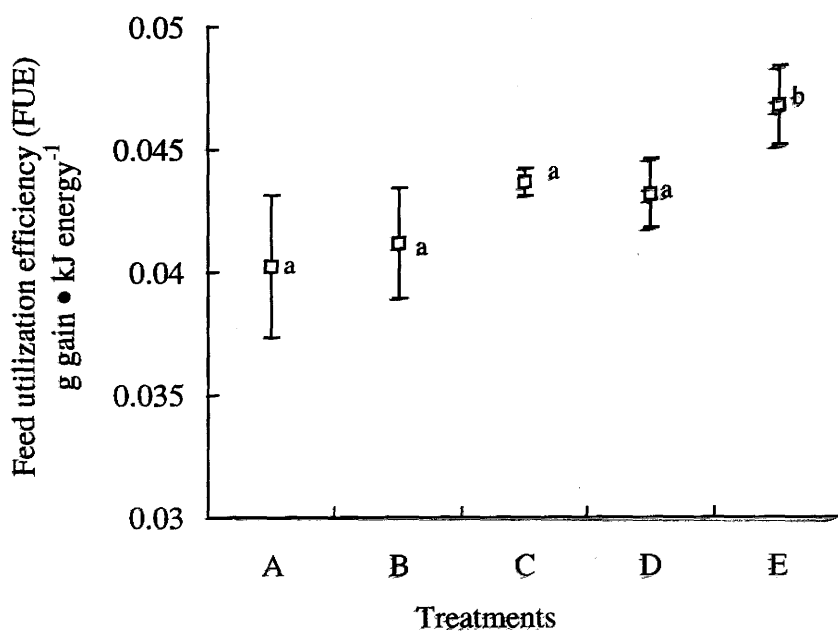


Figure 10.5 Feed utilization efficiencies ($\text{g gain} \bullet \text{kJ energy}^{-1}$) in different treatments. Error bars are 95 % CL. FUEs with same superscript are not significantly ($P < 0.05$) different. (See Table 10.1 for treatments' detail)

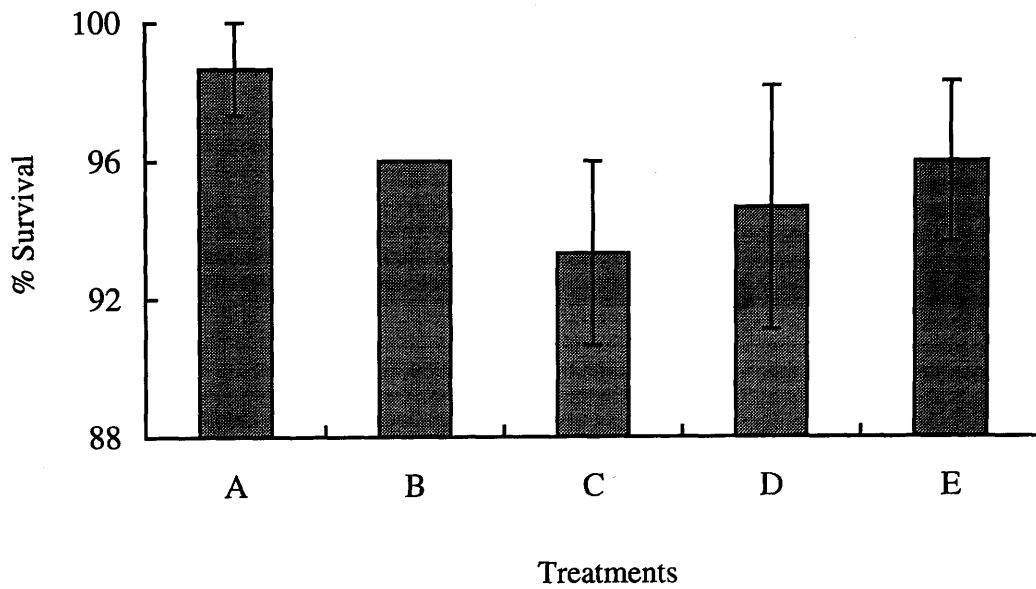


Figure 10.6 Mean survival (%) in different treatments over the experimental period. (See Table 10.1 for treatments' detail)

Night time feeding has been shown to reduce the FCR significantly in catfish, *Heterobranchus longifilis* (Kerdchuen and Legendre, 1991). From lowest FCR and highest Feed Utilization Efficiencies (FUEs) observed in Treatment E of the present study it would appear that the maximum advantage from feeding in *C. gariepinus* might be achieved with feeding during the night. In a 28 day experiment with *C. gariepinus* of initial weight 0.5 g, Hogendoorn (1981) observed the lowest FCR (0.75) when fish were fed during 12 h at night.

In conclusion, generally practised day time feeding with varying number of meal and equal meal size clearly justified previous observations of low growth performance, high food conversion rate and low feed utilization, although survival was not affected by the time of feeding. African catfish should be fed during the night or throughout 24 h, but not at a constant rate, or with a number of meals and at the same rate, but tracking varying feed demands. However, since 24 h feeding might prove more costly in a commercial fish farming situation, night time feeding is the preferred option.

Chapter 11

GENERAL DISCUSSION

11.1 INTRODUCTION

The objectives of the present study have been met in that a feeding strategy for fingerling *C. gariepinus* has been elucidated and that information on optimization of feeding and growth performance has been provided. Studies were conducted over two years on feeding and growth of *C. gariepinus* in a closed recirculating system in a controlled environment. As with every applied science the results of aquaculture research done in a laboratory must be applicable to the practical situation. The objective of this final chapter is to discuss the results of the project from a practical point of view and thus to address some of basic concerns of fish culturists.

11.2 CULTURE CONDITION

The broad tolerances this animal displays with regards to environmental factors (reviewed in Chapters 1 and 2) have made it a prime candidate for the development of an aquaculture industry wherever markets for its meat can be developed (Uys, 1989). A wealth of information is available concerning the optimum water quality, temperature and so on for this species, gathered with the aim of different types of cultures. Very few studies have focussed on density, light and shelter. However, like any other fish species, the growth and survival of African catfish can also be affected by their initial stocking density, photoperiod and provision of shelter (Chapter 2). The findings of chapter 4 show that in a field situation the provision of shelter and low light in conjunction with optimum density can be particularly effective in fry/fingerling facilities for *C. gariepinus*. The appropriate use of density, light and shelter can greatly enhance growth and reduce aggressiveness thus increasing survival rate in a catfish farm.

11.3 FEEDING AND GROWTH

Increasing environmental consciousness and financial stringency in the fish farming industry have put a premium on optimizing food utilization by fish in culture systems. It is therefore important that the accuracy of methods for determining the quantity of food intake is assessed. Rates of gastric evacuation were recognized by Ricker (1946) as having an important bearing on fish production in terms of estimating the daily ration. Various methods of estimating gastric evacuation of fish have been used by a number of researchers with varying degree of success. A radiographic method for studying trophic dynamics of fish has been described by Talbot and Higgins (1983) which incorporates the advantages of avoiding the need to force feed or sacrifice the fish and has proved successful on a range of species. The findings of Chapter 5 confirmed this method could accurately estimate gastric evacuation and food intake in African catfish in its normal feeding regime *i.e.*, without starving prior to or after presenting the feed. The method detailed in this chapter can be used both in laboratory and the field for successful trophic studies in *C. gariepinus* of different age groups.

11.4 QUANTITATIVE FEED ESTIMATION

The food intake of fish is controlled by routine need (metabolic score) and by the fullness of the stomach (Colgan, 1973). Routine need rises with food deprivation but at a progressively decreasing rate as the fish reacts physiologically and behaviourally to conserve its resources. The amount of food in the stomach of a fish at any instant in time varies as a function of the rates of food ingestion and evacuation and these rates are concomitant and interdependent. Voluntary food intake (appetite or food demand) is presumed to be zero when the stomach is full, insensitive of need but is greater than zero

with decreasing stomach content (evacuation). Various authors have shown that the appetite of a fish is inversely related to stomach fullness (Chapter 2). Elliott and Persson (1978) and Jobling (1981) discussed the various mathematical descriptions of gastric emptying curves used to estimate daily food consumption and some effects of different factors on evacuation.

Using the exponential inverse relationship between gastric evacuation and return of appetite, a simple model is proposed regarding the quantities to feed fingerlings and the frequency with which feed can be offered, in order to maximize intake (Tables 6.5 and 6.6). Based upon estimates of maximum stomach capacity as well as gastric evacuation rate it is observed that the feed intake (% bw day⁻¹) decreases from 10 to 5 % over the fingerling period and total consumption is maximized by frequent feeding over 24 h each day.

11.5 EFFECT OF FEED QUALITY AND PELLET SIZE

Much research has concentrated on elucidating the effects of factors such as fish size, feed type and size, meal size and temperature on gastric evacuation (Windell, 1978; Fänge and Grove, 1979; MacDonald *et al.*, 1982; Chapter 2). The effects of temperature and meal size can be avoided by feeding fish to satiation at the optimum temperature at which maximum feeding and fastest growth rate can occur. The effects of feed pellet size and varying feed quality (measured as difference in dietary energy) on gastric evacuation and growth were investigated in Chapters 7 and 8.

A clear pellet size-dependent growth in African catfish fingerlings was observed and the highest growth rate was associated with intermediate size of pellet (1.5 and 2 mm) (Chapter 7). The gastric evacuation rate of small food particles is faster than that of larger particles. When fish are offered frequent meals they will ingest more feed if fed small particles than large ones. However, both results in poorer growth performance in comparison with fish fed intermediate pellet sizes. This brings into question the economic advantage of feeding fish with any pellet size the fish can manipulate, a strategy which emphasizes optimization of feed intake at the possible expense of growth rate and food conversion.

Dietary energy content of food has been reported to influence the growth and feed intake of fish by many researchers (Chapters 2 and 8). African catfish, which were fed on diets of intermediate dietary energy (22.40 – 22.84 kJ g⁻¹) levels tended to grow faster. Both the high energy (23.16 kJ g⁻¹) and low energy (21.93 kJ g⁻¹) diet resulted in poorer growth performance and feed utilization efficiencies with high FCR compared with those fed the diet with intermediate energy level. Gastric evacuation rate decreased with increasing energy level and was more closely related to total energy and digestibility than with digestible energy.

11.6 DIEL RHYTHM

The development of fish farming anywhere is dependent upon the enterprise becoming more economically attractive and environmentally acceptable. In order to achieve this, both the growth performance of the fish and the reduction of effluent waste concentrations caused by un-ingested feed (also a source of lost revenue to the fish farmers) must be greatly improved. The most effective way to optimize the growth of fish and reduce water

pollution from its culture is to present food when the fish are most motivated to feed (Spieler, 1977; Parker, 1984; Seymour and Bergheim, 1991; Poxton, 1991; Handy and Poxton, 1993; Begout, 1995). A realistic and quantitative model of feeding rhythm for African catfish is presented in Chapter 9. This fish feeds predominantly at night and there is a clear and negative effect on feeding and movement of fish when feeding was restricted to only the light phase. In 24 h feeding fish showed two distinct peaks – the first just after the onset of the dark phase, between 2000 and 2300 h and the second before the onset of light (0600-0800 h). Total feed intake was more than two thirds of total the ration during night when fish had feed access throughout 24 h. When feeding was restricted to only the light phase, movement and feed intake were significantly lower than among fish with constant access to feed. The observed diel rhythm suggests that nocturnal *C. gariepinus* should be fed by night wherever possible.

11.7 CONCLUSION

Insufficient use of food and high food wastage often results from the use of improper feeding techniques, which do not consider the diel variations in appetite and feeding activity (Alanära, 1992). Meal timing plays a major role in food utilization by fish and may also affect growth rate and feed conversion efficiency (Boujard *et al.*, 1990). Using optimum pellet size and dietary energy level and by feeding following feeding rhythm, feed intake, feed conversion and growth performance can be largely improved in *C. gariepinus* (Chapter 11).

One of the primary goals of any aquaculture is to maximize production efficiency (Noakes and Grant, 1992). The feeding strategies detailed in this project may reflect a realistic

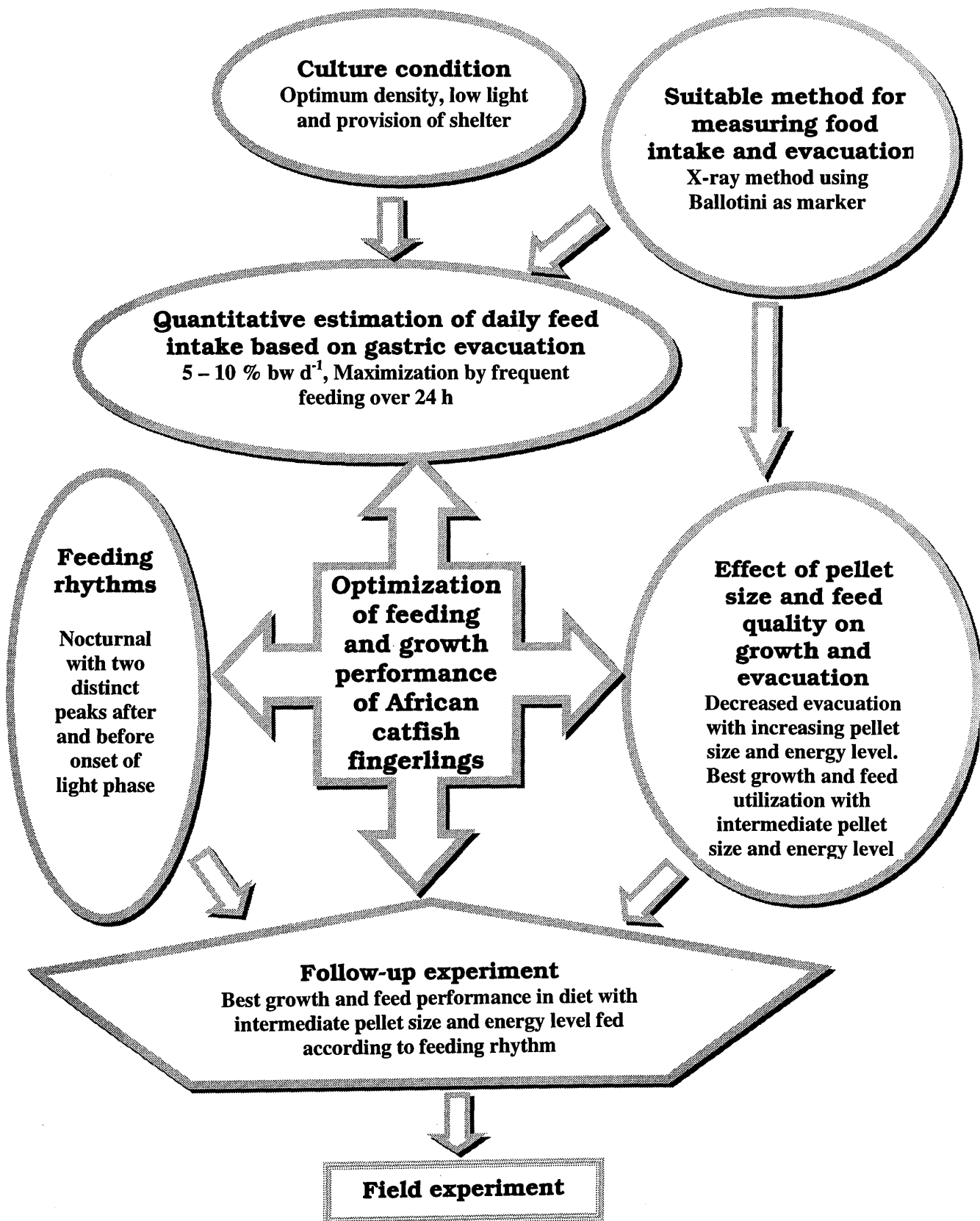


Figure 11.1 Flow diagram of the project on growth and feeding optimization of fingerling *Clarias gariepinus*

feeding pattern in African catfish and may help to maximize the production potential from the culture system (Figure 11.1). In the field, the methods can be successfully applied and take account of age, type of feed and prevailing environmental parameters.

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APPENDICES

Appendix 1



Plate 1 *Experimental fingerling rearing recirculation system (as described in 3.1)*

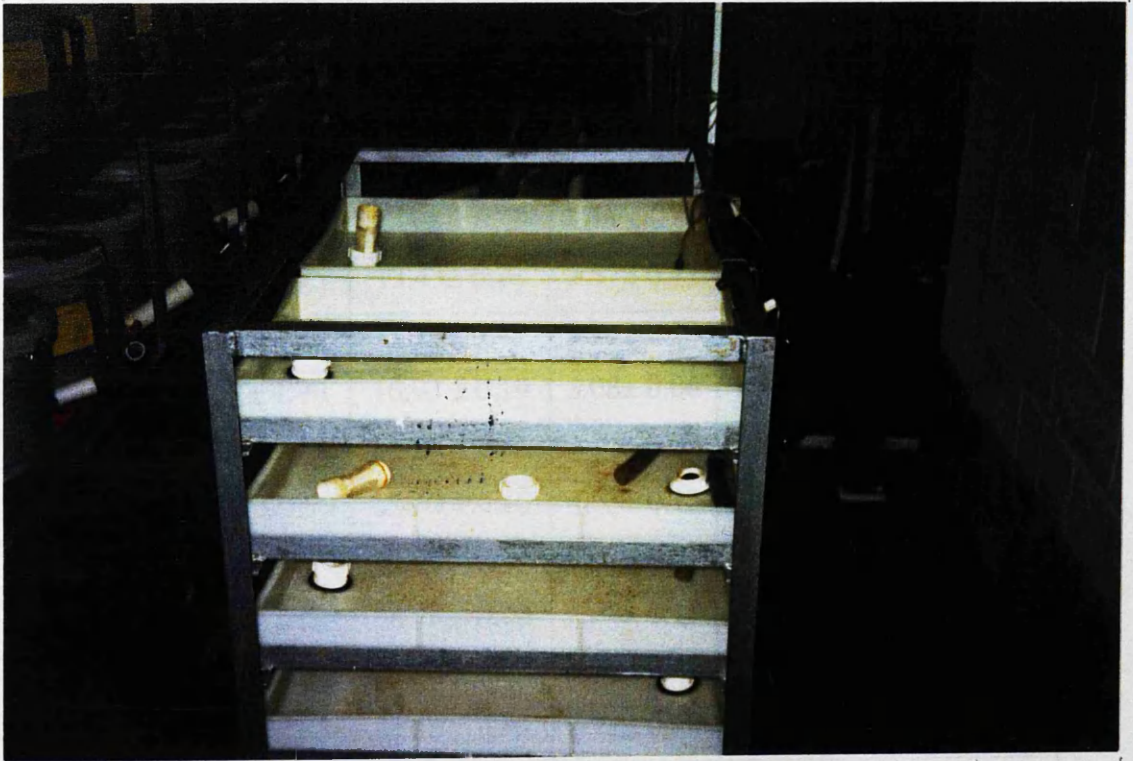


Plate 2 *Experimental egg incubation system (as described in 4.2.1)*

Appendix 2 *Total length, weight, width, mouth length and mouth width of 20 randomly selected fish over the 25-day period after hatching*

	$\bar{X} \pm CL$				
Day	Body			Mouth	
from hatching	Wt mg	TL mm	Width mm	ML mm	MW mm
01	3.47 ± 0.14	9.04 ± 0.14	1.49 ± 0.05	1.02± 0.01	0.46± 0.04
03	5.37 ± 0.21	10.24 ± 0.14	1.89 ± 0.04	1.13± 0.16	0.51± 0.13
04	8.25± 0.25	10.62 ± 0.24	2.02 ± 0.03	1.22± 0.11	0.52± 0.01
05	11.55 ± 0.56	11.70 ± 0.22	2.16 ± 0.05	1.29± 0.13	0.59± 0.08
06	14.53 ± 0.56	12.98 ± 0.23	2.53 ± 0.08	1.34± 0.03	0.64± 0.03
07	18.20 ± 0.95	13.43± 0.24	2.57± 0.11	1.57± 0.04	0.82± 0.03
08	26.20 ± 2.21	15.23± 0.45	2.76± 0.13	1.78± 0.06	0.88± 0.07
10	39.15 ± 1.70	16.93 ± 0.19	3.08± 0.06	1.83± 0.04	1.10± 0.04
12	55.90 ± 2.83	18.54 ± 0.31	3.36± 0.09	2.17± 0.06	1.19± 0.03
14	119.60 ± 5.99	24.50 ± 0.46	4.63± 0.13	2.67± 0.07	1.43± 0.04
17	244.20 ± 15.46	31.70 ± 0.91	6.01± 0.11	3.45± 0.11	2.00± 0.06
19	356.30 ± 21.93	34.78 ± 0.70	6.20± 0.15	4.01± 0.11	2.75± 0.08
21	458.60 ± 37.75	39.08 ± 0.99	7.25± 0.28	4.13± 0.16	3.28± 0.15
24	770.30 ± 43.53	45.15 ± 0.82	9.22± 0.23	4.97± 0.16	4.12± 0.10
25	790.30 ± 10.34	49.22 ± 0.91	9.56± 0.16	5.01± 0.34	4.18 ± 0.21

Appendix 3 *Feeding Artemia to the larvae of Clarias gariepinus*

1. After decysting the cyst of *Artemia* was stored in a highly saturated brine solution in a refrigerator at normal temperature (4 – 6 °C).
2. On day 3 (from hatching), at 08.00 h. the *Artemia* was taken out from the refrigerator and the brine was drained. Some *Artemia* was taken with fingertips and distributed in the water of the tank of larvae in a row.
3. *Artemia* was fed every 2 hours from 08.00 to 20.00.