ENVIRONMENTAL REQUIREMENTS FOR THE HATCHERY REARING OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (PISCES : CLARIIDAE) LARVAE AND JUVENILES.

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by

PETER JACOBUS BRITZ

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CONTENTS

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ABSTRACT

Inadequate seed production has historically been a factor limiting the commercial culture of Clarias gariepinus. The need to determine the environmental requirements of larvae was identified, in order to facilitate their successful mass rearing in hatcheries. The effects of key environmental factors on the growth, survival and aspects of the behaviour of C. gariepinus larvae and juveniles were investigated under controlled conditions. A strong emphasis was placed on the interpretation of the observed responses in terms of the natural history of the animal. Larvae were obtained by artificially inducing and spawning feral adult broodfish. Larval growth rates were highest in the temperature range 26-33°C, with a peak at 30°C. Survival of larvae was high between 22 and 33°C. The final temperature preferendum of juveniles, determined in a thermal gradient was 30°C, and it was concluded that this temperature is probably optimal for most of the physiological processes of the animal. Larvae and juveniles displayed strong negative phototaxis. When reared under different photoperiod regimes, larval growth increased with longer dark periods, however a similar rate of mortality was recorded in all photoperiod treatments. The provision of cover was found to enhance larval growth under conditions of continual light (24L/0D), however under conditions of continual darkness (0L/24D) similar growth rates were recorded regardless of the presence or absence of cover. All growth rates in continual darkness (0L/24D) were higher than those in continual light (24L/0D), regardless of whether cover was provided or not. It was concluded that a 0L/24D photoperiod is optimal for larval rearing. At salinities between 0 and 5 ‰ similar growth and mortality rates of larvae were

recorded. At 7.5 ‰ larval growth and survival rates were lower, and at 10 ‰ all larvae died within 48 hours. The 96h-LC50 for unionised ammonia was found to be 2.3 mg/l. The cytological effects of unionised ammonia were evident as degenerative changes in the gill and liver tissues. In an investigation of tank hygiene, it was found that larval growth and survival rates were highest in tanks not cleaned at all. As the frequency of tank cleaning increased, the growth and survival rates of larvae decreased. When reared at densities ranging from 45-450/l, larval growth was found to be density dependent. A model of hatchery productivity (number larvae of standard size produced/time/vol.) was developed based on the density dependent nature of larval growth. The model predicts that maximum productivity, will be achieved at a rearing density of 1400/l. The high growth and survival rates obtained over a broad range of each environmental parameter investigated serve to explain how larvae survive and grow in their unstable floodplain habitat in nature. The broad environmental tolerances of C. gariepinus are of benefit with respect to the culture of larvae, as successful larval rearing is possible over a fairly wide range of environmental conditions.

CHAPTER 1.

INTRODUCTION

The African sharptooth catfish, Clarias gariepinus (Clariidae : Siluriformes), is in many senses a remarkable beast, a statement immediately justifiable if one considers its phenomenal natural distribution, which ranges from the Cape Province, South Africa throughout Africa into Turkey and Asia-minor (Teugels 1986). This airbreathing (Moussa 1957) clariid species exists in diverse environments, ranging from temperate to tropical, and is represented in a correspondingly diverse array of aquatic faunal assemblages, from the species poor Orange river system (Skelton 1986) to species-rich Lake Malawi (Fryer and Iles 1972). Natural history studies (Greenwood 1955, 1957, Groenewald 1964, Clay, 1977a, Bruton 1978, 1979 a, b, c & d, Clay 1979, 1981) have shown that C. gariepinus is an extremely hardy and adaptable animal, efficiently able to exploit a wide variety of both animal and plant protein, under diverse environmental conditions. Furthermore, it is able to withstand adverse environmental conditions and habitat instability. Not surprisingly, this ubiquitous fish is perhaps the most important individual species in traditional subsistence, and modern feral African freshwater fisheries, being estimated to comprise some 20% of the total catch (Clay 1977).

Catfish of the genus Clarias, mainly C. gariepinus (recently synonymised with C. lazera and others, see Teugels 1986) and its closely related Asian counterparts C. batrachus and C. macrocephalus (hereafter collectively referred to as catfish), adapt well to artificial environments, and are rapidly gaining status as premier aquaculture species. Established catfish culture industries exist in Thailand (Sidthimunka 1972, Areerat 1987), Taiwan (Chen 1976) and the Philippines (Carreon, Estocapio, and Enderez 1976), and are developing rapidly in several African and European countries (Huisman and Richter 1987,

Wray 1987, personal observations in South Africa, Zambia, and Zimbabwe). Perhaps the most exciting feature of the catfish in terms of aquaculture, is its potential for highly intensive culture without prerequisite pond aeration or high water exchange rates, necessary for the intensive culture of other species (Sidthimunka 1972, Areerat 1987, Huisman and Richter 1987). This type of culture is primarily facilitated by the catfish's airbreathing ability and tolerance of poor water quality (Sidthimunka 1972, Babiker 1984, Clay 1979, Huisman and Richter 1987). The unique potential of airbreathing catfish for intensive culture is illustrated by production statistics from Thailand where yields ranging from 12.5 - 100 t/ha/yr are obtained in stagnant ponds (Panayotou, Wattanutchariya and Isvilanonda 1972, Sidthimunka 1972, Areerat 1987). Such figures stand in marked contrast to intensive channel catfish (Ictalurus punctatus) culture where maximum yields in stagnant ponds are of the order of 7 t/ha/yr, if mechanical aeration is employed (Wellborn and Tucker 1985).

In contrast to fish with a longer tradition of culture (e.g. carp and salmonid species), research into the biology of C. gariepinus has a relatively recent history, beginning in the 1950's (Greenwood 1955, 1956, 1957). Very little further work was done until the early 1970's when its aquacultural potential became evident, stimulating the initiation of intensive research programmes in various African countries, namely, the Central African Republic (C.T.F.T. 1972, Hastings 1973, De Kimpe and Micha 1974, Micha 1971, 1972, 1973, 1975, Kelleher and Vinke 1976, Richter 1976), Cameroon (Hogendoorn and Wieme 1975, Hogendoorn 1977, 1979, 1980, 1981, 1983, Hogendoorn and Vismans 1980, Hogendoorn and Koops 1983), South Africa (Van der Waal 1972, 1974, 1978, Schoonbee, Hecht, and Polling 1980, Hecht 1981, 1982, Hecht, Saayman and Polling 1982, Bok and Jongbloed 1984) and to a lesser extent in Kenya (Christensen 1981a&b), Zimbabwe (Clay 1977a, 1979, 1981) and Nigeria (Agunbiade 1977 and others). As a result, the understanding of the biology and culture of C. gariepinus has developed rapidly, and the number of catfish

related publications have increased exponentially, covering diverse research interests. Most publications have been aquaculture related reflecting the rapid development of the technology for its culture. The reasons for this rapid development have been the undertaking of comprehensive experimental aquaculture programmes, and the adaptation of existing culture techniques developed over longer periods for more established culture species.

Early experimental programmes revealed the excellent aquaculture potential of C. gariepinus (El Bolock and Koura 1960, El Bolock 1969, 1973, 1975, 1976, Van der Waal 1972, 1978, De Kimpe and Micha 1974, Kelleher and Vinke 1976, Richter 1976, Clay 1977, 1979, Hogendoorn 1983). A serious problem encountered in all of these studies was an inadequate production of fry. This factor has been cited as one of the primary reasons retarding the initiation of commercial catfish production in Africa (Kelleher and Vinke 1976, Hogendoorn 1977, Hecht 1985). Early attempts at fingerling production involved either natural or controlled spawnings in ponds or tanks (Van der Waal 1972, 1978, De Kimpe and Micha 1974, Nugent 1975, Micha 1975, Kelleher and Vinke 1976, Richter 1976, Hogendoorn 1977). Larvae were stocked directly into ponds without any intermediate nursing. Although good growth rates were obtained, unacceptably high larval mortalities ranging from 32.5 - 99.8% occurred (Kelleher and Vinke 1976, Hogendoorn 1980). The major causes of mortality were cited as cannibalism, lack of adequate nutrition, and predation by the African clawed toad, Xenopus laevis (Van der Waal 1972, 1978, Richter 1976, Kelleher and Vinke 1976, Hecht 1982, 1985). In view of these problems associated with the semi-extensive rearing of fry, it was independently stated by several workers that an adequate supply of fry could only be achieved through the development of intensive hatchery rearing techniques (Sidthimunka 1972, Carreon, Ventura and Amazan 1973, Carreon et al. 1976, Richter 1976, Clay 1977, Hogendoorn 1979, Hecht 1985).

The development of intensive fry rearing procedures for C.

gariepinus forms part of a world wide trend towards controlled seed production (EIFAC 1976, Huisman 1976, Coche and Bianchi 1979). This is because an inadequate supply of fry has almost universally been a major limiting factor in the development of the large scale culture of various species. For example, the catfish (C. batrachus) (Panayotou et al. 1972, Carreon et al. 1976), mullet (Mugil cephalus) (Liao 1976, Nash and Konigsberger 1981), milkfish (Chanos chanos) (Bardach, Rhyther and MacLarney 1972), eel (Anguilla species) (Japan Fisheries Association 1975), and tilapia hybrids (Mires 1983, Balarin and Haller 1982, Lovshin 1983), culture industries have all been constrained by a shortage of fry. The evolution of intensive fry rearing techniques usually involves the development of artificial spawning procedures, followed by larval feeds, and the creation of optimal artificial environments in hatcheries (Shehadeh 1979). The major breakthrough facilitating the development of modern aquaculture was the discovery that most species could be spawned artificially by means of hypophysation (the injection of pituitary gland homogenates or extracts) (Bardach et al. 1972, Huisman 1982). For example, the artificial hormone induced spawning of carp has facilitated its culture on a commercial scale in temperate areas such as Israel where natural spawning is less predictable (Bardach et al. 1972, Hepher and Pruginin 1977). Intensive rearing procedures in essence offer the culturist greater control over fingerling production, for example:

- The time of spawning can be controlled by the culturist, thus work can be planned in advance.
- Induced spawning eliminates environmental variables, i.e. spawning area, temperature, light and other climatic factors.
- Fish that will not spawn naturally can be induced to spawn.
- Culture ponds can be stocked with fry that are uniform in age and size.
- Disease transmission from broodstock to offspring, and predation by adults is minimised.
- Prophylaxis and treatment of disease is greatly

facilitated.

- Size grading of batches of larvae is easily performed minimising sibling cannibalism.
- Hatchery rearing eliminates losses due to natural predators.
- More efficient management is possible with regard to feeding, inventories, growth rate data and mortality records.

In the following section, the background to the undertaking of the present study is sketched by reviewing the development of intensive larval rearing techniques under controlled conditions for C. gariepinus.

Initial efforts by Van der Waal (1972, 1978) and Hogendoorn (1977) to artificially spawn, strip and incubate the eggs of Clarias gariepinus paved the way for the intensive rearing of larvae. Artificially induced spawning using C. gariepinus pituitaries was first carried out by Van der Waal (1972). Although hypophysation using Clarias pituitaries offers a reliable and convenient method of induction (Van der Waal 1972, Richter 1976, Carreon et al. 1976, Hecht et al. 1982, Viveen, Richter, Van Oordt, Janssen, and Huisman 1985) most research efforts have, however, concentrated on the use of other agents to induce spawning. Carp pituitaries have proven to be an effective inducing agent (Hogendoorn 1977, Hogendoorn and Vismans 1980), however the use of synthetic hormone treatments such as DOCA (desoxycorticosterone-acetate) (De Kimpe and Micha 1974, Hogendoorn and Wieme 1975, Micha 1975, Pham 1975, Richter and Van den Hurk 1982), human chorionic gonadotropin (HCG) (Eding, Janssen, Kleine Staarman, and Richter 1982) and progestagen (Richter 1985) have met with limited success. Recent experimental work has, however, shown that a pimozide/ LH-RH combination is an effective means of inducing spawning (De leeuw, Goos, Richter, and Eding 1985, Richter, Eding, Goos, De Leeuw, Scott and Van Oordt 1987). Pimozide is not, at this stage, commercially available and has not yet been approved for use on

fish (Richter et al. 1987). For the foreseeable future, therefore, hypophysation remains the only realistic alternative for the commercial culturist, particularly in Africa where commercial hormone preparations are not readily available.

Further developments with regard to artificial propagation have been the cryopreservation of sperm (Steyn, Van Vuren, Schoonbee, and Nai-Hsien Chao 1985, Steyn and Van Vuren 1987) and handstripping sperm from males (Van der Waal and Polling 1984). Sperm preservation will facilitate genetic selection and eliminate the uncertainties involved in spawning, caused by the seasonal nature of male gonadal development. Furthermore, the necessity of sacrificing a male with each artificial spawning is eliminated. The handstripping of males has, however, met with limited success and is not, at this stage, considered a practical technique for large scale propagation.

Early attempts by Van der Waal (1972, 1978) to incubate eggs in Zuger funnels failed due to the adhesive nature of the eggs. A good survival of eggs was, however, obtained by incubating them either in a monolayer on fine mesh (Van der Waal 1972, 1978) or in plastic trays in flowing water (Hogendoorn 1977). Schoonbee et al. (1980) developed a technique for removing adhesiveness using a modified Woynarowich solution (Woynarowich 1962), thereby facilitating successful incubation of eggs in funnels. Subsequent experience at the Rhodes University hatchery (unpublished) has shown that the incubation of eggs on series of vertical gauze screens, without removal of egg adhesiveness, is the most effective method of egg incubation.

Experimental attempts at controlled larval rearing in tanks and troughs, using various forms of zooplankton as feed, produced mixed results (Van Der Waal 1972, Jocque 1975, Pham 1975, Hogendoorn 1979, 1980, Hogendoorn and Vismans 1980). Investigations into the large scale production of C. gariepinus fry were initiated by Hecht (1981, 1982). Hecht (1982)

successfully reared larvae at high densities (250-300/l) with low mortality (2.7%) using relatively high water exchange rates (200 l/hour), in plastic bins (1150 x 1000 x 680 mm) maintained at 288 l capacity.

The development of dry larval feeds for C. gariepinus was a major breakthrough facilitating the intensive large scale rearing of larvae (Hecht 1981, 1982, Uys 1984, Uys and Hecht 1985), as the collection and/or culture of large quantities of live organisms as feed is a cumbersome and unreliable process (Appelbaum 1977). Hecht (1981, 1982) showed that a dry feed (torula yeast and fishmeal) or torula yeast in combination with live feed (Daphnia spp.) was superior to live feed alone. The dietary requirements of C. gariepinus larvae were investigated in greater detail by Uys (1984), culminating in the formulation of an artificial dry larval feed for the intensive rearing of catfish larvae (Uys 1984, Uys and Hecht 1985). More recently, decapsulated Artemia cysts have successfully been used as a dry larval feed (Verreth and Den Bieman 1987, Verreth, Storch and Segner 1987). However, the relatively high cost of this diet in comparison to the yeast/ fishmeal based diet formulated by Uys (1984), favours the use of the latter.

While considerable progress had been made with regard to artificial breeding and nutrition of catfish larvae, very little was known of their environmental requirements. This research need had been identified by several authors (Hogendoorn 1979, Safriel and Bruton 1984, Hecht 1985). Successful hatchery rearing of larvae depends primarily on nutrition, disease prophylaxis, and optimum environmental conditions (Shehadeh 1979). Disease has fortunately proven to be a relatively minor problem in the intensive rearing of C. gariepinus larvae (Van der Waal 1972, Huisman and Richter 1987). This study was undertaken with a view to determine the environmental requirements for the hatchery rearing of C. gariepinus larvae. It can be regarded as a necessary step in the development of basic hatchery procedures.

The study begins with an outline of the theoretical framework within which experimental hypotheses and designs were generated (chapter 2). A general description of hatchery procedures employed is presented in chapter 3. In the following chapters (4-6) experimental investigations into the effects of temperature, photoperiod, and salinity on growth, survival and various aspects of behaviour are described. In chapter 7, investigations into aspects of water quality are described and discussed. These include the determination of the lethal and cytological effects of ammonia, as well as an investigation to determine criteria for rearing container hygiene. In chapter 8, the effect of density on the large scale rearing of larvae was investigated and a model of hatchery production developed. Chapter 9 concludes with a general discussion and recommendations.

CHAPTER 2

THEORETICAL CONSIDERATIONS FOR EXPERIMENTAL DESIGN

In this discussion the theoretical framework within which the experiments were designed is developed.

The environment of a fish can be defined in terms of biotic and abiotic factors. The prime objective of the experimental work was to examine the qualitative and quantitative aspects of abiotic factors affecting the growth and/or survival of C. gariepinus larvae, in order to define the environmental requirements for their successful rearing in hatcheries. Biotic factors pertinent to the optimisation of the hatchery environment, for example, ration, feeding frequency, density and cannibalism have been the subject of previous and ongoing experimental studies (Hogendoorn 1981, Hogendoorn, Janssen, Koops, Machiels, Van Ewijk, and Van Hees 1983, Uys 1984, Machiels and Henken 1986, Hecht and Appelbaum 1987a&b, Uys, Hecht and Walters 1987, Verreth and Den Bieman 1987, T. Pienaar, Department of Ichthyology and Fisheries Science, Rhodes University, unpublished data). These were contextualised where appropriate in the discussions of the respective chapters.

Fry (1947, 1971) extended Blackman's (1905) classic concept of limiting factors by recognising that environmental factors act through metabolism on activity. He thus defined five simple conceptual categories into which abiotic factors may be classified according to their metabolic effects, namely:

1) Controlling Factors, which govern the rates of reaction of metabolic processes (e.g., temperature, pH).

2) Limiting Factors, which restrict the supply or removal of metabolites (eg. oxygen).

3) Masking Factors, which modify or prevent the effect of an

environmental factor through some regulatory device (e.g., temperature regulation by countercurrent heat flow as in warm-bodied fish.)

4) Directive Factors, which cue or signal the animal to select or respond to particular characteristics of the environment (eg. temperature preference, photoperiod induced gonadal maturation).

5) Lethal Factors, which cause a breakdown of metabolic functioning leading to death (eg. high temperatures, rising levels of ammonia, salinity).

A cursory examination of these categories reveals that environmental factors may act via a multiplicity of metabolic pathways and that it is possible to examine their effects at many levels (e.g. biochemical, cytological, organismal).

The effects of environmental factors on fish are most commonly, although not exclusively, measured in terms of growth and survival. This is undoubtedly because these measurements are the most meaningful biological parameters for the management of the aquaculture and fishing industries, for which most fish research is ultimately commissioned. Furthermore, growth and survival are simple measurements which represent the summation of diverse metabolic pathways. Growth and survival were thus adopted as the basic parameters for experimentally examining the effect of abiotic environmental factors on C. gariepinus in the present study.

The theoretical aspects of the effects of environmental factors on fish growth have been reviewed by Brett (1979). Abiotic factors, primarily temperature, light, salinity and oxygen interact with biotic factors, primarily ration, fish size, and competition, to determine a fish's scope for growth. Brett (op. cit.) defined various levels of growth, namely, maximum growth

(G-max), optimum growth (G-opt), zero growth (G-0) and negative growth (G-starve), which are all functions of ration. Hatchery rearing seeks to rear a fish, to a size suitable for stocking into production ponds, in as short a time as possible and therefore strives to attain G-max, which is primarily a function of maximum ration (R-max). In the present experimental series, therefore, fish were fed ad libitum as frequently as possible in an attempt to achieve G-max.

An organism responds to its environment as a whole, and there are inherent difficulties in experimental design when attempting to define the effect of a single environmental variable. Environmental factors may act in concert or independently on metabolic activity which may display a linear or non-linear response. The examination of the effect of an environmental parameter under controlled conditions in which other previously defined factors are held constant, does not necessarily mean that a similar qualitative result will be obtained, if the same experiment is repeated under a modified set of controlled conditions. This is because the effect of the factor being examined may be masked or altered by the modified regime of other controlled environmental factors. For example, the salinity tolerance of salmon is dramatically altered by different photoperiod regimes (Saunders, Henderson and Harmon 1985). Thus, in order to minimise experimental artifacts, and obtain meaningful generalisations regarding a particular factor, the design of controlled experiments in artificial environments should be informed by the biology of the organism as a whole, and in particular its natural history. It is therefore appropriate that relevant aspects of the ecology and life history of C. gariepinus are briefly considered in order to generate a framework within which to formulate experimental hypotheses and interpret results.

The most striking aspect of the ecology of C. gariepinus is its generalised nature, manifest in its ability to opportunistically exploit a wide range of environments ranging from stable to

unstable (Bruton 1979a&d). Characteristics facilitating this ability are its tolerance of environmental extremes (Clay 1977, Bruton 1978, Quick and Bruton 1984), euryphagy (Groenewald 1964, Van der Waal 1972, Bruton 1979b) and tolerance of poor water quality facilitated primarily by its ability to breathe air (Moussa 1957, Bruton 1979d). The "r-K" classification of MacArthur and Wilson (1967) and the altricial-precocial homeorhetic states developed by Balon (1981, 1983) are useful theoretical models for discussing the reproductive biology and early life history of C. gariepinus. According to these theories, C. gariepinus may be regarded "r"-selected and an altricial generalist. C. gariepinus can reproduce in their first year (Richter, Eding, Leuven and Van Der Wijst 1982, personal observations), have a relatively high fecundity, produce relatively small eggs, and exhibit no parental care (Greenwood 1955, Van der Waal 1972, 1974, 1978, Bruton 1979a, Clay 1979). Eggs hatch successfully over a wide range of temperature (17 - 32^{o}C) (Van der Waal 1972, 1978, personal observations). Hatching, although temperature dependent, usually occurs approximately 24 hours after fertilisation (at 24-26 ^{o}C) (Greenwood 1955, Van der Waal 1972, De Kimpe and Micha 1974, Richter 1976, Viveen et al. 1985). The offspring display a rapid development which includes an intermediate larval stage (indirect development) (Bruton 1979a). Spawning usually takes place in unstable environmental conditions associated with rain, rising water levels, and flood (Greenwood 1956, 1957, Holl 1966, 1968, Spinage 1971, Bowmaker 1973, Bruton 1979a). Mortality of offspring may be catastrophic (Greenwood 1955). The offspring, by exploiting newly inundated environments which are usually rich in food, operate temporarily in what MacArthur and Wilson term an 'ecological vacuum', almost free from inter- and intra-specific interactions. At this point the population of offspring is as close as it will ever be to the idealised "r" extreme of the "r-K" continuum. As time passes, biotic interactions, primarily predation and competition, become more intense shifting the population a little more toward the "K" endpoint. For example, density effects lead to competition for cover and food which

results in coeval sibling cannibalism (Hecht and Appelbaum 1987b). The phenomenon of cannibalism, which is intensified at high densities (e.g. under hatchery conditions) can thus be interpreted as a selective response to an increasingly "K"-selected environment. A relatively "r"-selected ecology predicts productivity, explaining in part, the relative abundance of C. gariepinus within its natural distribution (Clay 1977).

Considering thus the natural history of C. gariepinus, it was hypothesised that controlled experiments should, in general, reflect the broad tolerance that larvae display for most environmental factors in their characteristically unstable natural habitat. Specifically it was hypothesised that these tolerances would be manifest as good growth and survival over a broad range of each environmental factor (e.g. temperature, photoperiod, salinity) investigated under controlled conditions.

With regard to biotic factors, it was realised that high density hatchery rearing intensifies intra-specific interactions, which could result in depressed growth rates, cannibalism, and a possible masking of the effects of the abiotic parameters under experimental investigation. As far as was possible therefore, experiments examining the effects of abiotic parameters were designed to eliminate or minimise biotic influences, which could possibly act as masking factors. This was achieved by:

- Ad libitum feeding as often as possible in growth experiments in order to eliminate any differential effects ration size and feeding frequency might have introduced. A constant availability of food has furthermore been shown to minimise cannibalism (Hecht and Appelbaum 1987b).

- Stocking relatively low densities into experimental containers (10-20 individuals/litre) to minimise possible masking effects that intense intraspecific interactions may exert on growth and survival.

Furthermore, additional behavioural and/or cytological observations were made to verify trends in the growth/survival data.

While all of the experiments were designed to have a direct application with regard to fish culture, an explanation of the observed responses, in terms of the animals natural history, was emphasised. This was because the responses of a fish in artificial environments are often artifacts of interactions in nature (eg. predator avoidance), and can therefore only be explained by reference to their ecology. It is believed that this approach, often neglected in aquaculture research, facilitates a deeper understanding of the natural history of the animal, as well as, the development of more efficient culture techniques.

CHAPTER 3

GENERAL METHODS

The experimental phase of this study was conducted over three breeding seasons (1985-1987). Initial experiments were conducted during the first two seasons at the Rhodes University hatchery. Further experiments were conducted during the latter half of the second and third season at a commercial hatchery (Blyde River Aquaculture) in the Transvaal lowveld. The artificial propagation techniques employed, as well as the sampling and statistical methods used to monitor growth are described in this section.

COLLECTION AND CONDITIONING OF BROODSTOCK

The broodstock available at Rhodes University were second generation descendants of fish collected in the PK Le Roux dam on the Orange river, Cape Province and spawned at the Amalinda Provincial Fish Station near East London. To avoid the genetically unsound practice of inbreeding these fish, further broodstock were collected from below the Augrabies Falls on the lower Orange River in July 1985. The size of the Amalinda broodstock ranged from 400 - 2000g, while that of the Augrabies fish was generally larger ranging from 1000 - 6000g. Broodstock were brought to an early seasonal maturity (September) in both breeding seasons by maintaining them indoors, in warm water on a high protein diet.

In the lowveld, broodstock were obtained from a recreational dam near Phalaborwa on the Olifants river. These fish were ripe when caught and needed no conditioning prior to hypophysation. The size of fish ranged from 2000-5000g.

Due to the small size of the Amalinda fish, the eggs and sperm of several fish were mixed to produce batches of larvae large enough

for experimental purposes. The fish obtained from Phalaborwa, were however, large enough to individually provide sufficient gametes for experimental purposes. Full-sibling batches of larvae were therefore produced by spawning single pairs of these broodfish.

INDUCED SPAWNING

Spawning was induced by hypophysation using homoplastic pituitary glands, usually obtained from male fish killed during previous spawnings and stored in ethanol. A single dose of approximately 1.5 glands per fish (donors and recipients were approximately of equivalent weight) was administered. The glands were homogenised in distilled water and injected intramuscularly in the nape region. The fish were usually ready for spawning 12-14 hours after hypophysation at temperatures of 24-27^{o}C. This method of induction was found to be 100% successful, provided the eggs of the females were sufficiently ripe.

When the eggs became free running, they were striped into a dry plastic bowl. Sperm was obtained by dissecting the testes out of freshly killed males.

FERTILISATION AND INCUBATION OF EGGS

Fertilisation was effected by gently mixing the gametes and then adding a small quantity of water from the incubation trough. The gametes were then gently stirred for 60-90 seconds until the eggs had swollen sufficiently (so as not to fall through the 1mm mesh of the incubation frames) and were becoming sticky. They were then gently spread in a monolayer on plastic gauze frames (40cm X 15cm) and placed vertically in the incubation trough.

At the Rhodes University hatchery the eggs were incubated in a temperature controlled recirculating system with an ultra-violet water steriliser and biological filter (Fig. 1). At the Blyde river hatchery, eggs were incubated in a flow through

system, using water drawn from the Blyde river irrigation canal (Fig. 2).

Although the quality of this water was not analysed in detail, it was considered good as the water was clear, and low in nutrients, being drawn from the Blydepoort dam in a mountain catchment area. The pH of the water was neutral (pH 7).

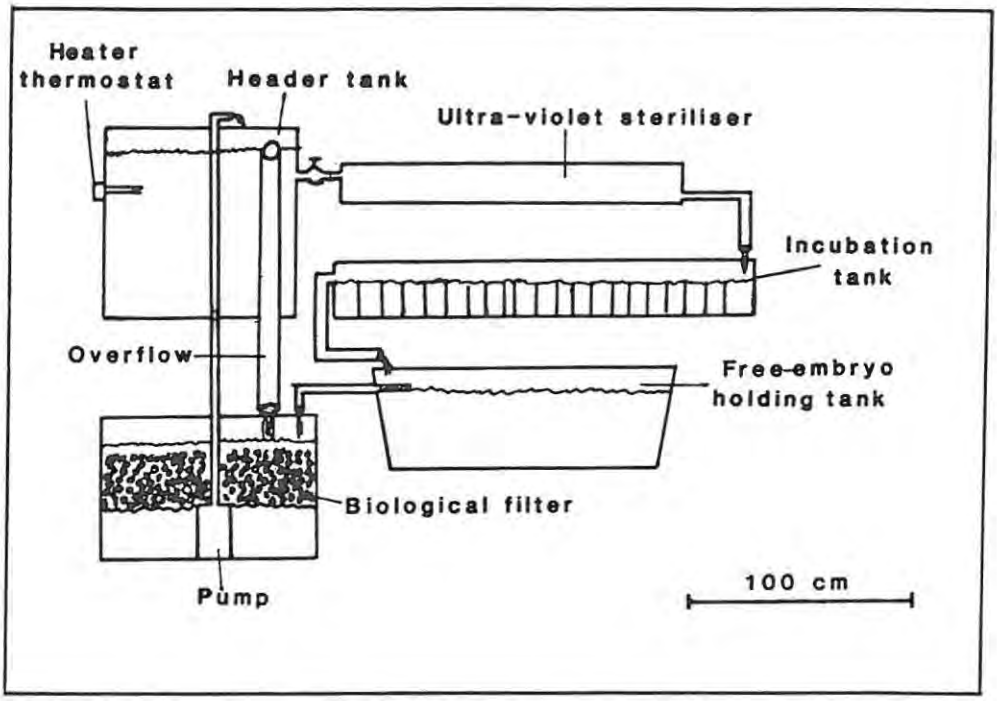

Figure 1. Egg incubation system used at Rhodes University, utilising recirculating water and ultra-violet sterilisation.

After hatching, which occurred 23 - 28 hours after fertilization at 23-27°C, the free embryos swam up and out of the incubation trough into secondary holding containers. Dead eggs and deformed free-embryos remaining in the incubation trough were then removed from the system. The free embryos were kept in the holding container until the commencement of exogenous feeding, whereupon they were counted into batches of 500 each and reared under the various experimental conditions.

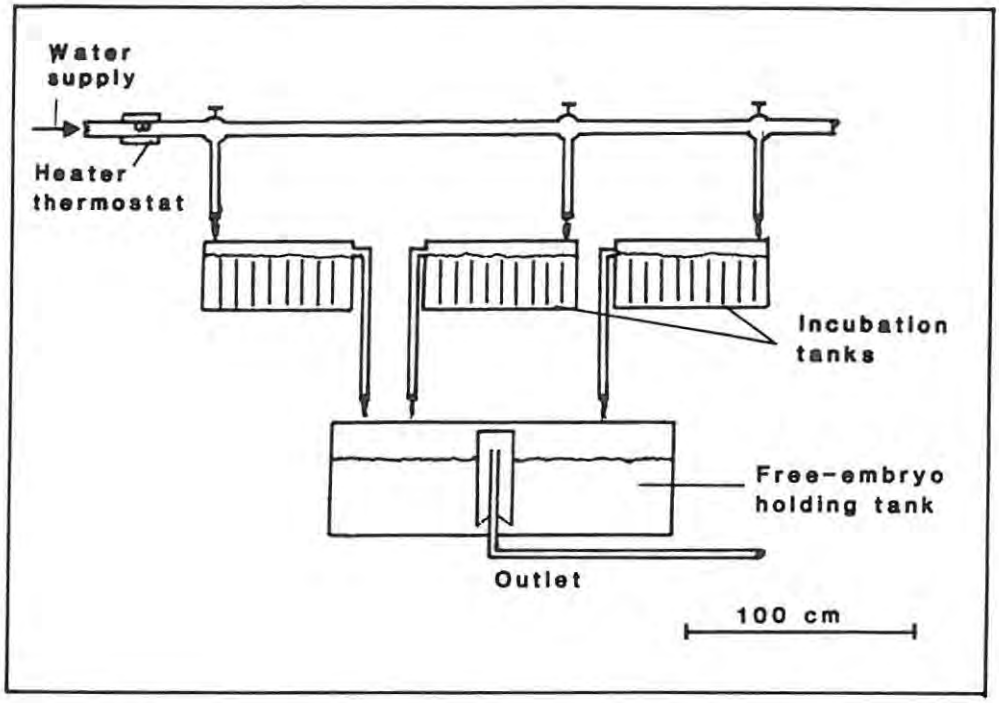

Figure 2. Egg incubation system used at the Blyde river hatchery utilising a "flow-through" water system.

REARING SYSTEMS

The rearing systems differed according to the requirements of each experiment, and are described in the various chapters.

MONITORING OF GROWTH AND SURVIVAL

The growth experiments were all initiated within 3 days after the start of exogenous feeding. At this stage larvae typically have a uniform mass of ca 10 mg, and a length of 7-8 mm. Samples of 30 fish were taken from each experimental tank every third or fourth day. The sampled fish were anaesthetised with benzocaine hydrochloride, preserved in a 5% buffered formalin solution, and subsequently measured and weighed. The duration of the growth experiments was between 12 and 16 days. Mortalities were

determined by removing and counting dead larvae on a daily basis, as well as by counting the surviving fish in each container at each sampling. At the termination of each growth experiment, larger samples of fish (60-100) were taken for measurement to facilitate more rigorous statistical analysis.

ANALYSIS OF DATA

Although the length and mass of the sampled fish were determined, length was chosen as the basis for presenting growth data. This was because length was considered to be a more "conservative" parameter than mass, in that the mean for the linearly increasing length parameter is subject to less variation than that of mass which increases by the cube. This is due to the variation about the mean being smaller for length, than for mass, although obviously, the absolute differences between the means are also smaller for the length parameter. In effect therefore, for samples of fish with a very variable size (a characteristic of cohorts of sibling catfish), the mean of length is less biased by extreme outlyers than that of mass. Furthermore, the mass of a fish can change dramatically over a short period of time, whereas length reflects the summation of physiological processes over a longer period. The length of larval catfish was found to increase in a linear fashion. Growth rates, were therefore determined from the slopes of regressions calculated from the sample means for length, observed for each treatment over the experimental period. For the sake of reference, regression equations for growth in terms of mass over the experimental period are included. These were calculated after cube root transformation of the mass data which produces a linear relation between body weight and the length of the culture period (Hogendoorn 1980, Verreth and Den Bieman 1987). The condition factor equation, $CF=100M/L^3$, was used to define differential trends in length and mass.

Size variation within cohorts of young sibling fish has been shown to be a factor which promotes cannibalism in several

species (Cooper 1937, Murphy 1949, Tarrant 1960, Wright 1970, Coutant and De Angelis 1983), including C. gariepinus (Hecht and Appelbaum 1987b). The size variation within the batches of larvae in the growth experiments was analysed to determine to what extent size variation is affected by different environmental factors. The sample variation about the mean was not considered an appropriate measure of size variation as this parameter increases proportionately with the sample mean, and therefore cannot be used to compare the size variation of groups with different means. Standard deviation expressed as a percentage of the mean, eliminates this bias and was adopted as a measure of size variation in this study using the following equation:

$$\text{Size Variation} = (\text{Sample standard deviation}/\text{Sample mean}).100$$

This equation was used to determine the size variation of all samples of larvae taken at the conclusion of each experiment.

STATISTICAL ANALYSIS

In all experiments, replicate treatments were combined if they were found to be not significantly different using a student t-test ($P<0.05$). To determine whether any significant differences were present between the different treatments in each experiment, analysis of variance in combination with Scheffe's range test was used.

A NOTE ON TERMINOLOGY

The definitions of Balon (1975) are used to describe the intervals in the development of C. gariepinus. Free-embryo is used here to describe the interval from hatching to the start of exogenous feeding. Larva describes the interval between the start of exogenous feeding and metamorphosis, and juvenile the interval from metamorphosis to sexual maturity.

CHAPTER 4.

TEMPERATURE PREFERENCE AND THE EFFECT OF TEMPERATURE ON THE GROWTH OF LARVAE AND JUVENILES

INTRODUCTION

Temperature is the single most important abiotic factor controlling the metabolism of obligate poikilotherms (Fry 1971, Beitinger and Fitzpatrick 1979). A knowledge of the physiological responses of a fish to temperature is therefore of vital importance to the fish culturist.

While aquaculture research is largely designed to have a direct application, it is vital to consider the natural history of an organism when designing experiments and interpreting results. C. gariepinus can be classified as r-selected (MacArthur and Wilson 1967), being in all respects a generalist. It can withstand an extremely wide range of environmental conditions, a fact which is reflected in its wide natural distribution (see chapter 1) It is extremely eurythermal, probably not encountering lethal temperatures throughout its distributional range. Donnelly (1973) observed that a diel temperature range of 13.5 to 27.5°C did not have any effect on the survival of adult fish and, under laboratory conditions, adults have been observed to tolerate temperatures as low as 6°C and as high as 50°C (Babiker 1984). Temperature profiles of the digestive enzymes of C. gariepinus have revealed that the animal is able to maintain high levels of digestive activity over an extremely wide range of temperature (10 - 40°C) (Uys and Hecht 1987). Eggs hatch successfully from 17-32°C (Van der Waal 1972, 1978, personal observations) and Greenwood (1955) found that larvae tolerated diel temperature fluctuations between 22 - 33°C in their natural habitat.

The objective of this study was to determine the optimum temperature requirements for the rearing of Clarias gariepinus larvae. Two complimentary methods were employed. Firstly, the larvae were grown at different temperatures, and secondly the final temperature preferendum of juveniles was determined.

The final temperature preferendum of a species is defined by Fry (1947) as; "a temperature around which all individuals will ultimately congregate, regardless of their thermal experience before being placed in the gradient" and "that temperature at which the preferred temperature is equal to the acclimation temperature". A considerable wealth of data, demonstrating final temperature preferenda to be innate, and species specific, supports Fry's hypothesis (Reynolds and Casterlin 1976, 1979, Magnuson and Beitinger 1978). Moreover, it is generally assumed that the final preferendum is the optimal temperature for most physiological functions of fish (Coutant 1975, Crawshaw 1977, Beitinger and Fitzpatrick 1979, Jobling 1981, Giattina and Garton 1982).

MATERIALS AND METHODS

The Effect of Temperature on Larval Growth

This experiment was conducted in a constant temperature room in the Department laboratories. At the onset of exogenous feeding, twelve batches of 500 larvae each were gradually acclimated at a rate of 1°C every two hours to 12 different experimental temperatures ranging from 22° to 33°C. The experimental containers consisted of twelve 180x40x30 cm glass aquaria holding 100 l of water each. Each tank was equipped with a 300 W thermoregulated immersion heater, and a box-type biological filter through which water was circulated by means of an air-lift. Cover was provided for the larvae in each tank in the form of 1.5m^2 of nylon shadecloth with 3 mm aperture mesh. The

containers were illuminated with neon light, and an 18L/6D photoperiod was maintained by mean of an electronic time switch. Fifty percent of the water was exchanged every fourth day at which time the bottom of each container was siphoned clean. Dechlorinated tap water was used in the experiment. All mortalities were recorded. Fish were fed ad libitum every three hours from 08h00 to 20h00. The fish were fed on the artificial dry feed described by Uys and Hecht (1985). In addition, at the first and last feeding every day the fish were given a quantity of live Daphnia.

Growth and survival in the experimental containers were monitored as described in the general methods section (chapter 3).

Temperature Preference of Juveniles

A horizontal thermal gradient was established in a glass tank divided by a series of baffles, made of dark tinted glass, into 12 chambers (Fig. 3). The temperature of the water flowing into the system varied between 22 and 23°C during the experimental period. The water was heated as it flowed through the chambers by means of 300W thermoregulated immersion heaters placed in each compartment. A temperature gradient of 1 ± 0.5°C was maintained in each of the twelve compartments, and in this way a thermal gradient with a range of 12°C was established. A flow rate of approximately 1 l/min was maintained. If desired the thermal gradient could be "shifted" by varying the flow rate. An airstone placed in each compartment prevented vertical temperature stratification and "smoothed" the gradient. A length of nylon net shadecloth was placed throughout the chamber as a "perching" substrate and to encourage the fish to move between compartments. The tank was illuminated with neon light, with an intensity at the water surface of 300 lux. A photoperiod of 18 hours light/ 6 hours dark was maintained by means of a time switch.

Twenty-five six week old juveniles (0.5 ± 0.15g) were placed in the gradient chamber corresponding to their acclimation

temperature (26°C). The number of fish and temperature in each of the 12 compartments was noted at two hourly intervals between 08h00 to 22h00 for the first 24 hours, and then at four hourly intervals between 08h00 and 22h00 for the following six days. Fish were fed twice a day with an equal amount of dry food in each compartment. Excess food was siphoned from the bottom of the chamber every morning. The perching substrate was removed and replaced daily to discourage the development of territoriality .

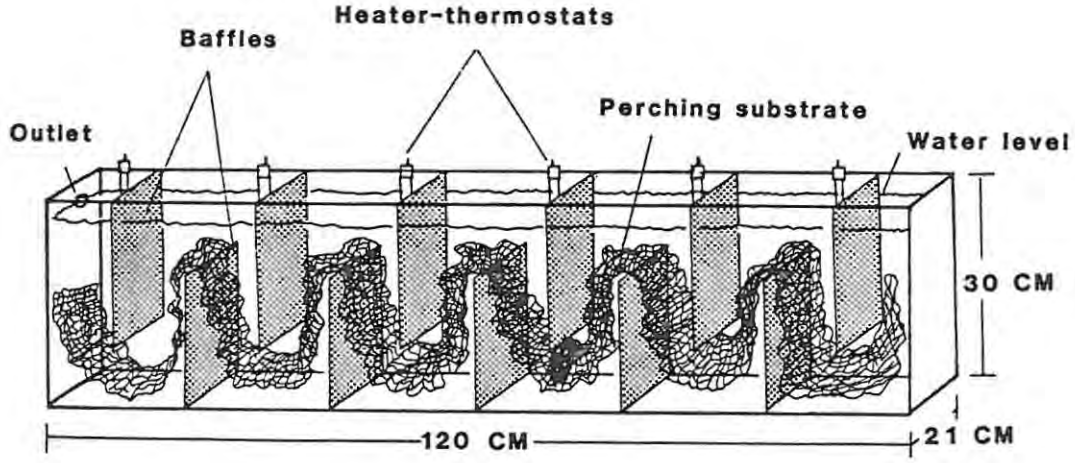

Figure 3. Horizontal thermal gradient chamber used to determine the temperature preference of Clarias gariepinus juveniles.

Note: The results of this study have already been published (Britz and Hecht 1987).

The Effect of Temperature on Larval Growth

The linear regressions from which the growth rates for each treatment were determined are presented in Table 1. A high growth rate was recorded over a fairly broad range of temperatures (26-33°C) with the highest growth rate at 30°C (Fig. 4).

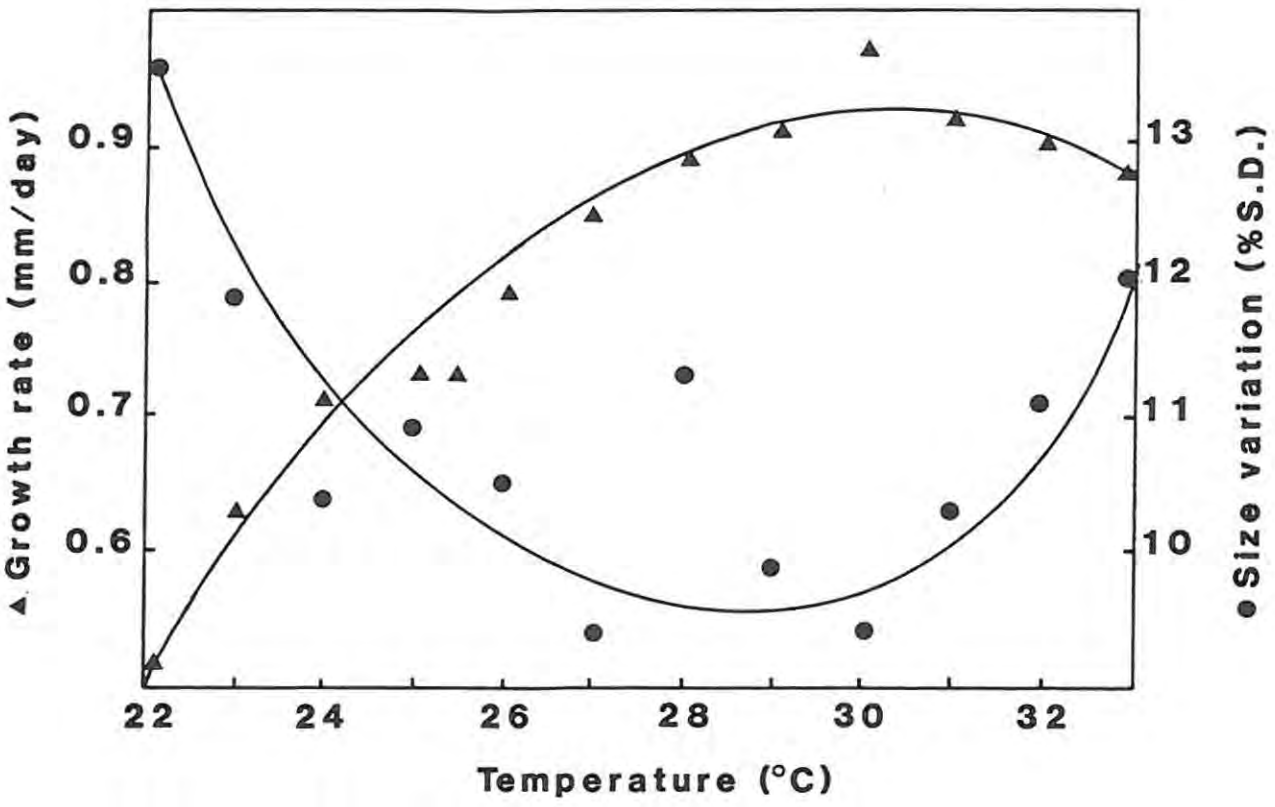

Figure 4. Growth rates and size variation of Clarias gariepinus larvae after 13 days rearing at different temperatures. For calculation of size variation, see chapter 3 (= growth rate, = size variation).

TABLE 1

Regression equations of length and weight-at-age of *Clarias gariepinus* larvae after 13 days rearing at different temperatures.

Temperature (°C)	Length (mm TL)/age (days)	r^2	Weight (mg)/age (days)	r^2
22	length=0.51age+5.98	0.93	weight=1.73+0.11age^3	0.88
23	length=0.63age+6.08	0.98	weight=1.81+0.14age^3	0.90
24	length=0.71age+5.94	0.97	weight=1.73+0.14age^3	0.94
25	length=0.73age+5.94	0.98	weight=1.69+0.16age^3	0.91
26	length=0.79age+6.14	0.99	weight=1.56+0.18age^3	0.92
27	length=0.85age+6.12	0.99	weight=1.56+0.19age^3	0.94
28	length=0.89age+6.03	0.99	weight=1.60+0.19age^3	0.97
29	length=0.91age+5.89	1.00	weight=1.37+0.20age^3	0.95
30	length=0.97age+5.98	1.00	weight=2.30+0.21age^3	0.97
31	length=0.92age+5.79	0.99	weight=1.40+0.20age^3	0.97
32	length=0.90age+5.68	0.99	weight=1.33+0.19age^3	0.95
33	length=0.88age+5.17	0.97	weight=1.06+0.19age^3	0.89

At temperatures below 26°C growth rates declined sharply. Statistical analysis (Table 2) revealed that the final lengths of larvae between 26 and 33°C were not significantly different ($P<0.01$), while those below 26°C were significantly lower ($P<0.01$) than those above.

Survival was good in all groups of fish. Total mortalities ranged from 15 to 25% (Table 3). However, approximately only a quarter of this mortality could be accounted for and it was assumed that the remainder was due to cannibalism.

Analysis of the growth data revealed that the size variation of larvae (Table 3) appeared to be affected by temperature (Fig. 4). Size variation was lowest over the temperature range at which the highest growth rates were recorded. At temperatures above and below this range size variation increased.

TABLE 2

Statistical analysis of mean final length after 13 days rearing at different temperatures, using ANOVA and Scheffe's range test. F-ratio = 22.7, significance level = 0.000. Asterisks in different columns denote significant differences between treatments, while those in the same column denote no significant differences. Significance level = 0.01.

Temperature (°C)	Scheffe's grouping		
22	*		
23	*	*	
24		*	
25		*	
26			*
27			*
28			*
29			*
30			*
31			*
32			*
33			*

TABLE 3

Condition factor and size variation of larval Clarias gariepinus after 13 days rearing at different temperatures.

Temperature (°C)	Mortality (%)	Condition factor	Size variation (%SD of x)
22	25	0.92	13.6
23	19	0.98	11.9
24	21	1.01	10.4
25	23	0.97	10.9
26	17	0.94	10.5
27	20	0.95	9.4
28	17	1.01	11.3
29	15	1.04	9.9
30	18	1.03	9.4
31	21	1.00	10.3
32	20	0.96	11.1
33	20	1.03	12.0

There was a weak relationship between the condition factor of the larvae and temperature at which they were reared (Table 3, Fig. 5). The condition factor was highest over the range of temperatures at which the highest growth rates were recorded.

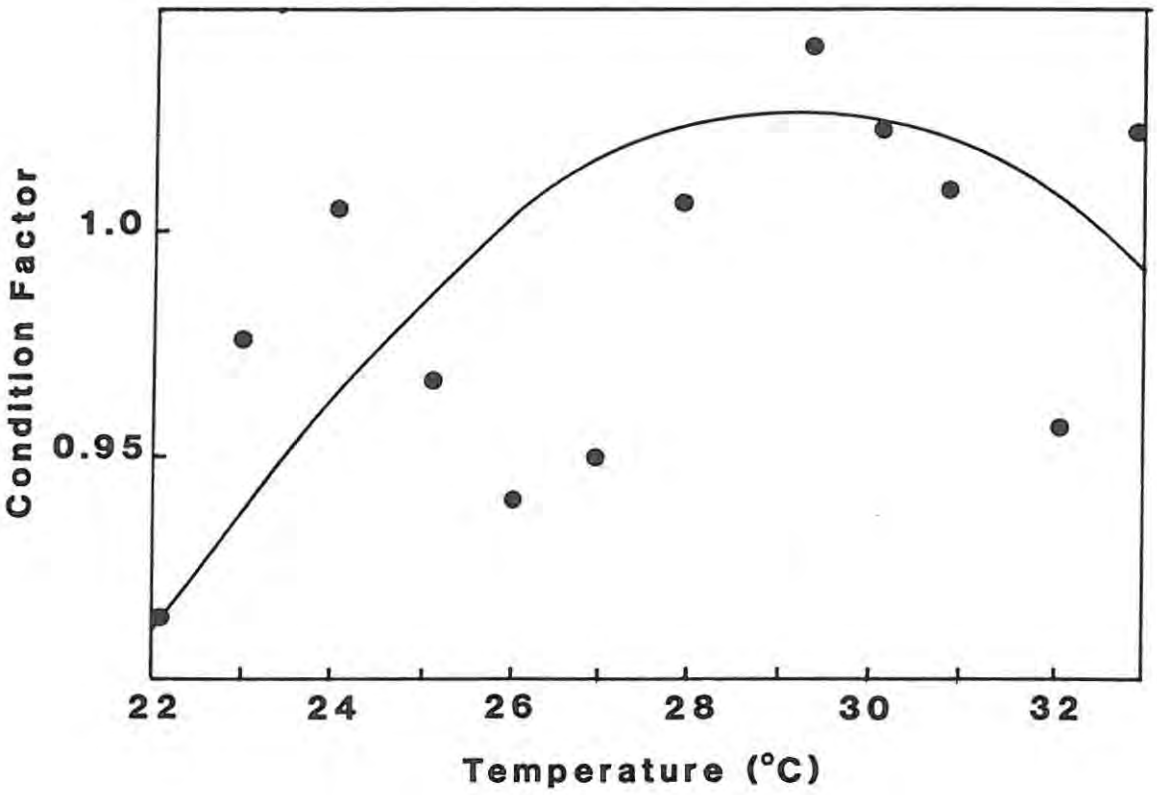

Figure 5. Condition factor of Clarias gariepinus larvae after 13 days rearing at different temperatures (curve fitted by eye).

Juvenile Temperature Preference

The optimal period for the determination of final temperature preferenda is between 24 and 96 hours after placement in the gradient (Reynolds and Casterlin 1979). This allows the fish to gravitate towards their final preferendum during the first 24 hours. In the present study, the final temperature preferendum, defined as the mode of the mean distribution of fish in the gradient between day 2 and 7, was found to be 30°C (Fig. 6).

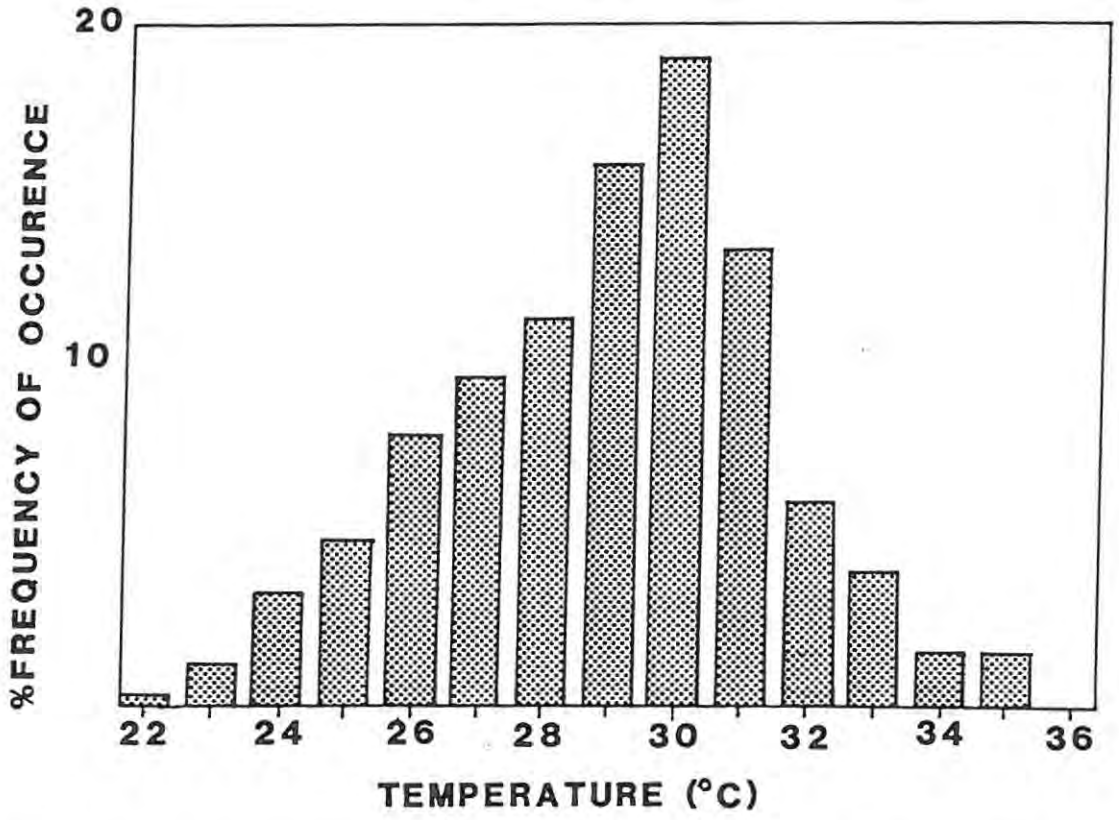

Figure 6. Mean distribution of Clarias gariepinus juveniles in a thermal gradient for 6 days following an initial acclimation period of 24 h. The mode of 30°C indicates the final temperature preferendum.

The distribution of fish in the gradient about the thermal preferendum had a negatively skewed distribution, there being a much higher frequency of occurrence in the 26-29°C range, than in

the 31-34°C range (Fig. 6).

The present experiment was not designed to determine the acute temperature preferendum, which is ideally determined within two hours of the placement of fish in a thermal gradient (Reynolds and Casterlin 1979). However, when the data collected over the first 24 hours were analysed certain acute effects were evident (Fig. 7). Two peaks in frequency of occurrence were present with one at 30 and another at 34°C, with a mean at 32°C.

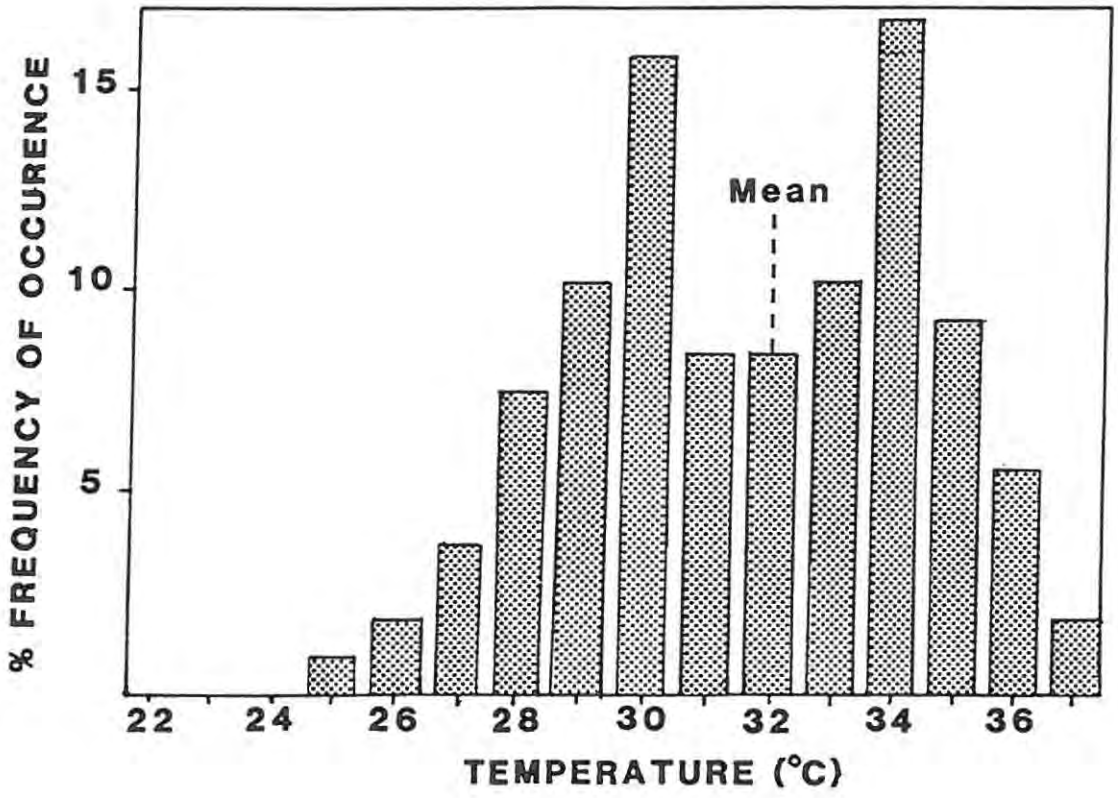

Figure 7. Distribution of Clarias gariepinus juveniles in a horizontal thermal gradient during the first 24 h after placement in the gradient. Note the bimodal distribution with the "initial overshoot" peak at 34°.

DISCUSSION

The present study revealed that larval growth rates were high over a wide range of temperature (26-32°C) and that survival remained high at temperatures over the entire experimental temperature range (22-33°C). These results complement previous observations (Greenwood 1955, Donnelly 1973, Van der Waal 1972, 1978, Babiker 1984) demonstrating C. gariepinus to be an extremely eurythermal species. The eurythermality of the larvae may be interpreted as an adaptation facilitating the reproduction of this fish, which spawns under unstable environmental conditions which accompany rain and flood (Greenwood 1957, Holl 1968, Bowmaker 1973). During this period temperatures may fluctuate widely, however the temperature tolerance of the larvae enables them to efficiently exploit the newly inundated floodplain environment, in which competition and predation are low and food resources probably abundant.

With regard to Clarias culture, the high growth rates and condition factor of the larvae recorded between 26 and 33°C indicate that this temperature range is optimal for larval rearing. The growth rates obtained in this study correspond well with the observations by Hogendoorn et al. (1983) who found that juvenile (0.5g) Clarias lazera (recently synonymised with Clarias gariepinus (Teugels 1986)) grew best between 27.5 and 32.5°C with satisfactory growth at 25°C. Although larvae were not grown at temperatures above 33°C in the present study, Hogendoorn et al. 1983 showed that growth rates decrease sharply above this level and that at 35°C some fish displayed scoliosis. (This deformity, which is commonly associated with heat stress, was probably caused by a lack of vitamin C).

The present results show that the size variation of larval C. gariepinus is affected by temperature. This has implications with regard to cannibalism, which is promoted by increasing size variation (Hecht and Appelbaum 1987b). Size variation was shown

to decrease as the temperature at which the highest growth rate was recorded (30°C) was approached. This implies that the rate of cannibalism would theoretically be lowest near the optimum temperature for growth. The only other observation found describing the effect of temperature on size variation was that of Coutant and DeAngelis (1983), who examined its effect on the size variation of largemouth (Micropterus salmoides) and smallmouth bass (M.dolomieui), two species in which size variation also promotes cannibalism. These authors (op. cit.) described a weak trend of increasing size variation with rearing temperature. This observation may however have been an analytical artifact, as the variation of the mean was used as a measure of size variation, which is a parameter which increases proportionately with the mean. The apparent increase in size variation with temperature noted by these authors may thus simply have been a reflection of the increasing mean size of the fish with increasing rearing temperature. The effect of temperature and other factors which may promote size variation between siblings of cannibalistic fish species remains an interesting question, deserving of further attention. This need is particularly relevant with respect to intensive fish culture, in which the role of cannibalism as a significant cause of mortality has received increasing recognition (see Hecht and Appelbaum 1987b).

The final temperature preferendum of 30°C corresponded exactly with the temperature at which the fastest growth was recorded. It was therefore concluded that this temperature is probably optimal for most physiological processes. This conclusion is supported by research into the feed requirements of C. gariepinus larvae and juveniles at different temperatures (Hogendoorn et al. 1983, Verreth and Den Bieman 1987). These authors found that feed conversion efficiency, protein efficiency ratio and growth rates were highest at 30°C.

The negatively skewed distribution of fish about the preferred temperature, observed in the present study (Fig. 6), is a

phenomenon that has been commonly observed in temperature preference studies on other species of fish as well as ectothermic species in other taxa (De Witt and Friedman 1979, Reynolds and Casterlin 1979). Similarly the growth rate of C. gariepinus larvae and juveniles appears to decrease more sharply above the optimum temperature for growth than below it (Hogendoorn et al. 1983, present results). Reynolds and Casterlin (1979) observed that the preferred temperature of warm water fish often lies within a few degrees of their avoided or upper lethal temperatures, and suggested that the upper thermal limits are more precisely thermoregulated than the lower limits and hence a skewed distribution is produced about the preferred temperature. De Witt and Friedman (1979) have, however, hypothesised that "negatively skewed temperature distributions have as their basis the regulation, not of temperature per se, but rather a physiological rate process whose rate is an exponential function of temperature". An example, cited by these authors, of a temperature dependent rate process is oxygen consumption which can be described by exponential parameters such as the Q_{10} or Arhenius U.

The mean preferred temperature (32°C), of C. gariepinus juveniles, during their first 24 hours in the gradient, was higher than the final temperature preferendum (30°C) indicating that certain acute effects were present (Fig. 7). Clay (1979) recorded a similar acute temperature preference (32.5±1.5 °C) for Clarias gariepinus juveniles, 60-120 minutes after placing the animals in a thermal gradient. These higher acute temperature preferenda are probably attributable to an "initial overshoot phenomenon" which is common in physiological processes following an acute temperature change. This has been observed in studies on several species of fish (Reynolds and Casterlin 1979). The acute temperature preference of fish, ideally determined within two hours of placing them into a gradient, is known to be influenced by acclimation temperature and other factors (Reynolds and Casterlin 1979) and does not necessarily reflect the optimum temperature for physiological processes. The final temperature

preferendum is therefore a more useful parameter for the fish culturist, seeking the optimum temperature to rear an organism.

On the basis of the present results, it was concluded that 30°C is the optimal temperature for larval rearing, however temperatures in the range 26-33°C are acceptable. The temperature tolerance of C. gariepinus is clearly of benefit to the fish culturist facilitating more flexibility with regard to temperature control in the larval rearing operation. This attribute could possibly preclude the installation of expensive equipment to regulate hatchery temperatures. For example, in the event of a temperature fluctuations, to which flow through systems drawing water at ambient temperatures are susceptible, the growth and survival of larvae would not be seriously impaired.

The high growth and survival rates of C. gariepinus larvae over a wide range of temperature shown in the present study lend support to the originally proposed hypothesis (chapter 3). Namely, that the wide environmental tolerances C. gariepinus displayed in nature will, under controlled conditions, be manifest as high growth rates and survival over a broad range of each environmental parameter.

CHAPTER 5.

THE EFFECT OF LIGHT ON THE BEHAVIOUR AND GROWTH OF LARVAE AND JUVENILES.

INTRODUCTION

Light is a powerful directive factor (sensu Fry 1971), cueing or synchronising the endogenous cycles of metabolism or activity in fish and other organisms. Most recent research into the effects of photoperiod and light intensity in fish have been investigations into their function as an environmental cue or "Zeitgeber" (Aschoff 1954), which synchronizes their physiological and activity rhythms (see Chovnick 1960, Schwassmann 1971, Thorpe 1978).

It is accepted that fish in freshwater as well as in the ocean show a cyclic daily activity pattern (Schwassmann 1971). An understanding of the nature of these activity patterns, could be of critical importance to the fish culturist striving to maximise the growth rate and survival of a species. The light - dark regime is the dominant factor affecting these diel activity patterns (Schwassmann 1971, Muller 1978, Eriksson 1978) and is thus deserving of attention when fundamental aquaculture research is undertaken.

Photoperiod and light intensity may affect the growth and survival of a species via a number of physiological pathways. Primarily they have been shown to affect the periodicity of endogenous physiological rhythms, but may also exert direct effects on the behaviour of an organism, not necessarily linked to any endogenous rhythm (Richus and Winn 1979). For example, it is known that rates of ontogenetic development (Eriksson & Lundqvist 1982, Saunders et al. 1985, Clarke and Shelbourn 1986), locomotor activity (Schwassmann 1971), gonadal development

(Baggerman 1979), thyroxine levels (Noeske and Spieler 1983), feeding behaviour (Schwassmann 1971, Tandler and Helps 1985), and stress responses (Billard et al. 1981), all of which can ultimately affect growth and survival, may be strongly influenced by photoperiod and light intensity. The growth rate of marine fish larvae has been found, almost universally, to increase with longer light photoperiods, as they rely almost entirely on vision to obtain food and cannot feed in the dark (Barahona-Fernandes 1979, Tandler & Helps 1985). Very few reports of the effect of light on the growth and survival of freshwater fish larvae exist, and those available show no clear trends. Meske (1985) examined the effect of photoperiod on the growth and survival of common carp (Cyprinus carpio), wels (Siluris glanis) and eel (Anquilla anquilla). Photoperiod had no effect on the growth and survival of carp and wels (a bottom dweller which shuns light), when fed on dry food. The wels would, however, only consume live food (larval Cyprinus carpio) in darkness. Meske (op. cit.) found that eels grew best with a 12 hour light / 12 hour dark photoperiod, if fed twice daily during the dark period, while shining weak torchlight into the tank. Unfortunately, no attempt was made to explain these interesting observations in terms of the natural history of these species and their application is thus limited. Saunders et al. (1985) demonstrated that the growth rate of hatchery reared atlantic salmon parr (Salmo salar) was highest under continuous light, but that their subsequent growth upon stocking into sea cages was depressed. Clarke and Shelbourn (1986) showed coho salmon fry (Onchorhynchus kisutch) reared with a delayed seasonal change in the photoperiodic regime grew five times as fast, and at a more even rate, than those reared under a natural photoperiod.

The different results obtained in these studies demonstrate that the simple empirical quantification of the effect of light, on the growth and survival of a species under culture conditions, does not necessarily lead to an explanation of the observed responses. To understand the effect of light on a species, and hence facilitate efficient aquacultural production, reference

must be made to its natural history and endogenous rhythms. In this study the effect of photoperiod and light intensity on the behaviour, growth and survival of larval and juvenile catfish, was examined and is interpreted in the context of the fish's ecology.

MATERIALS AND METHODS

Free-embryo phototaxis

The phototactic behaviour of a batch of approximately 30 000 free-embryos was observed in a series of three consecutive trials conducted 48-72 hours after fertilization.

After hatching (26-28 hours after fertilisation) in the recirculating incubation system (Fig. 1), the free-embryos were siphoned into an opaque, epoxy painted, rectangular glassfibre container (1200 X 600 X 500 mm) holding 80 l of water. Aerated water flowed through the container at a rate of 1 l/min and was recirculated through a gravel biological filter. The water temperature was maintained at 30^{o}C. All light intensities were measured at the water surface with a Gossen lux meter. Prior to the initiation of the trials the free-embryos were held under neon light, intensity 300 lux.

Trial 1. This initial trial was designed to determine the basic nature of free- embryo phototaxis. A beam of light, originating from a lamp with a 60 watt incandescent daylight bulb (2000 lux), was used to illuminate small areas (diameter 15cm) of the tank bottom. The response of the free-embryos was recorded as a negative or positive phototaxis.

Trial 2. In the light of the results of trial 1, this trial was

designed to determine the strength of the phototactic response at high light intensities. Aggregations of free-embryos in the container corners were successively exposed to light beam intensities ranging from 600 to 8000 lux and their rate of dispersal from the illuminated area was estimated. The rate of dispersal was defined as b the time it took for 50% of an aggregation of free-embryos to disperse. The stage at which 50% of an aggregation was dispersed was estimated visually. Although such a subjective measurement is open to error and criticism, it was considered adequate for the purposes of this investigation. After each exposure to the light beam the free-embryos were gently dispersed into the water column and then left to settle for fifteen minutes, in which time they reformed the aggregations in the corners.

Trial 3. This trail was designed to determine the strength of the phototactic response at low light intensities. The holding container was darkened by means of a black PVC cover and the free-embryos were left undisturbed for 16 hours to allow them to acclimate to the dark conditions. Using the procedure described for trial 2, the aggregations of free-embryos were exposed to light beam intensities ranging from 16 to 800 lux and their rate of dispersal estimated.

Juvenile Phototaxis.

The phototactic behaviour of five week old juveniles (0.18 g, 27 mm TL) was determined by placing them in a compartmentalised light gradient (Fig. 8). Each compartment was illuminated by a 60 watt incandescent daylight bulb, the intensity of which was controlled by means of a rheostat. Each compartment was lined with black PVC plastic to block out light from adjoining compartments and from the external environment. Small holes in the corners of each compartment allowed the fish to move between compartments. A liftable flap was cut into the PVC outside each compartment so that the fish could be observed. Hatchery water,

at 28°C, flowed through the container at a rate of 1 l/min. The water in each compartment was aerated.

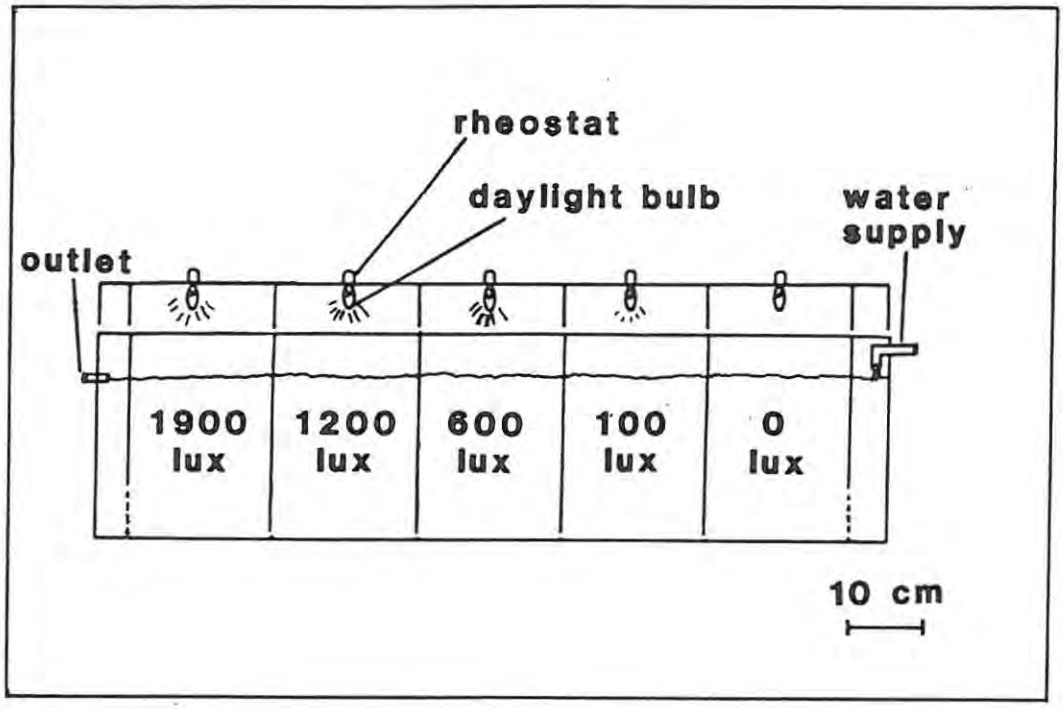

Figure 8. Light gradient chamber used to determine the phototactic response of Clarias gariepinus juveniles.

Prior to the start of the experiment the light intensity in all the compartments was set at 100 lux, and ten fish were placed in each compartment. They were then left for 12 hours to become accustomed to their new environment. The number of fish in each compartment was then recorded every hour for eight hours. Analysis of variance and Scheffe's range test revealed that there was no significant difference ($p<0.01$) in the distribution of fish between compartments. The light intensities were then adjusted to create a light gradient ranging from 0 to 1900 lux. The number of fish in each compartment was then recorded at three hourly intervals for fifteen hours daily. Every second day the light gradient in the tank was reversed.

The fish were fed a formulated larval feed (Uys and Hecht 1985), three times a day, which was distributed evenly in the chamber. Every day the bottom of each compartment was siphoned clean. The experiment was terminated after six days at which time the fish in each compartment were captured, anaesthetised and preserved in 5% buffered formalin for subsequent measuring and weighing.

The Effect of Photoperiod on Growth.

This experiment was performed in May 1988 (late summer) at the Blyde river hatchery. Growth was monitored and analysed as described in the general methods section (chapter 3). The growth and survival of replicate batches of larvae were investigated under photoperiod regimes of 0, 6, 12, 18, and 24 hours light per day. At the start of exogenous feeding, three days after fertilization, batches of 500 larvae were placed into square plastic bins (30 l capacity). The bins were illuminated with neon hatchery lighting (250 lux) and black PVC covers were used to regulate the photoperiod. A flow through system was used, at a rate of 2 l/min. Over the duration of the experiment, the water temperature was 24 $\pm$ 1^{o}C. The larvae were fed on dry larval feed, as formulated by Uys and Hecht (1985), every two hours for 18 hours each day. The experimental containers were siphoned clean every 48 hours.

The Effect of Cover on Growth in Light and Darkness

This experiment was performed in October 1987 (Spring) at the Blyde river hatchery. The growth and survival of larvae in the presence and absence of cover was monitored in continual light and darkness. At the start of exogenous feeding, replicate batches of 500 larvae were placed in rectangular plastic containers (60 l capacity, 20 cm water depth) half of which were darkened by means of black PVC covers. The open containers were illuminated with fluorescent light (250 lux). Cover, in the form of nylon netting (2 m^{2}, 3cm diameter mesh) was placed in half of the darkened and illuminated containers. Water flow was

maintained at 2 l/min., at a temperature of 23 ± 1°C. The larvae were fed formulated dry larval feed (Uys and Hecht 1985) which was supplemented twice daily with live zooplankton (Daphnia). Uneaten food and faeces were not removed during the experiment.

RESULTS

Free-Embryo Phototaxis

In trials 1-3, the free-embryos displayed a negative phototactic response which became stronger with increasing light intensity (Fig. 9).

At intensities above 50 lux it took less than a minute for 50% of an aggregation to disperse. From 50 lux down to 15 lux intensity, the 50% dispersal time increased markedly; however, even at the lowest intensity (15 lux) to which the free-embryos were exposed, a distinct negative phototaxis remained evident. As the light intensity to which an aggregation of larvae was subjected approached that of the acclimation intensity, so the strength of the phototactic response declined.

Juvenile Phototaxis

The juvenile catfish displayed a distinct preference for darkness, the highest mean frequency of fish being observed in the dark compartment (0 lux) (Fig. 10). ANOVA in combination with Scheffe's range test revealed that the percentage frequency of fish observed in the 0 lux and 100 lux compartments, was significantly higher ($p<0.01$) than in other compartments of higher light intensity (Table 4). It was noticed that most of the

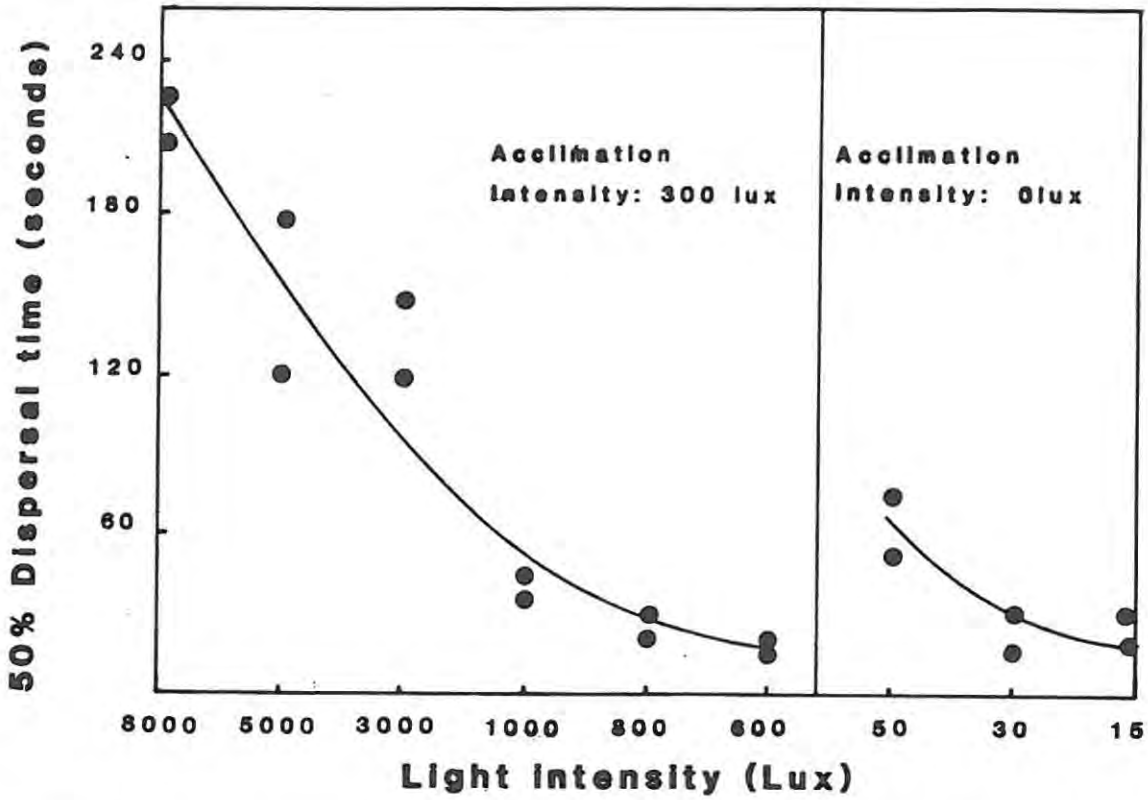

Figure 9. The strength of the negative phototactic response of Clarias gariepinus free-embryos subjected to different light intensities. 50% dispersal time was defined as the time taken for half an aggregation of larvae to disperse when subjected to light intensities higher than their acclimation intensity.

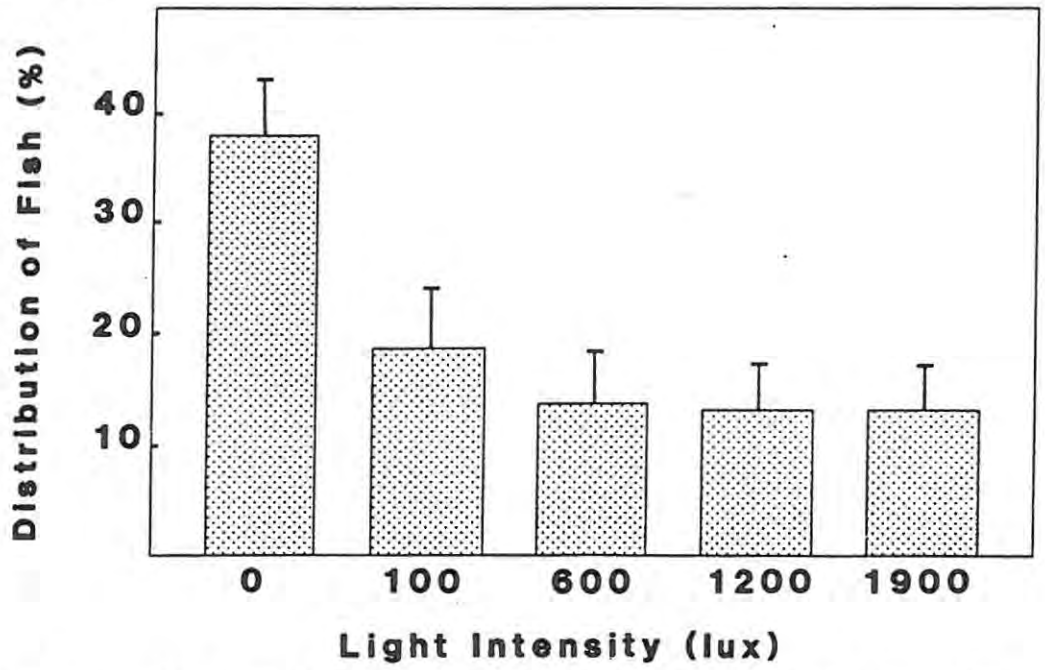

Figure 10. The mean distribution of Clarias gariepinus juveniles for a seven day period in a light gradient. (Half of the standard deviation of the mean is represented by the lines above the bars.)

TABLE 4

Statistical analysis of the mean distribution of fish in the light gradient over seven days, using ANOVA and Scheffe's range test. F-ratio = 103, significance level = 0.000. Asterisks in different columns denote significant differences, while those in the same column indicate no significant differences (P<0.01).

Light Intensity (lux)	Sheffe's Grouping		
0	*		
100		*	
600			*
1200			*
1900			*

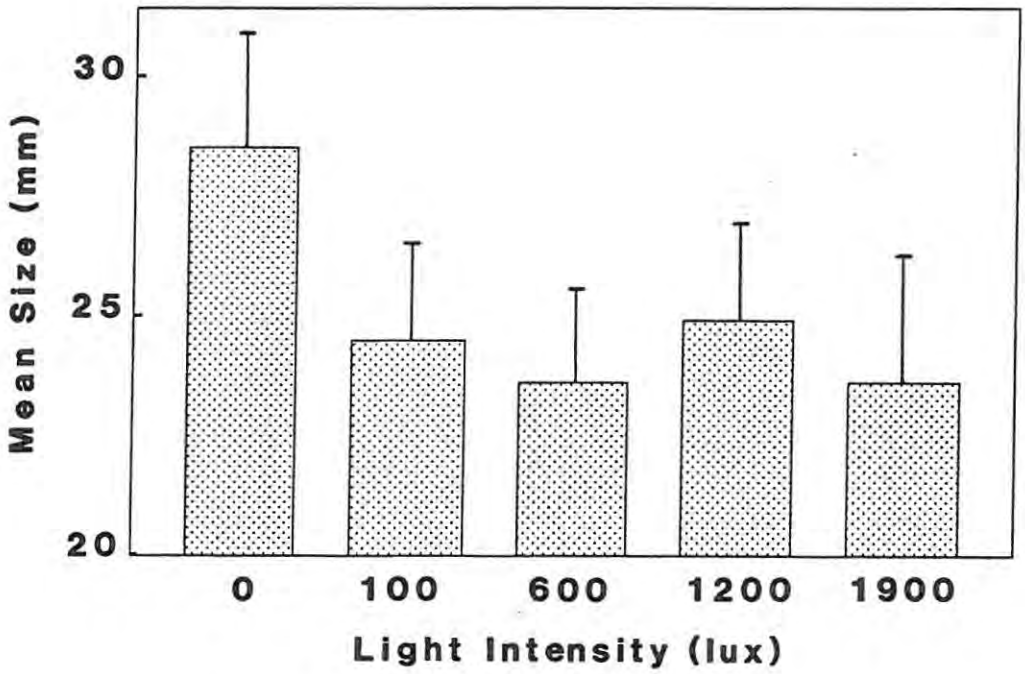

Figure 11. The mean size (mm) of Clarias gariepinus juveniles in the compartments of the light gradient at the end of a seven day period. (Half of the standard deviation of the mean is represented by the lines above the bars)

larger fish selected the darkened compartment (Fig. 11). These fish displayed a degree of territoriality occupying a 'home spot' or 'refuge', to which they would return when resting or upon being disturbed by the observer. Such refuges consisted of the corners and crevices created by the airstones, heater tubes, and container walls. Any other fish which approached would be chased from such a refuge.

Photoperiod and Growth

The data of the replicate treatments were pooled as a student t-test (Table 5) revealed that replicate sample means for length did not differ significantly ($p < 0.05$). The highest growth rates (see Table 6 for regression equations) were recorded for batches of fish reared with a 24D/0L photoperiod (continual darkness)(Fig. 12).

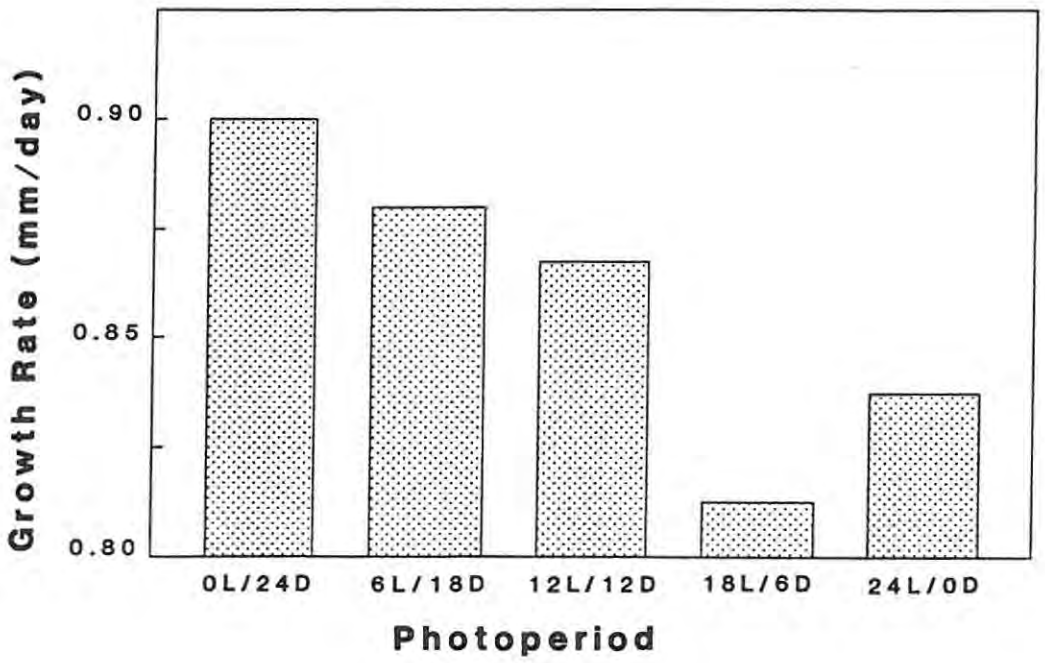

Figure 12. The growth rate of Clarias gariepinus larvae reared for 12 days under different photoperiod regimes.

TABLE 5

Student t-test analysis of replicate data for final mean length of larvae reared under different photoperiod regimes for 12 days.

Photoperiod	t-statistic	Probability
0L/24D	0.22	0.25
6L/18D	1.13	0.10
12L/12D	1.41	0.05
18L/6D	1.60	0.05
24L/0D	0.25	0.25

TABLE 6

Regression equations of length and weight-at-age of *Clarias gariepinus* larvae after 12 days rearing under different photoperiod regimes.

Photoperiod	Length (mm TL)/age (days)	r^2	Weight (mg)/age (days)	r^2
0L/24D	length=0.90age+8.80	0.99	weight=1.73+0.228age^3	1.00
6L/18D	length=0.87age+8.79	0.98	weight=1.73+0.223age^3	0.99
12L/12D	length=0.84age+8.91	0.98	weight=2.05+0.208age^3	1.00
18L/6D	length=0.73age+9.11	0.94	weight=2.80+0.167age^3	0.97
24L/0D	length=0.77age+8.80	0.98	weight=1.95+0.198age^3	0.99

Growth rates declined as the length of the light period increased. However, slightly higher growth rates were recorded in the 24L/0D treatment in comparison to the 18L/6D photoperiod. Statistical analysis (ANOVA and Scheffe's range test) revealed that the final mean length of larvae differed significantly between all treatments (Table 7).

TABLE 7.

Statistical analysis of mean final lengths after 16 days rearing under different photoperiod regimes, using ANOVA and Scheffe's range test. F-ratio = 102, significance level = 0.000. Asterisks in different columns denote significant differences between treatments, while those in the same column denote no significant differences. Significance level = 0.01.

Photoperiod	Scheffe's grouping				
0L/24D	*				
6L/18D		*			
12L/12D			*		
18L/6D				*	
24L/0D					*

A similar mortality was recorded for all treatments which ranged between 30% and 40% (Table 8). The condition factor of the larvae at the end of the experiment was highest in the treatments with longer dark periods (Table 8). The size variation of larvae did not, however, appear to be affected by the photoperiod treatment (Table 8).

Cover, Light and Growth

The data for replicate treatments were pooled as student t-test analysis (Table 9) revealed no significant difference ($p < 0.05$) between sample means for length. In the light treatments, higher larval growth rates (see Table 10 for regressions) were obtained in the containers provided with cover than those without (Fig. 13).

TABLE 8

Condition factor and size variation of larval Clarias gariepinus reared for 13 days under different photoperiod regimes.

Photoperiod	Mortality (%)	Condition factor (%)	Size variation (%SD of $\bar{x}$)
0L/24D	31	1.21	7.0
6L/18D	37	1.22	7.0
12L/12D	34	1.15	5.7
18L/6D	35	0.94	6.0
24L/0D	39	1.14	7.0

TABLE 9

Student t-test analysis of replicate data for final mean length of larvae reared under different photoperiod regimes in the presence or absence of cover for 13 days. D = 0L/24D photoperiod, L = 24L/0D photoperiod, COVER = cover present, NO-COVER = cover absent.

Treatment	t-statistic	Probability
D/COVER	0.62	0.25
D/NO-COVER	0.67	0.25
L/COVER	0.93	0.10
L/NO-COVER	1.49	0.05

TABLE 10

Regression equations of length and weight-at-age of Clarias gariepinus larvae after 13 days rearing in continual darkness or light in the presence or absence of cover. D = 0L/24D photoperiod, L = 24L/0D photoperiod, COVER = cover present. NO-COVER = cover absent.

Treatment	Length (mm TL)/age (days)	r^2	Weight (mg)/age (days)	r^2
D/COVER	length=1.38age+5.23	0.99	weight=1.16+0.31age^3	0.99
D/NO-COVER	length=1.35age+5.61	1.00	weight=1.37+0.30age^3	1.00
L/COVER	length=1.29age+5.28	0.99	weight=1.30+0.29age^3	0.99
L/NO-COVER	length=1.11age+6.06	1.00	weight=1.91+0.25age^3	1.00

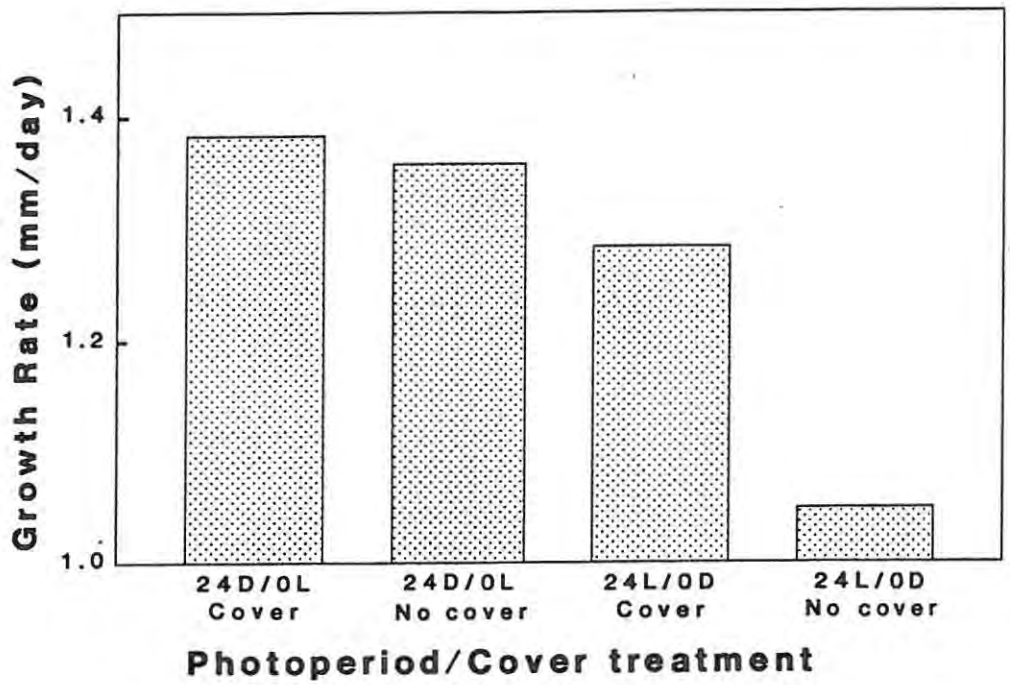

Figure 13. The growth rate of Clarias gariepinus larvae after 13 days rearing under different combinations of photoperiod and cover (nylon shadecloth netting).

These differences were reflected in the mean final lengths of the larvae which differed significantly ($p<0.01$ ANOVA and Scheffe's range test) between the two treatments (Table 11). In darkness, however, similar growth rates were recorded in the presence or absence of cover (Fig. 13) and there was no significant difference ($p<0.01$ ANOVA and Scheffe's range test) in the mean final length of larvae between the two treatments (Table 11).

TABLE 11.

Statistical analysis of mean final length after 16 days rearing in continual light or darkness, with and without cover, using ANOVA and Scheffe's range test. F-ratio = 40, significance level = 0.000. Asterisks in different columns denote significant differences between treatments, while those in the same column denote no significant differences. Significance level = 0.001. L = 24L/0D photoperiod, D = 0L/24D photoperiod, COVER = cover present, NO-COVER = cover absent.

Photoperiod	Scheffe's grouping		
D/COVER	*		
D/NO-COVER	*		
L/COVER		*	
L/NO-COVER			*

Furthermore, the growth rates of larvae in all the dark treatments were higher than those in the light, regardless of whether cover was provided or not (Fig. 13). These differences were reflected in the mean final lengths of the larvae which were significantly higher ($p<0.01$ ANOVA and Scheffe's range test) for those reared in darkness (Table 11).

A similar rate of mortality was recorded for all treatments, which ranged between 14-20 % (Table 12). The condition factor of larvae was slightly higher for those reared in darkness (Table 12), however the size variation of the larvae did appear to be affected by the experimental treatments (Table 12).

TABLE 12

Condition factor and size variation of Clarias gariepinus larvae after 13 days rearing in continual darkness or light in the presence or absence of cover. D = 0L/24D photoperiod, L = 24L/0D photoperiod, COVER = cover present, NO-COVER = cover absent.

Treatment	Mortality (%)	Condition factor	Size variation (%SD of $\bar{x}$)
D/COVER	14	1.08	12.9
D/NO-COVER	19	1.07	11.0
L/COVER	16	1.06	13.0
L/NO-COVER	20	1.06	11.9

DISCUSSION

Experimental evidence in this study has shown that C. gariepinus larvae and juveniles display a distinct negative phototaxis and increasingly higher growth rates with shorter photoperiods. Cover was found to enhance growth under light conditions. While these empirical results have a direct application in aquaculture, they can only be fully interpreted in the context of the animals observed natural history. Very few observations, particularly with regard to larvae, have however been made of the effect of photoperiod and light intensity on C. gariepinus.

Fish can be classified into diurnally active species, relying predominantly on cone vision, and nocturnal species which rely more on tactile, chemical or electrical senses (Schwassmann 1971). Two lines of evidence suggest that Clarias gariepinus falls into the latter category. Firstly, it appears that adult fish have a diel activity rhythm, being most active and feeding predominantly at night. This has been conclusively demonstrated by Bruton (1979c) in Lake Sibaya where C. gariepinus hunts most actively at night, migrating into shallow inshore waters to prey predominantly on cichlids. A similar nocturnal inshore migration of related clariid catfish was described by Coulter (1961) in Lake Tanganyika. The predominantly nocturnal activity of C. gariepinus can also be inferred from gill-net, long-line and

sportfishing catch statistics in other water bodies, for example, Lake Victoria (P.H. Greenwood, British Museum of Natural History, personal communication), Okavango river and delta (Merron 1988), Orange river system (personal observations), and Olifants River System (W. Uys, Rhodes University, personal communication), where catches are usually highest at night, particularly during the first four hours after sundown. Behavioural observations under controlled conditions, which show that Clarias juveniles are negatively phototactic (present study) and display enhanced activity in darkness (Britz and Pienaar in prep.), provide further evidence suggesting that C. gariepinus is nocturnally active. Secondly, C. gariepinus is anatomically better adapted to seek prey and avoid being preyed upon under conditions of low light or darkness. This is because its acuity of vision is very poor and the animal relies primarily on the tactile, chemosensory and electrosensory functions of its four pairs of circumoral barbels to detect food or prey, and to explore its physical environment (Lissman and Machin 1963, Bruton 1979b, Hecht & Appelbaum 1987b, personal observations). Hecht & Appelbaum (1987b) investigated the relative importance of eyes and barbels in prey capture by post-larval catfish and showed that they are tactile and possibly chemo-receptive rather than visual predators. The negative phototaxis of Clarias larvae, which are tactile feeders, therefore contrasts markedly with the almost universal positive phototaxis of planktonic marine fish larvae which are visual feeders. Bruton (1979b) provided conclusive evidence that the predation efficiency of adult Clarias gariepinus on Oreochromis mossambicus, a cichlid with very acute vision, is greatly enhanced at night. In addition, the tactile predation strategy of the catfish is reflected in the mode of prey capture which follows a search - contact - lunge/suck and capture sequence (Bruton 1979b, Hecht & Appelbaum 1987b). This is in stark contrast to visual predators which display a search - orientate - fix - strike and capture sequence, with a well-defined reactive distance (Vinyard and O'Brien 1976, Brownell 1985, Colgan, Brown and Borsatti 1986).

It should be noted that C. gariepinus is not exclusively active at night as it will opportunistically adopt a searching and feeding behaviour pattern if food or prey are detected in the light. This was shown experimentally by Bruton (1979b) in Lake Sibaya, and is demonstrated vividly in the annual catfish run in the Okavango river, Botswana (Merron, in press). Here, in a feeding frenzy which continues unabated day and night, the catfish move upstream in a dense mass devouring any prey encountered. A similar opportunistic daytime predation strategy by Clarias gariepinus has been observed in Lake Victoria. Here the fish congregate in high densities under the floating nests of breeding cormorants and prey on any chicks falling into the water (P.H. Greenwood, B.M.N.H, personal communication). Intensively fed larvae and juveniles reared in continual light, however, displayed reduced browsing behaviour (Britz and Pienaar, in prep.) and lower growth rates (present study) than in darkness, indicating that under culture conditions, light suppresses feeding activity.

Over the last 30 years a considerable amount of evidence has been accumulated demonstrating the diel activity patterns of fish to be primarily expressions of endogenous circadian rhythms, which are synchronised by environmental periodic factors (eg. light and temperature) called "Zeitgebers" (Schwassmann 1971, 1980). Under constant laboratory conditions from which periodic fluctuations have been eliminated, circadian activity rhythms usually continue with a free running period (t) which approximates the environmental period (T) of 24 hours (Kavaliers 1980). The present study was not designed to establish whether the diel activity of this species would, in continual light or darkness, continue with a free running period. It is, however, of interest to consider whether any evidence exists demonstrating the presence of an inherent, endogenous circadian rhythm in this species. No indication of a sustained diel activity pattern, in continual light or darkness, was observed in the present study or during previous general observations of hatchery reared larvae. Recent behavioural observations (Britz and Pienaar in prep.)

have, however, shown that territorial aggression gradually declines and becomes negligible over a seven day period in continual darkness. Conversely, a gradual increase in territorial aggression was observed in continual light over the same period. These gradual behavioural changes perhaps represent the breakdown of an inherent activity rhythm in the absence of the light "Zeitgeber". Further experimental work is thus required to establish conclusively whether a free running period, approximating 24 hours, exists or not in this species.

The negative phototaxis of C. gariepinus appears to be intimately linked to the extremely strong cover-seeking response of the animal under light conditions. In their natural habitat, larvae inhabit the cover provided by the rootstocks of semi-aquatic plants or bundles of flotsam and detritus on the edges of rivers and lakes (Bruton 1978). Laboratory observations without cover have shown that, in the light, the behaviour of larval and juvenile C. gariepinus is characterised by increased territorial aggression and darting movements (disturbed activity) interspersed with long rest periods, (Britz and Pienaar in prep.). The activity of larval and juvenile C. gariepinus in the light, appears therefore, to be part of an inherent behavioural strategy to avoid visual predators. Predation is considered to be a major factor structuring aquatic communities (Paine 1966, Connell 1975, Menge and Sutherland 1976, Zaret 1980), and a number of studies have demonstrated that habitat complexity may intimately affect predator/prey interactions, providing refuges for prey and reducing predator foraging efficiency (Vince, Valiela, Backus and Teal 1976, Stein and Magnuson 1976, Heck and Thoman 1981, Crowder and Cooper 1982, Savino and Stein 1982, Anderson 1984, Cook and Streams 1984, Wolf and Kramer 1987). In contrast to adult fish, which can be considered top predators, Clarias larvae are probably subject to an intense predation pressure in their natural habitat. This is firstly because the relatively fecund adults exhibit synchronous spawning, which results in high densities of larvae being concentrated in the marginal vegetation after spawning (Bruton

1979a, P.H. Greenwood, B.M.N.H., personal communication). Secondly, the larvae which are not very strong swimmers and have poor acuity of vision, are poorly equipped to visually anticipate and escape the approach of a predator. It is therefore of benefit for the larvae to seek refuge in shaded areas provided by cover during the daytime and to forage more widely for food at night. In so doing, they are able to avoid visual detection by predators, yet feed just as efficiently due to their tactile foraging strategy. This supposition is supported by the observations of Britz and Pienaar (in prep.) who showed that in darkness territoriality is reduced while browsing activity is increased. These observations are consistent with the generalisations drawn by Helfman (1986) who, in his review of the effect of light on the behaviour of fishes, stated: "In the wild, most of a fish's day appears to be spent either pursuing food or avoiding predators; many fish appear to separate the day into an active food gathering phase and a relatively inactive resting phase that is intimately linked with predator avoidance".

Cover seeking and increased territoriality during light periods have interesting implications with regard to intra-specific interactions. The habitat space available to larval catfish in the daytime is, in effect, drastically reduced as larval fish all tend to home in on suitable refuge areas, and are thus concentrated into a relatively small portion of the available three dimensional space. A limited number of refuges will tend to promote competition for occupancy and this would explain the increased territorial aggression observed under light conditions. Furthermore, it has been shown that the provision of shelter promotes territoriality during the light period (Hecht & Appelbaum 1987b). It appears that larger fish always tend to possess the prime refuges, a phenomenon that is always evident in larval and juvenile holding tanks (personal observations). It is further illustrated by the size distribution of fish in the light gradient experiment (Fig. 4) in which the dark compartment, which may be regarded as a refuge area, was monopolised by the larger fish. It has been shown that cannibalistic aggression is high

among larval and juvenile catfish (Hecht & Appelbaum 1987b) and the greater number of intra-specific contacts, as a result of refuge seeking behaviour, could potentially cause a significant increase in the rate of cannibalism. For example, a small fish seeking a refuge could be cannibalised when it invades the territory surrounding the refuge of a larger fish. Alternatively, a larger fish looking for a refuge may eat a smaller fish occupying a suitable refuge. The extent to which this effect is present in this species' natural habitat is, in the absence of field observations, purely speculative but will certainly be affected by the nature and extent of available natural cover. Further field work including behavioural observations are needed to quantify the extent of cannibalism in the wild.

With regard to the culture of C. gariepinus it is evident from the above discussion, that the rearing of larvae in light, without the provision of cover is highly stressful and explains the low growth rates recorded under these conditions (see results of the cover and photoperiod experiments). The provision of cover in light appears to alleviate this stress to a certain extent (evidenced by slightly better growth rates), however the higher growth rates obtained in darkness, regardless of the presence of cover, clearly demonstrate that this condition is optimal from a culture point of view. The similar growth rates obtained with or without cover in continual darkness, are explained in terms of the observed behaviour of the fish (Britz and Pienaar in prep). The predominantly resting behavioural mode, observed in the light, which is intimately linked to territoriality, is replaced by a predominantly active, foraging behavioural mode in darkness, with no territoriality. The presence of cover, which provides suitable territorial spaces, is therefore made redundant in the dark, accounting for the similar growth rates observed in the darkened containers in the presence or absence of cover. The increase in growth rate associated with a decreasing light period appears directly related to the increased foraging activity observed in the dark. As the dark period increases, more time is devoted to foraging resulting in a greater intake

of food and an enhanced growth rate.

On the basis of the observed growth rates, and the reduction of territorial and possibly cannibalistic aggression in the dark, it is recommended that Clarias larvae be reared, for culture purposes, in continual darkness. It should however be noted that successful larval rearing is possible under a variety of photoperiod regimes, as evidenced by the the good survival of larvae obtained for all photoperiod treatments.

CHAPTER 6.

THE EFFECT OF SALINITY ON LARVAL GROWTH AND SURVIVAL.

INTRODUCTION

In contrast to its extreme tolerance of most environmental factors (eg. temperature, low oxygen (Bruton 1979, Quick and Bruton 1984, Babiker 1984)) C. gariepinus is, however, a relatively stenohaline species not tolerating salinities above 10-15 ‰ (Nair 1969, cited in De Kimpe and Micha 1974, Clay 1977b, Babiker 1984, Chervinski 1984). Most inland bodies of fresh water have a measurable salinity, particularly in South Africa, where increasing salinity is a problem in certain rivers, and groundwater is often brackish with salinities of up to 12 ‰ (Viljoen, Meiring du Plessis and Hall 1984, S.A. Dept. Water Affairs unpubl. data 1987). While the lethal levels of salinity have been determined for Clarias juveniles and adults, very little is known about the effects of sub-lethal concentrations of salinity on the growth and survival of this species. Such knowledge is obviously an important factor if this species is to be considered for culture in areas with brackish water.

In the present study, the effect of salinity on the growth and survival of larval catfish was investigated in order to define a range of salinities suitable for larval rearing.

MATERIALS AND METHODS

Replicate batches of 500 larvae, spawned in fresh water (0 ‰), were acclimated to 0, 2.5, 5.0, 7.5, and 10 ‰ salinity by increasing the saline concentration of the medium by 2.5 ‰ every three hours. Appropriate amounts of NaCl were used to obtain the desired concentrations which were checked using a refraction salinometer. The experiment was conducted in 25 l epoxy painted glass-fibre containers which were aerated. The water temperature

was maintained at 27°C by means of thermostatically controlled immersion heaters. The experimental containers were illuminated with neon light (250 lux) and a photoperiod of 13L/11D was maintained with an electronic time switch. The larvae were fed every 2-3 hours from 08h00 to 22h00 with dry larval feed (Uys & Hecht 1985). This diet was supplemented with a single feed of live zooplankton (predominantly Daphnia) administered daily at 16h30. Every day uneaten food and faeces were siphoned off the bottom of the experimental containers and 80% of the water in each container was replaced.

The osmotic concentration of the plasma of C. gariepinus reared in fresh water was determined with a freezing point depression osmometer (Advanced Instruments model 3D Digimatic Osmometer). Ten adult fish (300-5000g) were anaesthetised with benzocaine-HCl and blood was drawn from the heart using a hypodermic syringe. The blood was immediately centrifuged and the layer of plasma drawn off. The plasma from each fish was divided into two sample tubes which were then analysed in the osmometer. Adults were used because the plasma samples obtainable from the juveniles were too small to be analysed in the osmometer available for the study. The plasma osmotic concentration of freshwater fish is, however, universally of a similar concentration and it was therefore considered a reasonable assumption that adult and juvenile C. gariepinus would have a similar plasma osmotic concentration.

RESULTS

Growth and Survival

The growth rate of the larvae for each salinity treatment was determined from the slope of a linear regression, calculated from the means for length of samples taken over the experimental period (Table 13).

TABLE 13

Regression equations of length and weight-at-age of Clarias gariepinus larvae after 16 days rearing at different salinities.

Salinity (‰)	Length (mm TL)/age (days)	r^2	Weight (mg)/age (days)	r^2
0.0	length=0.99age+5.31	0.96	weight=1.40+0.250age^3	0.98
2.5	length=0.98age+5.58	0.98	weight=1.73+0.251age^3	0.99
5.0	length=0.99age+5.35	0.98	weight=1.68+0.242age^3	1.00
7.5	length=0.94age+5.09	0.96	weight=1.15+0.229age^3	1.00
10.0	-	-	-	

Replicate data sets for each treatment were pooled, as student t-test analysis revealed that there was no significant difference between replicate means (Table 14).

TABLE 14

Student t-test analysis of replicate data for final mean length of larvae reared under different salinity treatments.

Salinity (‰)	t-statistic	Probability
0.0	0.55	0.25
2.5	0.05	0.25
5.0	0.13	0.25
7.5	0.15	0.25

The growth rates of larvae were similar up to a salinity of 5 ‰, but were slightly lower at 7.5 ‰ (Fig. 14). No growth data were obatined for the 10 ‰ treatment as all larvae died within 48 hours.

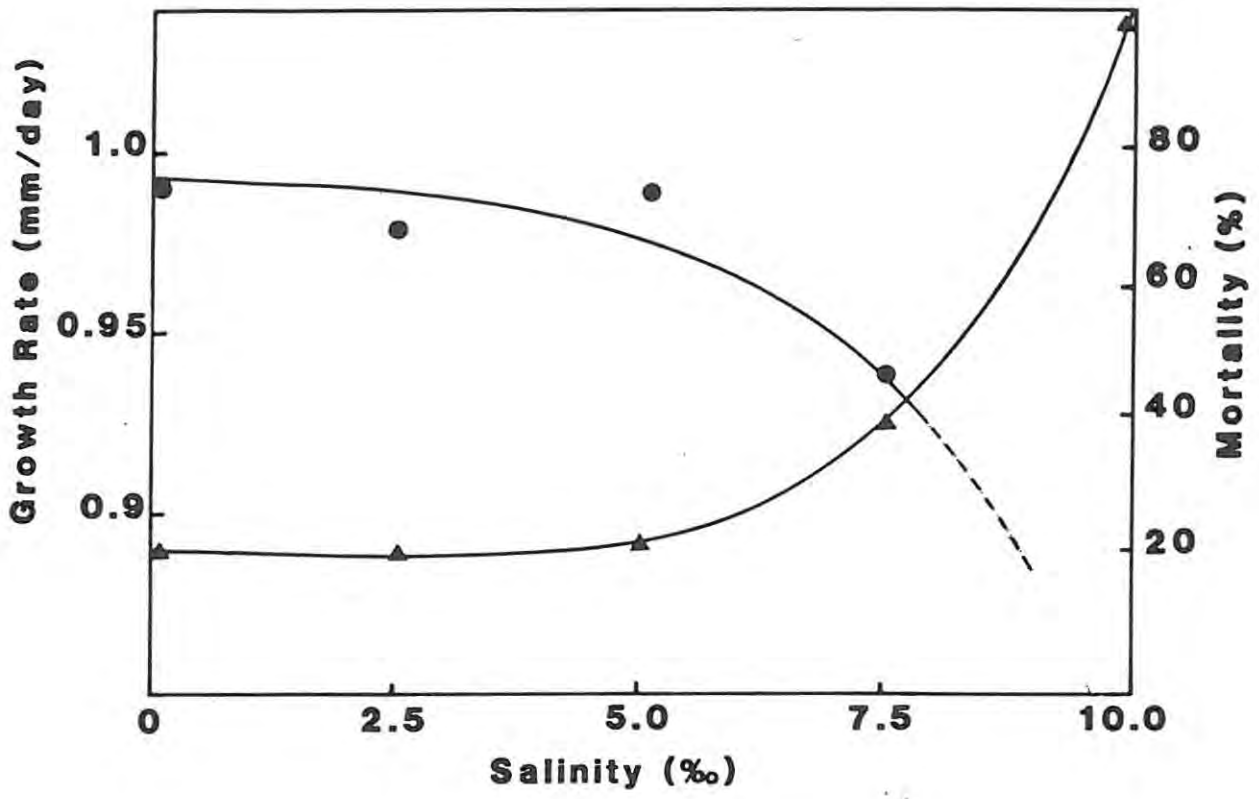

Figure 14. Growth and mortality rates of Clarias gariepinus larvae reared over a 16 day period at different salinities. (● = growth rate, ▲ = mortality).

Statistical analysis revealed that while the final lengths of larvae did not differ significantly between 0-5 ‰ , those recorded at 7.5 ‰ were significantly lower (table 15) than in the 0-5‰ range. At the higher salinities, the larvae were visibly thinner. This was reflected in their condition factor which decreased with increasing salinity (Fig 15, Table 16).

A similar mortality of larvae (15-20%) was recorded between 0-5‰ salinity, however, at 7.5 ‰ the mortality rate was higher (40%), and at 10‰ all larvae died within 48 hours (Fig. 14, Table 16).

TABLE 15

Statistical analysis of mean final length after 16 days rearing at different salinities, using ANOVA and Scheffe's range test. F-ratio = 3.18, Significance level = 0.024. Asterisks in different columns denote significant differences between treatments, while those in the same column denote no significant differences. Significance level = 0.01.

Salinity (‰)	Scheffe's grouping	
0.0	*	
2.5	*	
5.0	*	
7.5		*

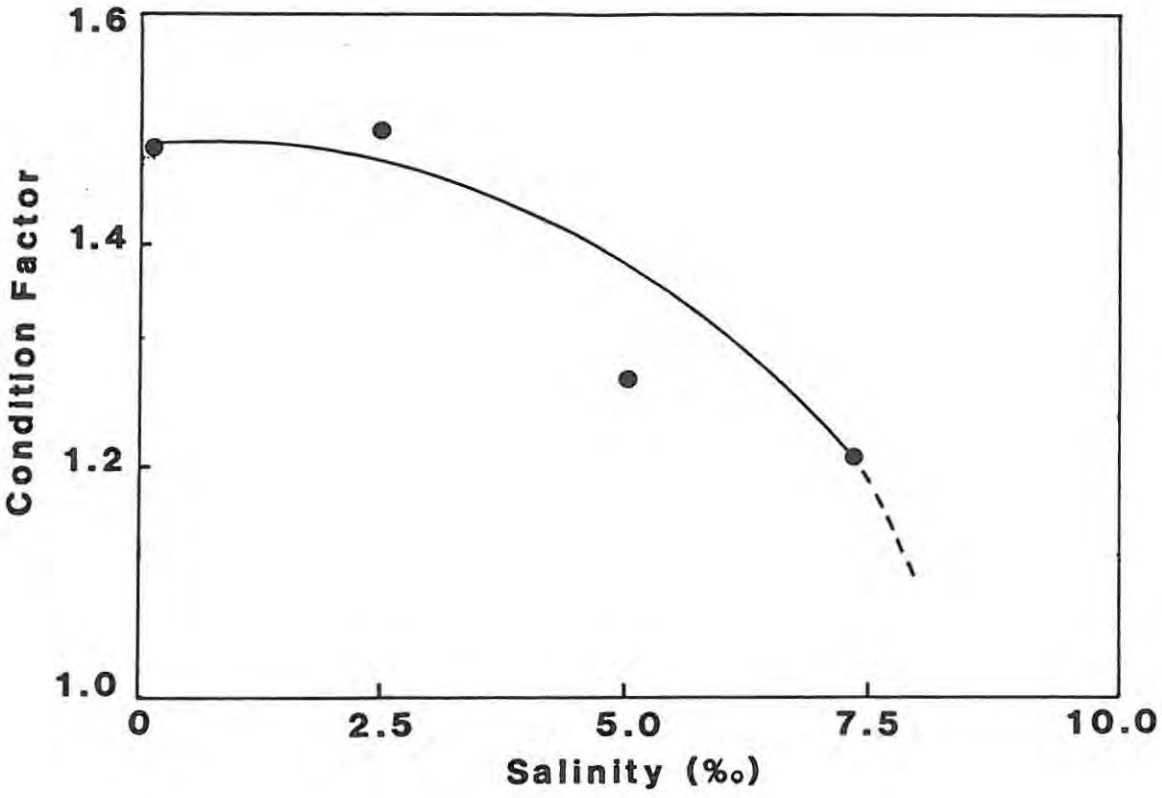

Figure 15. Condition factor of Clarias gariepinus larvae after 16 days rearing at different salinities.

TABLE 16

Condition factor and size variation of Clarias gariepinus larvae reared for 16 days at different salinities.

Salinity (‰)	Mortality (%)	Condition factor	Size variation (%SD of x)
0.0	20	1.34	13.7
2.5	23	1.4	15.5
0.5	21	1.24	13.8
7.5	40	1.21	13.3
10.0	100	-	-

Plasma Osmotic Concentration

The mean osmotic concentration of the plasma of ten adult fish was found to be 280 $\pm$ 20 mOsm/kg, which is equivalent to a salinity of 9.5 ‰ .

DISCUSSION

A number of studies on the effect of salinity on growth and survival of a variety of fish species exist, and have been subject to an extensive review by Brett (1979). Brett's analysis of existing data revealed that the highest growth rates, of the various species considered, clustered either around 0 ‰ (freshwater), or 10$\pm$2‰ , or 28-35‰ salinity. These clusters correspond roughly to three ecological groupings: 1) freshwater stenohaline and anadromous species, 2) euryhaline species, and 3) strictly marine species. The data of the present and other studies (Table 3) demonstrate conclusively that Clarias gariepinus falls into the freshwater stenohaline group. Decreasing growth rates with increasing salinity, as shown for Clarias larvae in the present study, appear to be characteristic of freshwater stenohaline fish (Brett 1979). It has been postulated that this is due to increasing maintenance requirements at higher salinities (Brett 1979, Kilambi 1980). Associated physiological effects observed with increasing

salinity are increased food intake but poor conversion efficiency, reduced oxygen consumption and weight loss (Arunachalam and Reddy 1979, Maceina and Shireman 1979, Kilambi 1980, Kilambi and Zdinak 1980, Maceina, Nordlie and Shireman 1980, Von Oertzen 1985). The decreasing condition factor of C. gariepinus larvae observed at higher salinities in the present study are probably a reflection of such effects.

With regard to salinity tolerance, Clarias larvae, juveniles and adults display a similar response to other freshwater stenohaline fish, in that they do not survive and grow for prolonged periods in salinities much above 10 ‰ for prolonged periods (Table 17).

TABLE 17

Effect of salinity on the growth and survival of freshwater stenohaline fish.

Species	Initial size (mm)	Initial size (g)	Best growth rate (‰)	Lethal salinity (‰)	Duration (Days)	Reference
Mystus vittatus	1.3-2.1	-	0	>10	30	Arunachalam & Reddy (1979)
Heteropneustes fossilis	adult(?)	-	-	>13.7	14	Parwez et al. (1979)
Heteropneutes fossilis	adult (?)		-	>10	6	Al Daham & Bhatti (1977)
Ictalurus punctatus	115-149	7.4-17.3	-	>12	40	Allen & Avault (1971)
Cyprinus carpio	hatched larvae		3	-	8	Lam & Sharma (1985)
Cyprinus carpio	-	-	-	17	-	Al Hamed (1971)
Ctenopharyngodon idella	100-120	-	-	12-14	4	Maceina & Shireman (1979)
Ctnopharyngodon idella	-	-	-	>9	-	Maceina et al. (1980)
Ctenopharyngodon idella	32-50	0.4-1.1	-	>9.75	30	Chervinski (1977)
Ctenopharyngodon idella	-	-	0	-	15	Kilambi (1980)
Ctenopharyngodon idella	-	40.8	-	>10.5	24	Cross (1970)
Hypothalmicthys molitrix	53-67	1.1-2.4	-	>7.8	2	Chervinski (1977)
Hypothalmicthys molitrix	35-55	(juvs.)	-	12-14	-	von Oertzen (1985)
Carassius auratus	84	12.1	1-6	>15	70	Canagaratnam (1959)
Carassius auratus	larvae		1-6	8	21	Murai and Andrews (1977)
Poecilia reticulata	70	-	7-12	-	40	Gibson & Hirst (1955)
Ictiobus cyprinellus	-	-	-	>9		Hollander & Avault (1975)
Rutilus rutilis	20-36	(juvs.)	-	12.5±1	>365	Schofer (1979)
Perca fluviatilis	adult (?)		-	12-16	>90	Lutz (1972)
Clarias gariepinus	60	2.6	-	>9.75	7	Chervinski (1984)
Clarias gariepinus	-	-	-	>11.6	-	Babiker (1984)
Clarias gariepinus	-	600-1500	-	>15	4	Clay (1977b)
Clarias gariepinus	-	-	-	>11.6	-	Nair (1969), cited in De Kimpe and Micha (1974)
Clarias gariepinus	larvae		0-5	8-10	16	Present study

The salinity tolerance of Clarias gariepinus appears to be dependent on the stage of development of the fish, since the tolerance of the larvae as observed in the present study was lower than that documented for juvenile and adult fish in other studies (Clay 1977b, Chervinski 1984). Similarly, an increase in salinity tolerance with stage of development has been documented for other freshwater stenohaline species, for example Ictalurus punctatus (Allen and Avault 1969) Buffalo fish (Hollander and Avault 1975) and roach Rutilus rutilus (Schofer 1979).

Most information regarding the osmoregulatory physiology of teleost fish is based on euryhaline fish, with the economically important salmonids having received most attention (Brett 1979, Goswami, Parwez and Sundaraj 1983, Norton and Davis 1977). Fish regulate their plasma ions such that the internal osmotic pressure of their body fluids is equivalent to approximately 10‰ salinity, with a range of 2‰ depending on tolerance, regulating capacity, and environmental salinity (Brett 1979, Holmes and Donaldson 1969). While a fairly large body of literature exists regarding osmotic and ionic regulation in fishes, explanation of the underlying mechanisms involved remain largely speculative (Evans 1980). It has been found that freshwater stenohaline fish are, in general, unable to maintain a constant concentration of body salts as the external salinity rises to concentrations isosmotic and hyperosmotic to their body fluid concentration (Goswami et al. 1983, Norton and Davis 1977, Maceina et al. 1980, Ellory, Lahlou, and Smith 1972). Body fluid osmolarity increases as the salinity of the external medium is increased and the salinity tolerance of these fish is thus probably limited by the maximum body fluid osmotic pressure in which the cells can function (Maceina et al. 1980). The results of the present study indicate that Clarias larvae do not survive and grow in salinities higher than those isosmotic with their body fluid (plasma) concentration (9.5‰).

The relatively low salinity tolerance of C. gariepinus has probably prevented its penetration of brackish waters in which more euryhaline species such as O. mossambicus exist.

The growth and survival of Clarias larvae in salinities up to 7.5‰ could facilitate the prevention and treatment of most freshwater ectoparasitic pathogens. At the Rhodes University hatchery ectoparasitic infections of Gyrodactylus, Dactylogyrus, and Icthyophthyrius infections have been successfully treated with salt. As a preventative measure against pathogenic infection larvae can be reared in low salinities (<5 ‰). This strategy could be particularly effective in recirculating rearing systems where the salinity of the system is easily regulated. If increasing the salinity of the rearing system is not desired or practical, for example in flow-through systems, the culturist could treat outbreaks of disease by subjecting the larvae to higher salinities (5-10 ‰) for short periods.

With regard to Clarias culture, it was concluded that salinities up to 5 ‰ are suitable for larval rearing with the optimum being 0-2.5 ‰. Salinities higher than 5 ‰ appear unsuitable for larval rearing due to declining growth rates and survival.

CHAPTER 7.

THE EFFECT OF TANK HYGIENE ON THE GROWTH OF LARVAE AND AN INVESTIGATION INTO THE ACUTE EFFECTS OF AMMONIA ON JUVENILES

INTRODUCTION

The two major water quality parameters limiting intensive fish culture are low dissolved oxygen, and the accumulation of nitrogenous wastes (primarily ammonia), which are the products of metabolism and/or food wastes (Westers and Pratt 1977, Holt and Arnold 1983, Soderberg, Flynn and Schmittou 1983). Poor water quality may affect the health of fish under intensive rearing conditions in two ways. Firstly, low dissolved oxygen or toxic substances may have a direct detrimental effect on metabolism, and secondly, poor water quality leads to stress which renders the fish more susceptible to disease. The culture of Clarias gariepinus is a relatively recent undertaking, and scant information exists in the literature regarding water quality criteria necessary for its intensive culture. In the present study certain aspects of water quality were investigated as a step towards defining water quality criteria necessary for the intensive rearing of this species.

Airbreathing catfish are renowned for their hardiness, tolerance of poor water quality, and ability to survive environmental extremes lethal to other fish species. These qualities endow the catfish with a unique potential for highly intensive pond culture, in that aeration or high water exchange rates, necessary for the intensive culture of other species, are not required (Areerat 1987, Huisman and Richter 1987). Dissolved oxygen levels are only limiting in the early larval stages before the suprabranchial organ becomes functional at approximately 3-4 weeks (Balon unpublished data 1987, personal observations). Adequate dissolved oxygen levels are, however, easily maintained in the hatchery environment by means of aeration or good flow rates. The airbreathing ability of the catfish therefore

facilitates extremely high stocking densities and yields per hectare. For example, in Thailand Clarias batrachus stocking densities of 100 000 - 500 000 and yields of 100 tons per hectare are reported for static pond culture (Panayotou et al. 1972, Sidthimunka 1972, Areerat 1987). The water quality parameter limiting intensive catfish production is, therefore, likely to be the level of ammonia in the water.

High levels of ammonia, sometimes encountered in aquacultural production, may cause suppressed growth (Brockway 1950, Kawamoto 1961, Burrows 1964, Larmoyeaux and Piper 1973, Holt and Arnold 1983, Colt and Tchobanoglous 1976, Alderson 1979, Sadler 1981), sub-lethal histopathological changes (Burrows 1964, Flis 1968a&b, Smart 1976, Thurston, Russo, Leudtke, Smith, Maine, Chakoumakos, Wang and Brown 1984) and even death. These effects usually occur under conditions of high stocking density and intensive feeding. For effective water quality management in intensive fish culture systems, it is therefore necessary that the levels of ammonia toxic to the cultured species be known. The levels of ammonia tolerated by catfish, although reputedly high, had not previously been quantified.

The toxicity of ammonia to fish is well documented (see review of Alabaster and Lloyd 1980). Aqueous ammonia exists in a dynamic, primarily pH dependent, equilibrium between the ionised (NH_4^+), and unionised form (NH_3) (Emerson, Russo, Lund and Thurston 1975, Alabaster and Lloyd 1980). The toxicity of ammonia depends principally on the NH_3 fraction, and NH_4^+ is considered relatively harmless (Wuhrman and Woker 1948, Downing and Merkens 1955, Lloyd 1961). The differential toxicity of the two forms of ammonia is ascribed to the ability of the uncharged, lipid soluble NH_3 molecules, to readily diffuse across the gill cell membranes, whereas the charged NH_4^+ molecules, which are hydrated and larger, do not readily pass through the charge lined micropores of the gill membrane (Fromm and Gillette 1968, Hampson 1976). High internal concentrations of NH_3 affect intracellular and blood pH, adversely affecting the oxygen binding capacity of

haemoglobin. Histopathological effects of NH_3 on gills include epithelial cell hyperplasia and hypertrophy, hemorraging, karyolysis, telangiectasis (dilation of capillaries forming a small tumor), cellular degeneration, separation of pillar cells, and congestion of blood in gill lamellae (Smith and Piper 1975, Smart 1976, Burkhalter and Kaya 1977, Thurston, Russo and Smith 1978, Redner and Stickney 1979).

The objective of the present study was to provide an initial definition of certain water quality criteria necessary for the intensive rearing of larval and juvenile C. gariepinus. Firstly, the acute effects of ammonia on C. gariepinus were investigated and secondly, larval growth was monitored in relation to tank hygiene.

MATERIALS AND METHODS

Ammonia Bioassay

Juvenile C. gariepinus, age 35 days (30 ± 5 mm TL, 5 ± 1 g) which had commenced airbreathing were used for the experiment. The fish had been reared at 28°C in the recirculating hatchery water system at Rhodes University, and fed ad libitum with live Daphnia in combination with a formulated dry larval feed (Uys and Hecht 1985).

Two trials were conducted to determine the 96-h median lethal concentration (96-h LC50) for NH3. The trials were designed as an initial "screening test" based on the standard fish toxicity testing procedures recommended by Alabaster and Lloyd (1982).

The trials were conducted under static bioassay conditions, in 30l epoxy painted fibreglass containers. Reagent grade ammonium chloride (NH_4Cl) was used as a source of un-ionised ammonia. Ninety-five percent of the toxicant solution in the experimental containers was replaced every 24 hours with freshly made up

solutions. Dechlorinated tap water (detailed analysis - Table 18) was used to make up the toxicant solutions. The experimental

TABLE 18

Analysis of water used for ammonia toxicity experiments.

Parameter	Analysis
Conductivity (ms/m)	51
Alkalinity $CaCo_3$ (ppt)	39
Cl (ppm)	110
Ca (ppm)	20
Mg (ppm)	9
Na (ppm)	63
K (ppm)	2.8
Fe (ppm)	trace
Mn (Ppm)	-
Zn (ppm)	trace
Cu (ppm)	-

water temperatures were maintained at 28°C, by means of thermostatically controlled immersion heaters. The containers were lightly aerated during the toxicity tests. No measurable loss of ammonia due to aeration was found during a 24 hour period, as reported by others (Gerking 1955, Colt and Tchobanoglous 1976). A 16L/8D photoperiod was maintained by means of an electronic time switch prior to, and during the experimental period.

The experimental treatments consisted of five concentrations of ammonium chloride equivalent to unionised ammonia concentrations ranging from 1 - 3 mg/l, and a fresh water control. Each treatment and the control were duplicated. Twenty fish were used for each treatment in the first trial and 28 fish per treatment in the second trial. Feeding was stopped 24h before the trial, and the fish were not fed during the trial period to prevent any possible changes in the toxicant concentration that might have been caused by the excretion nitrogenous metabolites. The mortality of fish was recorded every 12 hours.

Oxygen concentrations and pH were measured twice daily. Oxygen concentrations exceeded 90% saturation in all bioassays and pH

was constant at 6.76 $\pm$ 0.02. Water samples were taken daily before the toxicant solutions were replaced and their ammonia-nitrogen concentration determined using the Nesslerisation method (Golterman, Clymo and Ohnstad 1978). The fraction of unionised ammonia was calculated using the equations of Emerson, Russo, Lund and Thurston (1975).

Ultrastructural Effects of Ammonia

At the conclusion of the second trial, the gills and liver of two surviving fish from each experimental concentration were dissected out for ultrastructural examination of the toxic effects of ammonia. The gill tissues were washed in a phosphate buffer solution to remove external mucous, and fixed in a 2.5% glutaraldehyde solution in phosphate buffer (pH 7.3).

These tissues were prepared for transmission electron microscopy according to the procedures recommended by Cross (1985). Briefly, the tissues were secondarily fixed with a 1% buffered Osmium tetroxide (OsO_4) solution, followed by dehydration in an ethanol gradient. After treatment with propylene oxide (a transitional solvent), the samples were embedded in resin (TAAB/ARALDITE mixture). Sections were cut with an ultra-microtome, heavy-metal stained and examined with a transmission electron microscope (JEOL model JEM 100CX). Representative photomicrographs of tissues for each ammonia concentration were taken.

Tank Hygiene and Growth

The growth of larval C. gariepinus was monitored in replicate rearing containers subject to different cleaning intervals. Cleaning consisted of the removal of uneaten food and faeces (hereafter referred to as waste) by means of a siphon. The experimental treatments consisted of replicate batches of 500 larvae in containers cleaned at 24 and 48 hourly intervals with

a control which was not cleaned at all. The rearing containers consisted of rectangular plastic bins (60 l capacity) with a flow through water supply at a of rate 2l/min. . The larvae were fed ad libitum every two hours, for 18 hours a day, on a formulated larval feed (Uys and Hecht 1985). The rearing containers were illuminated with neon light (250 lux) and subject to a 24 hour light photoperiod (This photoperiod regime was used because the experiment was conducted prior to the photoperiod experiment, and at this stage a 24L/OD photoperiod was maintained at the Blyde river hatchery where this experiment was performed). Growth and survival was monitored as described in the general methods section (chapter 3). At the conclusion of the experiment, water samples were taken from each treatment for ammonia analysis.

RESULTS AND DISCUSSION

Ammonia Bioassay

Shortly before death, the fish were observed hanging vertically with their mouths at the water surface. This response, normally observed under conditions of low dissolved oxygen, indicates a probable impairment of respiratory function due to their exposure to ammonia. The 96 hour mortality and LC50 of the fish, calculated by probit analysis (Sprague 1973), is illustrated in Fig. 16. The 96h-LC50 was calculated to be 2.3 mg/l unionised ammonia. This figure is relatively high, being similar to those recorded for other 'coarse' fish (e.g. carp and tilapia), and considerably higher than the figures for the more sensitive salmonids (Table 19).

Toxicity bioassays in which death is used as a standard response unfortunately have a limited application in aquaculture. These standard tests which were primarily developed for pollution monitoring are, however, the only accepted

procedures currently available for comparing the toxicity tolerance of different fish species. With regard to fish culture,

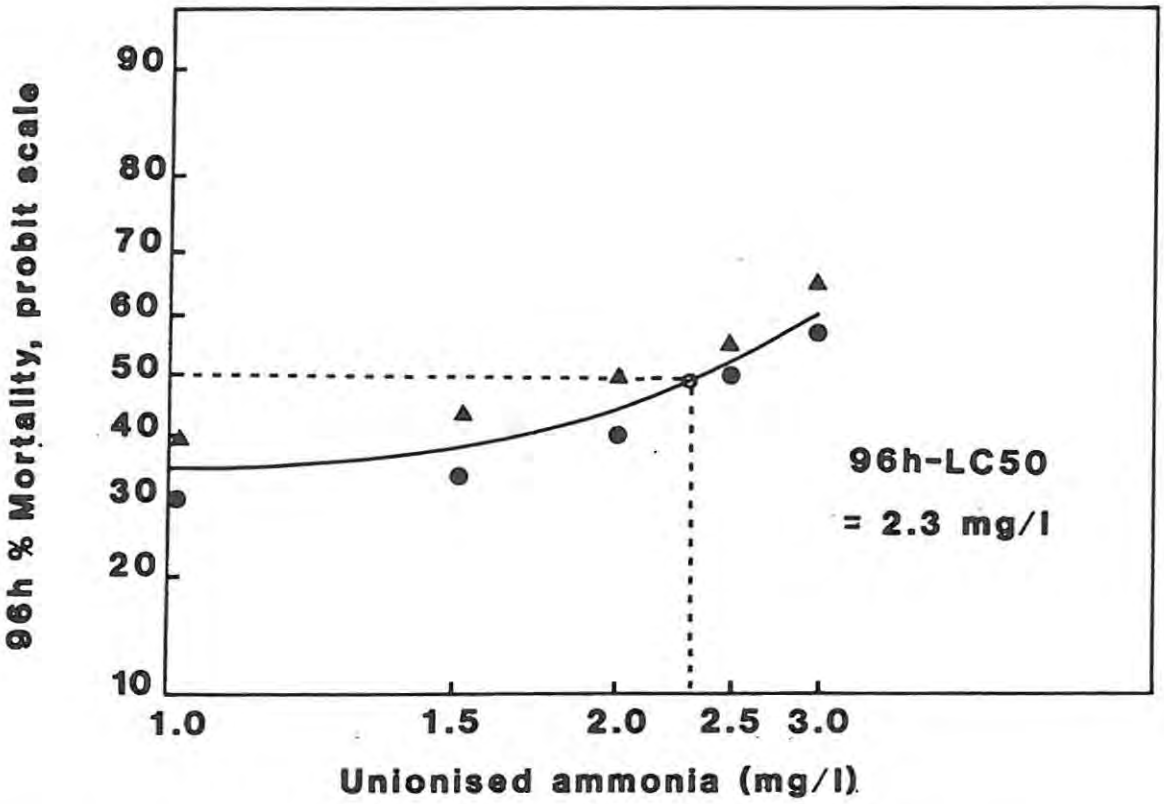

Figure 16. Mortality of Clarias gariepinus juveniles after 96 hours exposure to different concentrations of unionised ammonia, on a probability scale. Probit analysis (Sprague 1973) was used to determine the 96h-LC50. ▲ = trial 1. ● = trial 2.

TABLE 19

Median lethal concentrations (LC-50's) of un-ionised ammonia for selected fish species.

Species	LC 50 ($mg/l\ NH_3$)	Test Duration	Author
Cyprinus carpio	1.74	96 hrs.	Hasan & MacIntosh 1986
Tilapia aurea	2.4	48 hrs	Redner & Stickney 1979
Oreochromis mossambicus	2.2-2.5	96 hrs	Visser 1986
Ictalurus punctatus	1.6	96 hrs	Colt & Tchobanoglous 1976
Salmo gairdneri	0.41-0.45	500 min.	Lloyd & Herbert 1960, Alderson 1976
Salmo clarki	0.5-0.8	96 hrs	Thurston et al. 1978
Clarias gariepinus	2.3	96 hrs	Present study

the determination of median lethal concentrations of ammonia, should therefore be regarded as a first step in the quantification of its toxic effects. Follow-up studies examining the sub-lethal effects of ammonia, on growth and survival, are necessary for the establishment of minimum levels acceptable in the culture environment. This need is particularly pressing with regard to airbreathing catfish because of their demonstrated potential for intensive culture in static water. Based on the demonstrated relationship of sub-lethal levels to the LC-50 in other species, tentative levels of ammonia acceptable in the culture environment may however be suggested. For example, Colt and Tchobanoglous (1978) found that the growth of channel catfish Ictalurus punctatus, is reduced by 50% at 32% of the LC-50 and Lloyd and Orr (1969) showed that no adverse effects of ammonia were evident below 12% of the threshold LC-50 for rainbow trout (Salmo gairdneri). Upon reviewing the literature relating to ammonia toxicity, Alabaster and Lloyd (1982) observed that most fish species differ only slightly in their tolerance to prolonged ammonia exposure. These authors maintain that the more significant differences for short periods of exposure, are not great enough to justify different criteria for different species. They propose that a universal water quality criterion for ammonia based on a trout standard (0.025 mg/l NH_3), would not be too harsh for waters containing resistant species of coarse fish. Until further work examining the sub-lethal effects of ammonia on C. gariepinus is performed, it is therefore safe to assume that catfish larvae are not adversely affected below this level either.

The higher short term resistance to ammonia recorded for C. gariepinus, and other coarse fish (Alabaster and Lloyd 1982), is however important with respect to their culture as ammonia levels in culture ponds typically fluctuate and may reach high levels for short periods. For example, active photosynthesis by aquatic plants can cause the pH level of culture ponds to rise above 9.0 in the late afternoon, resulting in an un-ionised concentration of NH_3 two orders of magnitude higher than at pH 7

(Alabaster and Lloyd 1982, Tucker and Boyd 1985). This effect is well illustrated in static water channel catfish (Ictalurus punctatus) ponds where ammonia levels usually range between 0.0 - 0.2 mg/l NH_3, but may rise above 1.1 mg/l NH_3 on summer afternoons as a result of active photosynthesis (Tucker and Boyd 1985). While Alabaster and Lloyd's (1982) water quality standard is a useful one for defining the level of ammonia permissible in natural waters, it would appear too harsh a criterion for the pond culture of C. gariepinus and other species which are more resistant to the acute effects of ammonia. The definition of meaningful water quality criteria with respect to ammonia for the intensive culture C. gariepinus must thus ultimately be the product of further applied studies.

Cytological Effects of Ammonia

The effect of ammonia on the gill and liver tissue of Clarias gariepinus is shown in the TEM photomicrographs (Figs. 17 and 18). The liver is the main organ responsible for detoxification and the size of the hepatic cells reflects their physiological functional state (Hibiya 1982). The condition of the liver is thus indicative of the level of toxicant which an organism can tolerate. In the control the fish liver cells were small with shrunken ectoplasm indicating relative inactivity (Fig 17a). At NH_3 concentrations ranging from 1.0-1.5 mg/l (Fig. 17b-c) the liver cells displayed a dramatic increase in size with a great proliferation of mitochondria. These changes indicate that active detoxification of ammonia was probably occurring. At 2.0 mg/l (Fig. 17d) however, the liver cells appeared shrunken, some cellular separation had occurred and fewer mitochondria were present in the ectoplasm. These degenerative symptoms indicated that the liver was, at this concentration, unable to cope with the level of toxicant present. From 2.5-3.0 mg/l (Fig. 17 e-f) a complete degeneration of the liver tissue had set in. There was a gross separation of cells, shrinkage of the ectoplasm and degeneration of organelles.

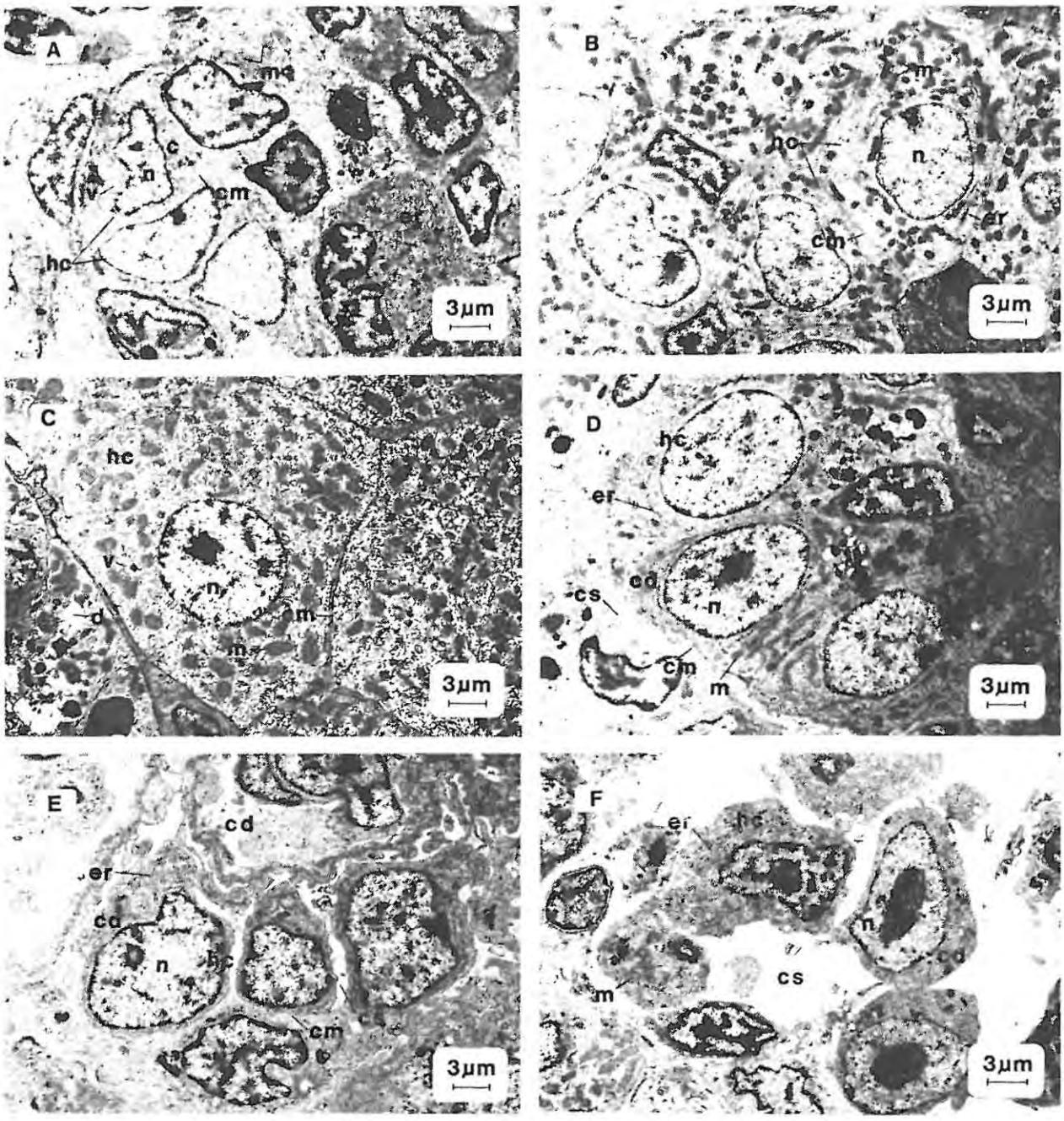

Figure 17. The effect of increasing concentrations of unionised ammonia on Clarias gariepinus liver tissue. Magnification for all photomicrographs (A - F) = 4800x. A = control (fresh water), B = 1.0 mg/l NH_3, C = 1.5 mg/l NH_3, D = 2.0 mg/l NH_3, E = 2.5 mg/l NH_3 and F = 3.0 mg/l NH_3. Note the increase in the size of the liver cells from A - C, and the shrinkage and degeneration of cells from D - F. (hc = hepatic cell, c = cytoplasm, n = nucleus, m = mitochondrion, er = endoplasmic reticulum, v = vacuole containing lipid or glycogen, cm = cellular membrane, d = damage as a result of sectioning the fixed tissue, cs = cellular separation, cd = cytoplasmic degeneration).

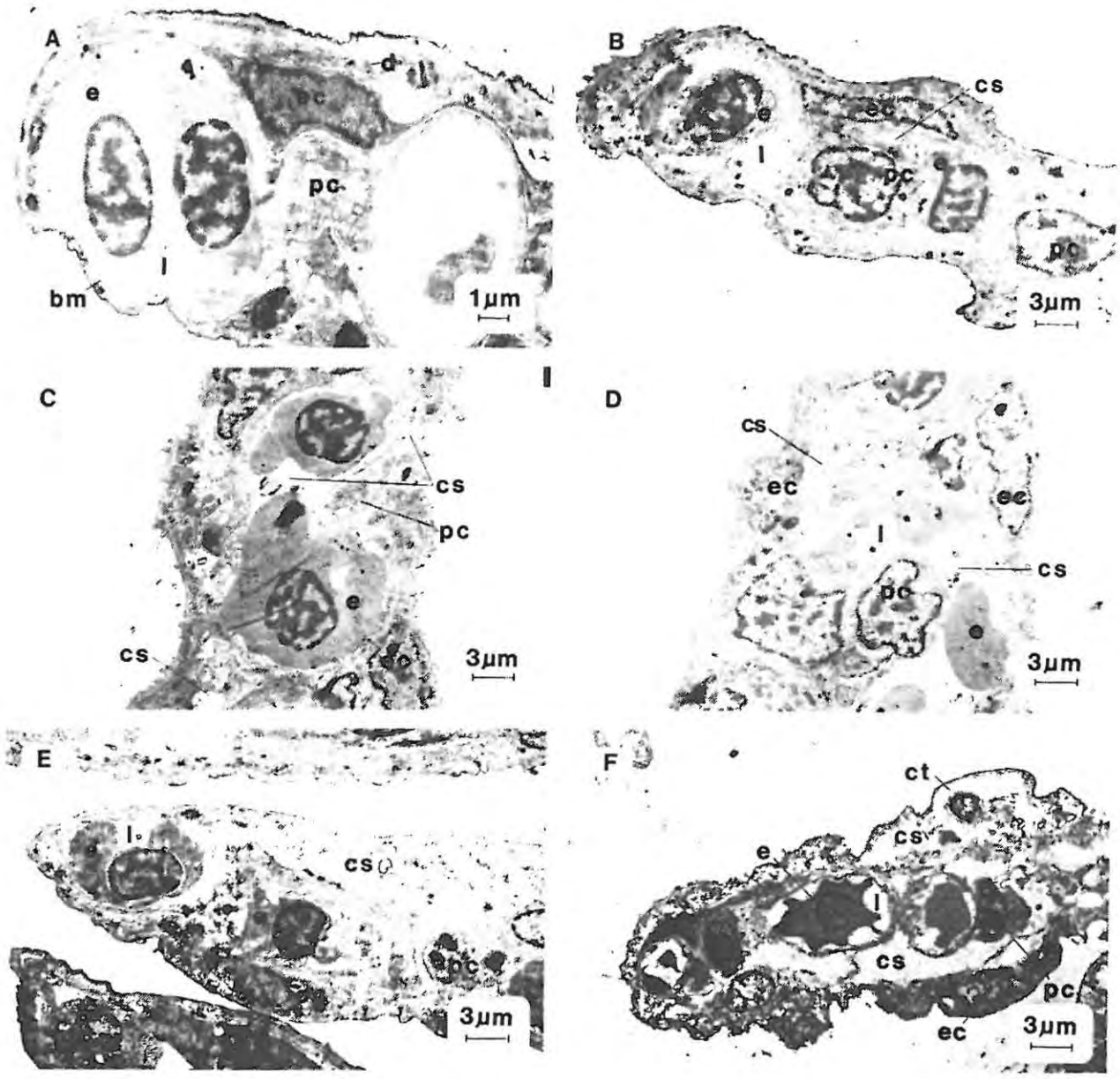

Figure 18. The effect of increasing unionised ammonia concentrations on Clarias gariepinus gill tissue. A = control (fresh water), B = 1.0 mg/l NH_3, C = 1.5 mg/l NH_3, D = 2.0 mg/l NH_3, E = 2.5 mg/l NH_3 and F = 3.0 mg/l NH_3. Magnification = 7200x for the control (A) and 4800x for all the ammonia treatments (B - F). (ec = epithelial cell, pc = pillar cell, e = erythrocyte, l = capillary lumen, d = desmosome, bm = basement membrane, cs = cellular separation, ct = cytolysis).

Normal liver functioning was, at this stage, probably terminally impaired.

The acute effects of ammonia on gill tissues were evident as degenerative changes (Fig 18 a-f). The control gill tissue represents the normal condition of the gills (Fig. 18a). At the lowest ammonia concentration (1.0 mg/l NH_3) the lamellar epithelial cell layer had separated slightly from the pillar cells in places (Fig. 18b). At 1.5 mg/l NH_3 (Fig. 18c) the separation was more advanced, sometimes disrupting the vascular canals, and hence hindering the passage of red blood cells. Similar, but more advanced, effects were evident for the 2.0 mg/l treatment (Fig. 18d). At 2.5-3.0 mg/l NH_3 (Fig 18 e-f) a general breakdown of the cellular structure had occurred. The separation of the outer endothelial and pillar cells was widespread, disrupting the thin blood/water barrier and probably impairing the diffusion of oxygen into the gill lamellae. Some erythrocytes appeared shrunken, cytolysis and general cellular degeneration were evident. The ability of the erythrocytes to absorb, bind and transport oxygen was probably severely impaired. These findings are largely consistent with the findings of similar studies on other species (Flis 1968a, Smart 1976, Thurston et al. 1978) which demonstrate that degenerative changes in gill tissues are characteristic of exposure to high levels of ammonia.

From the degeneration of gill tissue (Fig. 18 a-f) and the observed behaviour of the animals before death, it was concluded that asphyxia was probably the immediate cause of death.

Rearing Container Hygiene and Growth

Replicate data for the different treatments were pooled as student t-test analysis revealed that there were no significant differences ($p<0.05$) between replicates (Table 20). Contrary to expectation, larval growth rates (see Table 21 for regressions) and survival (Table 22) decreased with more frequent cleaning

TABLE 20

Student t-test analysis of replicate data for final mean length of larvae reared in containers subject to different cleaning intervals. Control = rearing container not cleaned during the experimental period.

Cleaning Interval (hours)	t-statistic	Probability
Control	1.05	0.05
48	1.51	0.05
24	0.91	0.10

TABLE 21

Regression equations of length and weight-at-age of Clarias gariepinus larvae after 12 days rearing in containers cleaned at different intervals. Control = rearing container not cleaned during the experimental period

Cleaning interval (hours)	Length (mm TL)/age (days)	r^2	Weight (mg)/age (days)	r^2
Control	length=0.86age+8.584	0.99	weight=1.16+0.211age^3	0.99
48	length=0.77age+8.94	0.99	weight=2.09+0.196age^3	0.99
24	length=0.73age+8.98	0.97	weight=2.41+0.189age^3	0.99

TABLE 22

Condition factor and size variation of larval Clarias gariepinus after 12 days rearing in containers cleaned at different intervals. Control = containers not cleaned during the experiment.

Cleaning interval (hours)	Condition factor	Size variation (%SD of $\bar{x}$)	Mortality (%)
Control	1.0	6.6	22
48	0.96	6.2	28
24	1.0	5.7	36

(Fig. 19). These differences were reflected in the mean final lengths of the larvae which differed significantly between treatments (Table 23).

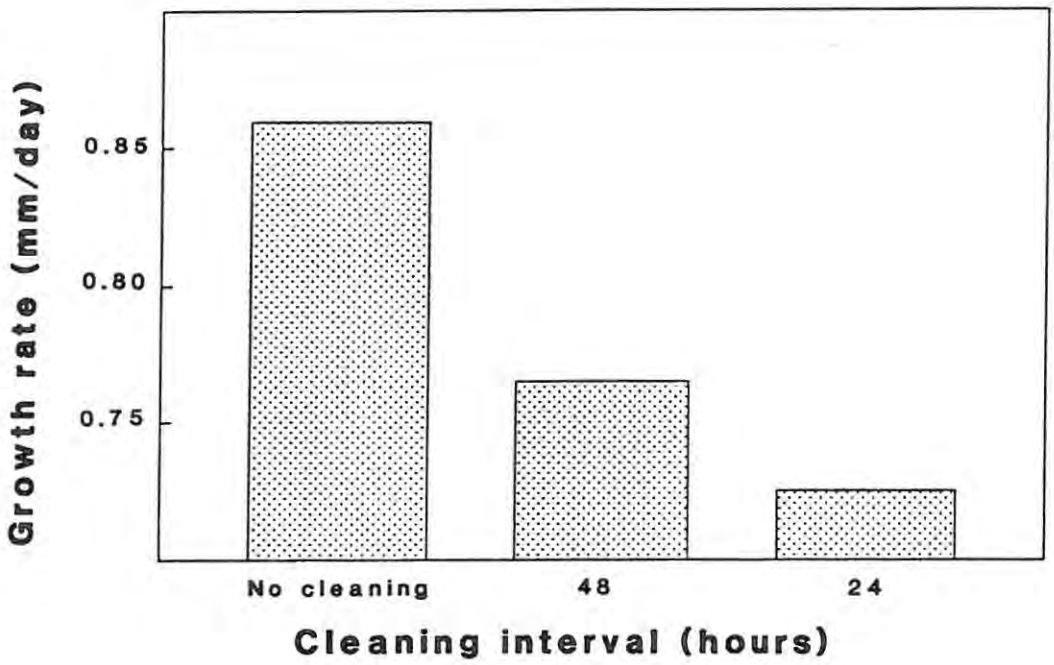

Figure 19. The growth rate of Clarias gariepinus larvae reared for 12 days in containers cleaned at different intervals. The control containers were not cleaned at all.

TABLE 23

Statistical analysis of mean final length after 12 days rearing using different cleaning intervals, using ANOVA and Scheffe's range test. F-ratio = 100, significance level = 0.000. Asterisks in different columns denote significant differences between treatments, while those in the same column denote no significant differences. Significance level = 0.01.

Cleaning interval (hours)	Scheffe's grouping		
control	*		
48		*	
24			*

The size variation and condition factor did appear to be influenced by the experimental treatments (Table 22). The levels of unionised ammonia recorded in the rearing containers (Table 24) were lower than the minimum levels which may adversely affect the growth of salmonids (Alabaster and Lloyd 1972).

TABLE 24

Levels of unionised ammonia recorded in larval rearing containers on the final day of the tank hygiene experiment.

Treatment (cleaning interval)	Ammonia (NH^3) (mg/l) ($\bar{x} \pm SD$)
No cleaning	0.0014 ± 0.0005
48 hours	0.0010 ± 0.0004
24 hours	0.0009 ± 0.0005

It therefore appears that the uneaten food and faeces (hereafter referred to as wastes) in the rearing containers did not adversely affect the larvae, but were in some way beneficial. There are three possible explanations for this result: 1) The larvae may have gained additional nutrition from these wastes, either because they were underfed, or because the wastes possessed an unidentified growth factor which enhanced their growth. Due to the intensive feeding regime employed it is unlikely that the larvae were underfed, and it was hypothesised that bacterial and/or fungal growth on the waste substrate may have enhanced the growth of the larvae. This hypothesis is supported by the recent work of Uys and Hecht (1987) who showed that C. gariepinus possesses exceptionally high levels of lysosyme in their digestive tract and is thus capable of utilising bacteria as a source of nutrition. 2) The waste may have provided cover for the larvae, which has been been demonstrated to enhance growth in conditions of continual light (chapter 5 this study). 3) The disruption of territories and feeding behaviour caused by frequent cleaning may be stressful, resulting in lower growth rates and increased mortality. To

validate the possible causes of this interesting result obviously requires further experimental investigation. It may, however, be concluded that food and faecal wastes in larval rearing containers, provided with a reasonable flow rate, do not alter the water quality in a way that is detrimental to larval growth and survival.

Conclusions

C. gariepinus is relatively tolerant of poor water quality, as evidenced in this study by its high tolerance for ammonia, and apparent imperviousness to food and faecal wastes in larval rearing containers. These results further illustrate the hardy nature of this animal, and explain, in part, its tolerance of poor water quality in its natural habitat. When exposed to lethal levels of unionised ammonia, asphyxia appears to be the immediate cause of death due to the degeneration of gill tissues and a subsequent impairment of gaseous exchange. Until further investigations into the sub-lethal effects of ammonia on C. gariepinus are performed, the universal ammonia water quality criterion for ammonia, of 0.025 mg/l NH_3, suggested by Alabaster and Lloyd (1982) may provisionally be adopted as a conservative upper limit for larval rearing. Ammonia levels remained well below this level in the larval rearing systems employed in this study and it was concluded that ammonia should not be a limiting or lethal factor with respect to C. gariepinus larval rearing.

CHAPTER 8.

THE EFFECT OF DENSITY ON GROWTH AND THE DEVELOPMENT OF A PRODUCTION MODEL FOR THE HATCHERY REARING OF LARVAE.

INTRODUCTION

At the conclusion of the experimental work described in the previous chapters, it was decided to establish what possible production could typically be expected for the large scale commercial rearing of larvae, applying the knowledge and techniques gained in the present and previous studies. When rearing larvae on a commercial, as opposed to an experimental, scale certain economically dictated compromises have to be made with respect to experimentally defined optimal rearing conditions. Hatchery production costs are primarily functions of time (e.g. labour, running expenses) and space (capital costs of the building, rearing containers etc.). Hatchery management therefore strives to produce the maximum number of larvae in as short a time, and in as small a volume of water, as possible. Hatchery productivity is thus defined here as the number of larvae reared to a given size, per unit volume, per unit time. Due to the density-dependent nature of C. gariepinus larval growth (Hecht and Appelbaum 1987a), hatchery productivity is critically affected by rearing density. Although a density of 300 individuals /l for the hatchery rearing of Clarias larvae has been suggested by Hecht (1982), no attempt has been made to determine the density at which hatchery production will be maximised. In the present study larvae were reared on a large commercial-scale at different densities, and a predictive model of hatchery production is developed, based on the density-dependent nature of C. gariepinus larval growth.

MATERIALS AND METHODS

The trial was carried out at the Blyde river hatchery, a commercial hatchery with a flow through water supply which had been in operation for less than a year. Broodstock in a ripe condition weighing 4-5 kg. were obtained from the municipal sewage ponds at Warmbaths, Transvaal. Two groups of females, five (group 1) and four (group 2) individuals respectively, were spawned on separate days in early summer (November), after the average water temperature in the hatchery had risen above 25°C. Spawning was effected as described in the general methods section (chapter 3). The batches of eggs obtained from individual females were not mixed and the hatched larvae from each female were thus reared as discrete groups. The batches of eggs were weighed, and the total number of eggs in each batch estimated from subsamples (2 g) in which the number of eggs had been counted. Hatching success was estimated as a percentage by counting the number of live eggs, in samples of 1000 eggs, on the incubation screens just prior to hatching. After hatching the larvae swam from the incubation troughs into circular plastic rearing containers (diam. 1.5 m, depth 30cm, vol. 540 l). The batches were reared at densities ranging from 39-454/l. Water was supplied at a rate of 3 l/min during the day, and 1.5 l/min at night to minimise the possibility of overflows due to blocked outlet screens. Due to material circumstances it was not possible to darken the rearing containers as desired, however the hatchery lighting was switched off, and the larvae were subject to a natural photoperiod (13L/11D) with diffuse daylight of low intensity (40-80 lux) during the light period. The water temperature in the hatchery was 27 $\pm$ 3°C, and displayed a daily range of 4°C, being coolest in the morning, and warmest at sunset. Exogenous feeding commenced on the third day after fertilization. The larvae were fed ad libitum every 2-3 hours for 18 hours a day, on dry larval feed (Uys and Hecht 1985). This feed was supplemented with Artemia nauplii for the first three days of exogenous feeding. The time from fertilization until the transfer of larvae out of the hatchery to the nursery ponds was 12 days. At the time of

transfer, each batch of larvae was netted and wet weighed. At the same time sub-samples of 200-400 larvae were weighed and counted, their mean weight calculated, and hence the survival in the batch as a whole estimated.

RESULTS AND DISCUSSION

The results of the rearing trial are summarised in Table 25. The total number of larvae harvested for both groups was 651000. The mean final weights of individual larvae ranged from 16-27 mg (Table 25) for the respective batches, a weight difference of 40 percent. Regression analysis revealed a strong negative correlation (r^2 = -0.8) between final mass and density (Fig. 20) indicating that growth is density dependent.

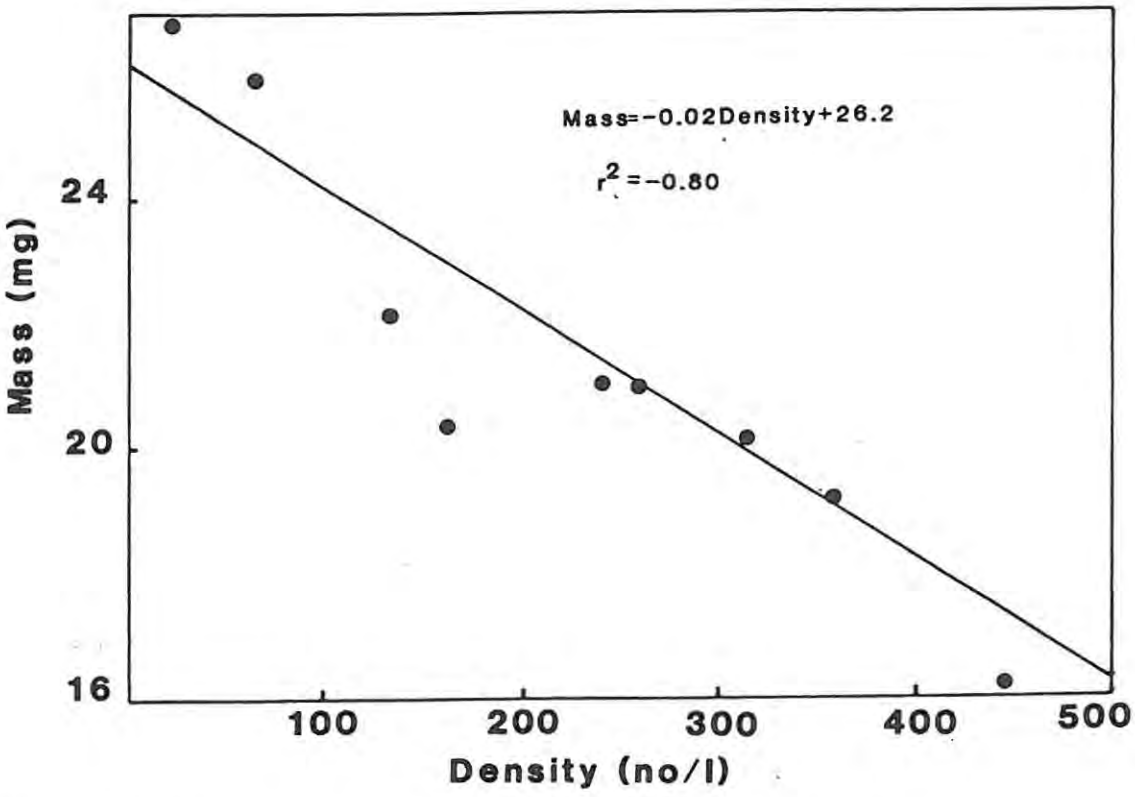

Figure 20. Linear regression of final mass on density for Clarias gariepinus larvae after twelve days rearing at different densities, illustrating density-dependent growth.

TABLE 25

Results of the large scale rearing trial performed over 12 days at the Blyde river hatchery.

Female	Egg wt. (g)	% Hatch	Number hatched (x1000)	Density (No/l)	Individual weight (mg)	No.larvae produced (x1000)	% survival
Group 1: Spawned 7 November 1987							
A	104	41	21	39	27	4	19
B	142	64	45	83	26	6	13
C	200	62	62	115	22	36	58
D	186	87	80	148	20	53	66
E	287	93	133	246	21	47	35
Total	673	-	275	-	-	136	-
Mean	224.3	80.6	91.6	126	21	45.3	53
S. dev.	61.6	18.9	37.7	70	2.7	20.5	20
Group 2: Spawned 18 November 1987							
A	401	69	138	256	21	69	50
B	913	73	333	308	20	213	64
C	405	97	196	363	19	99	51
D	533	93	245	454	16	124	52
Total	2252	-	912	-	-	505	-
Mean	563	82.8	228	423	19	126	54.3
S.dev.	208	12	72	132	1.8	54	5.7
Combined data: Group 1 and 2							
Total	2925	-	1187	-	-	641	-
Mean		75	139	274	21	72	45
S.dev.		17	97	140	3	61	17

The specific growth rate (G) of each batch was calculated (Table 26) and the relationship between specific growth rate and density was well described by a linear regression (Table 27). The density dependent growth rates observed here thus corroborate the observations of Hecht and Appelbaum (1987a) in Israel, who demonstrated that the growth of C. gariepinus larvae, reared at lower densities (5 - 20 /l), was density-dependent. Survival of larvae, however, appeared to be density-independent as there was

TABLE 26

Observed specific growth rates (G) of Clarias gariepinus larvae reared at different densities, nine days after the start of exogenous feeding.

Group number	Density (no./l)	FW (mg)	IW (mg)	G (% body wt./day)
1a	39	27	3.0	35.3
b	83	26	3.0	34.8
c	115	22	3.0	32.7
d	148	20	3.0	31.5
e	246	21	3.0	32.1
2a	256	21	3.0	32.1
b	308	20	3.0	31.5
c	363	19	3.0	30.8
d	454	16	3.0	28.4

FW = Observed final weight of larvae
IW = Initial weight of larvae at the start of exogenous feeding
G = 100.Ln(FW-IW)/T (where T = time in days)

TABLE 27

Regression of observed specific growth rates as a function of density.

Specific growth rate (G) = -0.0135density+35.17

R Squared	0.89
No. of Observations	9
Degrees of Freedom	7
Std Err of density Coef.	0.0048
Std Err of Y Est	0.73

no correlation between density and survival. The data in Table 25 do however suggest that survival may be a reflection of broodstock quality, as the number of eggs per female, hatching success and survival of larvae were higher for the Group 2 fish spawned on 18/11/87, than for group 1 fish spawned on 7/11/87. These differences are probably indicative of an improved condition of the broodstock during the 11 day interval between the two spawnings.

The artificial hatchery environment under which the larvae were reared can be considered close to optimal. Feeding level and frequency were high and no water quality or disease problems were experienced. Temperatures were within the optimum range recommended in this study, the larvae were subject to low light intensities during the light period and salinity was 0‰. Higher growth rates might, however, have been obtained had more live feed been available.

Given the above rearing conditions, the key variable affecting larval growth and hence hatchery production, thus appears to be density. A model of hatchery production, based on the inter-dependent relationship between density and growth, is developed below from which the optimum density for maximal production is predicted. A hatchery strives to produce a maximum number of larvae of a standard size, in a minimum volume of water, in as short a time as possible. Hatchery productivity may thus be measured as the number of larvae produced per unit time per unit volume and is described by the following equation:

$$P = \frac{N(i).M/V}{T}$$

where,

P = number of larvae/vol/time.
N(i) = initial number of larvae
M = mortality factor (N(i)/Number surviving)
T = Time to rear larvae to a standard size (days)
V = Rearing volume (litres)

Rearing time (T) is a function of the desired final size and growth rate, therefore:

$$T = \frac{FS}{G}$$

where,

G = growth rate (ln(mg) or mm /day)
FS = Final size (ln(mg) or mm)

Growth rate may be predicted from its observed relationship to density, which for the data obtained in this study was well described by a straight line regression equation (table 27).

$$G = b.D + C$$

where,

D = density (ind./ l)
b = regression coefficient
C = constant

By substituting these parameters, a predictive model for productivity as a function of density is derived:

$$P = \frac{N(i).M/V}{FS/(b.D+C)}$$

Using the specific growth rate/ density relation derived from the observed data in the present study (Table 27), maximum productivity was calculated from the above equation over a range of densities (Table 28) by setting mortality (M) at zero. Productivity, specific growth rate, and rearing time are illustrated in Figure 21 as functions of density. Upon calculating the observed production for the batches of larvae in the present study (Table 29), it was clear that maximum productivity was not reached at the experimental rearing densities (Fig. 21). The observed data points are significantly lower than the idealised productivity curve due to the relatively high mortality factor (Table 29). Assuming that the specific growth rate / density relationship remains linear, it is predicted that maximum productivity will be achieved at a density of 1400 /l. At this level, productivity is predicted to be 65 /l/day. At densities above 1400/l, productivity begins to decrease due to the exponentially increasing rearing time (Fig. 21).

TABLE 28

Predicted specific growth rate (G), rearing time (T) and productivity (P max), as functions of density.

Density (no/l)	(G) (% b.wt/d)	T (days)	P max (no/l/day)
10	35.0	11.6	8.9
100	33.9	11.9	8.4
200	32.4	12.2	16.3
300	31.1	12.6	23.7
400	29.8	13.1	30.6
500	28.4	13.5	36.9
600	27.0	14.1	42.7
700	25.7	14.6	47.8
800	24.4	15.2	52.3
900	23.0	16.0	56.2
1000	21.7	16.8	59.4
1100	20.3	17.7	62.0
1200	18.9	18.8	63.9
1300	17.6	20.0	65.0
1400	16.3	21.4	65.4
1500	14.9	23.1	65.0
1600	13.6	25.1	63.8
1700	12.3	27.5	61.8
1800	10.9	34.6	58.9
1900	9.5	34.5	55.1
2000	8.2	39.6	50.4

TABLE 29

Observed productivity (P obs) calculated from the productivity equation for groups 1 and 2.

Group	Density (no/l)	Mortality factor	P obs (no/l/day)
1 A	39	0.19	0.87
B	83	0.13	1.26
C	115	0.58	7.27
D	148	0.66	10.27
E	246	0.35	9.24
2 A	256	0.5	13.65
B	308	0.64	20.73
C	363	0.51	19.04
D	454	0.52	22.44

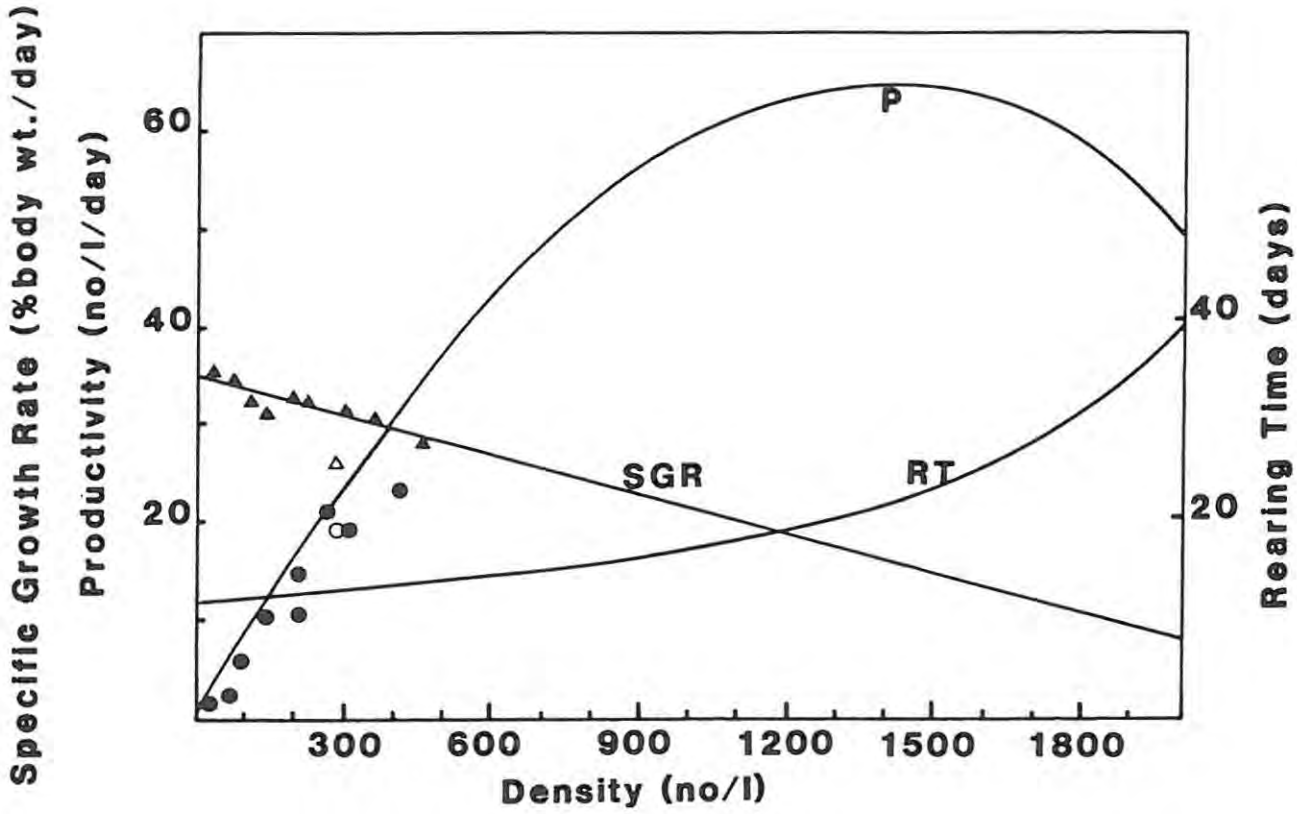

Figure 21. Specific growth rate (SGR), hatchery productivity (P), and rearing time (RT) of Clarias gariepinus larvae as functions of density. For calculation of these parameters see Tables 27 and 28 and the text.
▲ = $SGR_{(obs)}$ (Table 26), ● = $P_{(obs)}$ (Table 29).
△ = SGR (Hecht 1982), ○ = P (Hecht 1982).

To produce the maximum number of larvae per unit time and volume it thus pays to increase density to a point where productivity begins to decrease due to:

1) declining growth rates and hence increased rearing time or
2) reduced survival or
3) reduced fitness of larvae,

however the third point is not included as a factor in the productivity equation.

The strength of a model of course depends on the assumptions, and the observed data, upon which it is based. Whether the relationships developed above are valid at higher densities can only be confirmed by further investigations. The only record of rearing densities of C. gariepinus larvae similar to the present

study were those of Hecht (1982) who experimentally reared larvae at 300/l (temperature 23.5 $\pm$ 5°C). Hecht (op. cit.) observed low mortalities (<10%) and obtained his highest growth rates using a diet similar to the one fed to the larvae in the present study (torula yeast and live feed). The specific growth rate and productivity values obtained in this study lies close to the idealisd curves derived from the present data (Fig. 21) and thus add weight to the predictive value of these relations. Due to the absence of any observations of the growth of rate of larvae at densities higher than 450/l, the predicted density for maximum productivity (1400/l) should be regarded as extremely tentative.

In conclusion it may be stated that given optimal rearing conditions, the two most significant factors affecting hatchery productivity are mortality and density. The productivity equation developed here takes these two factors into account and therefore is a potentially useful tool for hatchery management. For example, it is evident from the model that maximum productivity was not achieved at rearing densities employed in the present and previous studies. Furthermore, the density at which maximum productivity is predicted (1400 /l), provides a useful guideline for hatchery management, and is a testable figure, upon which further investigation may be based. Another aspect of the model useful for management, is its ability to predict rearing time, to a given size, as a function of density. Using the production figures of the present study in combination with the production model, it is now possible for prospective culturists to make realistic projections regarding the planning of a hatchery to suit their production requirements.

CHAPTER 9.

CONCLUDING DISCUSSION

The findings of this study largely corroborate the originally proposed hypothesis (chapter 2), that controlled experiments should reflect the broad tolerances larvae display for most environmental factors in their characteristically unstable natural habitat. Specifically, these tolerances were manifest as:

1) high growth rates over a fairly wide range of temperature, photoperiod and salinity, as well as in relatively poor water quality, and;
2) good survival well outside the ranges at which optimum growth was recorded for each of these parameters.

These results obtained under controlled conditions thus complement previous field observations on the breeding biology and early life history of C. gariepinus which were cited in chapter 2. The high larval growth rates recorded over a wide range of environmental conditions help to explain how C. gariepinus exists successfully in the diverse physical environments within its natural distributional range. The good survival of larvae observed outside its environmentally optimal ranges for growth, reflects their ability to cope with the instability of the floodplain habitat, which may unpredictably fluctuate between optimal and adverse environmental conditions. If these observations are considered in terms of the conceptual life history models invoked in chapter 2, they are consistent with the classification of C. gariepinus as an altricial generalist (sensu Balon 1981, 1983) and as having a strongly r-selected ecology (sensu MacArthur and Wilson 1967) in its early life history stages. If the wide environmental tolerances and breeding biology of C. gariepinus are considered, together with its qualities of euryphagy and tolerance of poor water quality,

C. gariepinus may be characterised as being an efficient opportunist and survivor, being well equipped to exploit whatever resources are available under favourable conditions, and to survive under adverse conditions. These characteristics thus serve to explain its vast and ubiquitous natural distribution throughout Africa and Asia.

The high growth rates and survival recorded over a wide range of environmental conditions, are clearly advantageous with respect to the culture of C. gariepinus. Although an optimum condition was found to exist for each environmental parameter examined, the high growth rates obtained about these optima indicate that successful larval rearing is possible within fairly broadly defined ranges of temperature, photoperiod, salinity and water quality, as opposed to a narrowly defined set of environmental conditions. A further advantage that the environmental tolerances of C. gariepinus larvae hold for fish culture, is that, should one or more of the environmental parameters deviate from the range of optimal growth, the survival of larvae should not be seriously impaired. Optimum environmental conditions, for C. gariepinus larval rearing, are thus defined below as a series of optima with acceptable ranges of deviation for the various environmental parameters:

Temperature - The optimum temperature for growth appears to be 30°C, however temperatures in the range 26-33°C yield acceptable growth. At temperatures below this range, growth rates decrease but survival is still good.

Photoperiod - A 0L/24D photoperiod (continual darkness) appears optimal. Although larval growth decreases with longer light photoperiods, survival is good. In light conditions, the provision of cover enhances growth.

Salinity - A salinity range of 0-2.5%. appears to be optimal, however larval growth is acceptable up to 5%. salinity. Survival is good up to 7.5 %.

Oxygen - Although the effect of oxygen on larval growth and survival has not been quantified, well oxygenated water is recommended. This is easily achieved by means of aeration or good flow rates.

Water Quality - C. gariepinus display a high tolerance for ammonia, with a 96h-LC50 = 2.3 mg/l NH_3. Under normal hatchery rearing conditions, ammonia does not appear to be a limiting or lethal factor. Furthermore, the cleaning of larval rearing containers appears unnecessary, because uneaten food and faeces do not appear to adversely affect water quality to an extent that is detrimental to the larvae.

Density - Growth is density dependent, and the optimum density for intensive larval rearing must therefore be a compromise, determined by a cost-benefit analysis. The model developed in chapter 8 predicts that hatchery production increases up to a density of 1400 individuals/l.

The preceding discussions illustrate that natural history studies were vital to the interpretation of the responses of C. gariepinus, obtained under artificial environmental conditions in the present study. This underlines the necessity for fundamental research into the biology of an organism, for the development of techniques for its culture. Conversely, the present study, although by definition was of an applied nature, has contributed towards the understanding of the natural history of C. gariepinus, through the interpretation of results in terms of the animals ecology.

In the analysis of growth data in this study, size variation within batches of larvae was examined as a factor which might influence cannibalism. The only environmental factor which was found to influence size variation significantly, was that of temperature. The size variation of larvae recorded in the different growth experiments, however, differed markedly between experiments (Table 30).

TABLE 30

Ranges of size variation recorded in experiments examining the effect of environmental factors on growth. (Aug. = Augrabies, Am. = Amalinda, Pal. = Phalaborwa)

Environmental factor	Size variation (% sd)	Broodstock origin	Date spawned	Broodstock condition
Temperature	9.4 - 13.6	Aug.♂ x Am. ♀	4/11/1985	gravid
Salinity	13.7 - 15.5	Am.♂ x Am. ♀	16/03/1986	gravid (small fish)
Photoperiod	5.7 - 7.0	Pal.♂ x Pal. ♀	26/04/1987	gravid
Tank hygiene	5.7 - 6.6	Pal.♂ x Pal. ♀	26/04/1987	gravid
Photoperiod/cover	11.9 - 13.0	Pal.♂ x Pal. ♀	16/10/1987	not fully gravid (early season)

The large differences in size variation recorded for larvae spawned from different broodstock, and at different times of season, suggest that the size variation of the larvae was influenced primarily by the origin and condition of the broodstock, rather than by the environmental conditions under which they were reared. These results reveal the necessity for research into the genetic selection, as well as broodstock conditioning.

With the conclusion of the present study, it may safely be stated that the technology for Clarias seed production has been developed to a stage where it can no longer be regarded as a major factor impeding the development of commercial catfish culture. The development of this technology has been extremely rapid in comparison to other cultured finfish, and reflects the rational and committed research base from which it has proceeded. While a basic research foundation has been laid facilitating the

intensive rearing of Clarias larvae, their exists a vast potential for improving and refining the technology for the mass rearing of larvae. Such further development must, however, largely be the product of experience as the needs and nature of the developing industry become evident.

Given the fledgling nature of the catfish culture industry in southern Africa, it is appropriate at this stage, to consider the economic implications of intensive larval rearing for the future of catfish culture. Due to the capital and running costs of a hatchery, seed production forms a significant proportion of the costs of a culture operation. A major running expense of hatchery is that of wages because the larval rearing operation is labour intensive, requiring skilled management. At current prices, it is estimated that the cost of seed will form 15-25% of production costs if purchased from fingerling producers (W. Uys, Rhodes University, personal communication 1987). A certain economy of scale thus applies with regard to catfish production as a whole. While it is cost effective for large scale farms to invest in a hatchery, employing management and labour, it is probably more economic for the small scale producer to purchase fingerlings from larger producers for growout. It has become clear that successful larval rearing requires careful management, technical competence, and experience. This may favour the establishment of specialist seed producers, to supply fingerlings for grow-out. This trend is already evident in the Netherlands where the catfish culture industry has divided itself into specialist fingerling producers and relatively small-scale growout operations (Huisman and Richter 1987).

In conclusion, the objectives of the study have been met. The effects of key environmental factors on C. gariepinus larvae have been quantified, and environmental requirements for hatchery rearing defined. Furthermore, a contribution has been made to the understanding of the natural history of C. gariepinus through the interpretation of results in terms of the animal's ecology. In

the short term, the empirical results of this study have a direct application with respect to the commercial mass rearing of larvae. In the longer term, it is hoped that the synthesis of knowledge and concepts provided by this study will have heuristic value, facilitating the development of more efficient rearing techniques and providing a basis for further investigations into the natural history of C. gariepinus.

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