A COMPARISON OF THE AQUACULTURE POTENTIAL OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) AND ITS HYBRID WITH *HETEROBRANCHUS LONGIFILIS* VALENCIENNES, 1840 IN SOUTHERN AFRICA.

THESIS

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For my parents, Keith and Dawn Oellermann

ABSTRACT

The aim of this study was to evaluate the aquaculture potential of a hybrid between the two largest African silurid catfish, *Heterobranchus longifilis* (HL) and *Clarias gariepinus* (CG). A morphometric, meristic and karyological study showed that the hybrid shared some of the physical characteristics of both *C. gariepinus* and *H. longifilis*, while its chromosome complement and fundamental number (2N = 54, FN = 95), was intermediate between that of *H. longifilis* (2N = 52, FN = 92) and *C. gariepinus* (2N = 56, FN = 97). The HLxCG cross could therefore be regarded as a true hybrid. For the characters tested, no morphological or karyological differences were apparent between the HLxCG hybrids produced in West Africa and those produced in southern Africa. In southern Africa, the HL♂xCG♀ cross had higher fertilization and hatching rates than pure strain *H. longifilis*, *C. gariepinus* or the reverse cross. There was no difference in the survival of the HL♂xCG♀ hybrid larvae and *C. gariepinus* larvae up to the onset of exogenous feeding. Pure strain *H. longifilis* juveniles had a faster growth rate than the *H. longifilis* ♂ x *C. gariepinus* ♀ juveniles, but the hybrid always grew at a faster rate than *C. gariepinus* or the reverse cross. The HL♂xCG♀ cross was consequently chosen as the hybrid with the greatest potential for siluroid aquaculture in southern Africa. The HL♂xCG♀ hybrid showed evidence of partial gonadic, gametic and post-zygotic sterility in both sexes. The hybrid was not completely sterile, as it was artificially induced to spawn, and a small number of viable F_2 hybrid and F_1 hybrid x *C. gariepinus* larvae were produced. However, in the light of its probable reproductive strategy, it is highly unlikely that the hybrid would pose an ecological risk to the southern African region. The *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid was compared to *C. gariepinus* for selected water quality preferences and tolerances. The hybrid had a wider temperature preference (28 °C to 34 °C) than *C. gariepinus* (28 °C to 30 °C), but appeared to be more dependent on aerial respiration than *C. gariepinus*. The air-breathing frequency of hybrid fish began to increase at dissolved oxygen concentrations below 3.8 $mg.\ell^{-1}$, while *C. gariepinus* only showed an increase in air-breathing frequency at concentrations below 3.0 $mg.\ell^{-1}$. The hybrid was more tolerant of un-ionised ammonia (96-hour LC_{50} = 9.1 $mg.\ell^{-1}$) than *C. gariepinus* (96-hour LC_{50} = 6.5 $mg.\ell^{-1}$), but their 96-hour LC_{50} salinity tolerances were similar (10.8-11.0 $g.\ell^{-1}$). The *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid had a higher fillet yield (43.9 %) than *C. gariepinus* (38.9 %), but the crude protein content and amino acid profile of the two groups were similar. Catfish are traditionally grown in earthen ponds under semi-intensive conditions in southern Africa, at around 4 kg of fish per cubic meter of water ($kg.m^{-3}$). However, the hybrid could tolerate densities of up to 415 kg of fish per cubic metre of water, if the water was exchanged hourly ($kg.m^{-3}.hr^{-1}$), and the density at which yield was optimised was rounded off to 400 $kg.m^{-3}.hr^{-1}$. The high threshold density and water quality tolerances of the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid indicates that it is ideally suited for highly intensive aquaculture. It was concluded that it would be more productive to farm the HG♂xCG♀ hybrid on an intensive basis in southern Africa, than it would be to farm *C. gariepinus* in the traditional manner.

CONTENTS

CHAPTER 1. INTRODUCTION

The African catfish, *Clarias gariepinus* is one of the most suitable species for aquaculture in the world (Hecht *et al.*, 1995). It is a fast-growing species which attains a maximum size in excess of 30 kg (Bruton, 1976). It's distributional range includes temperate and tropical environments (Skelton and Teugels, 1992), and it can withstand adverse environmental conditions and relatively poor water quality, mainly due to its ability to breathe both atmospheric and aquatic oxygen (Moussa, 1957). It is an omnivorous scavenger, a filter-feeder and a predator (Murray, 1975; Bruton, 1979a; Spataru *et al.*, 1987), which hunts its prey alone or in orchestrated packs (Bruton, 1979a; Merron, 1993). *Clarias gariepinus* is highly fecund and easily spawned under captive conditions (Britz, 1991). In certain areas within its natural distribution range it is a prized eating fish. In Nigeria, for example, the demand for *C. gariepinus* is greater than the supply (Haylor, 1987), and is sold at four times the price of tilapia (Goddard, 1987).

Clarias species, particularly *C. batrachus*, *C. fuscus* and *C. macrocephalus* are farmed widely in Asian countries such as Thailand, the Philippines, Indonesia, India, China and to a limited extent in Taiwan (Carreon *et al.*, 1976; Areerat, 1987; Zheng *et al.*, 1988; FAO, 1992; Hecht *et al.*, 1995). *Clarias gariepinus* is farmed mainly in Africa and Europe, although it is now also receiving attention in India, China, and has recently been introduced into Brazil. In Europe it is farmed in the Netherlands and in Belgium (Huisman and Richter, 1987), and in Africa it is farmed at various levels in Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Congo, Egypt, Gabon, Ghana, Guinea, Ivory Coast, Kenya, Lesotho, Mali, Nigeria, Rwanda, South Africa, Swaziland, Zaire, Zambia and Zimbabwe (Anon., 1989; FAO, 1992; FAO, 1994, Hecht *et al.*, 1995). The total production of *C. gariepinus* in Africa for 1993 was estimated at about 4500 tonnes (Hecht *et al.*, 1995). The world production of *Clarias* species in 1992 was about 83 000 tonnes (FAO, 1994).

The first 10 tonnes of *C. gariepinus* were produced in South Africa in 1987. By 1990 production had increased to 1400 tonnes, but fell back to some 350 tonnes in 1993 (Hecht *et al.*, 1995). By 1994 the combined production of *C. gariepinus*, *Cyprinus carpio* and *Oreochromis* species was only about 200 tonnes (Kaiser, 1995). The decline in production has principally been due to poor consumer demand and acceptance of the fish (Kenmuir, 1993) and increasing feed costs (a threefold increase in the price of feed over four years) leading to reduced profit margins (Uys, 1993; Hecht *et al.*, 1995). With hindsight market resistance was inevitable; the entire industry was based on and driven by scientific research (Hecht *et al.*, 1995) with no objective consideration of the true demand for the product in southern Africa.

Fish make up a comparatively small portion of the diet of South African people; about 4.5 to 6.0 $kg.capita^{-1}.year^{-1}$ (R. Williams, Irvin & Johnson (Pty) Ltd., pers comm.), compared to the 9 $kg.cap^{-1}.yr^{-1}$

for developing countries and 26 $kg.cap^{-1}.yr^{-1}$ for developed countries (Laureti, 1992). The demand for fish in South Africa is presently met by the marine capture fisheries. Researchers have predicted that the global production of existing capture fisheries will not be able to meet the rising demand for fish protein within the next decade or so (New, 1991). The maximum yield of these fisheries is finite, therefore aquaculture will become increasingly more important in providing the world with fish protein (New, 1991). Uys (1993) has noted that when the marine fish supplies eventually fail to meet the demand in southern Africa, *C. gariepinus* will be the aquaculture species most likely to fill the void. Nevertheless, in South Africa at present it is still cheaper to harvest fin fish from the sea than it is to farm them on land.

An aquaculture product simply cannot compete in the southern African fish market unless it is perceived to be of greater value than the other species available to the consumer (eg. trout), or is more competitively priced than the fish produced by the marine fisheries. Manicom *et al.* (1992) have advocated that a specialist demand for catfish be created by marketing them as an extremely high quality product and targeting the upper income consumer. The alternative approach would be to reduce production costs as much as possible so that catfish can be sold at a more competitive price. This goal could be achieved in two ways; by increasing on-farm production efficiency, or by manipulating the productivity of the farmed species.

The productivity of a fish can be increased (for a constant feed input) by genetic manipulation through breeding programs, intraspecific crossbreeding, and interspecific hybridization (Tave, 1986; Dunham *et al.*, 1987). The most common method used is to improve growth rate by mass selection, where the largest individuals of a year class are selected to produce the next generation (Dunham *et al.*, 1987; van der Walt *et al.*, 1993). This technique assumes that changes in growth rate are heritable traits for the species, and not just the result of particular environmental conditions. Gjedrem's (1983) review of heritability in important aquaculture species showed that selection for improved growth rate resulted in varying degrees of success in different groups of fish. The ictalurid catfish showed high levels of heritability for improved growth rate, while the tilapiines showed almost none. The salmonids and cyprinids showed some heritability for this trait (Gjedrem, 1983). Research by Grobler *et al.* (1992) and van der Walt *et al.* (1993) has shown that the growth rate of *C. gariepinus* can be improved through selection and breeding programs, as rapid growth appears to be associated with a specific genetic marker for this species.

The intraspecific crossbreeding of strains of a species sometimes leads to the phenomenon of heterosis, whereby the growth of the hybrid strain is faster than that of the parent strains (Shull, 1948; Falconer, 1989). This technique has been used successfully for many species, including the cichlid *Oreochromis niloticus*, the cyprinid *Cyprinus carpio*, the salmonid *Oncorhynchus mykiss*, and the catfish *Ictalurus punctatus* (Smitherman and Dunham, 1985; Horstgen-Schwark *et al.*, 1986; Tave *et al.*, 1990;

Wohlfarth, 1993). Intraspecific crossbreeding of the different populations of *C. gariepinus* occurring in Africa may also produce a faster growing strain for aquaculture.

Heterosis can also occur when different species are hybridized. Most research on artificial interspecific hybridizations has been performed on the salmonids, with conflicting results as to the presence or extent of growth heterosis occurring in the various crosses (Chevassus, 1979). Heterosis for growth has been reported in other groups of fishes, including the genera *Ictalurus*, *Lepomis*, *Morone*, *Oplegnathus*, *Pomoxis* and *Stizostedion* (Tave and Smitherman, 1982; Harada *et al.*, 1986; Siegwarth and Summerfelt, 1990; Hooe and Buck, 1991; Oppenborn and Gouldie, 1993; Tidwell and Webster, 1993). Heterosis has also been suggested to occur in the clariid *Clarias gariepinus* hybrids with *C. macrocephalus* (Little and Griffiths, 1992) and *Heterobranchus bidorsalis* (Salami *et al.*, 1993).

In 1984 two clariid catfish species, *Heterobranchus longifilis* and *C. gariepinus* were successfully hybridized (Hecht and Lublinkhof, 1985) and have since been produced at the Mubuyu Farms hatchery, in Zambia, on an annual basis.

Heterobranchus longifilis is a fast growing, omnivorous clariid catfish which grows larger than *C. gariepinus*, commonly exceeding 30 kg (Bruton, 1976) and reaching a maximal weight of about 50 kg (Bell-Cross and Minshull, 1988). The females are highly fecund, and can be artificially spawned with relative ease ((Legendre, 1986; Nwadukwe, 1993). Unlike *C. gariepinus*, its distribution is limited to tropical Africa (Skelton and Teugels, 1991). These fish can survive in brackish water of up to 10 parts per thousand (Legendre, 1991) and their ability to breathe air allows them to survive in water with low concentrations of oxygen (Hecht and Lublinkhof, 1985). Although little is known about the environmental tolerances of *H. longifilis*, it has shown great potential for aquaculture (Hecht and Lublinkhof, 1985; Bruton, 1988; Legendre *et al.*, 1992).

Hecht and Lublinkhof (1985) found that the hybrid grew faster than *C. gariepinus*, and speculated that the hybrid was monosexual or sterile. Subsequent observations on hybrid survival, growth and attempted husbandry were recorded as a matter of routine by hatchery and fish farm staff at Mubuyu Farms during the following years until 1990. Hecht *et al.* (1991) then compared the survival and growth of the *H. longifilis* and *C. gariepinus* early juveniles to that of their reciprocal hybrids. They found that the *C. gariepinus* ♂ x *H. longifilis* ♀ hybrid had a significantly lower egg survival rate to hatching than the reciprocal hybrid and the two parental strains. They also found that while the hybrid early juveniles did not show heterosis, they grew faster than the *C. gariepinus* early juveniles. The results of their investigation was the impetus which gave rise to the initiation of this study in 1991.

In 1992 a suite of papers was published by a research group based in the Ivory Coast describing the morphology, growth rate, reproduction, karyology and genetics of the reciprocal hybrids of the same parent species, *H. longifilis* and *C. gariepinus* (Legendre *et al.*, 1992; Teugels *et al.*, 1992a; Teugels

et al., 1992b). There were surprising differences in some aspects of the research reported for the West African (Ivory Coast) hybrids and the southern African (Zambian) hybrids. For example, in West Africa the *C. gariepinus* ♂ x *H. longifilis* ♀ hybrid had a better survival rate and grew faster than its reciprocal hybrid, but in southern Africa it was the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid which grew fastest and had the best survival rate.

Also, the West African hybrid males were sexually mature after one year and the females reached maturity during the second year, at a body mass in excess of 800 g (Legendre *et al.*, 1992). Between 1990 and 1992 an annual attempt was made to induce the southern African hybrids to spawn during their anticipated natural breeding season. However, by the time the Ivory Coast hybrid research papers were published, no southern African hybrid eggs had been produced, nor had hybrid semen been used to successfully fertilize *C. gariepinus* or *H. longifilis* eggs (Mubuyu Farms hatchery records, 1985-1992). These differences may be a manifestation of the genetic disparity between one or both of the parent species from the two regions.

Nevertheless the *H. longifilis* x *C. gariepinus* hybrids from both regions grew faster than *C. gariepinus*; thus this hybrid may be vitally important to the southern African aquaculture industry as a means to improve production.

For a catfish farm to be economically viable in South Africa, the ratio between economic return and input costs has to be maximized. In this region the catfish farms are mostly semi-intensive, and are based on the guidelines set out by Hecht *et al.* (1988). The production ponds are about 1000 m^2 in size, containing catfish at a density of about 4.0 $kg.m^{-2}$ (Hecht *et al.*, 1995). Even with yields of up to 40 tonnes per hectare, catfish farming has not proved to be a viable economic proposition in South Africa. This has given rise to the need for greater intensification and reduction in production costs.

Intensive catfish culture is usually carried out in tanks, ranging in size from 1 to 4 m^3, at densities of 14 $kg.m^{-3}$ (Diana & Fast, 1989) to 40 $kg.m^{-3}$ (Viveen *et al.*, 1990). Ultra-intensive culture was first reported from the Netherlands, where Huisman and Richter (1987) and Bovendeur *et al.* (1987) reached catfish densities of 360 $kg.m^{-3}$ and 436 $kg.m^{-3}$ respectively in 0.9 m^3 tanks in an experimental recirculating system. The estimated yield from these tanks was more than 1000 $kg.m^{-3}.year^{-1}$, compared with a maximum of about 5 $kg.m^{-3}.year^{-1}$ for semi-intensive fish farms.

Very little follow-up research has been reported in the literature on ultra-intensive catfish farming. In South Africa, only two commercial fish farmers have experimented with this technology. The Silver Creek fish farm grew *C. gariepinus* at densities ranging from 250 to 350 $kg.m^{-3}$ (T. Hecht, Dept. Ichthyology & Fisheries Science, Rhodes University, pers. comm.) while W. Uys (Blyde River Aquaculture, pers. comm.) attained densities in excess of 650 $kg.m^{-3}$ in 3.0 m^3 concrete tanks at Blyde

River Aquaculture. These high densities did not seem to hinder the growth of the fishes or increase their mortality rate. In fact Bolnick (in Gittens, 1991) speculated that catfish at high densities were less aggressive, which could have a positive impact on their growth and mortality. Uys (pers. comm.) predicted that the only limiting factor to growth at these high densities would be access to food due to crowding.

It is clear that the full production potential of clariid catfish farming southern Africa has not been explored. The possible increase in production resulting from the farming of the *H. longifilis* x *C. gariepinus* hybrid, instead of *C. gariepinus*, coupled with ultra-high density culture, may be sufficient to make catfish farming economically viable in this region.

The principle aim of the following study was to investigate the aquaculture potential of the *H. longifilis* x *C. gariepinus* hybrid in southern Africa, and to compare this to the potential of *C. gariepinus*. To achieve this objective the fertilization rate, hatching success and larval survival rates and the growth rates of the hybrid and *C. gariepinus* were compared. The *H. longifilis* x *C. gariepinus* hybrid was tested for positive heterosis for growth and reproductive sterility.

The hybridization of two species causes an increase in heterozygosity in their hybrid. Heterozygosity results in the dominance of beneficial genes over recessive, deleterious genes at the gene loci (Falconer, 1989), which may allow the hybrid to have greater environmental tolerance ranges than its parent species (Bettoli *et al.*, 1985; Ma and Yamazaki, 1986). The hypothesis that the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid's water quality tolerances would be different to those of *C. gariepinus* was tested for temperature, oxygen concentration, un-ionized ammonia concentration and salinity.

The growth of many species of fish is influenced by the density at which they are raised. The density beyond which their growth is impaired is called the threshold density (Piper *et al.*, 1982). It was hypothesised that the *H. longifilis* x *C. gariepinus* hybrid could tolerate particularly high densities, and a series of experimental production trials were conducted in an attempt to define the hybrid's threshold density.

The adult *C. gariepinus* and hybrids were also tested for differences in fillet yield, protein content and amino acid profile once they attained a marketable size, as hybridization may have had an effect the quality of the flesh of the catfish.

Firstly however, it was important to ascertain whether the West African hybrids and the southern African hybrids had the same phenotype and shared the same genotype. This necessitated an investigation for disparities in morphology and karyology between the West African and southern African *H. longifilis* x *C. gariepinus* hybrid groups.

CHAPTER 2. THE MORPHOLOGY AND KARYOLOGY OF SOUTHERN AFRICAN *CLARIAS GARIEPINUS*, *HETEROBRANCHUS LONGIFILIS*, AND THEIR F_1 HYBRID.

2.1. INTRODUCTION

The southern African *H. longifilis* x *C. gariepinus* hybrids described by Hecht and Lublinkhof (1985) and Hecht *et al.* (1991) had different characteristics to the West African hybrids described by Legendre *et al.* (1992). For example, the *C. gariepinus* ♂ x *H. longifilis* ♀ hybrid had a significantly lower hatching rate than the reciprocal or pure strain crosses in southern Africa, but in West Africa this difference was not apparent. In West Africa the *C. gariepinus* ♂ x *H. longifilis* ♀ hybrid grew at a faster rate than its reciprocal hybrid and the pure strain parental crosses, while in southern Africa both hybrid crosses grew at an intermediate rate to the fast growing *H. longifilis*, and the slower growing *C. gariepinus*. A series of attempts at backcrossing *C. gariepinus* eggs with hybrid sperm at the Mubuyu Farms hatchery failed, and hybrid females were never induced to produce eggs (Mubuyu Farms hatchery records, 1985-1992). These results appeared to support the hypothesis that the hybrid catfish were effectively sterile (Hecht and Lublinkhof, 1985) but Legendre *et al.* (1992) showed that the hybrids produced in West Africa were, to a limited extent, fertile.

The differences between the southern African *H. longifilis* x *C. gariepinus* hybrids and the West African hybrids may be a consequence of genetic variation in the parental fish from these localities. The *H. longifilis* used as parental stock in West Africa and in southern Africa occurred almost at the opposite extremes of their natural distribution (Fig. 2.1).

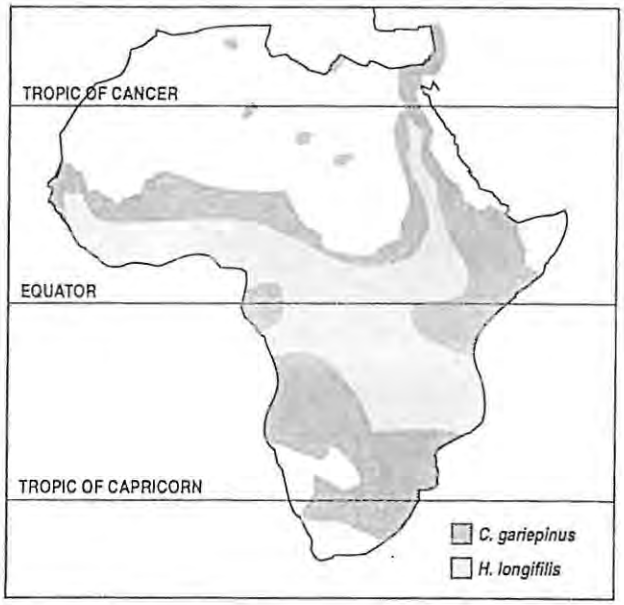

Figure 2.1. The overlapping distribution of *Clarias gariepinus* and *Heterobranchus longifilis* in Africa (summarized from Teugels *et al.*, 1990 and Skelton and Teugels, 1992).

Although *C. gariepinus* has a pan-African distribution, clinal variations in morphological characters (eg. vomerine tooth plate length, gill raker counts) have been reported for this species, from northern to southern Africa (Teugels, 1986). The southern African population was originally described as *Clarias mossambicus* by Peters in 1852 (Skelton and Teugels, 1991) and was considered a discrete species for many years. Teugels (1982) showed that the differences between *C. mossambicus* and *C. gariepinus* could be ascribed to clinal variation, and synonymised the two species. Teugels (1982) also synonymised *C. lazera* with *C. gariepinus*.

To confirm that these "species" were indeed conspecific, Ozouf-Costaz *et al.* (1990) undertook a karyological study of specimens from each of the *C. lazera*, *C. mossambicus* and *C. gariepinus* populations. They found that the karyotypes of the three populations were identical. While karyotypes seldom indicate genetic variations within the DNA of the chromosomes, they are species specific, and thus are useful taxonomic tools (Amemiya *et al.*, 1992). The karyotypes of the West African hybrid and its parent species were studied by Teugels *et al.* (1992). These karyotypes were prepared following the methods of Ozouf-Costaz *et al.* (1990), based on the nomenclature of Levan *et al.* (1964).

It was hypothesised that the genotypic differences between the parent species from the two regions may have been of sufficient magnitude to result in hybrids with different phenotypic characteristics. For this reason a morphological and karyological comparison of the hybrid and parental species' populations in West and southern Africa was undertaken, to find out whether they differed racially.

Morphological data have previously been used to determine which parent species their hybrids most closely resemble (Dunham *et al.*, 1982; Crivelli and Dupont, 1987). The meristic and morphometric character averages of the southern African group were used to this end, by employing Witkowski and Blachuta's (1980) hybrid index.

A series of multiple discriminant analyses were applied to the morphological data of the southern African fish, to amplify the phenotypic differences between *C. gariepinus*, *H. longifilis* and their F_1 hybrids. Morphological data for the West African hybrid were also included in a discriminant analysis, to test whether the discriminant functions could distinguish between the West and southern African hybrid groups.

2.2. MATERIALS AND METHODS

Morphometric and meristic methods

A morphometric and meristic analysis was carried out on *H. longifilis*, *C. gariepinus* and their F_1 hybrids. A preliminary study of 10 fish from each reciprocal hybrid cross showed that there were no

morphological differences between the two groups. Individuals from either of the two crosses are therefore simply referred to as "hybrids". Legendre *et al.* (1992) also reported that no morphological differences were discernable between the reciprocal hybrids.

The hybrid and *C. gariepinus* specimens used were farm-reared fish, obtained from the Mubuyu Farms hatchery in Zambia (16°09' S, 27°48' E). *Heterobranchus longifilis* specimens were collected from the Zambezi and Kufue river confluence (16°06' S, 28°54' E) using rod and line (Table 2.1). All specimens were measured shortly after they were killed, and no preserved specimens were used.

Table 2.1. The number, size, sex and locality of the hybrid and parental specimens collected for the morphological study.

Species	Locality	n	TL (mm) min	TL (mm) max	Mass (g) min	Mass (g) max	Sex
Hybrid	Mubuyu Farm	36	52	695	2	2600	18J, 13M, 5F
C. gariepinus	Mubuyu Farm	30	95	605	8	1290	11J, 9M, 10F
H. longifilis	Mubuyu Farm	8	54	172	2	54	5J, 1M, 2F
	Kafue/Zambezi*	22	768	1446	3500	26500	0J, 4M, 17F

* Kafue/Zambezi River confluence; J = juvenile, M = male, F = female

The morphometric measurements and meristic counts used in this study were based on those employed by Teugels (1986) to revise the systematics of the genus *Clarias*. The technical conventions are described in Teugels (1986). Morphometric dimensions were measured using vernier slide callipers and a stainless-steel tape measure, to the nearest 0.5 mm. The measurements were standardized as a percentage of head length (Fig. 2.2) or standard length (Fig. 2.3).

The meristic counts included the number of branchiostegal rays, dorsal rays, anal rays, pelvic rays and pectoral rays. Gill rakers were not counted due to their clinal variability. Ten hybrids were x-rayed to count the number of vertebrae, which was compared to the vertebral counts of the West African hybrids, reported by Legendre *et al.* (1992).

The mean, minimum and maximum morphometric percentages and the minimum and maximum meristic counts for each group were compared. Where possible these values were compared to the morphometric and meristic values published by Teugels (1986), Teugels *et al.* (1990) and Legendre *et al.* (1992) for these species.

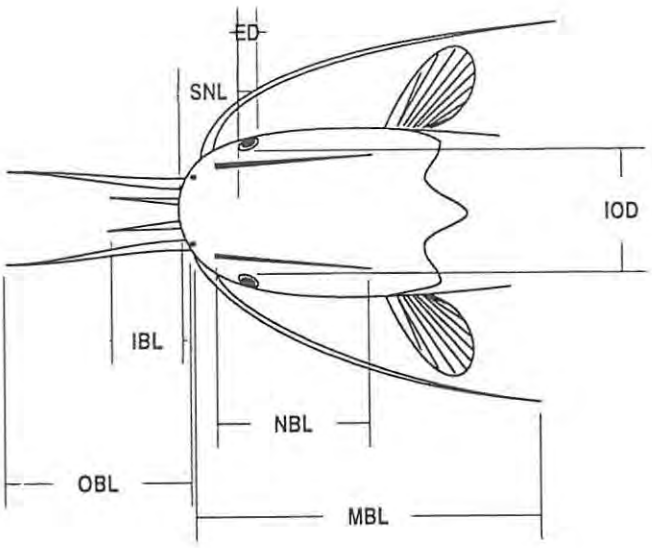

Figure 2.2. Morphometric measurements, standardized as a percentage of head length; where SNL = snout length, ED = eye diameter, IOD = interorbital distance, NBL = nasal barb length, MBL = maxillary barb length, OBL = outer mandibular barb length and IBL = inner mandibular barb length (modified from Teugels, 1986).

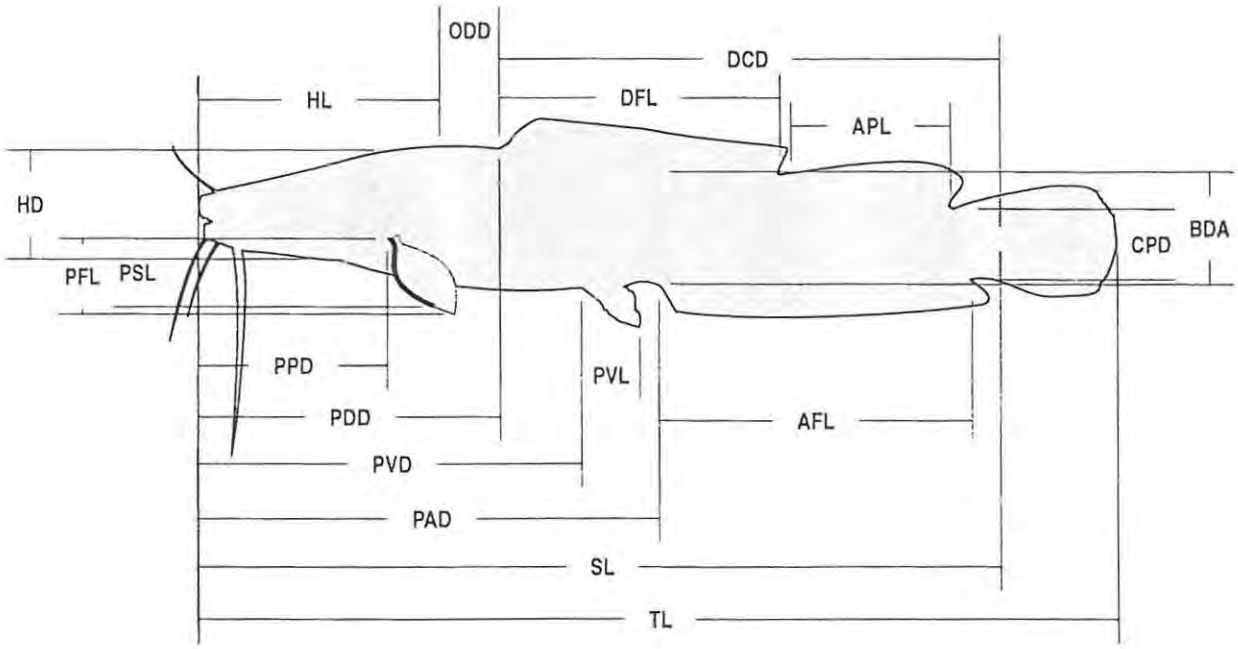

Figure 2.3. Morphometric measurements, standardized as a percentage of standard length; where TL = total length, SL = standard length, HL = head length, HD = head depth, PDD = predorsal distance, PAD = preanal distance, PVD = prepelvic distance, PPD = prepectoral distance, DFL = dorsal fin length, AFL = anal fin length, PVL = pelvic fin length, PFL = pectoral fin length, PSL = pectoral spine length, DCD = dorsal to caudal fin distance, ODD = occipital process to dorsal fin distance, CPD = caudal peduncle depth, BDA = body depth at anus and APL = adipose fin length.

In order to determine whether the hybrid was morphologically biased toward one or another of the parent species, a hybrid index was calculated, according to the equation developed by Witkowski and Blachuta (1980):

$$\text{Hybrid Index} = \frac{H - M_1}{M_2 - M_1} \times 100$$

where M_1 = the means of characters of *H. longifilis*, M_2 = the means of characters of *C. gariepinus* and H = the means of characters of the hybrid.

The characters were categorized following Crivelli and Dupont (1987) according to their hybrid index values:

Intermediate	=	45 - 55
C. gariepinus-like	=	> 55
H. longifilis-like	=	< 45
hybrid-specific	=	< 0 or > 100

The hybrid index of all the characters was used to determine which of the two parent species the hybrid most resembled.

The discriminating power of the meristic and morphometric characters used to characterise the three groups of catfish was tested using the multiple discriminant analysis programme of the StatGraphics^R version 7.00 statistical software package. The objective of the discriminant analysis was to weight and linearly combine all the independent characters of the individual fishes in some fashion so that the groups of fish were as statistically distinct as possible (Klecka, 1975). Probability distribution curves were fitted for each group within a character set, thus an unknown individual could be assigned to a known group of fish if its character values fell within the probability curves of that group (Jackson, 1983).

The discriminant analysis tests the predicting power of the probability curves by reassigning the original known values to groups on the basis of their probability curves. The character values are then expressed as discriminant scores. These scores are the distances from the values within a group's probability curve to the point where the curve intersects with another group's probability curve. The discriminant function is the algorithm which best describes the discriminant scores of the individual fishes for all the morphological characters used. The first discriminant function maximises the ratio of between-groups to within-groups variability, while the second function maximises the residual between-groups to within-groups variability (Dillon and Goldstein, 1984).

Discriminant function analyses were carried out for the two species and their hybrid using three sets of data; meristic counts, morphometric measurements standardized as a percentage of head length, and morphometric measurements standardized as a percentage of standard length. Dorsal ray counts, dorsal fin length and adipose fin length were the only characters not used in the discriminant function analysis. These characters were all related to the presence or absence of the adipose fin, which is the primary morphological difference between the three groups of fishes. If these characters were included in the discriminant function analysis, they would be heavily weighted, as their variation would be the largest in the data set. This would be counter-productive, as we are already aware of this difference between the groups. By excluding these characters, other discriminating factors may be found for the three groups.

A final discriminant analysis was carried out using both sets of morphometric data for the three southern African groups. The maximum, mean and minimum morphometric values of the reciprocal West African hybrids reported by Legendre *et al.* (1992) were also included, as a fourth group.

Karyological methods

Chromosome spreads were isolated from six *H. longifilis* and *C. gariepinus*, and 12 hybrids. As all the specimens were juveniles (between 10 g and 100 g), their sex could not be accurately determined.

The fish were removed from their holding tank on the evening before the chromosomes were extracted, and placed into a preparation tank. The following morning a 0.1 mg per 100 g body weight of colchicine solution (0.1 % w/v, made up with distilled water) was injected into the dorsal musculature of the fish. The fish were returned to the preparation tank and allowed to rest for two hours, after which they were killed by instantaneous decapitation.

The chromosomes were isolated according to the method described by Collares-Pereira (1992), as follows. The liver was dissected from a fish and placed into a watch glass containing a 0.075 M KCl solution, for 20 minutes. This hypotonic solution caused the liver cells to swell. The cells were then disassociated by gently rubbing the liver tissue on a fine grater mounted on the watch glass. A bulb pipette was used to draw up the liver cell suspension, and this was placed into a centrifuge tube.

The cell suspension was centrifuged for 10 minutes at 1000 rpm. The supernatant was then poured off, and the resultant pellet of liver cells was gently resuspended in fresh 4 % KCl solution. The cell suspension was then re-centrifuged. This process was repeated twice.

Once the supernatant had been poured off for the third time, a 3:1 absolute ethanol to glacial acetic

acid fixative was very slowly and gently added to the cells in the centrifuge tube. They were resuspended in the solution and centrifuged for 5 minutes at 800 rpm. This process was repeated twice, whereafter the cells were resuspended in fresh fixative and stored in sealed test tubes at 6 °C.

Glass slides were cleaned in a solution of HCl (1.0 M) before preparation of the chromosome spreads. A bulb pipette was used to draw up some of the fixative containing the liver cells from the test tube. A clean glass slide was then passed through steam, and while a thin film of water began to condense on the slide two drops of the cell solution were allowed to fall onto the slide, from a height of approximately 50 mm. The slides were dried overnight on a slide warmer.

A 4 % v/v Giemsa solution was used to stain the chromatin material of the cells. This solution was prepared by diluting standard Giemsa solution with boiling water. The diluted solution was then filtered through filter paper at least three times while still warm. Slides were stained in the cooled solution for five minutes, after which excess stain was washed off under a gentle flow of tap water.

Once the stain was dry the slides were examined using a Nikon Optiphot binocular microscope, at 400 to 1000 x magnification. To ascertain the diploid chromosome number for *C. gariepinus*, *H. longifilis* and their hybrid, the chromosomes of at least 30 spreads were counted per species.

Slides of chromosome preparations from the West African *C. gariepinus* ♂ x *H. longifilis* ♀ hybrid population were provided for comparative purposes by G. Teugels (Royal African Museum, Tervuren, Belgium).

Chromosome spreads were photographed under oil at 1000 x magnification, using 50 ASA black and white print film. The photographs of the twenty most well defined chromosome spreads for each group of fish were then scanned into a Map and Image Presentation System (MIPS), which is a computerized image enhancing facility. The chromosome images were contrast-enhanced and enlarged, and the arm lengths of the chromosomes were measured using electronic callipers.

Ozouf-Costaz *et al.* (1990) karyotyped *C. gariepinus* by dividing the chromosomes into groups on the basis of their centromere index (C);

$$C = \frac{s}{s + l} \times 100$$

where s = short arm and l = long arm of the chromosome.

Chromosomes with a centromere index of 46-50 were classed as metacentric, while submetacentric chromosomes had a C value of 35-45 and acrocentric chromosomes had a C value of less than 35. I attempted to karyotype the three catfish groups according to this method, so that the karyotypes of

the present study would be directly comparable to those of *C. gariepinus*, *H. longifilis* and the reciprocal hybrids published by Teugels *et al.* (1992a).

However, the variation in the number of chromosomes allocated to the three arm-ratio categories proved to be very high, even when using chromosome spreads from the same specimen. The categories were too specific for the shape variation and measurement errors intrinsic to chromosome analysis. Each chromosome consists of a single, long strand of DNA densely folded around a scaffold-like core (Babu and Verma, 1987). The DNA condenses into the characteristic chromosome shape during the metaphase of a cell undergoing mitosis. The extent to which a chromosome condenses depends on the type and amount of mitotic inhibitor absorbed by a cell, and the method by which the cell is fixed and prepared for analysis. These factors make the size, and to some extent the shape of the chromosomes quite variable (Denton, 1973).

For this reason some ichthyological karyologists prefer to use the simpler, more robust method of categorising chromosomes into bi-armed or uni-armed groups (Schwartz and Maddock, 1986; Oellermann, 1990). This was the method ultimately used in this investigation. While in theory the karyotype of a species is fixed, in practice measurement error and other types of experimental error lead to chromosomes being incorrectly categorized, thus these values need to be treated statistically.

The fundamental number of a species refers to the number of chromosome arms present in a species' chromosome complement. The fundamental number is at best an estimate based on the average bi- and uni-armed chromosomes of a species.

The average number of bi-armed and uni-armed chromosomes for 20 spreads per species were used to estimate the Fundamental Number (FN) of *C. gariepinus*, *H. longifilis* and their hybrid as follows:

$$FN = 2(n_1) + n_2$$

where n_1 was the number of bi-armed chromosomes and n_2 was the number of uni-armed chromosomes. The catfish groups were then compared on the basis of their fundamental numbers.

Voucher specimens of the southern African *C. gariepinus*, *H. longifilis* and reciprocal hybrid populations used in the above morphological and karyological studies were fixed in 10 % formaldehyde and preserved in 50 % propanol. These specimens have been placed into the JLB Smith Institute of Ichthyology's fish collection, under RUSI accession numbers 47287, 47288, 47289 and 47290.

2.3. RESULTS

There were differences in the coloration of the fish. *Heterobranchus longifilis* specimens were always uniform brown to dark brown in colour dorsally, while their ventral coloration varied from white to cream. Occasional patches of dark grey or black were also apparent. The coloration of *C. gariepinus* varied a great deal; while most of the fish had a marbled pattern, uniformly jet black or light grey specimens were also encountered. The ventral surface of *C. gariepinus* was usually lighter than the dorsal surface. Most of the hybrids also showed this marbled pattern, but were coloured in shades of dark brown to yellow rather than grey to black. There were some exceptions to this rule, where hybrids showed identical coloration to *C. gariepinus*.

Morphometrics and meristics

The vertebral counts of the two groups of hybrids were very close. The West African hybrid had a vertebral count ranging from 56 to 59 (Legendre *et al.*, 1992), while that of the southern African hybrid ranged from 55 to 57. The minimum and maximum meristic values for the *C. gariepinus*, *H. longifilis* and hybrid populations are presented in Figure 2.4. The mean, minimum and maximum morphometric percentages of head length and standard length are shown in Figures 2.5 and 2.6. Where the data is available from the literature, the values of the West African hybrid and parent species are included in the figures.

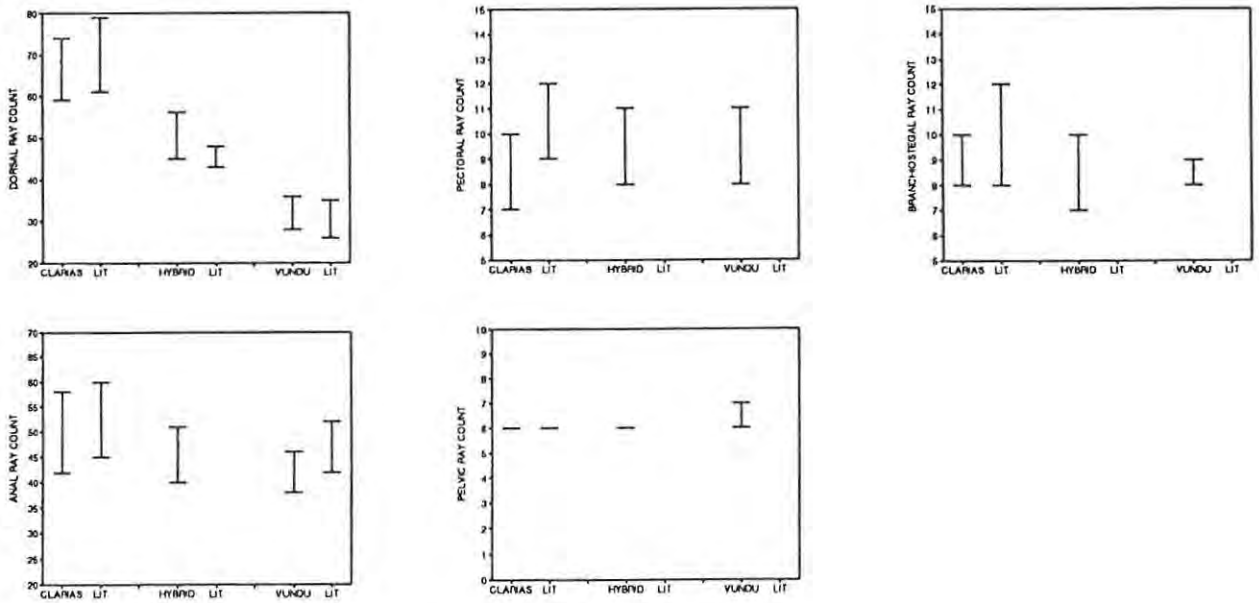

Figure 2.4. Comparison of the minimum and maximum meristic counts for *C. gariepinus* (CLARIAS), *H. longifilis* (VUNDU) and their F_1 hybrid (HYBRID), in this study and from the literature (LIT: Teugels, 1986; Teugels *et al.*, 1990; Legendre *et al.*, 1992).

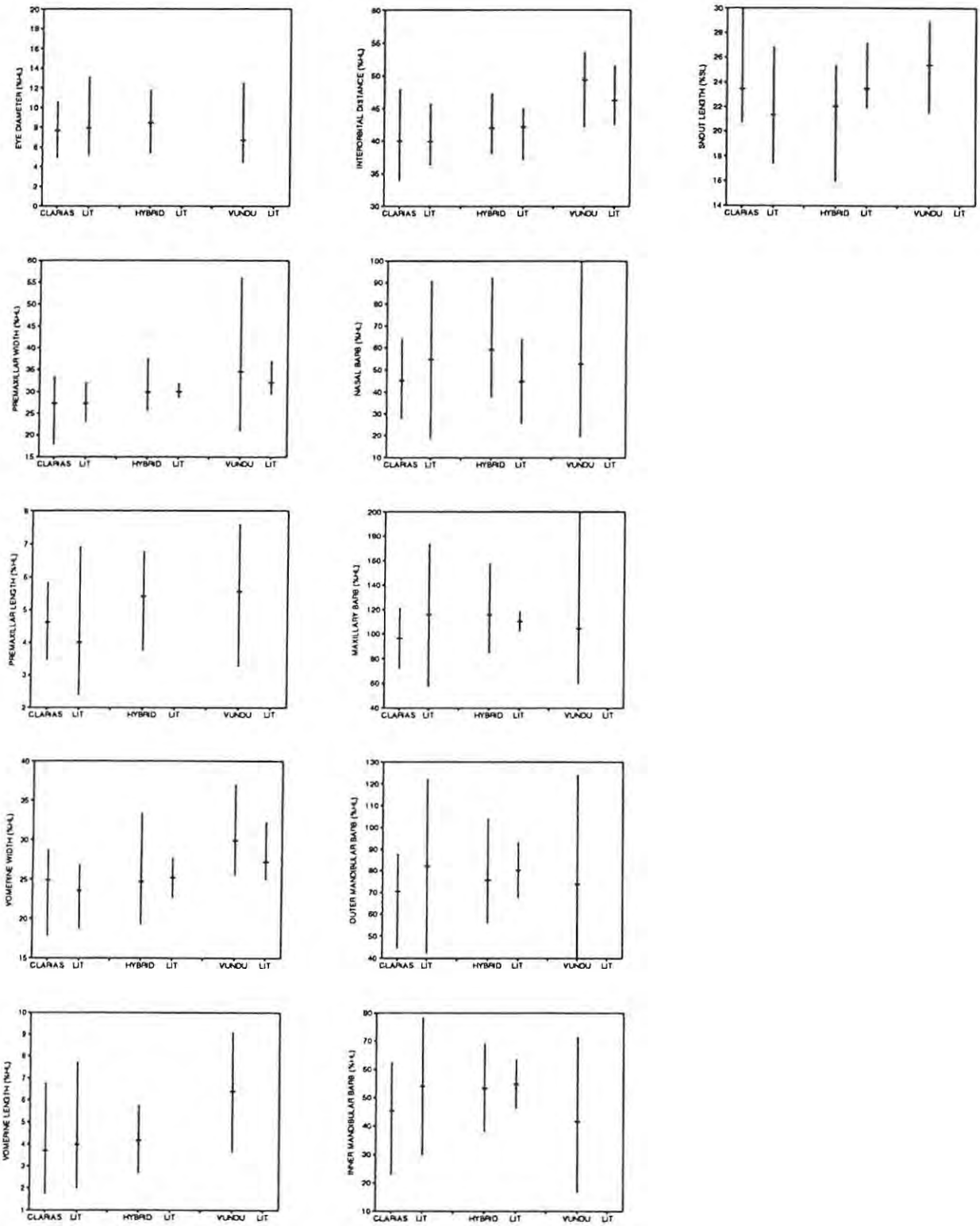

Figure 2.5. Comparison of the mean, minimum and maximum morphometric characters (% Head length) of *C. gariepinus* (CLARIAS), *H. longifilis* (VUNDU) and their F_1 hybrid (HYBRID), in this study and from the literature (LIT: Teugels, 1986; Teugels *et al.*, 1990; Legendre *et al.*, 1992).

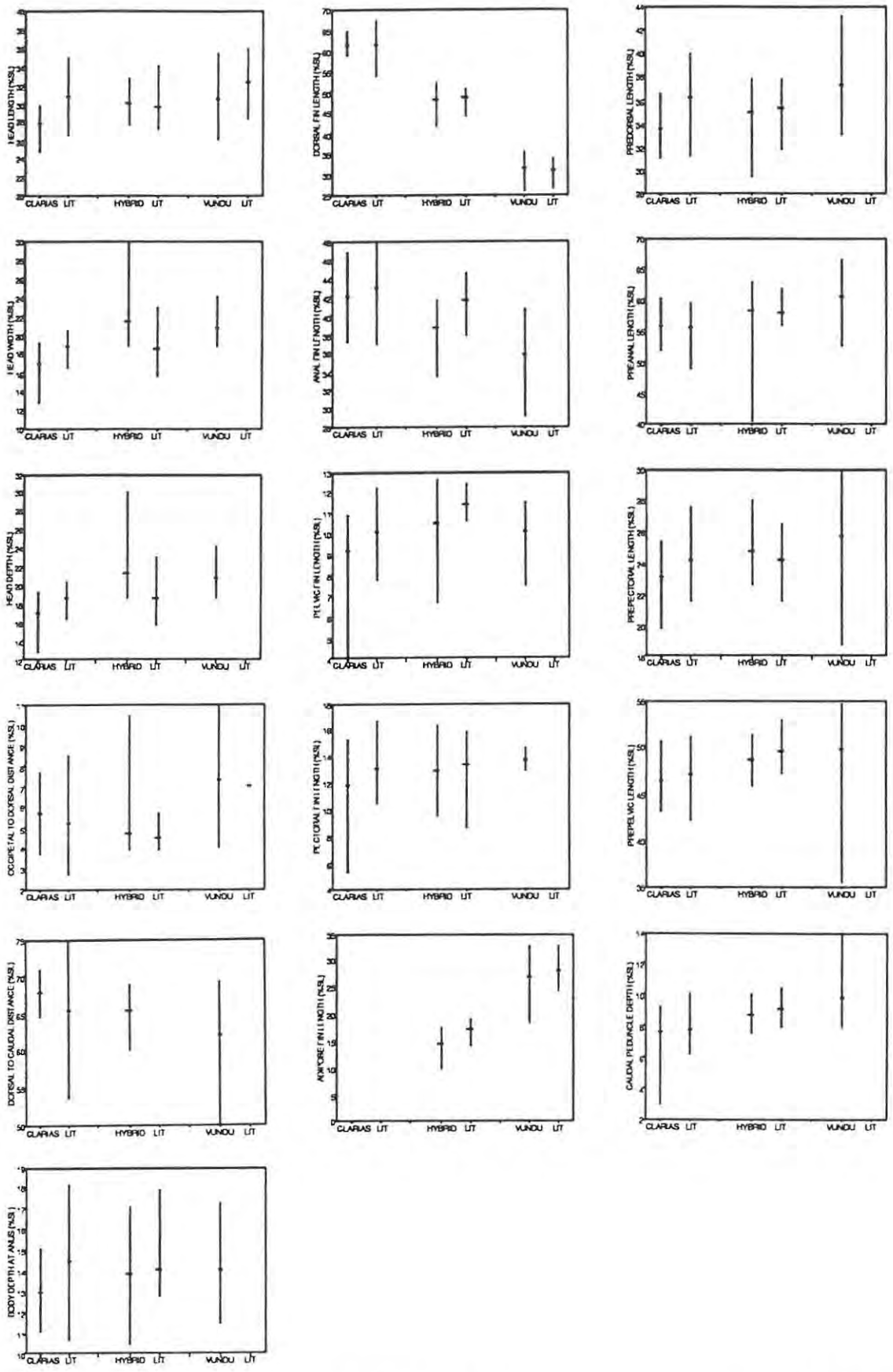

Figure 2.6. Comparison of the mean, minimum and maximum morphometric characters (% Standard length) of *C. gariepinus* (CLARIAS), *H. longifilis* (VUNDU) and their F_1 hybrid (HYBRID), in this study and from the literature (LIT: Teugels, 1986; Teugels *et al.*, 1990; Legendre *et al.*, 1992).

The hybrid index of the means of the characters showed that the hybrid was intermediate to the parent species for five of the characters. The hybrid shared nine characters with *C. gariepinus* and seven with *H. longifilis*, while six were specific for the hybrids (Table 2.2).

Table 2.2. The hybrid index scores for the means of the meristic and morphometric characters.

Character	Intermediate	*C. gariepinus*	*H. longifilis*	Hybrid
Dorsal rays	53			
Anal rays			36	
Pelvic rays			33	
Pectoral rays				108
Branchiostegal rays		86		
Snout length				172
Interorbital length		77		
Eye diameter				212
Premaxilla width		60		
Premaxilla length			24	
Vomerine width		94		
Vomerine length		80		
Head length			11	
Head width	55			
Head depth		79		
Body depth			9	
Caudal peduncle depth		59		
Predorsal distance		66		
Pre-anal distance		58		
Prepelvic distance			32	
Prepectoral distance			43	
Occipital to dorsal distance				156
Dorsal fin length	55			
Anal fin length	50			
Pelvic fin length				-60
Pectoral fin length				-22
Pectoral spine length	55			

The most obvious difference in physical appearance between *C. gariepinus*, *H. longifilis* and the hybrid was the size of the adipose fin. Adipose fin length also had an effect on the dorsal fin length and dorsal ray counts of the fish, thus these obvious differences between the groups were removed from their respective data sets, and the discriminating power of the rest of the characters were tested in a series of discriminant function analyses.

The first discriminant function of the meristic counts contained 95.8 % of discriminant information in the data set and was useful in separating the *C. gariepinus* and *H. longifilis* individuals into distinct groups, but the hybrid range overlapped both groups (Fig. 2.7).

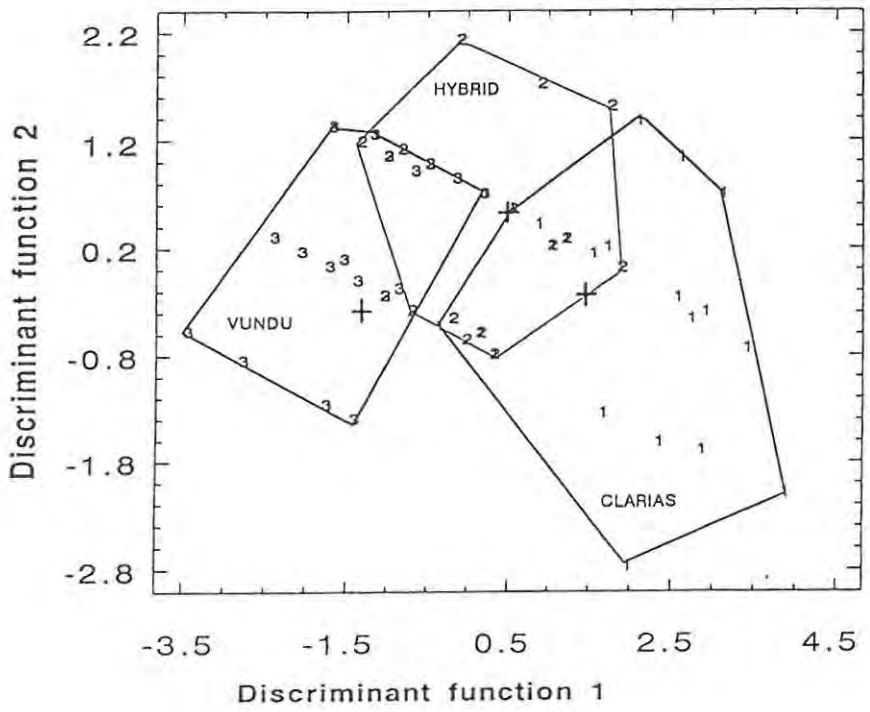

Figure 2.7. Scatterplot of *C. gariepinus* (1), hybrid (2) and *H. longifilis* (3) values and group centroids (+) for discriminant function one and two of the meristic data set.

The algorithm derived for the meristic discriminant analysis was more successful at discriminating between *H. longifilis* and the hybrid than between *C. gariepinus* and the hybrid (Table 2.3). This means that for the meristic characters used in the discriminant function analysis, the hybrid was closer to *C. gariepinus* than to *H. longifilis*.

Table 2.3. The percent individuals correctly assigned to their groups by the meristic discriminant functions.

	Predicted Group (percentage)		
Actual Group	*C. gariepinus*	Hybrid	*H. longifilis*
C. gariepinus	66.67	33.33	0.00
Hybrid	16.67	66.67	16.67
H. longifilis	0.00	20.00	80.00

The first discriminant function of the morphometric measurements standardised to head length contained 90.2 % of the discriminant information of the data set. Even so, the *C. gariepinus* and hybrid individuals could not be separated along this axis (Fig. 2.8). The *H. longifilis* specimens were well

discriminated from the other groups for this function. The hybrid and *C. gariepinus* groups were separated to some extent by the second discriminant function, which only contained 9.7 % of the discriminant information.

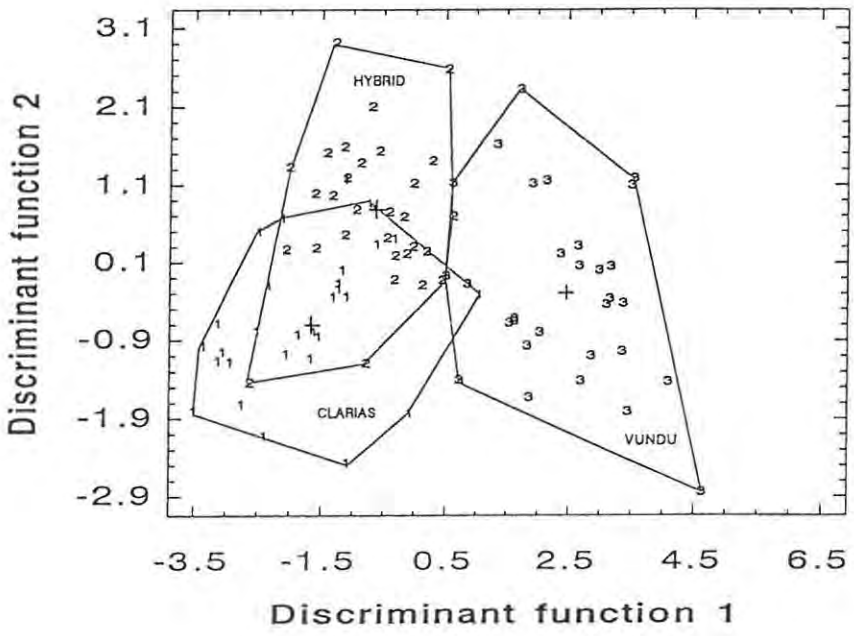

Figure 2.8. Scatterplot of *C. gariepinus* (1), hybrid (2) and *H. longifilis* (3) values and group centroids (+) for discriminant function one and two of the morphometric (% Head length) data set.

By combining the discriminant information of both discriminant functions, the discriminant analysis proved to be remarkably accurate at predicting the correct group to which an individual fish belonged (Table 2.4).

Table 2.4. The percent individuals correctly assigned to their groups by the head length-standardised morphometric discriminant functions.

	Predicted Group (percentage)		
Actual Group	*C. gariepinus*	Hybrid	*H. longifilis*
C. gariepinus	83.33	13.33	3.33
Hybrid	13.33	86.67	0.00
H. longifilis	0.00	6.67	93.33

There was a slight overlap in the range of *C. gariepinus* and *H. longifilis* specimens for the first discriminant function of the morphometric data converted to a percentage of standard length (Fig. 2.9). However, the hybrid specimens occurred well within the range of both parent species for discriminant function one. The three groups could not be separated using discriminant function two. Discriminant function one and two explained 87.2 % and 12.8 % of the variation in this data set respectively.

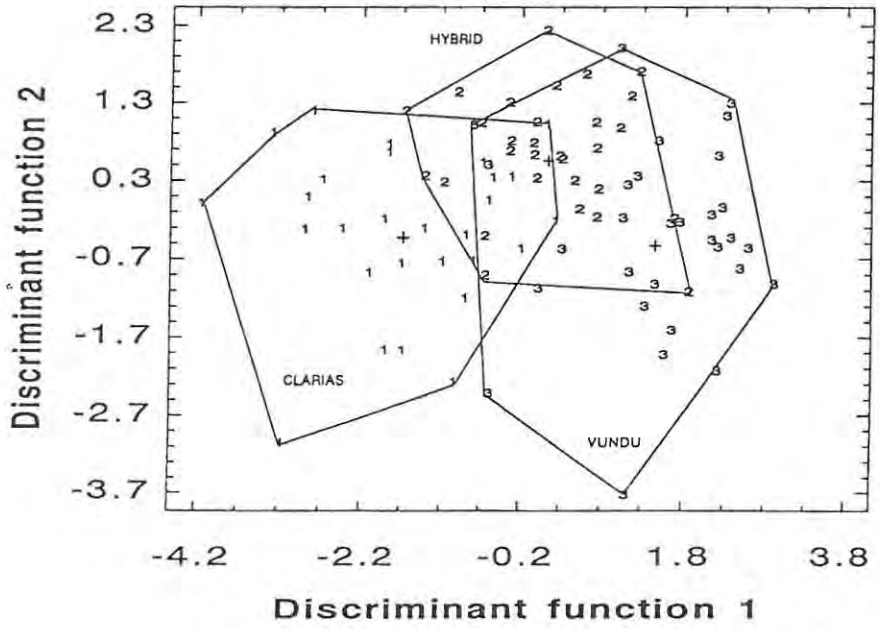

Figure 2.9. Scatterplot of *C. gariepinus* (1), hybrid (2) and *H. longifilis* (3) values and group centroids (+) for discriminant function one and two of the morphometric (% Standard length) data set.

The discriminant function algorithms were very successful at re-assigning the specimens to their correct groups (Table 2.5).

Table 2.5. The percent individuals correctly assigned to their groups by the standard length-standardized morphometric discriminant functions.

	Predicted Group (percentage)		
Actual Group	*C. gariepinus*	Hybrid	*H. longifilis*
C. gariepinus	76.67	23.33	0.00
Hybrid	16.67	73.33	10.00
H. longifilis	3.33	10.00	86.67

In the final discriminant function analysis, characters from both the head length and standard length-standardized data sets were used to discriminate between the three southern African groups and a fourth group of West African hybrids. The West African hybrids were distinct from the *C. gariepinus* and *H. longifilis* groups for both discriminant functions one and two, but could not be separated from the range of the southern African hybrid group (Fig. 2.10).

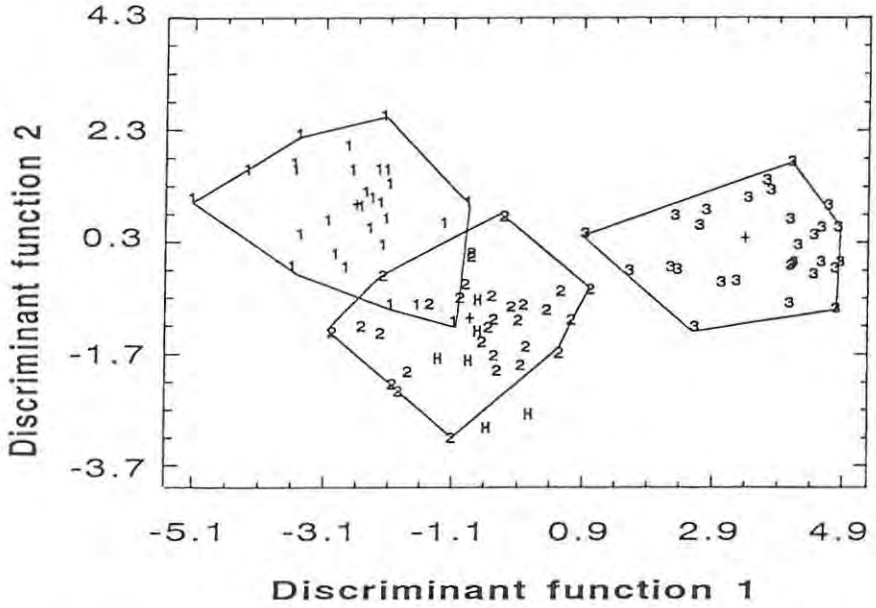

Figure 2.10. Scatterplot of *C. gariepinus* (1), southern African hybrid (2), *H. longifilis* (3) and West African hybrid (H) values and group centroids (+) for discriminant function one and two of the combined morphometric data set.

Karyology

The diploid chromosome numbers of *H. longifilis* and *C. gariepinus* were different (Fig. 2.11), but their fundamental numbers were remarkably similar for species of different genera. The fundamental numbers of the West African and southern African hybrids' chromosomes were identical (Table 2.6).

Table 2.6. The fundamental number (FN), mean bi-armed and uni-armed chromosome numbers and their standard deviations, and diploid chromosome number for *H. longifilis*, *C. gariepinus* and hybrids from southern Africa and West Africa.

Species	FN	Bi-armed	Uni-armed	Std. Dev.	2N
H. longifilis	92	40	12	±1.6	52
C. gariepinus	97	41	15	±1.6	56
Hybrid (southern Africa)	95	41	13	±1.1	54
Hybrid (West Africa)	95	41	13	±2.0	54

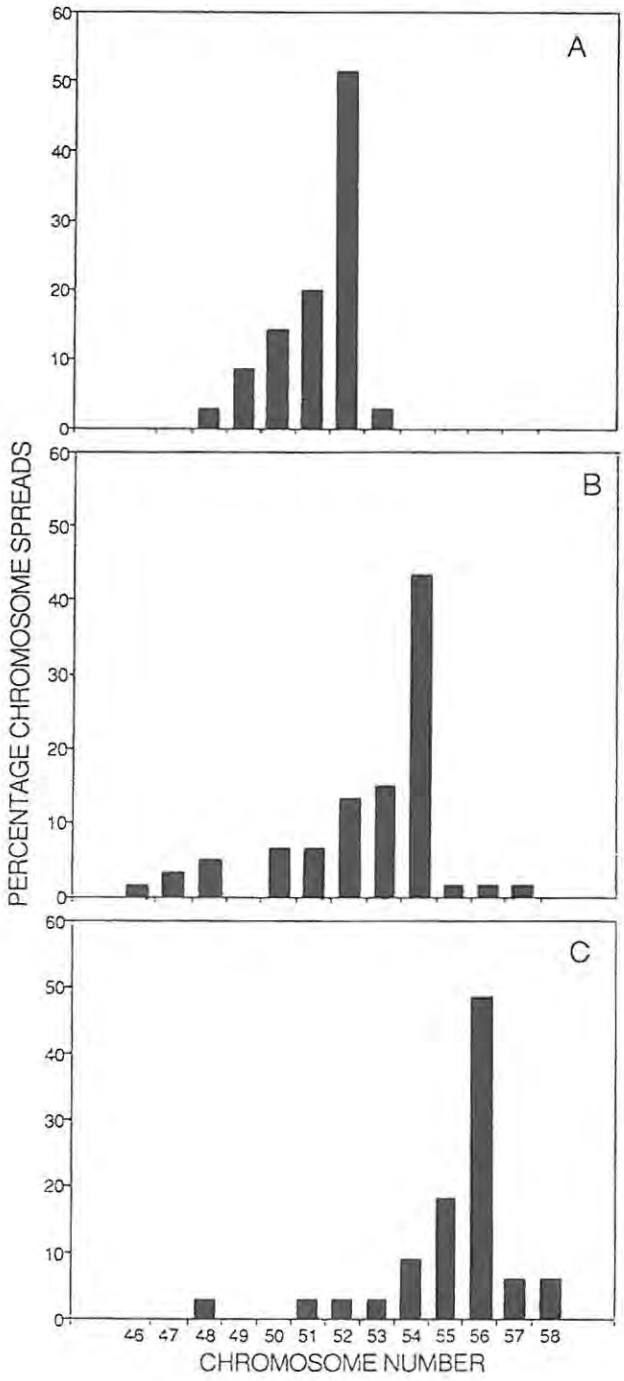

Figure 2.11. The modal diploid chromosome number for *H. longifilis* (A), hybrid (B), and *C. gariepinus* (C) (n = 30 spreads/species).

Clarias gariepinus had a diploid (2N) number of 56 chromosomes and *H. longifilis* had a 2N of 52 chromosomes. The somatic cells of the hybrids contained an intermediate number of 54 chromosomes. The hybrid chromosome complement had one more bi-armed and uni-armed chromosome than *H. longifilis*, and two uni-armed chromosomes less than *C. gariepinus*.

2.4. DISCUSSION

The morphometric and meristic values reported in the literature (Teugels, 1986; Teugels *et al.*, 1990; Legendre *et al.*, 1992) for *C. gariepinus*, *H. longifilis* and their hybrid were remarkably similar to the values observed for these fish in the present study. In fact, it was impossible to distinguish between the West African and southern African *H. longifilis* x *C. gariepinus* hybrids using standard meristic and morphometric methods, multiple discriminant analysis or karyology. For all intents and purposes the two populations were morphologically identical. Therefore it is parsimonious to assume that the differences reported between the two groups were not due to genotypic differences.

However, while the genome is the genetic blueprint for a species, the "blueprint" can be interpreted in different ways during the epigenesis of an organism. Epigenesis has been defined by Balon (1983) as "... the interaction of developmental elements leading to the construction of a phenotype", and includes the interaction of gene products at the cellular, tissue and organ level. The local environment in which an organism is raised has an effect on these epigenetic interactions, causing changes in morphology, physiology and ethology (Balon, 1983; Cowley and Atchley, 1992).

The two groups of hybrid catfish were spawned and raised under very different environmental conditions. The West African hybrids were spawned in tap water and raised in slightly brackish lagoon water in the tropics of the northern hemisphere (Legendre *et al.*, 1992). The southern African hybrids were spawned and raised in fresh river water in the temperate region of the southern hemisphere. The fishes from the two regions were therefore exposed to differences in climate, water quality, food and photoperiod.

Dunham (1986) noted that while genotype-environment interactions were minor in strains and selected lines of the channel catfish *Ictalurus punctatus*, they were important in crossbred, hybrid or triploid catfish. For example, the *I. punctatus* x *I. furcatus* hybrid showed heterosis when grown in ponds, but it grew slower than *I. punctatus* when reared in tanks (Smitherman and Dunham, 1985).

Maternal effects may have also played a role in the apparent phenotypic (excluding morphological) differences between the West and southern African hybrids. Maternal effects are the *ex-genetic* influences that a mother may have on her progeny, including her responses to environmental conditions during reproduction (Cowley and Atchley, 1992). For example, Legendre *et al.* (1992) hypothesised that egg quality had an effect on the hatching rates and early larval survival of the West African hybrids. The *H. longifilis* brooders used to produce the West African hybrid were descendants of stock that spontaneously colonised the fish ponds of the Layo Research Station, while the *C. gariepinus* brooders had to be brought in from an experimental fish farm elsewhere in the Ivory Coast (Legendre *et al.*, 1992). The opposite was true at the Mubuyu Farms hatchery in Zambia. The *C. gariepinus* brooders

used to produce the southern African hybrid had spontaneously colonised the farm's fish ponds, while the *H. longifilis* brooders had to be collected from the Zambezi River.

Thus, the phenotypic differences reported between the two hybrid populations were most likely the result of different environmental influences on the hybrids and their parents, rather than racial differences between the West and southern African *C. gariepinus* and *H. longifilis* groups.

The meristic and morphometric characteristics of *C. gariepinus* and *H. longifilis* were very similar. The only significant differences in the data set related directly to the presence or absence of the adipose fin. Teugels *et al.* (1990) emphasized the close relationship between the *Heterobranchus* genus and the subgenus *Clarias (Clarias)* based on morphological and osteological data. However, the meristic and morphometric characters used in this study contained much valuable information when combined in a multiple discriminant analysis. The specimens of the *H. longifilis* and *C. gariepinus* groups could be separated with at least 96 % accuracy for all the data sets tested.

The intermediacy of morphometric and meristic characters has long been the basic criterion used to recognise hybrids (Hubbs, 1955). However, not all of the morphological characters of the *H. longifilis* x *C. gariepinus* hybrid showed intermediacy to those of the parent species. The hybrid index indicated that the hybrid shared more morphological characteristics with *C. gariepinus* than with *H. longifilis*. While the hybrid specimens always occurred between, and usually overlapped, the parent species' ranges on the scatterplots of their first and second discriminant function scores, the hybrids were not as well separated from the *C. gariepinus* group as they were from the *H. longifilis* group. This was particularly true for the meristic data set, where more than 33 % of the hybrids were incorrectly identified as belonging to the *C. gariepinus* group. If more than 20 % of the specimens are misclassified, the groups cannot be regarded as separate for that particular data set (Harrell and Dean, 1988).

While it is tempting to infer from this information that the hybrid was more like *C. gariepinus* than *H. longifilis* (particularly for head characteristics), the hybrid index can only be used to compare the morphological characters singularly, and not as a whole. Hybridization does not lead to a simple blending of inheritance; other genetic systems such as dominance and epistasis lead to at least some non-intermediate hybrid characters (Taylor *et al.*, 1986). If a different set of morphological features were chosen to characterize the groups, the hybrid could just as well seem closer to *H. longifilis* than to *C. gariepinus*.

Teugels *et al.* (1992b) used electrophoresis to compare the West African hybrid and its parent species at the genetic level. They found that the gene frequencies of the hybrid were intermediate between those of *C. gariepinus* and *H. longifilis*, for the enzyme systems tested.

The hybrid chromosomes also showed intermediacy to those of the parent species. The hybrid's chromosome number and fundamental number were mid-way between those of *H. longifilis* and *C. gariepinus*. If the chromosome complements of the parent species are similar enough, the two haploid sets of chromosomes donated by the parents can combine in a normal, diploid fashion, and the hybrid would show intermediacy between the parent species. However, other combinations of hybrid chromosomes may occur, particularly if the chromosome numbers of the parent species do not match. For example, Ma and Yamazaki (1986) found that the *Oncorhynchus masou* x *O. gorbuscha* hybrid chromosome complement consisted of combinations of either 52 or 59 chromosomes.

The different combinations of chromosomes can be reflected in the morphology of the hybrids. Tarnchalanukit (1986) reported that the intergeneric *Clarias macrocephalus* x *Pangasius sutchi* hybrid had four discrete morphotypes. Na-Nakorn *et al.* (1993) found that this hybrid had three possible chromosome complements. One type of complement was diploid, consisting of a set of haploid chromosomes from each of the parent species, while another type was triploid, consisting of a haploid set from *P. sutchi* and a diploid set from *C. macrocephalus*. A third type was also diploid, but only contained *C. macrocephalus* chromosomes, probably arising from gynogenesis.

The low variance about the mean values of the bi- and uni-armed chromosomes for the *H. longifilis* x *C. gariepinus* hybrid showed that the hybrid chromosome complement was as stable as that of its parent species. It is highly unlikely that different combinations of chromosomes occurred in these hybrids. The bi- and uni-arm chromosome analysis showed that the hybrid had most probably inherited a complete haploid (N) chromosome set from each of its parents, ie. *C. gariepinus* (N) = 21 + 7, *H. longifilis* (N) = 20 + 6, therefore the hybrid (2N) = 41 + 13 = 54. Teugels *et al.* (1992a) came to the same conclusion when they karyotyped the West African hybrid and its parent species.

Based on the morphological and karyological characteristics of the hybrids and their parent species it could be concluded that the *H. longifilis* x *C. gariepinus* cross is a true hybrid, in that it shows intermediacy with *H. longifilis* and *C. gariepinus*. It can also be concluded that the West African and southern African hybrid populations belong to the same group. The reasons for the apparent differences in larval and juvenile survival, growth and fertility of the two hybrid populations is therefore most probably be due to the different environments in which the hybrids were propagated.

It is clear that in order to gain an accurate picture of the aquaculture potential of the hybrid in southern Africa, it has to be tested under the environmental conditions occurring within the region.

CHAPTER 3. FERTILIZATION, HATCHING AND LARVAL SURVIVAL RATES OF THE *HETEROBRANCHUS LONGIFILIS* X *CLARIAS GARIEPINUS* HYBRID.

3.1. INTRODUCTION

Natural hybridization arises frequently in wild fishes. Chevassus (1979) estimated that 5000 to 6000 hybridizations have occurred. Many of these hybrids occurred because the natural habitat of the parent species was disturbed, through water impoundments, river flow changes, species translocation and the introduction of exotic species. For instance, the cyprinid species *Barbus aeneus* and *B. kimberleyensis*, which occur sympatrically in the Orange and Vaal rivers of southern Africa, have hybridized in the Hardap dam in Namibia (Gaigher, 1976, van Vuuren *et al.*, 1989). The cyprinid fishes are particularly susceptible to hybridization. Other examples of interspecific and intergeneric hybridization in the cyprinids can be found in the genera *Abramis*, *Alburnus*, *Blicca*, *Chondrostoma*, *Luxilus*, *Rudd*, *Rutilus*, *Scardinius* and *Vimba* (Brassington and Ferguson, 1976; Kozlov and Scherbina, 1976; Collares-Pereira and Coelho, 1983; Blachuta and Witkowski, 1984; Elvira *et al.*, 1990; Golubtsov *et al.*, 1990; Meagher and Dowling, 1991).

The Salmonidae are also susceptible to hybridization. For example, hybridization has occurred between the brown trout (*Salmo trutta*) of Europe and the Atlantic salmon (*Salmo salar*) of North America, when the latter was translocated to the United Kingdom and Sweden (Hurrel and Price, 1991; Jansson *et al.*, 1991; Jordan and Verspoor, 1993). Hybrids have also been reported in the genera *Salvelinus* and *Oncorhynchus* (Sheridan,1981; Hammar *et al.*, 1989).

Natural hybridization has been reported in many other families of fish, including the Anthidae, Catostomidae, Characidae, Cichlidae, Cottidae, Lutjanidae, Moronidae, Percidae, Pomacanthidae, Triglidae and Tetraodontidae (Nelson, 1973; Daget and Kouassi, 1978; Strauss, 1986; Conde, 1989; Abe and Kanai, 1990; Kuiter, 1990; Raesly *et al.*, 1990; Loftus, 1992; McClure and McEachran, 1992; Waldman and Bailey, 1992).

Salmonid fishes have been artificially hybridized since the late 19th century (Green, 1881; Day, 1882) and by the 1970's, more than 600 research papers had been published on the topic (Dangel *et al.*, 1973). Other families of fish in which artificial hybridization has been accomplished include the Acipenseridae, Catostomidae, Centrarchidae, Cichlidae, Cyprinidae, Oplegnathidae, Percidae and Siluridae (see Table 4.1).

If natural hybridizations have taken place within the siluroid catfish group, they must be rare occurrences, as no reports of this could be found in the available scientific literature. Some success has been achieved with the artificial hybridization of siluroid catfish at the interspecific and intergeneric

levels, but little success has been achieved at the interfamilial level. For example, the *Pangasius sutchi* x *Clarias batrachus* hybrid had a 10.7 % hatching success compared to its parent species (Tarnchalanukit, 1986). The *Pangasius sutchi* x *Clarias macrocephalus* hybrid had a hatching rate of 81 %, but only 1.8 % of the deformed larvae survived (Na-Nakorn *et al.*, 1993). The *Heteropneustes fossilis* x *Clarias batrachus* hybrid had both poor egg and larval survival, with a hatching rate of 2.82 % and a larval survival rate of only 0.02 % (Mukhopadhyay and Dehadrai, 1986).

At the interspecific level, *Clarias gariepinus* has been hybridized with *C. fuscus* in China (Zheng *et al.*, 1988) and with *C. macrocephalus* in Thailand (Jantrarotai *et al.*, 1994). The *C. batrachus* x *C. macrocephalus* hybrid is currently the favoured catfish for aquaculture in Thailand, because it combines the preferred taste of *C. batrachus* with the faster growth of *C. macrocephalus* (Little & Griffiths, 1992).

The intergeneric hybrids of *Clarias gariepinus* x *Heterobranchus bidorsalis* and *C. gariepinus* x *H. longifilis* have both been hailed as important species for aquaculture in Africa (Hecht and Lublinkhof, 1985; Salami *et al.*, 1993). However, Hecht *et al.* (1991) reported that the hatching rate of the *C. gariepinus* ♂ x *H. longifilis* ♀ cross was significantly lower than the hatching rates of the parent species and the reciprocal cross. Hecht *et al.* (1991) speculated that this difference was due to genetic changes resulting from hybridization. On the other hand, the *C. gariepinus* ♂ x *H. longifilis* ♀ hybrids produced in the Ivory Coast did not have a lower hatching rate than the reciprocal cross or the parent species (Legendre *et al.*, 1992).

In order to evaluate the success of the hybridization of *H. longifilis* and *C. gariepinus* in southern Africa, it was necessary to compare the fertilization and hatching rates of the reciprocal hybrids with those of *H. longifilis* and *C. gariepinus.*

However, hatching rates are not always a good indication of a successful hybridization, as the hatched embryos may not survive (Mukhopadhyay & Dehadrai, 1986; Tarnchalanukit, 1986). For this reason the survival rates of the *H. longifilis* x *C. gariepinus* hybrid larvae were compared to those of *C. gariepinus* larvae, once they had begun exogenous feeding.

3.2. MATERIALS AND METHODS

Heterobranchus longifilis was hybridized with *C. gariepinus* during the summers of 1992, 1993 and 1994, at the Mubuyu Farms hatchery in Mazabuka, Zambia. The *H. longifilis* brood stock were collected by hook and line at the confluence of the Kafue and Zambezi rivers, and ranged in size from 6.0 kg to 20.0 kg. The *C. gariepinus* brood stock were from domesticated farm fish, whose parental stock originally colonised the fish ponds at Mubuyu Farms. They ranged in size from 330 g to 700 g.

The spawning and rearing protocol was identical for all the hybridization attempts carried out at Mubuyu Farms hatchery, and is briefly described below.

Homogenized pituitary glands were used for hypophysation in all of the hybridization experiments, as described in Britz (1991). The same spawning procedure was followed each time, as described in Hecht *et al.* (1988). Prior to spawning, pituitary glands were removed from sexually mature *C. gariepinus* (not gender specific). These glands were homogenized in distilled water (1.0 gland/mℓ) and injected intermuscularly into the ripe female catfish. A half-dose starter injection was given to very large *H. longifilis* females (>7.5 kg) approximately 6-7 hours before the primary dose was administered. At a constant temperature of 28 °C, the females could be successfully stripped 12-14 hours after the primary injection.

Just prior to spawning, the males were killed and their testes removed and stored in 0.7% saline solution. The sperm remained viable for at least three days in this solution if kept refrigerated at a temperature below 6.0 °C.

Care was taken to strip the eggs from the females only when their eggs were running freely from the genital opening. The females with running eggs were towelled dry, and pressure was applied to the abdomen so that the eggs were extruded from the genital opening in a stream. The eggs were caught in a clean, plastic bowl. At the first sign of blood the extrusion of eggs was stopped, and the females were returned to their holding tanks.

The eggs of at least three *C. gariepinus* females were mixed, and divided into two bowls. This procedure was repeated with the eggs of the *H. longifilis* females. The sperm of a single male *C. gariepinus* was used to fertilize a bowl of *C. gariepinus* eggs and a bowl of *H. longifilis* eggs. The remaining eggs were fertilized with the sperm of a single male *H. longifilis*. In future these crosses will be indicated as follows:

H. longifilis sperm x *H. longifilis* eggs = HL♂xHL♀
C. gariepinus sperm x *C. gariepinus* eggs = CG♂XCG♀
H. longifilis sperm x *C. gariepinus* eggs = HL♂XCG♀
C. gariepinus sperm x *H. longifilis* eggs = CG♂XHL♀

The testis was held above the eggs and an incision was made along the distal margin. The semen was allowed to drip into the bowl as the testis was squeezed. The eggs and sperm were then gently mixed using a soft plastic spatula. Water was then added to the bowl to activate the sperm. After the eggs and sperm had been thoroughly mixed with water the eggs were allowed to stand for one minute, after which they were carefully poured onto 1.5 mm mesh frames. The eggs adhered to the mesh frames,

which were hung vertically in the incubation troughs. One trough was used per cross. The 2500 mm long troughs contained 180 ℓ of water, which was replaced at a rate of 100 $\ell.hr^{-1}$. The hatchery water temperature was maintained between 27.5 °C and 29 °C for the duration of all the experiments.

Fertilization and hatching rate

A number of eggs (approx. 100-200) from each of the above crosses were allowed to adhere to large petri dishes containing water from the incubation troughs. Three petri dishes of eggs for each cross were floated in their respective troughs. The petri dishes were removed from the troughs and the eggs were examined under a dissecting microscope every hour. The number of fertilized eggs and unfertilized eggs were counted after two hours, to calculate fertilization rates. The eggs were considered to be fertilized following Bruton (1979b) and Zaki and Abdula (1984), when the fertilized eggs had an almost transparent greenish-orange yolk, and cell division was clearly visible in the orange blastodisc. The yolk of the unfertilized eggs turned an opaque yellow.

Approximately half the water in the petri dishes was exchanged every 30 minutes. After each examination, they were refloated in the troughs. Using this method, the number of live and dead eggs were monitored, until the surviving eggs had hatched in each petri dish. The fertilization and hatching rates in each petri dish were calculated as a percentage of the total number of eggs in each petri dish.

The 1992, 1993 and 1994 hatching and fertilization rate data sets for the four crosses were compared using a two-way analysis of variance (ANOVA). Relationships within the data sets were established using the 95 % Tukey multiple range test.

Larval survival rate

Once the eggs from the 1994 fertilization trial had hatched, 90 *C. gariepinus* larvae were divided equally amongst three glass aquaria. The same number of HL♂XCG♀ hybrid larvae were placed into three identical aquaria. These small aquaria each held 1 ℓ of incubation trough water, and were fixed in an incubation trough. The water in the aquaria was exchanged at a rate of 0.2 $\ell.hr^{-1}$. Air was gently bubbled into the aquaria through diffusers, and the water temperature was maintained at 28 ± 1 °C. The surviving larvae in the aquaria were counted on the day after the larvae showed signs of exogenous feeding. The survival rates of the hybrid and *C. gariepinus* larvae were compared using a Kruskal-Wallis non-parametric test.

3.3. RESULTS

Fertilization and hatching rates

The fertilization and hatching rate data collected at Mubuyu Farms showed a great degree of variation. However, this variation was found to occur between the annual trials rather than between the crosses. The overall fertilization and hatching rates of the *H. longifilis*, *C. gariepinus* and reciprocal hybrid crosses were significantly different ($P < 0.05$) for all of the hybridization trials carried out (Table 3.1).

Table 3.1. Mean fertilization and hatching rates of the four crosses for the three hybridization trials.

Trial	Mean fertilization rate (% ± sd)	Mean hatching rate (% ± sd)
1992	86.0 ± 4.9[a]	45.5 ± 6.1[a]
1993	76.3 ± 7.4[b]	32.5 ± 9.6[b]
1994	95.0 ± 3.4[c]	67.2 ± 6.6[c]

[a] Superscript denotes homogenous groups

There was no significant difference ($P > 0.05$) between the fertilization or hatching rates of the *H. longifilis*, *C. gariepinus* or the CG♀XHL♀ cross for the three hybridization trials. However, the HL♂xCG♀ hybrid had a significantly higher ($P < 0.05$) hatching rate than *H. longifilis*, and a significantly higher fertilization rate ($P < 0.05$) than the rest of the crosses (Table 3.2).

Table 3.2. Mean fertilization and hatching rates of the three hybridization trials for the four crosses.

Cross	Mean fertilization rate (% ± sd)	Mean hatching rate (% ± sd)
HL♂xHL♀	81.8 ± 8.2[a]	44.5 ± 16.7[a]
HL♂xCG♀	93.3 ± 3.9[b]	55.0 ± 8.3[b]
CG♂xHL♀	83.7 ± 9.0[a]	49.6 ± 19.9[ab]
CG♂xCG♀	85.1 ± 10.0[a]	48.8 ± 15.2[ab]

[a] Superscript denotes homogenous groups

Larval survival rate

The *C. gariepinus* and hybrid larvae began to show signs of exogenous feeding 3 days after hatching. After four days post hatching the survival rate of the *C. gariepinus* larvae was found to be excellent in all the replicate aquaria (Table 3.3). The hybrid larvae showed a large variation in survival rate between the three replicate aquaria, but there was no overall difference ($P > 0.05$) in survival between the *C. gariepinus* larvae and the HL♂xCG♀ hybrid larvae.

Table 3.3. Survival rates of *C. gariepinus* and HL♂xCG♀ hybrid larvae after the initiation of exogenous feeding (day 4 post hatching).

Cross	Survival Rate (%)			
	Replicate 1	Replicate 2	Replicate 3	Mean (% ± sd)
C. gariepinus	93.3	100.0	96.7	96.7 ± 2.7[a]
HL♂xCG♀ hybrid	100.0	80.0	63.3	81.1 ± 15.0[a]

[a] Superscript denotes homogeneous groups

3.4. DISCUSSION

One of the major constraints to the more widespread farming of clariid catfishes in Africa is the lack of good quality seed stock (Haylor, 1992). Thus, the fertilization, hatching and larval survival rates of the *H. longifilis* x *C. gariepinus* hybrid are important factors to consider when appraising the hybrid's aquaculture potential.

The hybridization of the southern African populations of *H. longifilis* and *C. gariepinus* was very successful. The fertilization and hatching rates of the reciprocal hybrids were at least as high as those of the two parent species, and there was no significant difference ($P > 0.05$) between the survival rate of *C. gariepinus* larvae and HL♂xCG♀ larvae, post exogenous feeding.

Contrary to Hecht *et al.*'s (1991) observations, the hatching rate of the CG♂xHL♀ hybrid was not significantly different to the hatching rates of the other crosses carried out at the Mubuyu Farm hatchery. However, the HL♂xCG♀ cross had a significantly higher fertilization rate than the other crosses, and its hatching rate was significantly higher than that of *H. longifilis* in this study. This may be an indication that the HL♂xCG♀ cross has a genetic advantage for egg survival, compared to the other crosses, with a greater potential for aquaculture in Africa than its reciprocal cross. However, no genetic-based differences in hatching rate were observed between the parent strains and hybrid crosses spawned in West Africa (Legendre *et al.*, 1992).

The fertilization and hatching rates of the catfish larvae hybridized at Mubuyu hatchery fluctuated widely between the three consecutive annual trials. To some extent, this variation in breeding success was also apparent for the three consecutive annual *H. longifilis* x *C. gariepinus* hybridizations carried out in West Africa (FIG.1; Legendre *et al.*, 1992). The best hatching rates for the *H. longifilis*, *C. gariepinus* and reciprocal hybrids spawned at the Mubuyu Farms hatchery were obtained in 1994, and compare favourably with the hatching rates of other clariid catfish reported in the literature (Table 3.4).

Table 3.4. Hatching rates in some clariid catfish species.

Species	Hatching rate	Reference
Clarias batrachus	60-75 %	Viveen *et al.* (1990)
C. macrocephalus	82 %	Tarnchalanukit (1986)
C. batrachus ♂ x *C. macrocephalus* ♀	75 %	Tarnchalanukit (1986)
C. macrocephalus ♂ x *C. batrachus* ♀	71 %	Tarnchalanukit (1986)
Heterobranchus longifilis	63 %	Nwadukwe (1993)
H. longifilis	83 %	Legendre *et al.* (1992)
H. longifilis	66 %	This study, 1994 trial
Clarias gariepinus	71-79 %	Richter *et al.* (1985), Hecht *et al.* (1991)
C. gariepinus	66 %	This study, 1994 trial
H. longifilis ♂ x *C. gariepinus* ♀	68 %	Legendre *et al.* (1992)
C. gariepinus ♂ x *H. longifilis* ♀	75 %	Legendre *et al.* (1992)
H. longifilis ♂ x *C. gariepinus* ♀	70 %	This study, 1994 trial
C. gariepinus ♂ x *H. longifilis* ♀	65 %	This study, 1994 trial

From Table 3.4 it seems acceptable to assume that the average hatching rate of clariid catfish, including the *H. longifilis* x *C. gariepinus* hybrid, is between 60 and 80 %. The survival of post exogenous feeding larvae is high in *C. gariepinus* (Table 3.5), and it is likely that similar survival rates can be anticipated for the hybrid.

Table 3.5. Examples of survival rates reported for *C. gariepinus* larvae.

Age (post hatching)	% Survival	Reference
4 days	97 %	This study
10 days	96 %	Verreth & van Tongeren (1989)
14 days	64-96 %	Verreth *et al.* (1987)
15 days	> 80 %	Haylor (1992)
26 days	94-99 %	Awaiss *et al.* (1993)
30 days	50-96 %	Hogendoorn (1980)

In conclusion, the hybrid did not show a reduction in fertilization or hatching rates compared to *C. gariepinus*. On the contrary, the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid showed the best hatching and fertilization rate at the Mubuyu hatchery, in southern Zambia. In other words, the hybridization of *C. gariepinus* with *H. longifilis* did not compromise the quantity or quality of the catfish seed stock.

CHAPTER 4. GROWTH AND HETEROSIS.

4.1. INTRODUCTION

Some artificial hybrid crosses have been very successful in aquaculture, showing superior growth or survival rates compared to their parent species. The physiological advantages acquired through hybridization have been variously described as heterozygosis, hybrid luxuriance, hybrid vigour or heterosis (Shull, 1948).

Shull (1948) preferred to use the word "heterosis", as the other terms had been used in conjunction with aspects of genetic inheritance theory. He wanted to describe the phenotypic manifestations of hybrid vigour, without implying it's theoretical genetic mechanisms (Bowman, 1959). If hybridization were simply the mixing of two genotypes, with no genetic effects, the physiological characteristics of the hybrids would be an average of the characteristics of the parent species. Heterosis is a measure of how much the reciprocal hybrids deviate from this average, for a particular physiological characteristic. This deviation can be positive or negative (Sheridan, 1981; Ayles and Baker, 1983), although the term "heterosis" is usually used to describe the positive deviation.

Traditionally, the genetic mechanism used to explain positive heterosis has been that of dominance (Bowman, 1959). Assuming that deleterious genes are mostly recessive in a species' genotype (through natural selection), they can only be expressed when their alleles are homozygous. Even if both sets of parent genes are homozygous for a particular locus, there is a good chance that they will be heterozygous in the hybrid genome. Heterozygosity results in the dominance of beneficial genes over recessive, deleterious genes at the gene loci (Falconer, 1989).

Theoretically, the best genotype for a particular phenotypic expression would be a set of dominant homozygous alleles. Sometimes, however, the heterozygous alleles occurring at a locus in a hybrid are even more superior in merit than the dominant parental homozygotes. This effect is called over-dominance, and can give rise to positive heterosis as well (Sheridan, 1981). Heterosis may also arise from the interaction between genes occurring at different loci in the hybrid genome (Sheridan, 1981). This non-additive (ie. non-heritable) interaction is called epistasis (Falconer, 1989).

It is highly probable that there is no single genetic explanation for heterosis, but that it is a combination of the above factors, as well as other forms of genetic interaction (Bowman, 1959; Sheridan, 1981).

Artificial hybridization has been induced in diverse groups of fish, and has resulted in various types of heterosis. While natural interspecific hybridization appears to be rare in the siluroid catfishes, artificial hybridization is possible at both the interspecific, intergeneric and interfamilial levels (see Chapter 3),

often leading to heterosis for growth (Table 4.1).

Some examples of positive heterosis resulting from artificially induced interspecific hybridizations are provided in Table 4.1.

Table 4.1. Examples of artificial interspecific hybridization, and the types of positive heterosis arising from the crosses.

Species	Heterosis	Reference
Salmonidae		
Salmo trutta x S. salar	Growth	McGowan & Davidson (1992)
S. trutta x Selvelinus fontinalis	Growth, Survival	Suzuki & Fukuda (1971)
S. trutta x Selvelinus alpinus	Growth	Refstie & Gjedrem (1975)
S. trutta x Selvelinus malma	Growth	Refstie & Gjedrem (1975)
S. trutta x Selvelinus pluvius	Growth, Survival	Suzuki & Fukuda (1971)
S. trutta x Oncorhynchus kisutch	None	Blanc & Chevassus (1982)
S. salar x Oncorhynchus gorbuscha	None	Loginova & Krasnoperova (1982)
Salvelinus fontinalis x S. pluvius	Growth, Survival	Suzuki & Fukuda (1971)
S. fontinalis x Salvelinus namaycush	Growth	Spangler & Berst (1976)
S. fontinalis x Oncorhynchus mykiss	None	Blanc & Chevassus (1982)
S. fontinalis x Oncorhynchus masou	Survival	Suzuki & Fukuda (1971)
S. pluvius x Oncorhynchus rhadurus	Growth, Survival	Suzuki & Fukuda (1971)
Oncorhynchus mykiss x O. kisutch	None	Blanc & Chevassus (1982)
O. kitsuch x O. tsawytscha	None	Blanc & Chevassus (1982)
O. masou x O. gorbuscha	Growth, Salinity	Ma & Yamazaki (1988)
O. masou x O. rhodurus	Growth, Survival	Suzuki & Fukuda (1971)
O. nerka x O. keta	Growth	Suzuki & Fukuda (1971)
Cyprinidae		
Gnathopogon elongatus x C. carassius	None	Kasama & Kobayasi (1990)
Hypophthalmichthys moltitrix x C. carpio	Sterile	Bakos *et al.* (1978)
H. nobilis x Ctenopharyngodon idella	Sterile	Wiley & Wike (1986),
H. nobilis x C. idella	Thermal tolerance	Bettoli *et al.* (1985)
Rutilus rubilio x Alburnus alburnus	Growth	Crivelli & Dupont (1987)
Capoeta damascinus x Barbus canis	None	van Vuren & Fishelson (1990)
Luxilus cornutus x L. cerasinus	None	Meagher & Dowling (1991)
Notemigonus crysoleucus x Scardinus erythrophthalmus	Sterile	Tave *et al.* (1993)
Siluridae		
Clarias macrocephalus x C. batrachus	None	Tarnchalanukit (1986)
C. macrocephalus x Pangasius sutchi	None	Na-Nakorn *et al.* (1993)
C. gariepinus x C. fuscus	Growth, Survival	Zheng *et al.* (1988)
C. gariepinus x Heterobranchus longifilis	Growth	Legendre *et al.* (1992)
C. gariepinus x H. bidorsalis	Growth	Salami *et al.* (1993)
C. batrachus x Heteropneustes fossilis	None	Mukhopadhyay & Dehadrai(1986)
Ictalurus punctatus x I. furcatus	High density growth	Dunham *et al.* (1990)
	Growth, Dress out, Feeding, Catchablity	Tave & Smitherman (1982)

Table 4.1 cont'd. Examples of artificial interspecific hybridization, and the types of positive heterosis arising from the crosses.

Species	Heterosis	Reference
Cichlidae		
Oreochromis mossambicus x O. honorum	Monosexual (male)	Hickling (1962)
O. mossambicus x O. niloticus	Salinity	Villegas (1990)
O. niloticus x O. aureus	Monosexual (male)	Hulata *et al.* (1983)
Percidae		
Morone saxatilis x M. chrysops	Growth, Survival	Oppenborn & Goudie (1993)
M. saxatilis x White perch	None	Hodson *et al.* (1987)
Lepomis cyanellus x L. macrochirus	Growth, FCR	Tidwell & Webster (1993)
Other		
Pomoxis annularis x P. nigromaculatus	Growth	Hooe & Buck (1991)
Huso huso x Acipenser ruthenus	None	Arefjev (1989)
Oplegnathus fasciatus x O. punctatus	Growth, Survival	Harada *et al.* (1986)
Catostomus catostomus x C. commersoni	None	Nelson (1973)
Brachydanio frankei x B. rerio	None	Kavumpurath & Pandian (1991)
Stizostedion vitreum x S. canadense	Growth, Survival	Siegwarth & Summerfelt (1990)
	FCR, Handling, Decreased aggression	Malison *et al.* (1990)

Hecht and Lublinkhof (1985) hinted at the possibility of growth heterosis in the *H. longifilis* x *C. gariepinus* hybrid. Later, Hecht *et al.* (1991) reported that while the larvae of the reciprocal hybrids grew faster than *C. gariepinus*, they did not grow as fast as the larvae of *H. longifilis*, thus demonstrating that the hybrids did not show positive growth heterosis. However, from the data published by Legendre *et al.* (1992; FIG. 1) it was calculated that the West African *H. longifilis* x *C. gariepinus* hybrids showed substantial heterosis for growth. In this chapter, the southern African *H. longifilis* x *C. gariepinus* hybrids were tested for growth heterosis, by comparing the growth of *H. longifilis*, *C. gariepinus* and their reciprocal hybrids under controlled conditions.

The growth rate of the HL♂xCG♀ hybrid juveniles was also compared to that of *C. gariepinus* juveniles under controlled conditions for at least six months, in an attempt to quantify the growth performance of the hybrid, relative to *C. gariepinus*. Remarkable growth rates have been reported for *C. gariepinus* under ideal conditions (Mortimer, 1964; van der Waal, 1970, De Kimpe and Micha, 1974; Hecht and Britz, 1988; Kuiper, 1993). However, clariid catfish growth is highly variable (Clay, 1984; Quick and Bruton, 1984). For example, Mortimer (1964) reported that *C. gariepinus* attained 900g in their first year, but in the wild this species achieves an average mass of only about 66 g after one year (data from El Bolock, 1972; Willoughby and Tweddle, 1978; Bruton and Allanson, 1980; Clay, 1982; Clay, 1984; Quick and Bruton, 1984). Thus it would be difficult to quantify the growth of the hybrid in

anything but relative terms.

4.2. MATERIALS AND METHODS

Heterosis

The *H. longifilis* x *C. gariepinus* hybrid was tested for growth heterosis at the Mubuyu Farms hatchery in Zambia. Two experiments were carried out, approximately one year apart, during successive breeding seasons (November to January).

The hybrid and pure strain catfish were produced using large *H. longifilis* brood stock (10 kg to 26 kg) collected from the Zambezi River, and domesticated *C. gariepinus* (400 g to 1500 g) raised at the Mubuyu fish farm. Spawning and rearing methods used were the same as those outlined in Chapter 3.

In the first heterosis experiment, two hundred 14 day old juveniles from each of the HL♂xHL♀, CG♂xCG♀, HL♂xCG♀ and CG♂xHL♀ crosses were stocked into four identical 24 m^3 outdoor nursery ponds (water temperature = 28 ± 1 °C). They fed on the naturally occurring zooplankton, supplemented with 48 % protein trout starter granules, fed at 7h00 and 17h00 daily. After 64 days the ponds were drained, and all the surviving fish were measured for total length, and individually weighed to 0.1 g.

The second experiment was carried out one year later, in the same way as the first, except that the fish were harvested, weighed and measured after 77 days. The average temperature of the pond water was 27 ± 1 °C.

At the end of the experiments the average total lengths of the reciprocal F_1 hybrids and the pure strain parental species were used to ascertain whether the *H. longifilis* x *C. gariepinus* hybrids displayed heterosis for growth. For the purposes of this study heterosis is defined as "the growth performance of reciprocal hybrids relative to that of the mean of the growth of their parent groups". Heterosis is expressed as a percentage using the following formula (based on Tave, 1986):

$$\text{Heterosis (\%)} = \frac{((H_1 + H_2)/2)-((P_1 + P_2)/2)}{(P_1 + P_2)/2} \times 100$$

where: H_1 & H_2 = average total length of reciprocal hybrids
P_1 & P_2 = average total length of parent groups

Differences between the total lengths of the fish from each of the four groups were tested using a one-way analysis of variance, and the relationships between the groups were analysed using a 95% Tukey multiple range test, for both trials.

HL♂xCG♀ hybrid and *C. gariepinus* growth comparison

Two comparative growth trials on *C. gariepinus* and the HL♂XCG♀ hybrid were undertaken at Rhodes University. The fishes were spawned at the Mubuyu hatchery, and 14 day old juveniles were air-freighted to the Rhodes University aquaculture facility in sealed plastic bags, which contained oxygen and water. On arrival they were transferred to four 560 ℓ circular plastic tanks, where they were fed on an alternating diet of *Daphnia* and an artificial larval catfish food (Uys and Hecht, 1985) three times per day. The tanks were linked to a recirculating system, where the water passed through an 8.0 m^3 submerged biofilter, and was stored in an 8.0 m^3 reservoir. The water exchange rate in the tanks was 500 $\ell.hr^{-1}$. The entire system was contained within a 30 m x 7 m plastic "UV-Dek" agricultural tunnel.

In the first growth trial, a random sample of 20 *C. gariepinus* and 20 HL♂xCG♀ hybrid juveniles were removed from the holding tanks, and were placed into a 30 ℓ tank, 30 days after hatching. The tank was divided in half by a partition consisting of 1 mm mesh, with the *C. gariepinus* on one side of the partition and the hybrids on the other side. The mesh allowed the water in each tank to circulate between the compartments, while restraining the fish to one compartment. Two additional tanks were set up in an identical way as replicates. The tanks were linked to a recirculating system which consisted of a 6.25 m^3 trickle filter and a 2.0 m^3 water storage tank. A constant water temperature was maintained by thermostatically controlled immersion heaters, at 28.0 ± 1.0 °C. The water in each of the compartmentalised tanks was replaced at a rate of 60 $\ell.hr^{-1}$.

The fish were fed to satiation on a formulated diet (Uys and Hecht, 1985) at 9h00 and 18h00 every day, except on the days when they were weighed and measured. Excess food and waste products were removed from the tanks every morning, prior to feeding.

The fish were measured (to the nearest 0.1 mm) and weighed (to the nearest 0.01 g) at the start of the experiment, and then once a week, for five weeks. Growth curves of total length at age were plotted for the two groups of fish, and the relationships were modeled using least-squares linear regression. The slopes of the regression models were compared using a one-tailed F-test, based on the analysis of co-variance. As the hybrid juveniles were already larger than the *C. gariepinus* juveniles 30 days after hatching, the Specific Growth Rates (SGR) of the fish were also compared (Legendre *et al.*, 1992), using the Kruskal-Wallis nonparametric analysis.

Specific growth rate:

$$SGR\ (\%) = \frac{\ln(W_1) - \ln(W_0)}{dt} \times 100$$

where: W_0 & W_1 are the initial and final average mass of the fish in the tank in grams, and dt is the number of days of the experiment.

The second growth trial carried out at Rhodes University was designed in exactly the same way as the first, except that the fish at this stage were 81 days old. Twenty *C. gariepinus* and 20 HL♂xCG♀ hybrid juveniles were randomly selected from the holding tanks and were introduced into three compartmentalised replicate tanks. The second growth comparison was carried out following the same protocol as the first.

The fish were measured and weighed once a week for the first four weeks of the second growth trial, whereafter they were left undisturbed in the tanks (except for feeding and waste removal) for a further two months, until the end of the experiment.

In order to compare the condition factors of the HL♂xCG♀ hybrids and *C. gariepinus* reared under the same conditions, length and weight data were collected for larval, juvenile and adult fish at the Mubuyu Farms fish farm in Zambia and at the Rhodes University aquaculture facility in South Africa. These data were plotted, and regression analyses were carried out to determine the curves of best fit for the length-weight relationships of the two groups of fish. The weight data were then log-transformed, so that the regression slopes could be compared by an analysis of co-variance.

4.3. RESULTS

Heterosis

There was a significant difference ($P < 0.05$) between the growth rates of all the groups of fish in the first heterosis trial. The *H. longifilis* juveniles grew at a faster rate than the reciprocal hybrids and *C. gariepinus* juveniles. The HL♂xCG♀ hybrid had the second best growth rate, while pure *C. gariepinus* grew at a slower rate than the CG♂xHL♀ hybrid. This pattern was repeated in the second trial, where the *H. longifilis* juveniles grew at a significantly faster rate than the *C. gariepinus* and CG♂xHL♀ hybrid juveniles ($P < 0.05$). Although *H. longifilis* appeared to grow at a faster rate than the HL♂xCG♀ hybrid, the difference was not significant ($P > 0.05$). Positive heterosis for growth was not apparent for the *H. longifilis* x *C. gariepinus* hybrid juveniles in either of the trials (Table 4.2).

A heterosis value of 0 % would have meant that the growth rates of the reciprocal hybrids were a result of a simple mixing of the growth rates of the parent species. The negative heterosis value for the *H. longifilis* x *C. gariepinus* hybrids indicated that some form of negative interaction had occurred between the parental genes found at different loci in the hybrid genome (Sheridan, 1981). This negative interaction appeared to have a more pronounced effect on the growth of the CG♂xHL♀ cross than on the growth of the HL♂xCG♀ cross.

Table 4.2. The mean total length at age attained by *H. longifilis*, *C. gariepinus* and reciprocal hybrid juveniles, and the calculated heterosis for the growth trials carried out at Mubuyu Farms hatchery, in Zambia.

Trial	Age (days)	HL♂xHL♀ (mm ± sd)	HL♂xCG♀ (mm ± sd)	CG♂xHL♀ (mm ± sd)	CG♂xCG♀ (mm ± sd)	Heterosis (%)
Trial 1	78	146 ± 39[a]	116 ± 28[b]	83 ± 15[c]	67 ± 30[d]	-6.4
Trial 2	91	79 ± 17[a]	69 ± 10[a,b]	64 ± 20[b]	62 ± 16[b]	-5.6

[a] Superscripts denote homogeneous groups

HL♂xCG♀ hybrid and *C. gariepinus* growth comparison

The HL♂xCG♀ juveniles grew at a significantly faster rate than the *C. gariepinus* juveniles. The difference between the slopes of the *C. gariepinus* and hybrid growth models were significant ($P < 0.05$) for both trials (Fig. 4.1 and 4.2). The SGRs of the hybrid were also significantly higher ($P < 0.05$) than those of *C. gariepinus* for both trials (Table 4.3).

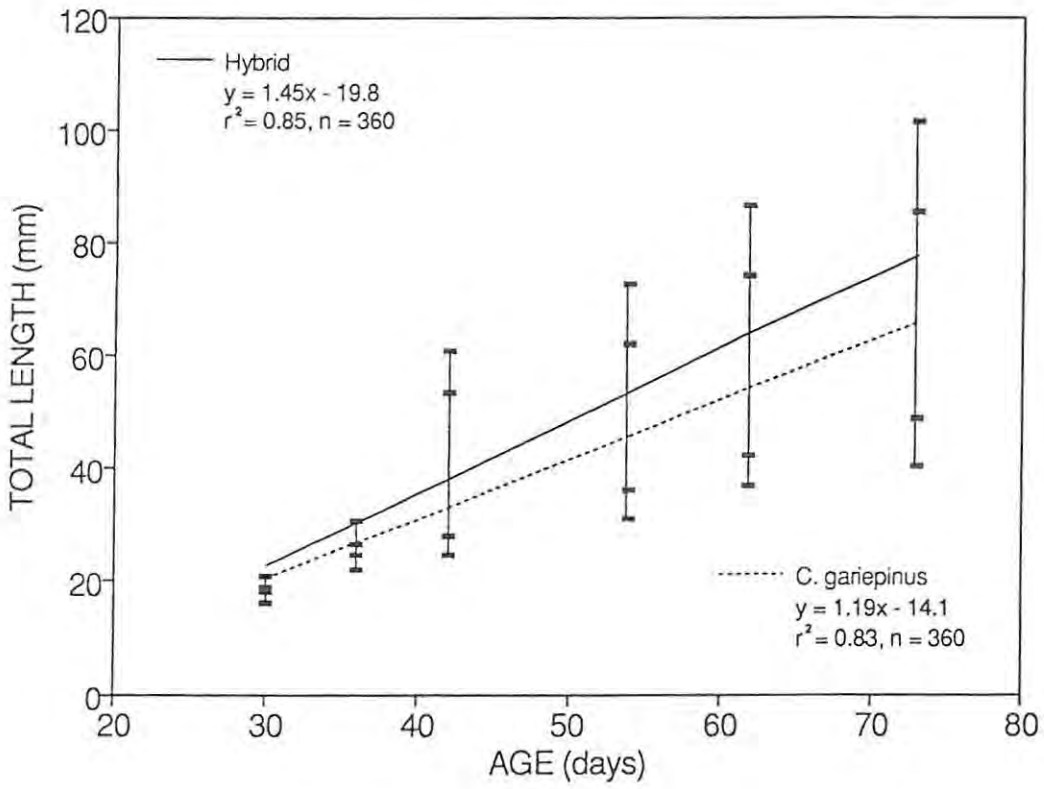

Figure 4.1. The growth of *C. gariepinus* and HL♂xCG♀ hybrid juveniles from 30 to 74 days post hatching.

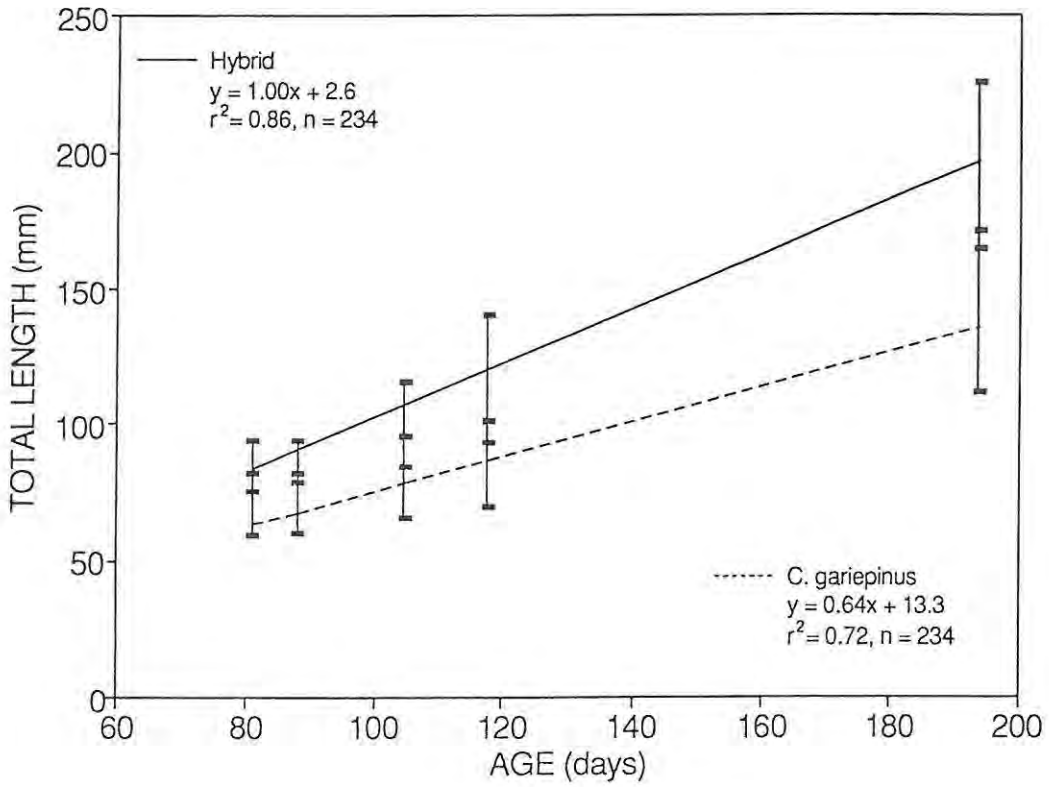

Figure 4.2. The growth of *C. gariepinus* and HL♂xCG♀ hybrid juveniles from 81 to 194 days post hatching.

Table 4.3. The growth interval, initial and final mass and specific growth rates (SGR) of the *C. gariepinus* and HL♂xCG♀ hybrid juveniles, for the two growth comparisons.

Age (days)	Species	Initial mass (g, $\bar{x} \pm$ sd)	Final mass (g, $\bar{x} \pm$ sd)	SGR (% g.day^{-1}, $\bar{x} \pm$ sd)
30 - 73	*C. gariepinus*	0.04 ± 0.01	2.5 ± 1.5	9.87 ± 0.05[a]
	HL♂XCG♀ Hybrid	0.06 ± 0.02	4.6 ± 2.7	10.34 ± 0.25[b]
81 - 194	*C. gariepinus*	2.6 ± 0.8	19.1 ± 10.5	1.7 ± 0.06[c]
	HL♂XCG♀ Hybrid	6.0 ± 1.4	65.2 ± 24.8	2.1 ± 0.06[d]

[a] Superscript denotes homogeneous groups

There was no significant difference ($P > 0.05$) between the slopes of the length-weight regressions of the *C. gariepinus* and HL♂xCG♀ hybrids, cultured at Mubuyu Farms in southern Zambia and at Rhodes University in South Africa (Fig. 4.3 and 4.4). The geometric regression equation provided the lines of best fit, which are presented in Table 4.4.

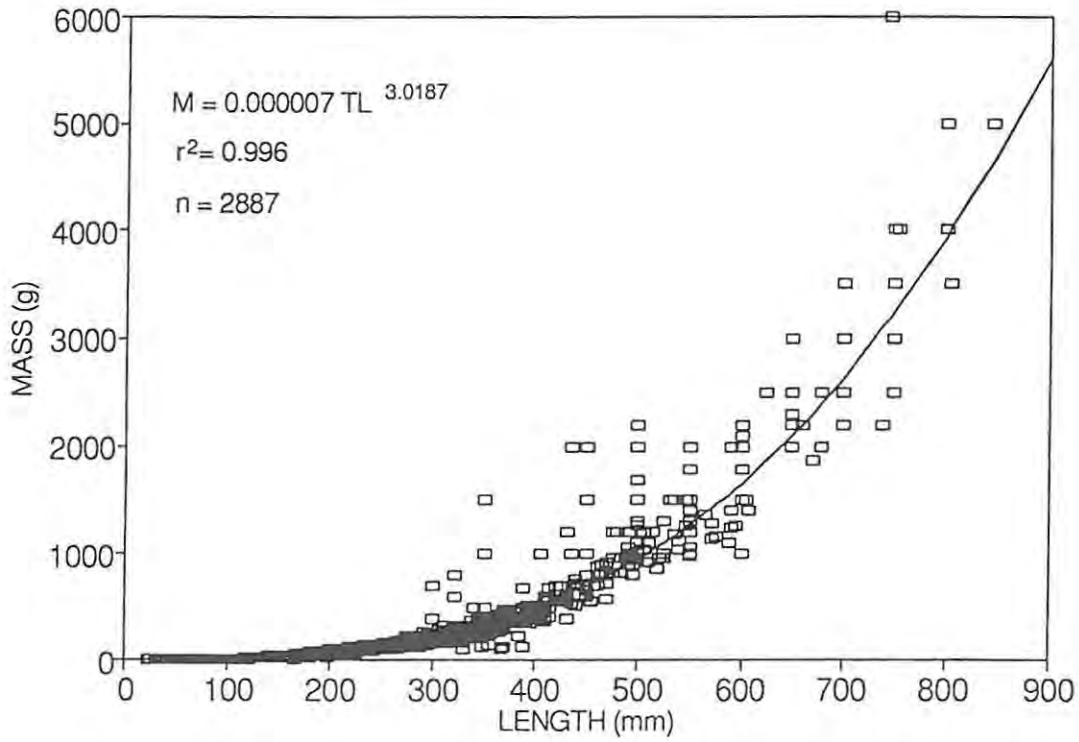

Figure 4.3. The length-weight relationship for *C. gariepinus* raised at Mubuyu Farms, Zambia and at Rhodes University, South Africa.

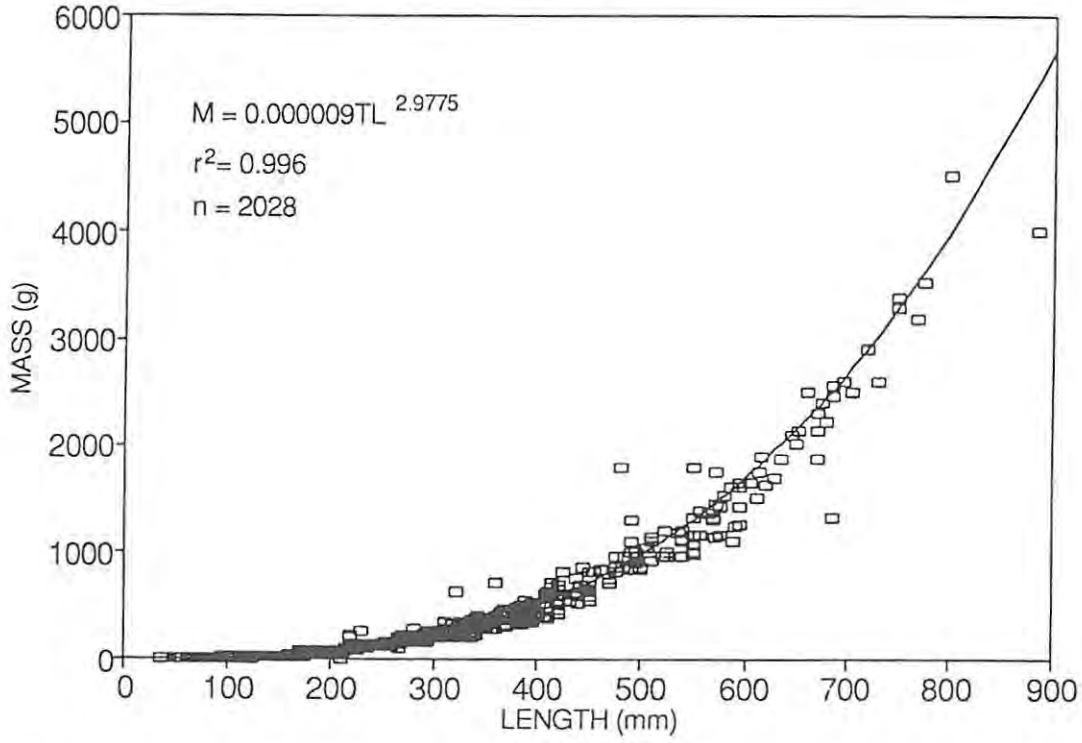

Figure 4.4. The length-weight relationship for the HL♂xCG♀ hybrids raised at Mubuyu Farms, Zambia and at Rhodes University, South Africa.

Table 4.4. Length-weight relationship equations for *C. gariepinus* and HL♂xCG♀ hybrids raised at Mubuyu Farms in Zambia and Rhodes University, South Africa.

Species	Regression equation	n	r^2
C. gariepinus	$M = 0.000007TL^{3.0187}$	2887	0.996
HL♂XCG♀ Hybrid	$M = 0.000009TL^{2.9776}$	2028	0.996

4.4. DISCUSSION

The *H. longifilis* x *C. gariepinus* reciprocal hybrids showed negative heterosis for growth under the environmental conditions experienced in southern Zambia. This supports the observation of Hecht *et al.* (1991). Based on their data (Table 1, Hecht *et al.*, 1991), the hybrids had a negative growth heterosis value of -5.9 % after 47 days, under similar experimental and seasonal conditions. This value was intermediate between the two growth heterosis values of -5.6 % and -6.4 % reported for the hybrid in the present study, 78 and 91 days after hatching.

However, if the average masses of the West African *H. longifilis*, *C. gariepinus* and reciprocal hybrids reported by Legendre *et al.* (1992, TABLE II) are used in the heterosis equation, the West African hybrids showed positive heterosis for growth. The difference in heterosis for growth between the West African and the southern African hybrids could be due to several factors. For example, Smitherman and Dunham (1985) found that the *Ictalurus punctatus* x *I. furcatus* hybrid showed positive growth heterosis when reared in ponds, but it had negative growth heterosis when reared in tanks. Thus it is clear that the rearing environment can influence the expression of heterosis in a hybrid.

Another factor which effects the measure of heterosis displayed by a hybrid is the amount of inbreeding that has occurred within the populations of its parent species. Inbreeding is the opposite of heterosis (Falconer, 1989), as it leads to an increase in the number of homozygous genes occurring at specific loci, and a concomminant increase in the expression of recessive, deleterious genes. The amount of heterosis shown by a hybrid is thus dependent on the degree of inbreeding that has taken place in the parent strains (Falconer, 1989). Catfish raised on fish farms are notoriously inbred, as they are usually reproduced from a small population of domesticated broodstock (van der Walt *et al.*, 1993). The *C. gariepinus* broodstock used to produce the southern African hybrids came from a line of at least eight generations of domesticated stock, but the *H. longifilis* broodstock were from the wild. The *H. longifilis* brooders used to produce the West African hybrids were second generation domesticated stock, but their parental stock had colonised the fish ponds of the Layo research facility in the Ivory Coast at least

eight years previously (Legendre, 1983; Legendre *et al.*, 1992). The fish in these ponds showed a loss of genetic variability compared with wild *H. longifilis* (Agnese *et al.*, 1995). The West African *C. gariepinus* broodstock were obtained from an experimental fish culture station in the Ivory Coast, and were most likely also inbred.

Refstie and Gjedrem (1975) noted that differences in the performance of interspecific hybrids occurred when different populations of the same parental species were used. Chevassus (1979) concurred with their assertion, stating in his review of salmonid hybridization that "a study of various works dealing with the same hybridization produces very heterogeneous results." He ascribed this variability to a lack of strict methodology, as well as intraspecific variability, particularly when different populations were used.

Thus the differences in heterosis between the *H. longifilis* x *C. gariepinus* hybrids in West and southern Africa are probably due to a combination of factors, including the amount of inbreeding that has occurred in the parent populations, the genetic variance between the West African and southern African *H. longifilis* and *C. gariepinus* populations, and local and regional environmental differences.

Clearly, heterosis can only refer to the very localized circumstances of the populations of fish in question, and is a concept entirely based on the fish farmer's perceived requirements from the hybrid product. One of the needs of the catfish farming community in southern Africa is a fish that reaches harvest size faster than *C. gariepinus*. Therefore from a farming perspective the hybrid has a significant advantage over pure strain *C. gariepinus*.

The HL♂XCG♀ cross grew faster than the reciprocal hybrid cross. The HL♂xCG♀ cross also appeared to have better fertilization and hatching rates than the CG♂xHL♀ cross (see Chapter 3). The fact that the HL♂xCG♀ hybrid outperformed the CG♂xHL♀ hybrid has distinct advantages for aquaculture in the southern African region. It means that cryo-preserved *H. longifilis* sperm can be used to fertilize locally available *C. gariepinus* females, without the need to keep *H. longifilis* broodstock outside of their natural distribution range (Fig. 2.1). This is particularly important from a conservation aspect. The large, fecund *H. longifilis* adults would cause an ecological disaster if they escaped from an aquaculture facility in southern Africa, and were able to establish a breeding population outside of their natural distribution range. For these reasons it was decided to concentrate on the aquaculture potential of the HL♂xCG♀ hybrid cross in southern Africa.

The absolute growth rates of the fish in the two comparative growth trials between *C. gariepinus* and the HL♂xCG♀ hybrid were low. This was most likely a consequence of experimental conditions. Nevertheless, the results clearly showed that the hybrid continued to out-perform *C. gariepinus* well after six months of development.

In all instances, the HL♂xCG♀ hybrids have been reported to grow at a faster rate than *C. gariepinus* (Hecht and Lublinkhof, 1985; Hecht *et al.*, 1991; Legendre *et al.*, 1992), but the relative difference between their growth rates was not constant. A comparison of the mean total lengths at age attained by the HL♂XCG♀ hybrid and *C. gariepinus* juveniles in this study and reported in the literature are presented in Table 4.5. The percent difference in total length of the HL♂XCG♀ hybrid and pure strain *C. gariepinus* juveniles at the end of each comparison were found, and summarised in Table 4.5. In each of the comparisons, the hybridized eggs and pure strain eggs were spawned by the same *C. gariepinus* females. Thus it was assumed that there was no difference between the initial length of the larvae of the two groups of fish at hatching (Legendre *et al.*, 1992) in each of the comparisons.

Table 4.5. The difference between the mean total length at age of *C. gariepinus* and the HL♂XCG♀ hybrid for a range of growth comparisons.

Age (days)	Final length (mm)		Difference	Reference
	Hybrid	*C. gariepinus*		
47	70	52	25.7 %	Hecht *et al.*, 1991
73	82	72	12.2 %	Present study
78	116	67	42.2 %	Present study
91	69	62	10.1 %	Present study
98	109	74	32.1 %	Hecht & Lublinkhof, 1985
113	200	142	29.0 %	Present study
309	421*	333*	20.9 %	Legendre *et al.*, 1992

* Calculated from mass, $M = 0.00001TL^3$

The HL♂XCG♀ hybrid attained a total length of between 10.1 % and 42.2 % larger than *C. gariepinus* at the termination of the growth comparisons presented in Table 4.5. The variation in the difference between the growth of these two groups of fish did not appear to be related to age or size. The variation was most likely a result of the different experimental and environmental conditions experienced by the fish during the different comparisons. It has previously been noted in this chapter that the environment can influence the extent of the expression of a desirable trait (such as enhanced growth) in a hybrid (Smitherman and Dunham, 1985).

There was no difference between the length-weight relationships of the HL♂XCG♀ hybrids and *C. gariepinus* spawned and reared at Mubuyu Farms and Rhodes University. The length-weight relationships of both hybrid and pure-strain populations were comparable to those of other *C. gariepinus* populations in southern Africa, reported in the literature (Table 4.6). The length-weight equation has been used to indicate the condition of the fish in a population (Quick and Bruton, 1984). Although some authors have used the a-statistic (intercept) or the b-statistic (coefficient of slope) of the equation to

indicate condition, both statistics need to be considered for an interpretation of the results to be meaningful (Bolger and Connolly, 1989). For this reason the expected mass of a hypothetical fish measuring 100 mmTL was calculated for each population in Table 4.6. The calculated weights at a length of 100 mm for the hybrids and *C. gariepinus* from Mubuyu Farms and Rhodes University were 8.2 g and 7.7 g respectively. These values compare favourably with other populations of *C. gariepinus* in southern Africa.

Table 4.6. Length-weight relationships for populations of *C. gariepinus* from various localities in southern Africa, and the expected weight at 100 mmTL for fish from each population.

Locality	Regression	r^2	n	M(100 mmTL)	Reference
Lake Sibaya	$M = 0.000040TL^{2.869}$	0.92	355	10.0 g	Bruton, 1979b
Lake Kyle	$M = 0.000017TL^{2.802}$	0.96	1447	6.8 g	Clay, 1984
Lake McIlwaine	$M = 0.000012TL^{2.879}$	0.99	263	6.9 g	Clay, 1984
Rynfield Dam	$M = 0.000007TL^{2.804}$	0.99	19	4.5 g	Clay, 1984
Gariep Dam	$M = 0.000014TL^{2.940}$	0.98	30	10.6 g	Clay, 1984
Elands R.	$M = 0.000007TL^{2.993}$	?	605	6.8 g	van der Waal, 1972
Lake Kariba	$M = 0.000011TL^{3.007}$	0.91	146	11.4 g	Clay, 1984
Crocodile R.	$M = 0.000006TL^{3.015}$	0.95	27	6.4 g	Clay, 1984
Hardap Dam	$M = 0.000004TL^{3.071}$	0.95	139	5.6 g	Gaigher in Quick & Bruton, 1984
Phongolo Floodplain	$M = 0.000003TL^{3.169}$	0.98	165	6.5 g	Kok in Bruton, 1979b
P.K. le Roux Dam	$M = 0.000002TL^{3.228}$	0.99	242	5.7 g	Quick & Bruton, 1984
Mean:	$M = 0.000011TL^{2.973}$			7.4 g	

The mean coefficient of the slope of the length-weight relationship of the southern African populations of *C. gariepinus* and the HL♂XCG♀ hybrid was approximately b = 3.0, which indicates that their growth was isometric; that is, they did not experience a change in body form or specific gravity as they increased in size (Ricker, 1975). Thus the exponent 3 can be used to transform the linear dimensions of length to the cubic dimensions of weight for *C. gariepinus* and the HL♂XCG♀ hybrid in southern Africa (Ricker, 1975). This is valuable knowledge when comparing the growth data of these fish reported in the literature, as it is often recorded in only length or weight.

In conclusion, the southern African *H. longifilis* x *C. gariepinus* hybrids did not show heterosis for growth, although the HL♂xCG♀ hybrid grew significantly faster than *C. gariepinus*. The pure *H. longifilis* strain had the fastest growth rate of all the crosses, but this large, fecund species can not be farmed in southern Africa for environmental conservation reasons. However, the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid could be considered for aquaculture in the region if it were sterile. This question is addressed in Chapter 5.

CHAPTER 5. THE QUESTION OF STERILITY IN THE *HETEROBRANCHUS LONGIFILIS* ♂ X *CLARIAS GARIEPINUS* ♀ HYBRID.

5.1. INTRODUCTION

The hybrid offspring of two species can have diverse genotypes. If the haploid genomes of the male and female combine, a diploid hybrid is produced. If the genomes combine but the second polar body of the egg is retained, then a triploid hybrid genome is produced. Normally the micropyle of an egg limits the number of sperm entering the egg (in most species only one sperm is permitted to reach the egg). The micropyle is not always successful at this function when the sperm of a different species is the invader. Fertilization of the egg by more than one sperm (polyspermy) can occur, resulting in a polyploid hybrid genome. Lastly, if the genetic material of either the male gamete (androgenesis) or female gamete (gynogenesis) is inactive or lost in the egg after fertilization, the offspring are not hybrids at all, but clones of the genetically active parent strain.

Gynogenesis arising from hybridization has been found in many cyprinids, including *Carassius auratus*, *Menidia clarkhubbsi*, the *Phoxinus eos* x *P. neogeous* cross, some species of the Cobitidae family, and several members of the genus *Poeciliopsis* (Echelle and Mosier, 1982; Vasil'yev, 1985; Dawley *et al.*, 1987; Abramenko, 1990). The catfish hybrid *Clarias batrachus* ♂ x *Heteropneustes fossilis* ♀ (Chaudhuri and Mandal, 1979) may in fact have been an example of androgenesis, as the hybrid showed both the internal and external characteristics of *C. batrachus*, but none of *H. fossilis*.

The hybrid's genotype can therefore have a significant effect on its reproductive potential. For example, if the genome is aneuploid (i.e. contains an odd-numbered chromosome complement) it cannot be separated into two equal groups during the anaphase of meiosis, causing the gametes to have differing chromosome numbers. This usually leads to very low fertilization rates or sterility. Examples of triploid hybrids have been found in the *Ctenopharyngodon idella* x *Aristichthys nobilis* cross (Marian and Krasnai, 1978) and the *Ctenopharyngodon idella* x *Cyprinus carpio* hybrid (Vasil'yev *et al.*, 1975).

However, an euploid genome (i.e. having an even-numbered chromosome complement) does not always indicate a fertile hybrid. Differences in the karyotypes of the parent species can cause hybrid sterility, as well as differences at the gene level. Chevassus (1983) described a continuum of possible reproductive states that could be found in a hybrid, from the normal maturation of both sexes, through zygotic sterility (viable gametes are produced by the hybrids and fertilization takes place, but for some reason the zygote aborts), gametic sterility (abberations in sperm and egg number, size and shape) to gonadic sterility (atrophied gonads with a few sexual cells at early stages of gametogenesis).

Examples of fertile hybrids can be found in groups as diverse as the salmonid *Salmo clarki* x

Oncorhynchus mykiss and *Salvelinus fontinalis* x *S. namaycush* crosses, the crappie *Pomoxis annularis* x *P. nigromaculatus* hybrid, the sturgeon *Huso huso* x *Acipenser ruthenus* hybrid and the plaice/flounder *Pleuronectes platessa* x *Platichthys flesus* hybrid (Chevassus, 1979; Lincoln, 1981; Rohrer and Thorgaard, 1986; Arefjev, 1989; Hooe and Buck, 1991).

The bass hybrid *Morone saxatilis* x *M. chrysops* produced viable offspring, but their hatching rate was only about 10%. This was probably a consequence of zygotic impairment (Smith and Jenkins, 1984).

Examples of gametic sterility can be found in the salmonid hybrid *Salmo salar* x *S. trutta* (Chevassus, 1979). The eggs of the *Oncorhynchus masou* x *Salvelinus fontinalis* hybrid were also abnormal, and only 10-15 % of the females developed mature ovaries. The rest had atrophied gonads with a gonadosomatic index of only 10% of that of the parent strains (Suzuki and Fukuda, 1973). Gonadic sterility has been reported in the cyprinid *Barbus longiceps* x *Capoeta damascina* hybrid, where gametogenesis in the atrophied gonads never progressed beyond the spermatid or early vitellogenic stage (Stoumboudi *et al.*, 1992).

Hybridization does not always have the same effect on the testes or ovaries of the hybrids. For example, the female cyprinid hybrid *Barbus barbus* x *B. meridionalis* developed viable ova in mature ovaries. However, the testes of the males could not produce sperm, even though they appeared to be morphologically normal (Phillippart and Berrebi, 1990).

Occasionally, more than one of the above types of genetic combinations occur when two species are hybridized. The hybridization of the catfish *Clarias macrocephalus* and *Pangasius sutchi* led to normal diploid hybrids, triploid hybrids and gynogenetic types, all occurring in fishes from the same spawning (Na-Nakorn *et al.*, 1993).

Hybrids frequently deviate from the theoretical sex ratio of 1:1 (Chevassus, 1983). Extreme examples of this deviation can lead to an effectively sterile monosex hybrid population, as has been reported for the all-female *Cyprinus carpio* x *Hypophthalmichthys molitrix* hybrid and the all-male tilapia *Oreochromis aureus* x *O. niloticus* hybrid (Bakos *et al.*, 1978; Hulata *et al.*, 1983).

In the light of the above review, it is vitally important to evaluate the reproductive potential of the *Heterobranchus longifilis* x *Clarias gariepinus* hybrids in southern Africa. Hecht and Lublinkhof (1985) reported that the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid, produced in Zambia, was possibly all-male. Subsequently it has been found that the hybrid has a sex ratio of about 1:1. However, the females showed no visible gonadal development after three years (T. Hecht, pers. comm.; Mubuyu Farms hatchery records, 1985-1991). Moreover, while the HL♂xCG♀ hybrid males had seemingly well developed testes after their first year, attempts to backcross hybrid males with *C. gariepinus* females

during the 1985 to 1991 breeding seasons failed (T. Hecht, pers. comm.).

From the above observations it may be hypothesised that the southern African HL♂xCG♀ hybrid is gonadically sterile. The hypothesis was tested in this study, by comparing the reproductive potential of the hybrid with that of *C. gariepinus*.

5.2. MATERIALS AND METHODS

Gonad development, sperm counts and sperm viability, and testicular ultrastructure

In order to assess the reproductive potential of the HL♂xCG♀ hybrid, its gonadal development, spermatozoa counts, spermatozoa viability and testicular ultrastructure were compared to that of *C. gariepinus*. Gonadal development was expressed in terms of the gonadosomatic index (see below). The pure strain *C. gariepinus* and the HL♂xCG♀ hybrids were raised at the Rhodes University aquaculture facility for three years. During each of the three breeding seasons (November to February) between 10 and 20 fish from both sexes of the two groups were sampled for analysis. Their gonadosomatic index (GSI) was calculated as follows:

$$\text{GSI (\%)} = \frac{\text{mass of gonad}}{\text{total mass of fish}} \times 100$$

The GSI for the males was calculated for the testicular lobes only; the seminal vesicles were excluded. One lobe of the testis from each fish was preserved in Bouin's solution. Those testicular lobes which were not fixed were used to determine sperm counts for one-year old, two-year old and three-year old *C. gariepinus* and hybrid males. An incision was made along the distal margin of the testicular lobe and the semen was allowed to flow out into test tubes. The *C. gariepinus* sperm was diluted 100 times in distilled water, while the hybrid sperm was diluted 10 times. The solutions were well mixed and a pipette was used to draw up 1 mℓ of the sperm solution, which was released onto a Neubauer Haemocytometer and viewed under a Nikon Optiphot phase-contrast microscope at 400 x magnification. Duplicate total sperm counts were undertaken for each fish. The sperm counts of one, two and three year old *C. gariepinus* and HL♂xCG♀ hybrid males were compared by analysis of variance. Relationships within the data set were analyzed using a 95 % Tukey multiple range test.

The percentage of live to dead spermatozoa present in the semen of the two year old *C. gariepinus* and HL♂xCG♀ hybrid males was ascertained using samples of the diluted semen described above. Two minutes after the semen had been diluted with distilled water, the spermatozoa were stained with eosin and nigrosin (Blom, 1950). The two minute exposure time to water allowed enough time for the spermatozoa to become active in the water. The dead spermatozoa were stained red by the eosin-nigrosin, while the live sperm were not stained, but had a greenish hue under the phase-contrast

microscope (Blom, 1950). The number of live and dead spermatozoa were estimated with the aid of a Neubauer Haemocytometer, and the counts were duplicated for each fish. Sperm viability was calculated as follows:

$$\text{Sperm viability} = \frac{\text{live spermatozoa}}{\text{live + dead spermatozoa}} \times 100$$

The sperm viability of the two year old *C. gariepinus* and HL♂xCG♀ hybrid males were compared using a Kruskal-Wallis nonparametric test.

A histological study was undertaken to compare the structure of the testes of *C. gariepinus* and the HL♂xCG♀ hybrid. Tissue samples from the testicular lobes (fixed in Bouin's solution) of the two-year old and three-year old *C. gariepinus* and hybrid males were prepared for histological examination by light microscopy. The tissue samples were dehydrated in an ethanol series and imbedded in wax. The wax-embedded testes were sectioned using a microtome, and 5.0 μm sections were mounted on glass slides and stained with Mallory's trichrome stain.

The slides were examined and photographed using a Nikon Optiphot light microscope. Spermatozoa abundance was estimated for the two and three year old fishes, at 1000 x magnification, using an oil immersion objective lens. The spermatozoa abundance in the lobules of the testes were divided into five categories (R. Bernard, Dept. Zoology and Entomology, Rhodes University; pers. comm.). These were;

0 = No spermatozoa found in the lobules of the testis
1 = Clumps of $\leq$10 spermatozoa found in the lobules
2 = Clumps of $\leq$100 spermatozoa found in the lobules
3 = Spermatozoa abundant, but lobules not full
4 = Lobules packed with spermatozoa

It was clear from the light microscopy study that the cellular structure of the *C. gariepinus* and hybrid testes was different. In order to clarify these differences, the testes of *C. gariepinus* and the HL♂xCG♀ hybrid were examined using transmission electron microscopy (TEM). Portions of the testicular tissue of the two and three year old fish were prepared for TEM following the method described in Cross (1985). The tissue was fixed in 2.5 % glutaraldehyde in a 0.1 M phosphate buffer. It was then washed with the buffer and secondarily fixed with 1.0 % buffered osmium tetroxide (OsO_4). After a further wash in the phosphate buffer, the tissue was dehydrated in an ethanol series (30 % to absolute ethanol). The tissue was then placed into a series of propylene oxide-resin baths, starting with pure propylene oxide, and ending with pure resin. The resin consisted of a mixture of TAAB 812 and Araldite CY 212, recommended by Cross (1969). The resin was irradiated for 36 hours at 60 °C. An LKB UM III ultramicrotome was used to section the embedded tissue, which was stained with uranyl acetate and

lead citrate.

Photomicrographs of hybrid and *C. gariepinus* testes were taken using a JEOL TEM 100 CX II transmission electron microscope, at different magnifications. The photomicrographs were used to compare the ultrastructure of the testes of the two and three year old hybrid and *C. gariepinus* males.

The seminal vesicles of the hybrid and *C. gariepinus* males used in the above studies were weighed and recorded as a percentage of total body weight. Samples of fluid from the seminal vesicles of five hybrid and five *C. gariepinus* males were examined for the presence of spermatozoa, using a Nikon Optiphot phase contrast light microscope. The fluid from the vesicles was highly viscous, and had to be diluted 1:100 with distilled water.

Hybrid reproduction and backcrossing trials

Three independent attempts were made to backcross one, two and three year old HL♂xCG♀ hybrid males with *C. gariepinus* and *H. longifilis* females, in 1992, 1993 and 1994. In all three attempts, batches of *C. gariepinus* and *H. longifilis* eggs were each fertilized with the sperm of two to four HL♂xCG♀ hybrid males, and with conspecific sperm as a control. Compared to *C. gariepinus* females, there appeared to be no significant ovarian development in the hybrid females, therefore no attempts were made to backcross these fish. The induced spawning procedure for all the backcross attempts was identical to that described in Chapter 3.

In February 1994, an attempt was made to backcross two year old HL♂xCG♀ hybrid females with *C. gariepinus* and HL♂xCG♀ hybrid males. Nine HL♂xCG♀ hybrid females and six two year old *C. gariepinus* females were injected with a catfish pituitary suspension, following the methods described in Chapter 3. The eggs obtained from the two groups of females were divided into two batches each, and were fertilized with either the combined semen of two *C. gariepinus* males or the combined semen of two HL♂xCG♀ hybrid males. The fertilized eggs were transferred onto gauze frames (see Chapter 3) and suspended in 90 ℓ incubation troughs. The incubation troughs were linked to a recirculating system. The water temperature of the system was maintained at 28 ± 1 °C, and the flow rate through the troughs was 100 ± 15 $\ell.hr^{-1}$.

Egg development was monitored using samples of 100 to 200 eggs from each cross, which were allowed to adhere to petri dishes. Three petri dishes of eggs per cross were floated in each of the incubation troughs. Approximately half the water in the petri dishes was exchanged every 30 minutes. The dishes were removed from the troughs every hour, and the eggs were observed under a dissecting microscope. The number of live and dead eggs were recorded. The number of eggs that survived to hatching in each petri dish was recorded as a percentage of the total number of eggs in the dish.

The free-swimming larvae in the incubation troughs were collected in glass aquaria. Twenty-four hours after hatching, the total number of larvae in each of the aquaria were counted. The surviving larvae were counted again on day four post hatching, after they had begun exogenous feeding.

5.3. RESULTS

Gonad development, sperm counts and sperm viability, and testicular ultrastructure.

Gonad development in the HL♂xCG♀ hybrid and *C. gariepinus* males was found to be rapid. Based on their GSI values, both groups of males were mature at the end of their first year. There was no significant difference ($P > 0.05$) between the GSI values of the two species at different ages. The mean GSI values for the males ranged between 0.8 % and 1.2 %, and their GSI values did not change significantly ($P > 0.05$) as the fish aged. Although the one year old hybrids showed much variation in GSI, this diminished as the fish aged (Fig. 5.1).

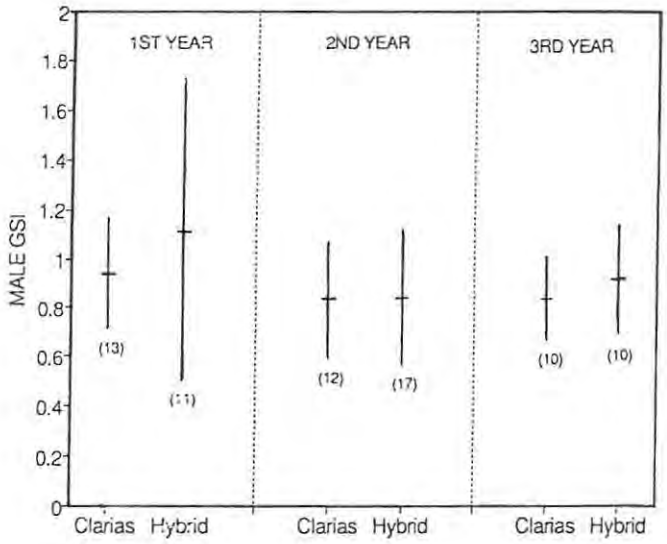

Figure 5.1. The gonadosomatic index of *C. gariepinus* and HL♂xCG♀ hybrid males over three years (vertical bars indicate standard deviation; sample number in parentheses).

The *C. gariepinus* females reached sexual maturity within their first year. The average GSI value for these one year old fish was 15 %. In contrast, there was little or no ovarian development in the hybrid females of the same age. During the second and third breeding seasons, the GSI values of the hybrid females never exceeded 4.6 %, with an average of 2.0 % for both seasons. The GSI values of the *C. gariepinus* females, on the other hand, increased to an average of 18 % in the third year (Fig. 5.2). Thirty percent of all the female hybrid ovaries examined at Mubuyu Farms in Zambia and at the Rhodes University aquaculture facility during the study (n = 70 fish) showed no ovarian development at all.

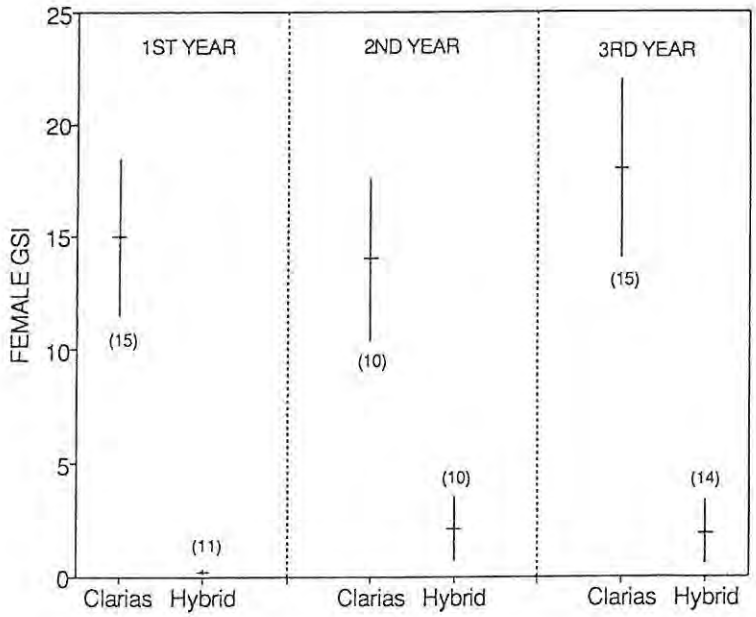

Figure 5.2. The gonadosomatic index of *C. gariepinus* and HL♂xCG♀ hybrid females over three years (vertical bars indicate standard deviation; sample size in parentheses).

The sperm counts of both the hybrid and *C. gariepinus* males increased with age (Table 5.1). However, the hybrid semen was at least 1000 times more dilute than the *C. gariepinus* sperm.

Table 5.1. Sperm counts of one, two and three year old *C. gariepinus* and HL♂xCG♀ hybrid males.

Age	Species	n	Total length (millimetres)	Total mass (grams)	Sperm conc. (10^9/mℓ)
1 yr	*C. gariepinus*	10	420 ± 30	484 ± 95	4.6 ± 1.9
	Hybrid	10	390 ± 20	389 ± 63	0.0018 ± 0.001
2 yr	*C. gariepinus*	10	486 ± 47	745 ± 301	6.1 ± 3.3
	Hybrid	10	574 ± 30	1428 ± 273	0.0025 ± 0.002
3 yr	*C. gariepinus*	10	576 ± 38	1177 ± 245	7.0 ± 2.6
	Hybrid	10	674 ± 44	2300 ± 430	0.0046 ± 0.006

± standard deviation

The sperm viability of the two year old hybrid males was significantly ($P < 0.05$) lower than that of *C. gariepinus* males. An average of only 29.9 ± 19.5 % of the HL♂xCG♀ hybrid spermatozoa were alive, compared to 83.5 ± 7.4 % for the two year old *C. gariepinus* males (Fig. 5.3).

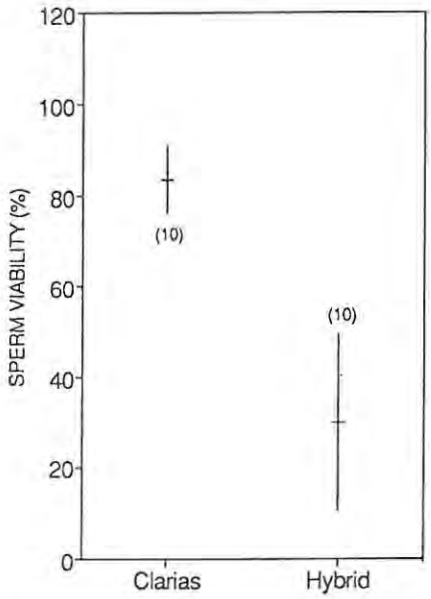

Figure 5.3. The mean sperm viability of two year old *C. gariepinus* and HL♂xCG♀ hybrid males (vertical bars indicate standard deviation; sample number in parentheses).

The hybrid sperm viability figures may be over-estimated. Two distinct sizes of "spermatozoa" were observed while counting the live and dead spermatozoa of the hybrid on the haemocytometer. The larger type were not observed while counting *C. gariepinus* spermatozoa. The larger cells may have been spermatids which were released prematurely into the lumen of the hybrid testes.

There were clearly discernable physical differences between the HL♂xCG♀ hybrid testes and the *C. gariepinus* testes. The hybrid testes were invariably very turgid, and were translucent pinkish in colour. The *C. gariepinus* testes were more flaccid, and ranged from creamy white in colour along their distal margins to a beige interior. The lobes of the hybrid testis were often mismatched in shape and size, and sometimes only one lobe was present. Patches of black necrotic tissue occurred in some of the hybrid testes examined.

The most obvious difference between the *C. gariepinus* and HL♂xCG♀ hybrid testes, when observed under the light microscope, was the difference in spermatozoa abundance. The lumen of the seminiferous lobules within the *C. gariepinus* testes were packed with spermatozoa, while those of the hybrid testes only contained small clumps of spermatozoa, if any at all (Fig. 5.4).

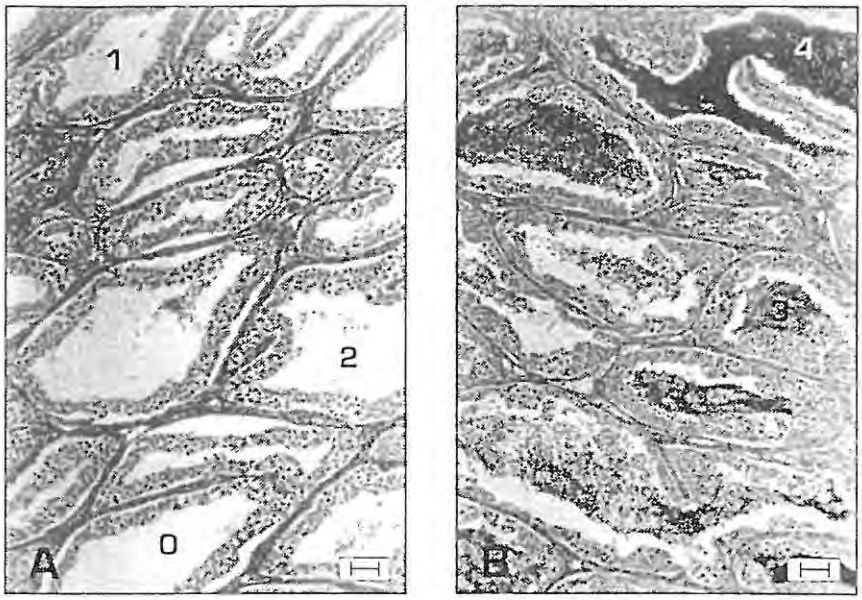

Figure 5.4. Spermatozoa abundance in hybrid (A) and *C. gariepinus* (B) testes. The numbers refer to abundance ratings (see text). Bar = 10 μm.

None of the hybrid testes (n = 20) scored a mean spermatozoa abundance rating of more than '2', and in about 40 % of the hybrid testes of both years no sperm could be located (Table 5.2). The *C. gariepinus* testes, on the other hand, were always rated at '3' or higher, and the spermatozoa were more evenly distributed throughout the sectioned areas of the testes.

Table 5.2. Spermatozoa abundance and distribution in the lumen of the testes of two and three year old HL♂xCG♀ hybrid and *C. gariepinus* males.

Abundance	Two year old		Three year old	
	Hybrid n = 10	*C. gariepinus* n = 10	Hybrid n = 10	*C. gariepinus* n = 10
	(%)	(%)	(%)	(%)
0	40	0	44	0
1	20	0	12	0
2	40	0	44	0
3	0	20	0	60
4	0	80	0	40

The transmission electron microscope photomicrographs showed that the hybrid testes and the *C. gariepinus* testes were quite different at the cellular level. Sections through the *C. gariepinus* testes showed normal spermatogenesis and spermiogenesis occurring, as described for *Clarias batrachus* by Lehri (1967) and for *C. lazera* (synonymised with *C. gariepinus* by Teugels, 1986) by Rizkalla (1970).

The testis of *C. gariepinus* consisted of closely packed seminiferous lobules (Nagahama, 1983). The lobules were separated by a thin layer of fibrous connective tissue. The outer layer of the lobules consisted of residual germ cells and large spermatogonia (Fig. 5.5) surrounded by packets or cysts of developing spermatogenic cells. Each cyst is the product of one spermatogonium, and contains the primary spermatocytes (Fig. 5.5) which undergo meiotic division to form the smaller secondary spermatocytes. The secondary spermatocytes are short-lived, experiencing a further meiotic division to produce spermatids. The spermatids undergo spermiogenesis, the process by which the cytoplasm and nuclear material reorganize in the cell. The nuclear material compacts into a dome-shape (u-shape in cross-section), and mitochondria collect at the mouth of the nuclear dome. The nuclear material compacts further, and a flagellum develops from the mouth of the nuclear dome. The cell is now described as a spermatozoon (Fig. 5.5). During the transformation from spermatids to spermatozoa the cyst ruptures, and the cells are released into the lumen of the seminiferous lobule.

The sections through the HL♂xCG♀ hybrid seminiferous lobules showed spermatogonia and apparent spermatocytes, but little further development. While the nuclear material of the *C. gariepinus* spermatocytes was spread evenly throughout the nucleus, the nuclear material in the hybrid spermatocytes was clumped together in one half of the nucleus, or in a u-shape along the nuclear membrane. The cells eventually lysed or degenerated in some way, without evolving further than the spermatocyte stage (Fig. 5.6).

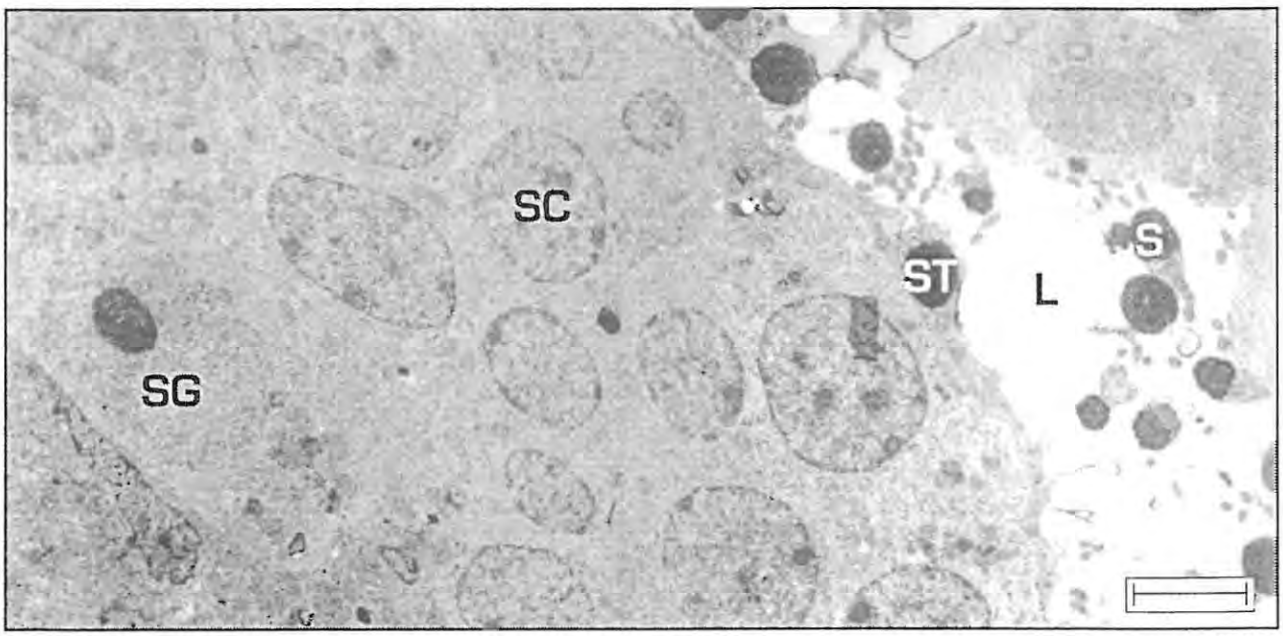

Figure 5.5. A section through a seminal lobule of a *C. gariepinus* testes, showing spermatogonia (SG), spermatocytes (SC), spermatids (ST), spermatozoa (S) and the lumen (L). Bar = 1.0 μm.

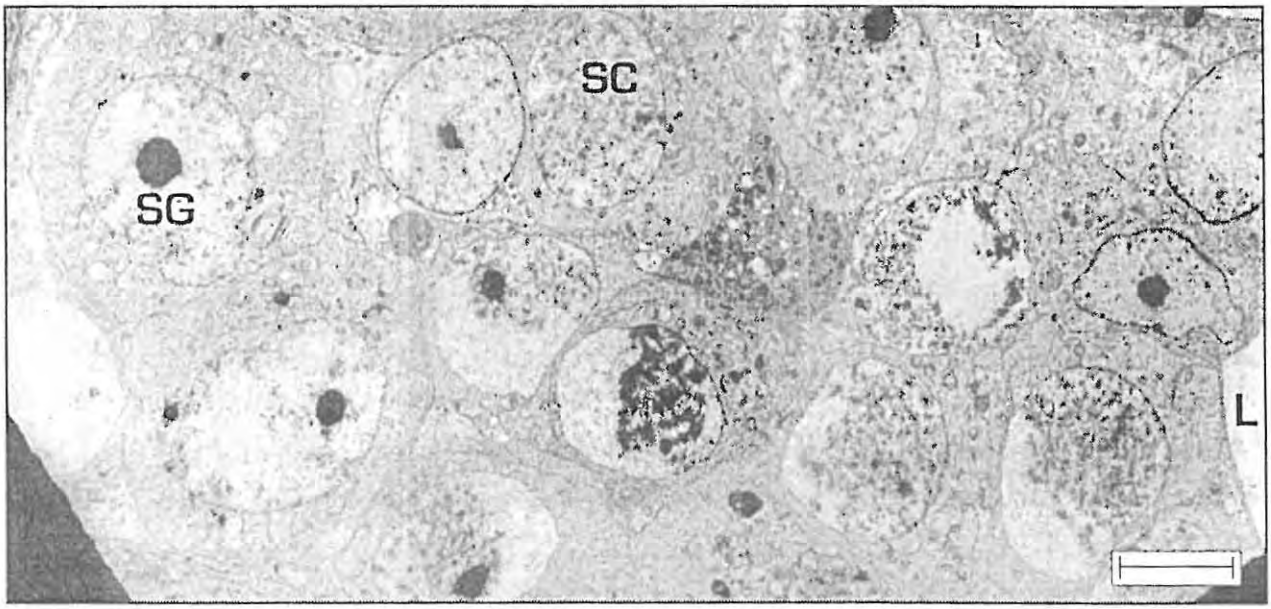

Figure 5.6. A section through a seminal lobule of a HL♂xCG♀ hybrid testes, showing spermatogonia (SG), spermatocytes (SC), and the lumen (L). Bar = 1.0 μm.

Spermatids were not observed in the sections of hybrid testes viewed under the transmission electron microscope, although one large spermatozoon was found in the lumen. Although the spermatozoon had a flagellum (dissappearing out of the plane of the section), its head was about 1.5 times larger than that of the *C. gariepinus* spermatozoa, and it contained many more mitochondria (Fig. 5.7).

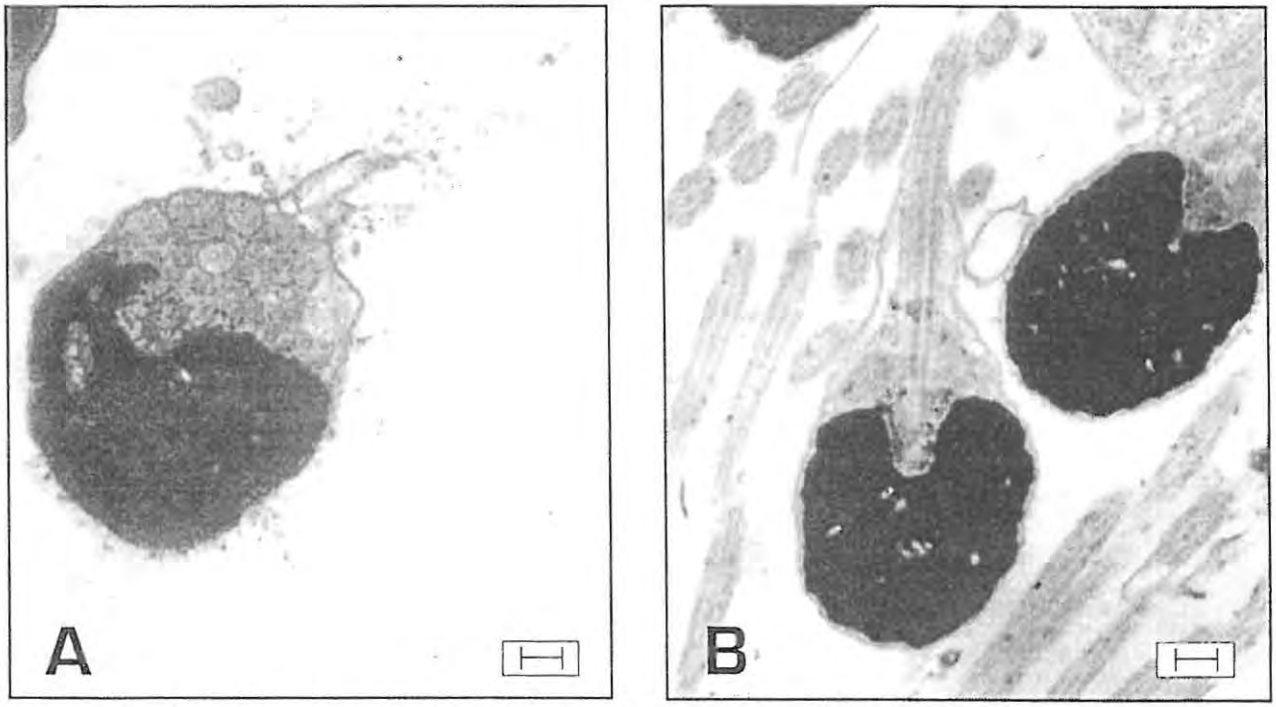

Figure 5.7. Examples of HL♂xCG♀ hybrid (A) and *C. gariepinus* (B) spermatozoa. Bar = 0.1 μm.

The seminal vesicles of the hybrid males were similar in appearance to those of the *C. gariepinus* males, but were significantly larger ($P < 0.05$). The seminal vesicles weighed an average of 1.22 ± 0.40 % of the total mass of the hybrid males, but only 0.78 ± 0.23 % of the total mass of the *C. gariepinus* males. The contents of the seminal vesicles appeared to consist primarily of cell debris. No intact spermatozoa were observed in the fluid of the seminal vesicles of either group of males.

Hybrid reproduction and backcrossing trials

The first three attempts to backcross HL♂xCG♀ hybrid sperm with *C. gariepinus* and *H. longifilis* eggs were unsuccessful. The only successful backcross trial was carried out in February, 1994. Six out of nine HL♂xCG♀ hybrid females and five out of six *C. gariepinus* females injected with catfish pituitary suspension were induced to spawn, after 10 hours. The hybrid females produced an average of 30 grams of eggs per kilogram of fish, while the *C. gariepinus* females produced an average of 150 grams of eggs per kilogram of fish.

The fertilization and hatching rates of the reciprocal backcross eggs were remarkably low, compared to those of the pure strain *C. gariepinus* larvae (Table 5.3). The fertilization and hatching rate of the F_2 hybrid eggs were even lower, which indicated that the gametes of both male and female HL♂xCG♀ hybrids were of poor quality. The survival rates of the reciprocal backcross larvae to day four post hatching were very low as well, compared to the pure strain *C. gariepinus* larvae. The survival rate of the F_2 hybrid larvae was less than half that of the backcross larvae (Table 5.3).

Table 5.3. The fertilization, hatching and survival rate of *C. gariepinus*, F_2 hybrid and HL♂xCG♀ hybrid x *C. gariepinus* backcross larvae.

Cross	% Fertilization	% Hatched	% Survival to Day 4
CG♂xCG♀	77.4	46.1	80.0
HYB♂xCG♀	18.7	5.4	28.0
CG♂xHYB♀	23.2	2.7	27.0
HYB♂xHYB♀	10.5	1.7	9.3

5.4. DISCUSSION

Most of the *H. longifilis* and *C. gariepinus* males and the *C. gariepinus* females raised at the Rhodes University aquaculture facility reached sexual maturity within their first year, while the *H. longifilis* females attained maturity during their second year. Small amounts of viable eggs could be induced from HL♂xCG♀ hybrid females during their second breeding season, as Legendre *et al.* (1992) found with

the CG♂xHL♀ hybrid females from West Africa. The successful backcross trial in this study showed that some of the HL♂xCG♀ hybrid eggs were viable. The hybrid eggs were successfully fertilized with hybrid sperm and *C. gariepinus* sperm, giving rise to a few viable larvae.

According to their GSI values, the male HL♂xCG♀ hybrids reached sexual maturity during their first year in southern Africa, and the sperm of two year old hybrids was used to successfully fertilize *C. gariepinus* eggs. Some viable larvae were produced using combinations of both male and female hybrid gametes, thus the *H. longifilis* ♂ x *C. gariepinus* ♀ catfish cross is not a completely sterile hybrid.

Nevertheless, both hybrid sexes showed symptoms of gonadic and gametic sterility, as described by Chevassus (1983). In most of the seminiferous lobules of the hybrid testis, gametogenesis was arrested at the spermatocyte stage, but some spermatozoa were found in the lumen. Spermatozoa counts showed that the HL♂xCG♀ hybrid males were producing only about 0.04 % the amount of spermatozoa produced by *C. gariepinus* males. The spermatozoa count of the hybrid and *C. gariepinus* males increased with age. The average spermatozoa counts for *C. gariepinus* over the three age groups compared favourably with the literature, but the average HL♂xCG♀ hybrid spermatozoa concentration was an order of magnitude less than that reported by Legendre *et al.* (1992) for the reciprocal hybrid (Table 5.4).

Table 5.4. Comparison of *C. gariepinus* and hybrid sperm counts in the literature and in this study.

Species	Stock	Sperm conc.	Reference
C. gariepinus	South Africa	6.200 x 10^9/mℓ	Steyn & van Vuuren (1987)
C. gariepinus	South Africa	5.900 x 10^9/mℓ	This study
C. gariepinus	West Africa	3.450 x 10^9/mℓ	Legendre *et al.* (1992)
CG♂xHL♀ hybrid	West Africa	0.057 x 10^9/mℓ	Legendre *et al.* (1992)
HL♂xCG♀ hybrid	South Africa	0.003 x 10^9/mℓ	This study

The concentration of spermatozoa reported for the southern African hybrid semen may be an overestimation, as the "large spermatozoa" observed in the hybrid semen were most probably prematurely released spermatids. Moreover, the spermatozoa of the two year old hybrid males were only 36 % as viable as the two year old *C. gariepinus* spermatozoa.

Spermatozoa storage in the seminal vesicles has been reported in *Clarias batrachus* and *C. lazera* (now synonymised with *C. gariepinus*; Teugels, 1986) by Nawar (1959) and Nair (1965). However, there were no intact spermatozoa to be found in the seminal vesicles of either the hybrid or *C. gariepinus* males investigated. This observation was also made by Rennie (1981). It is more likely that the seminal

vesicles have a secretory role (Sundararaj, 1958; Nagahama, 1983), producing a steroid pheromone which acts as a sex stimulant (Resink *et al.*, 1989).

The ovaries of the HL♂xCG♀ hybrids exhibited a high level of gonadic sterility. The average GSI value of the mature hybrid females was eight times less than that of the mature *C. gariepinus* females. Thirty percent of the hybrid females examined showed no ovarian development at all. Legendre *et al.* (1992) found that some of the oogonia of the CG♂xHL♀ hybrid ovaries had pycnotic nuclei, with a higher proportion of atretic follicles than *C. gariepinus*. Most of the follicles were arrested at the first stage of vitellogenesis. Similar abberations were most likely the cause of the severely reduced egg development in the HL♂xCG♀ hybrid females in southern Africa.

Evidence for some degree of gametic sterility, in both male and female HL♂XCG♀ hybrids, was found in the very low hatching rates of the F_2 hybrid and backcrossed eggs (Tables 5.3 & 5.4). Overall, the backcrossed and F_2 hybrid eggs had a hatching rate at least 10 times lower than that of pure strain *C. gariepinus* eggs.

In the wild, *C. gariepinus* adults spawn in the unstable environs of marginal areas of recently inundated floodplains (Bruton, 1979b). They are non-guarding, open substrate spawners (Balon, 1984). The larvae are exposed to habitat desiccation and high levels of predation (Bruton, 1979b). The early larval mortality of fishes following this type of reproductive strategy is very high (Balon, 1985), thus the females need to be highly fecund. The sperm concentration in the semen of profligate spawners also has to be high to successfully fertilize eggs in the wild. It has been calculated, for example, that salmon (*Salmo salar*) require approximately 1.9 x 10^6/mℓ spermatozoa to fertilize one egg under these conditions (Aas *et al.*, 1991). If the hybrids were to have the same reproductive strategy as *C. gariepinus*, the possibility of escapee HL♂xCG♀ hybrids from aquaculture operations forming competitive breeding colonies or backcrossing with indigenous populations of catfish seems extremely remote. The *C. gariepinus* courtship rituals are complex (see Bruton, 1979b for a full description) and an ethological study is necessary to ascertain whether the hybrids would recognize, interpret and react to the *C. gariepinus* courtship behaviour.

Aside from low hatching rates, the survival rates of the hybrid offspring were much lower than those of the *C. gariepinus* larvae. The backcrossed larvae had a survival rate three times lower than that of the *C. gariepinus* larvae to day four post hatching, while the survival rate of the F_2 hybrid larvae was eight times lower than for *C. gariepinus* larvae. The lower survival rates of the hybrid larvae were most likely the result of larval deformities caused by genetic abberations. For example, Legendre *et al.* (1992) found that 89.6 % of the CG♂xHL♀ hybrid/*C. gariepinus* backcross larvae were deformed, while 83.5 % of the F_2 hybrid larvae were deformed.

In conclusion, the HL♂xCG♀ hybrid was not sterile. However, when the constraints of partial gonadal sterility (low gamete production) and partial gametic sterility (low viability) are considered in the light of the hybrid's probable reproductive strategy, the southern African HL♂xCG♀ hybrid cannot be considered a threat to natural ecosystems. Before further conclusions can be drawn as to hybrid sterility in practical terms, an ethological study is necessary, to ascertain the reproductive potential of the *H. longifilis* x *C. gariepinus* hybrids in the wild.

CHAPTER 6. SOME PHYSICAL AND CHEMICAL WATER QUALITY PREFERENCES AND TOLERANCES OF *CLARIAS GARIEPINUS* AND ITS HYBRID WITH *HETEROBRANCHUS LONGIFILIS*.

6.1. INTRODUCTION

Available surface water is limited in most of the southern African region. The groundwater is often brackish, and salination has occurred in some of the rivers (Viljoen *et al.*, 1984; Anon, 1987). Thus, freshwater aquaculture in this region needs to utilize the available freshwater as efficiently as possible. Water re-use aquaculture systems coupled to intensive fish culture methods would seem to be ideal for this region. However, it is difficult to maintain good water quality at high densities of fish (O'Sullivan and Purser, 1993), particularly ammonia and oxygen levels. On the other hand, one of the advantages of recirculating systems is a greater degree of control over water quality parameters such as temperature (O'Sullivan and Purser, 1993). This means that the water temperature of the culture system can be maintained at the optimum temperature for growth of the cultured species.

For the above reasons, it was decided to examine the oxygen, ammonia and salinity tolerances of *C. gariepinus* and the HL♂xCG♀ hybrid, as well as their temperature preferenda.

Temperature

The body temperature of poikilothermic fish is dependent on environmental temperature, usually remaining within 1 °C of the ambient water temperature (Reynolds *et al.*, 1976). Temperature is important to fish for two principal reasons. It determines the rate of the metabolic reactions of the fish, and it regulates the structural integrity of the molecular (proteins and nucleic acids) and macromolecular (biological membrane) structures within the fish (Hazel, 1993). The temperature at which a fish functions most efficiently is called its optimum temperature (Jobling, 1981). To maximize productivity, fish farmers need to know the optimum temperature at which to raise their fish.

When placed in a temperature gradient, fish tend to congregate around an innate, species specific temperature, termed the preferred temperature or thermal preferendum (Reynolds and Casterlin, 1979; Britz and Hecht, 1987; Deacon and Hecht, 1995). A fish may not always choose to be at its optimum metabolic temperature in its natural environment, as temperature also effects gas solubility, food availability, habitat conditions, predators, *etc.* (Koppelman *et al.*, 1988). However, in controlled laboratory conditions the temperature at which fish congregate within a temperature gradient is a good indication of their optimum temperature (Giattina and Garton, 1978; Jobling, 1981; Coutant, 1987). After examining the temperature preference and optimum temperature for growth of about 50 species of fish, Jobling (1981) determined that the temperature preference of a fish is a good indication of its optimum temperature. However, the optimum temperature for growth is usually slightly lower than the

fish's temperature preference.

Fish have the ability to acclimate to non-optimal temperatures if they experience these conditions for extended periods. Acclimation is a response by a fish which enables it to tolerate a change in a single factor (e.g. temperature) in its environment (Allaby, 1985). This is accomplished by a compensatory change in metabolic rate associated with adaptive changes in cellular biosynthesis (Svirskiy and Golovanov, 1991).

When a fish is introduced into a temperature gradient it will gravitate to a temperature influenced by its previous thermal history, irrespective of its optimum temperature preference. This temperature is described as the fish's acute thermal preferendum (Jobling, 1981). In time however, the fish will move to its species specific temperature preferendum. Thus Fry (1947) introduced the concept of the final thermal preferendum of a species, which he described as "... the temperature around which all individuals will ultimately congregate, regardless of their thermal experience before being placed in the gradient."

Many authors have defined the temperature preference of a species as a single value characterized by some form of central tendency (Reynolds and Casterlin, 1979). Jobling (1981) considered this approach to be unrealistic, as fish in a temperature gradient tend to undertake exploratory excursions into regions of temperature higher or lower than the mean final temperature preference. Jobling (1981) suggested that the final temperature preference of a species be expressed as a zone of temperature preference.

Most fish can tolerate a wide range of temperatures outside of their zone of final temperature preference. However, beyond a certain point, an increase or decrease in temperature is lethal. This point is called the Critical Thermal Maximum or Minimum (CTM). The CTM has no unequivocal definition (Alabaster and Lloyd, 1980), and has been variously described as the point at which the fish loses equilibrium (Jobling, 1981) or dies (Bettoli *et al.*, 1985). The CTM has also been used as the equivalent of a temperature LC_{50} (Alabaster and Lloyd, 1980).

Clarias gariepinus can tolerate remarkable extremes in temperatures. It can survive temperatures as low as 7 °C and as high as 38 °C with acclimation (Klyszejko *et al.*, 1993). The critical thermal minimum and maximum for this species is 6 °C and 50 °C respectively, under laboratory conditions (Babiker, 1984). Hogendoorn *et al.* (1983) observed that juvenile *C. gariepinus* grew best in a temperature range from 27.5 °C to 32.5 °C.

Nothing is known of the temperature preference of *H. longifilis*. The fact that this species is geographically restricted to a more tropical distribution (Fig. 2.1) suggests that its critical thermal minimum and maximum temperatures should theoretically be higher than those of *C. gariepinus*. The

optimum preferred temperature of *H. longifilis* is therefore also likely to be different to *C. gariepinus*. Even if the preferred temperatures of both species were known, it would be impossible to predict the optimum temperature preference of their hybrid. Some hybrids have shown heterosis for temperature tolerance (Bettoli *et al.*, 1985), while others preferred temperatures intermediate to or similar to the parent species (Peterson *et al.*, 1979; Koppelman *et al.*, 1988).

The optimum temperature preference of the HL♂xCG♀ hybrid has important implications for aquaculture in southern Africa. If the fish require warmer water than *C. gariepinus* to reach their full growth potential, the hybrid may have no economic advantage over *C. gariepinus* for aquaculture in the region.

Britz and Hecht (1987) found that the final temperature preference of *C. gariepinus* larvae and early juveniles (30 °C) coincided with their optimum temperature for growth. Thus the final temperature preference of the hybrid is likely to provide a good estimate of its optimum temperature for growth. In this study, an attempt was made to define the hybrid's zone of temperature preference, and to compare it to that of *C. gariepinus*.

Oxygen

The ability to tolerate extremely low oxygen concentrations is one of the primary reasons why *C. gariepinus* has been hailed as an excellent aquaculture candidate (Sidthimunka, 1972); particularly for high density aquaculture (Huisman and Richter, 1987). These fish have a bimodal gaseous exchange capacity. Gills are used to extract oxygen from water, while accessory air-breathing organs extract oxygen from the atmosphere (Johansen, 1970). The accessory respiratory organs of *C. gariepinus* consist of the anterior and posterior labyrinthine organs (also known as the arborescent or dendritic organs), which extend into a suprabranchial chamber from the second and fourth gill arches, and the suprabranchial chamber membrane which lines the surface of the chamber (Maina and Maloiy, 1986). Fan-like branchial extensions, formed by modified gill filaments, cover two narrow apertures through which air enters and exits the suprabranchial chamber from the gill chamber (Moussa, 1956; Hughes and Datta Munshi, 1973).

During aquatic respiration, oxygenated water is forced through the gills using the mechanism of the buccal and opercular "respiratory pump" as described in Shelton (1970). *Clarias gariepinus* air-breathes in the following way (summarised from Vandewalle and Chardon, 1991);

1. As the fish begins to rise to the surface, air is forced out of the suprabranchial organ by the contraction of the posterior suprabranchial muscles, and the concomminant expansion of the water-filled opercular cavities. The air bubbles force their way past the branchial fans, through the suprabranchial apertures into the opercular cavities.

2. While still rising, the fish adducts its operculi and lifts the hyoid bars, forcing the air bubbles out through the opercular slits.

3. On reaching the surface, the fish takes air into its buccal cavity by lowering and abducting the hyoid bars. The operculi are also abducted, so that the water in the buccal cavity (and some of the air as well) flows into the opercular cavities. The fish then closes its mouth and dives.

4. Elevation of the hyoid bars raises the pressure in the buccal cavity and opens the epibranchial fan covering part of the suprabranchial aperture; the concomminant relaxation of the posterior suprabranchial muscles and the branchial levator muscles cause a low pressure in the suprabranchial chamber, allowing air to flow into the chamber.

5. Excess air escapes through the opercular slits. The fish reaches the bottom and aquatic ventilation continues through buccal and opercular activity.

The gills of air-breathing fish need to be continuously flushed with water, even in anoxic conditions, so that nitrogenous metabolites and CO_2 can be excreted, and ionic and osmotic regulation can take place (Graham and Baird, 1984).

The suprabranchial accessory respiratory organs of clariid fish are highly efficient. Maina and Maloiy (1986) found that they contributed 88 % to the total oxygen diffusing capacity of *C. gariepinus*, while the gills contributed only 12 %. This may explain why active adult *C. gariepinus* are obligate air-breathers (Moussa, 1957; Bruton, 1988).

The oxygen diffusing capacity of the gills, accessory respiratory organs and the skin of *C. batrachus* respectively contributed 10 %, 88 % and 2 % of the total diffusing capacity of the respiratory organs (Datta Munshi and Hughes, 1992). The extent to which respiration occurs through the skin of *C. gariepinus* has not been studied, but is probably similar to that of *C. batrachus*.

No studies have yet been carried out on the air-breathing capabilities of *Heterobranchus longifilis*; but their accessory respiratory organs are anatomically very similar to *C. gariepinus* (Teugels *et al.*, 1990). Dissections of the HL♂xCG♀ hybrid accessory respiratory organs showed that their gross morphological structures were indistinguishable from those of *C. gariepinus* (P.H. Greenwood, British Museum of Natural History, pers. comm.).

Even though the accessory respiratory organs of these catfishes are similar in structure, the various species may have disparate oxygen depletion tolerances, depending on the oxygen-binding ability of the haemoglobin in their blood. An air-breathing species with haemoglobin that has a high oxygen

affinity would only need to supplement its aquatic respiration with aerial respiration at low oxygen concentrations. For example, the *Ictalurus punctatus* x *I. furcatus* hybrid could tolerate lower oxygen levels than *I. punctatus*. Dunham *et al.* (1983) attributed this ability to the slightly different structure of the hybrid's haemoglobin.

The ability of *C. gariepinus* to breathe atmospheric oxygen allows it to survive in anoxic water. However, in order to breathe air, a fish has to rise to the surface of the water. There are several studies which suggest that rising to the surface has energetic costs for most air-breathing aquatic organisms (Arunachalam *et al.*, 1976; Vivekanadan and Pandian, 1977; Feder and Moran, 1985; Pandian and Marian, 1985; Bevan and Kramer, 1987; Haylor, 1992).

Under conditions of ultra-high density aquaculture (see Chapter 8) a fish rising to air-breathe often has to force its way to the surface. The normally territorial *C. gariepinus* change their behaviour at ultra-high densities, and literally "school up" on top of each other (Hecht *et al.*, in prep.). The need to air-breathe disturbs this concentrated school, and aggressive encounters ensue when a fish tries to re-enter the school (pers. obs.).

If the oxygen content of the water has an effect on the air-breathing frequency of the fish, it may be advisable to keep the oxygen levels in ultra-high density tanks as high as possible to minimise stress and promote growth. In the following study, the hypothesis that oxygen concentration effects air-breathing rate is tested, for the hybrid and for *C. gariepinus*.

Ammonia

Ammonia is the primary product of nitrogen metabolism in teleost fishes (Foster and Goldstein, 1969). It is produced by the metabolic activity of nerve and muscle tissue, the de-amination of amino acids by the liver, and the activity of enzymes produced by the microflora of the gut (Fromm and Gillette, 1968). The majority of the ammonia output (approximately 90 %) is excreted at the gills in freshwater teleosts (Sayer and Davenport, 1987).

It is well documented that ammonia is toxic to teleost fish (Smith and Piper, 1975). Total ammonia is the sum of the ionic NH_4^+ and un-ionized NH_3 species, which exist in equilibrium. The equilibrium is influenced primarily by pH and temperature in freshwater; the concentration of NH_3 increases as these parameters are increased (Emerson *et al.*, 1975). The toxicity of ammonia to fishes has been attributed to NH_3, as this gaseous molecule is readily soluble in the lipids of cell membranes and can diffuse into the fish without active transport (Hampson, 1976). The ionic NH_4^+ molecules are larger, hydrated and charged, and need to be actively transported through the charge-lined micropores of the hydrophobic components of the gill membrane (Hampson, 1976).

Ammonia is excreted from a fish across a transbranchial ammonia gradient, moving from the high ammonia concentration of the fish's blood to the lower ammonia concentration of the surrounding water (Wilson and Taylor, 1992).

When the ammonia concentration of the water is higher than that of the blood, NH_3 diffuses into the fish. Blood ammonia concentrations increase at first, then stabilize. If the ammonia concentration of the water is substantially higher, the ammonia in the blood will stabilize at a concentration lower than that of the water (Wilson and Taylor, 1992). Thus it is evident that ammonia is actively removed from the blood of the fish against the concentration gradient. The mechanism of this active excretion is probably based on an ion pump, where ammonia in the form of NH_4^+ ions is exchanged for Na^+ or H^+ ions across the gill epithelial apical membrane (Cameron, 1986; Randall and Wright, 1987; Wilson and Taylor, 1992).

Long-term exposure of fish to reversed (water to blood) ammonia concentration gradients have led to varied manifestations of chronic toxicity, including slower growth rates, blood acidemia and tissue damage (Smith and Piper, 1975; Sousa and Meade, 1977; Alderson, 1979; Cruz and Enriquez, 1981). Acute toxicity results in convulsions and death, probably due to the disruption of many membrane processes through the substitution of potassium ions by NH_4^+ (Randall and Wright, 1987).

A common way to compare ammonia tolerance in fish is to use the bioassay method, developed by the American Public Health Association for pollution control (APHA, 1976). Fish are exposed to different concentrations of ammonia for a specific duration, preferably 96 hours (Sprague, 1973). The mortality of the fish at each ammonia concentration is compared, usually on a log-probability graph, and the concentration is found at which 50 % mortality would have occurred. This value is described as the median lethal concentration or LC_{50} of ammonia for the tested species. The ammonia LC_{50} values reported in the literature almost always refer to un-ionized ammonia (NH_3) concentrations, not total ammonia. Most species of fish have LC_{50} values within the range 0.2 mg.ℓ^{-1} to 2.0 mg.ℓ^{-1} NH_3 (Alabaster and Lloyd, 1980). The tilapiines and air-breathing clariid catfish are exceptional, having LC_{50} values greater than this range.

Oppenborn and Gouldie (1993) found an interesting phenomenon when they compared the chronic and acute effects of ammonia on the striped bass *Morone saxatilis* and the *M. saxatilis* x *M. chrysops* hybrid bass. The hybrid had a significantly lower NH_3 tolerance than *M. saxatilis*, and could not maintain a constant blood ammonia concentration as the ambient ammonia concentrations were raised.

A species must have a high ammonia tolerance to be a successful candidate for ultra-high density aquaculture. The amount of ammonia in a recirculating fish farm depends on an equilibrium between its rate of production by the farmed organisms, its oxidation by bacterial activity, and the water

exchange rate (Hampson, 1976). Under ultra-high density aquaculture conditions ammonia production is high, while total water volume is low. Exchange rates are limited by the efficiency of the biological filters, so the ammonia in the recirculated water builds up to high ambient concentrations. If the HL♂xCG♀ hybrid were unable to tolerate high concentrations of ammonia, it would be unsuitable for ultra-high density aquaculture. In the following study the ammonia tolerance of the hybrid and *C. gariepinus* was tested and compared, using the median lethal concentration protocol.

Salinity

Clarias gariepinus is a stenotopic freshwater species. While adult *C. gariepinus* have been observed to enter and forage in estuaries, they move back into freshwater when the salinity increases above 10 $g.\ell^{-1}$ (Whitfield *et al.*, 1981). Mass mortalities of *C. gariepinus* have been recorded when sudden increases in salinity occurred, and the fish were unable to escape (Blaber, 1981). Under experimental conditions *C. gariepinus* has been reported to have a salinity tolerance of between 8.5 to 15 $g.\ell^{-1}$ (Table 6.1).

Table 6.1. The salinity tolerances of *C. gariepinus* reported in the literature.

Salinity	Stage	Experimental procedure	Reference
8-10 $g.\ell^{-1}$	larvae	16 day growth trial	Britz & Hecht, 1989
9.8 $g.\ell^{-1}$	juveniles	168 hour survival trial	Chervinski, 1984
10.0 $g.\ell^{-1}$	adults	field observation	Whitfield *et al.*, 1981
15.0 $g.\ell^{-1}$	adults	serial salinity increases	Clay, 1977

However, salinities as low as 2.5 $g.\ell^{-1}$ have an adverse effect on *C. gariepinus* larvae and adults. Above this salinity the larvae lose condition (Britz and Hecht, 1989), and the adults do not initiate breeding activity (Clay, 1977).

Legendre *et al.* (1992) found that two day old *C. gariepinus* larvae had a 100 % mortality when placed into lagoon water at a salinity of 8 $g.\ell^{-1}$, while 1.8 % of the *H. longifilis* larvae survived. They also noted that juvenile *H. longifilis* had a higher survival rate (96 %) than *C. gariepinus* (53 %) held at 3.0 $g.\ell^{-1}$, although they were hesitant to attribute the difference in survival entirely to salinity. Legendre (1983) contended that *H. longifilis* adults would be highly suitable for brackish water culture in salinities of up to 10 $g.\ell^{-1}$.

The ictalurid catfish hybrid *Ictalurus furcatus* x *I. punctatus* had a salinity tolerance of 14.6 $g.\ell^{-1}$ over 96 hours, compared to 13.2 $g.\ell^{-1}$ for pure strain *I. punctatus* (Stickney and Simco, 1971). Allen and Avault (1971) compared the salinity tolerance of the parent strains, and found that *I. furcatus* was

slightly more tolerant to brackish water than *I. punctatus*.

It was therefore postulated that the HL♂xCG♀ hybrid may also be more tolerant of salinity than *C. gariepinus*. This hypothesis was tested in the following study.

6.2. MATERIALS AND METHODS

Temperature preference

Clarias gariepinus juveniles ($\bar{x}$ = 48.3 ± 6.4 mmTL, 1.1 ± 0.4 g) were acclimated to a temperature of 28 °C in an 80 ℓ glass aquarium connected to a biological filter. After seven days 20 fish were removed and placed into a temperature gradient chamber. The test chamber consisted of a glass tank divided into compartments by a series of baffles (Fig. 6.1). A variable control 200 W aquarium heater was placed into each of the compartments. An air stone was placed under each heater, so that the rising bubbles dispersed the heated water evenly within each of the compartments.

Water was allowed to flow into one side of the chamber, and was drained off from the opposite end. By adjusting the heaters and the water flow rate through the chamber, a temperature gradient was generated. The temperature in each compartment varied by 1 °C as ambient temperatures fluctuated during the course of a day, providing an overall temperature range of 25 °C to 33 °C. A thermometer was placed in each of the compartments to monitor the water temperature.

To prevent the fish from being disturbed, the test chamber was surrounded by black curtaining. A timed overhead light source (7300 lux, calculated as in Casewell, 1993) provided a photoperiod of 12 hours light and 12 hours dark. The fish were fed every morning (09h00) and evening (18h00), by sprinkling feed pellets evenly over all of the compartments.

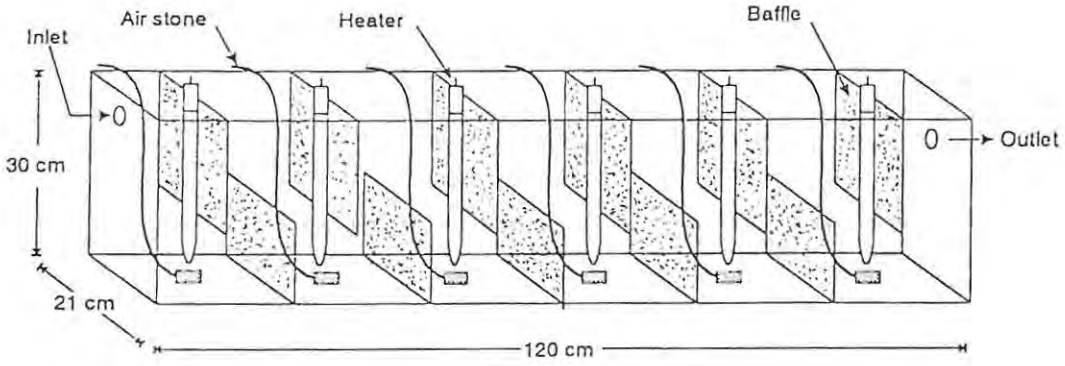

Figure 6.1. A schematic representation of the thermal gradient tank.

The temperatures of the compartments were measured to the nearest 1.0 °C, and the number of fish congregated at each temperature in the gradient were recorded, at random intervals, three times daily during the six days. As the temperature in each compartment varied during the day, the number of fish were recorded per temperature, *not* per compartment. The number of fish occurring at each temperature was recorded as a percentage of the total number of fish observed in the chamber. The three observations were averaged for each day. The daily averages from the six days of the trial were then pooled for each temperature.

After six days, the fish were removed, the chamber was cleaned and the temperature gradient reset. Three replicates were performed using equally sized *C. gariepinus* juveniles. A one-way analysis of variance was carried out to test that no significant differences ($P > 0.05$) in frequency of occurrence existed between the replicated trials for each temperature in the gradient. The replicated frequency data were pooled for each temperature. The differences in the frequencies of occurrence of the *C. gariepinus* juveniles in the temperature gradient were tested for significance ($P < 0.05$) by analysis of variance. Relationships within the data set were analyzed using a 95 % Tukey multiple range test.

After the experiments had been completed, the chamber was emptied, cleaned, and restocked with 20 HL♂xCG♀ hybrid juveniles (50.5 ± 11.0 mmTL, 1.32 ± 1.0 g). The hybrids were exposed to the same conditions (25 °C to 33 °C) as the *C. gariepinus* juveniles in the chamber, and were observed following the same protocol for six days. Three replicate hybrid temperature gradient trials were undertaken and the data were analyzed as for *C. gariepinus*.

Because the hybrids did not show a normal distribution in the 25 °C to 33 °C temperature gradient, a further experiment was carried out, but at higher temperatures, over eight days. The temperature gradient in the second series of trials ranged from 31 °C to 35 °C; in all other respects the trials were set up, executed and analyzed in the same way as the previous experiments.

The effect of oxygen concentration on air-breathing frequency

Hybrid and *C. gariepinus* juveniles were kept in an 80 ℓ holding tank connected to a biological filter, at a temperature of 28.0 ± 1.8°C. One fish was removed from the holding tank at a time and placed into a glass respirometer (Fig. 6.2) containing one litre of holding tank water, raised to a temperature of 30.0 ± 0.8 °C. It was important to standardize water temperature for all the respirometer experiments, as the amount of oxygen available to the fish for aquatic respiration is dependent on water temperature. A temperature of 30 °C was chosen as it corresponded to the mean optimum preferred temperature as determined in the previous experiment. The temperature was maintained by placing the respirometer on a heated magnetic stirrer; the gently rotating stirrer ensured that stratification of temperature and oxygen did not occur.

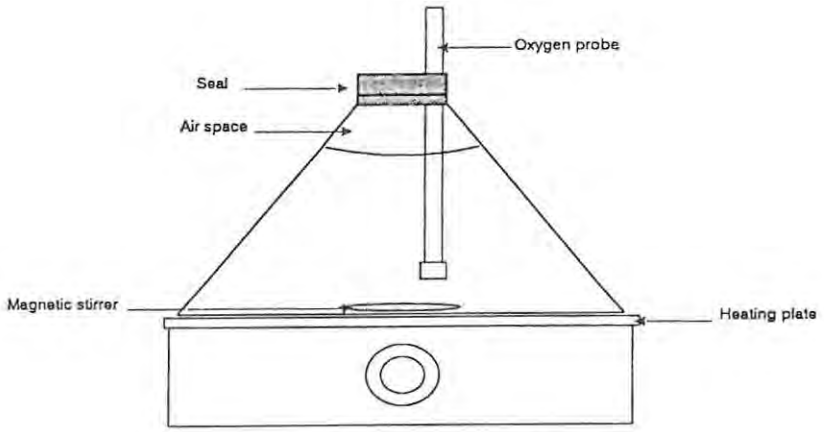

Figure 6.2. A schematic representation of the respirometer.

The fish was allowed to acclimatize in the respirometer for 30 minutes, during which time air was bubbled into the water through a diffuser. The diffuser was then removed, and an OxyGuard^{R} Handy Mk II combination oxygen/temperature probe was inserted into the water, and the respirometer was sealed. Approximately 40 cm^3 of air was left between the seal and the water surface, so that the catfish could air-breathe. The oxygen concentration, water temperature and number of times the fish surfaced to air-breathe were recorded every five minutes. Each run was terminated after 75 minutes.

Ten HL♂xCG♀ hybrids ($\bar{x}$ = 178 ± 28 mmTL, 40.1 ± 17.8 g) and 10 *C. gariepinus* ($\bar{x}$ = 173 ± 18 mmTL, 34.7 ± 12.8 g) were monitored in this way. The individual trials were carried-out at random times during the day or night.

The air-breathing rate of individual fishes varied greatly, depending on their activity. To compare changes in the air-breathing rate of the experimental fish over time (irrespective of their individual breathing tempo) these data had to be standardised. The number of times a fish surfaced to air-breathe during a five minute interval was transformed to a percentage of the total number of air-breaths taken by that fish during the 75 minute trial. The oxygen concentration of the water and the air-breathing frequencies of the 10 hybrid and 10 *C. gariepinus* juveniles during the five minute intervals were then pooled for each species, for statistical analysis. An analysis of variance was carried out for each group, to test if there was a significant change ($P < 0.05$) in the frequency of air-breathing of the fish in the respirometer over time. Tukey multiple range tests were used to analyze the relationships within the two data sets.

The mean oxygen concentration of the water in the respirometer and the mean air-breathing frequency of the fish at each of the five minute intervals was plotted on a graph for both groups of fish, so that

the relationship between air-breathing frequency and oxygen concentration could be visualised.

Median lethal ammonia concentration

Fifty HL♂xCG♀ hybrid and 50 *C. gariepinus* juveniles of approximately 120 mmTL (110 g) were kept in 80 ℓ glass aquaria for ten days, at densities of about 24 g fish/litre of water. The water temperature of the tanks was kept at 29.0 ± 1.0 °C, and on the tenth day the pH of the water was measured to be 8.7, NH_3 concentration was 0.04 mg.ℓ$^{-1}$ and the oxygen content was 6.40 mg.ℓ$^{-1}$.

After a 48 hour starvation period, five hybrids and five *C. gariepinus* were placed in each of eight 60 ℓ polyurethane bags containing 10 ℓ of water. There was no significant difference ($P > 0.05$) in average size of the hybrids and *C. gariepinus* within and between the bags. The bags contained phosphate-buffered tap water at a pH of 7.0. Prior to use, the tap water was allowed to stand in a 200 ℓ barrel for 10 days, while air was bubbled through it to remove all traces of chlorine.

Ammonium chloride (NH_4Cl) was added to the water in sufficient quantities so that the water in the replicated bags contained NH_3 at 2.5, 5 and 15 mg.ℓ$^{-1}$, according to the calculations of Emerson *et al.* (1975). No NH_4Cl was added to two bags, which were used as controls. Other ammonium salts have been used as a source of NH_3, including ammonium bicarbonate, ammonium monohydrogen phosphate and ammonium sulphate; but Thurston and Russo (1983) found that there was no difference in their toxicity to fish, compared to ammonium chloride.

Approximately 40 ℓ of air was pumped into the bags. The necks of the bags were then sealed using elastic bands. It was necessary to keep the bags sealed so that the high concentrations of gaseous ammonia in some of the experimental bags could be maintained, with as little loss of this gas as possible to the atmosphere. The bags were floated in a large water bath, which was set at temperature of 31 °C. These relatively high temperatures were necessary to regulate the ammonia equilibrium in the bags, increasing the amount of the NH_3 species present in the water, at a constant pH of 7.0.

The bags were opened every 24 hours to remove dead fish and to take a water sample, after which the bags were re-inflated with fresh air. The experiment was run over 96 hours, according to the accepted method for ascertaining the median lethal concentration (96 hour LC_{50}) of ammonia for organisms (Sprague, 1973).

Ammonia concentration was measured in millivolts using an Orion^R ammonia electrode, and converted to milligrams total ammonia per litre (mg.ℓ$^{-1}$) using a standard calibration curve. Un-ionized ammonia (NH_3) was recalculated as a function of temperature and pH, using the equations of Emerson *et al.* (1975). All electrode readings were verified using the Nesslerization method. Oxygen concentration and

water temperature were measured using an OxyGuard® Handy Mk II portable oxygen probe. Nitrite and nitrate concentrations were measured using a Hach DR-EL/4 apparatus, while an Orion® probe was used to ascertain the pH of the water.

The percent mortalities of *C. gariepinus* juveniles and hybrid juveniles after 96 hours were plotted against NH_3 concentration on a log-probit scale. The median lethal NH_3 concentrations were read off the graph following Sprague (1973) and Sokal and Rohlf (1973). After the experiment the surviving fish were returned to the holding tank and observed for a further seven days.

Median lethal salinity

Fifty HL♂xCG♀ hybrid and 50 *C. gariepinus* of approximately 200 mmTL (58 g) were held in 80 ℓ glass aquaria linked to a recirculating system for ten days, at a density of about 40 g fish/litre of water. Temperature was kept constant at 28.0 ± 0.3 °C. On the tenth day, the following water quality values were recorded for the holding tank system; pH = 7.7, NH_3 concentration = 0.001 mg.ℓ$^{-1}$, oxygen concentration = 6.6 mg.ℓ$^{-1}$, salinity = 0.0 g.ℓ$^{-1}$ and $CaCO_3$ = 140.0 mg.ℓ$^{-1}$.

After a 48 hour starvation period, five hybrids and five *C. gariepinus* were placed in each of eight 60 ℓ polyurethane bags containing 10 ℓ of water. There was no significant difference ($P > 0.05$) in average size of the hybrids and *C. gariepinus* within and between the bags. The bags contained tap water which had been kept in a 200 ℓ barrel for 10 days, through which air had been bubbled to remove any trace of chlorine. Evaporated marine salt was added to the replicated bags so that the water in the bags had a salinity of 4, 8 or 12 g.ℓ$^{-1}$. No salt was added to two bags, which were used as controls.

Approximately 40 ℓ of air was pumped into the bags. The bags were then sealed using elastic bands, and were floated in a large water bath, which was set at a temperature of 28°C. They were opened every 24 hours to remove dead fish and to take a water sample, after which the bags were re-inflated with air. The experiment was run over 96 hours, according to the accepted method for ascertaining the median lethal concentration (96 hour LC_{50}) of toxic substances for aquatic organisms (Sprague, 1973).

Salinity was measured using an Enco® salinity probe, and all measurements were verified using a refraction salinometer. Oxygen concentration and water temperature were measured using an OxyGuard® Handy Mk II portable oxygen probe. Nitrite and hardness ($CaCO_3$) concentrations were measured using a Hach DR-EL/4 spectrophotometric water quality analyzer, and an Orion® probe was used to ascertain the pH of the water.

The median lethal salinity for the hybrid and *C. gariepinus* juveniles was found using log-probit graphs

as in the ammonia experiment. The percent mortalities of *C. gariepinus* juveniles and hybrid juveniles after 96 hours were plotted against salinity on a log-probit scale. The median lethal salinity and standard deviation were read off the graph following Sprague (1973) and Sokal and Rohlf (1973). After the experiment the surviving fish were returned to the holding tank and observed for a further seven days.

To test the instantaneous salinity tolerance of the catfish juveniles, five *C. gariepinus* juveniles ($\bar{x}$ = 81.6 ± 8.8 mmTL, 3.5 ± 1.1 g) and five HL♂xCG♀ hybrid juveniles ($\bar{x}$ = 86.4 ± 7.2 mmTL, 5.1 ± 0.9 g) were introduced into 5.0 ℓ glass aquaria containing distilled water. Each aquarium were placed onto a heated magnetic stirring apparatus. The heating plate kept the water in the aquarium at a temperature of 28.0 ± 0.5 °C. The magnetic stirring rod was set to revolve just fast enough to ensure complete mixing of the water in the aquarium. The fish were allowed to settle in the aquaria for one hour, after which 2.0 g.ℓ^{-1} salt was added to the water. Thereafter, the salinity of the water was increased by 2.0 g.ℓ^{-1} every 60 minutes, until the fish began to die. A control aquarium was set up in the same way as above, but salt was not added to the water. The experiment was replicated. The surviving fish were removed from the aquaria after the trials, and were placed in a recovery tank containing fresh water.

6.3. RESULTS

Temperature preference

The only difference in the replicates of the *C. gariepinus* temperature preference experiment occurred at 32 °C, where the frequency of occurrence of fish at this temperature was significantly higher for replicate two (Table 6.2).

Table 6.2. The difference in the frequency of occurrence of *C. gariepinus* in the 25-33 °C temperature gradient for three replicates.

Temperature (°C)	R.1 (%)	R.2 (%)	R.3 (%)	Mean (% ± sd)	*P*(0.05)
25	0.0	1.6	2.6	1.4 ± 1.1	0.49
26	8.1	5.7	5.9	6.6 ± 1.1	0.86
27	6.3	6.1	9.6	7.3 ± 1.6	0.45
28	19.4	17.1	17.7	18.1 ± 1.0	0.98
29	16.3	19.5	16.4	17.4 ± 1.5	0.25
30	23.0	17.7	19.1	19.9 ± 2.3	0.77
31	10.6	13.2	11.5	11.8 ± 1.1	0.34
32	9.7	12.4	8.6	10.2 ± 1.6	0.02*
33	6.6	6.8	8.6	7.3 ± 0.9	0.35

* Significant difference

As the overall difference between the replicates was not significant, the data was pooled. The *C. gariepinus* juveniles showed a normal distribution within the temperature gradient of the pooled data. Most of the fish congregated in the 28 °C to 30 °C zone, occurring with less frequency in the higher or lower ranges of the gradient (Fig. 6.3). The frequency at which the fish occurred in the 28-30 °C temperature zone was significantly higher ($P < 0.05$) than at any of the other temperatures (Table 6.3). It is clear from Table 6.3 that the zone of temperature preference for the *C. gariepinus* juveniles was between 28 and 30 °C.

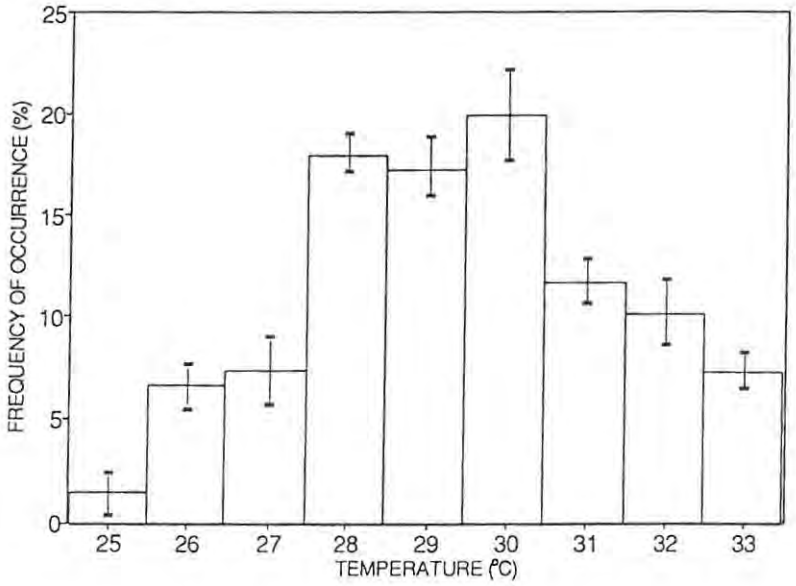

Figure 6.3. The frequency of occurrence of *C. gariepinus* juveniles at temperatures within a 25 °C to 33 °C thermal gradient.

Table 6.3. A Tukey multiple range test depicting the water temperatures at which *C. gariepinus* juveniles occurred in similar frequencies. Homogeneous groups are linked by the same characters.

Temperature (°C)	Mean frequency (%)	Homogeneous groups
25	1.4	a
26	6.6	a b
27	7.3	a b c
28	18.1	d
29	17.4	d
30	19.9	d
31	11.8	c
32	10.2	b c
33	7.3	a b c

The daily variation in the frequency of occurrence of the hybrids in the 25-33 °C temperature gradient was high for all the replicates. When the replicates were compared using an analysis of variance, no significant differences were found between the data sets (Table 6.4).

Table 6.4. The difference in the frequency of occurrence of the hybrid in the 25-33 °C temperature gradient for three replicates.

Temperature (°C)	R.1 (%)	R.2 (%)	R.3 (%)	Mean (% ± sd)	*P*(0.05)
25	4.2	7.3	6.5	6.0 ± 1.3	0.65
26	6.3	4.2	4.9	5.1 ± 0.9	0.83
27	10.1	9.3	9.8	9.8 ± 0.3	0.94
28	13.0	14.3	8.5	12.2 ± 2.7	0.14
29	10.5	11.4	12.0	11.3 ± 0.6	0.90
30	20.5	13.4	16.8	16.9 ± 2.9	0.54
31	12.5	11.9	16.5	13.6 ± 2.0	0.16
32	16.6	14.6	11.8	14.4 ± 2.0	0.27
33	16.7	13.5	13.2	14.5 ± 1.5	0.79

As there were no statistical differences between the replicates, the frequency of occurrence data were pooled. The pooled hybrid distribution frequency was quite different to that of *C. gariepinus* (Fig. 6.4). The hybrids had a similar lower temperature preference limit of 28 °C, but unlike *C. gariepinus* they showed no upper limit in the 25-33 °C temperature gradient (Table 6.5).

Table 6.5. A Tukey multiple range test depicting water temperatures at which hybrid juveniles occurred in similar frequencies. Homogeneous groups are linked by the same characters.

Temperature (°C)	Mean frequency (%)	Homogeneous groups
25	6.0	a
26	5.1	a
27	9.8	a b c
28	12.2	b c d
29	11.3	b c d
30	16.9	b d
31	13.6	b c d
32	14.4	b c d
33	14.5	b c d

These results indicated that the upper limit of the preferred temperature zone of the hybrid was higher than the range of the temperature gradient. The distribution of the fish in the gradient was skewed toward the higher temperatures.

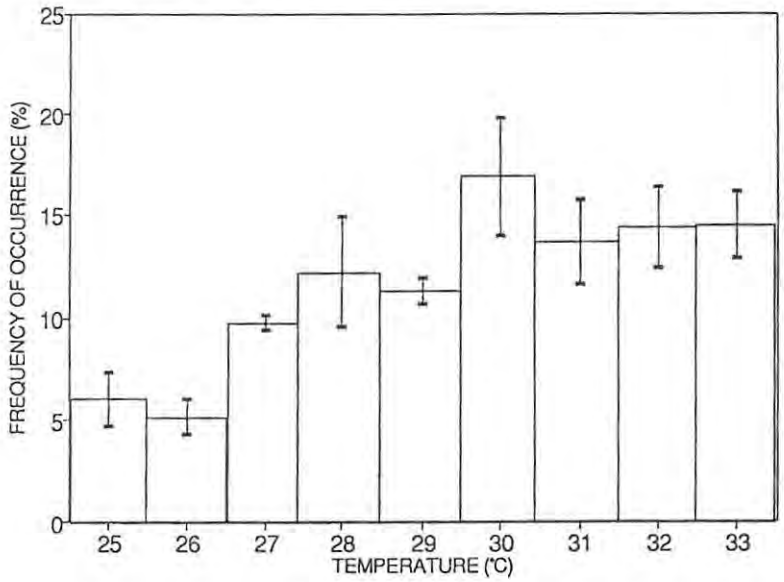

Figure 6.4. The frequency of occurrence of hybrid juveniles at temperatures within a 25 °C to 33 °C thermal gradient.

In the second hybrid temperature gradient (31-35 °C), there was a significantly higher occurrence of fish at 32 °C in the third replicate than in the other replicates (Table 6.6).

Table 6.6. The difference in the frequency of occurrence of the hybrid in the 31-35 °C temperature gradient for three replicates.

Temperature (°C)	R.1 (%)	R.2 (%)	R.3 (%)	Mean (% ± sd)	*P*(0.05)
31	23.8	20.6	19.5	21.3 ± 1.8	0.59
32	8.2	15.5	23.9	15.8 ± 6.4	0.04*
33	14.0	23.5	19.1	18.8 ± 3.9	0.42
34	13.1	18.6	21.2	17.6 ± 3.4	0.87
35	9.3	21.8	16.3	15.8 ± 5.1	0.45

* Significant difference

As this was the only statistical difference between the three replicates, the frequency of occurrence data were pooled. There was no significant difference ($P > 0.05$) in the frequency of occurrence of the hybrids at any of these higher temperatures for the pooled data over the eight days of the experiment (Fig. 6.5).

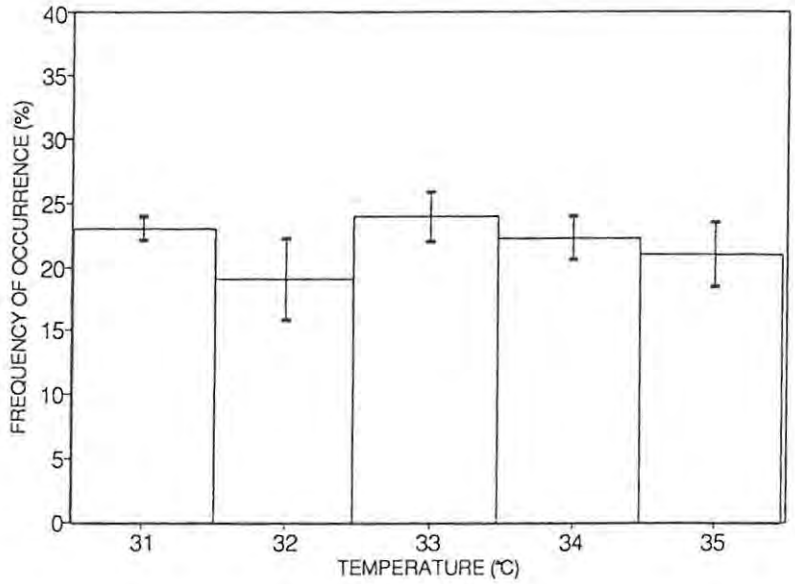

Figure 6.5. The frequency of occurrence of hybrid juveniles within a 31 °C to 35 °C thermal gradient.

The daily distribution of the fish in the 31 °C to 35 °C temperature gradient were compared using an analysis of variance, and a 95 % Tukey multiple range test. It was discovered that the sample day had a significant effect ($P < 0.05$) on the distribution of the hybrids at the lowest (31 °C) and highest (35 °C) temperatures (Fig. 6.6). The scatterplot showed that the hybrids congregated at 31 °C with increasing frequency as the experiment progressed (Fig. 6.6), while their frequency of occurrence at 35 °C decreased accordingly.

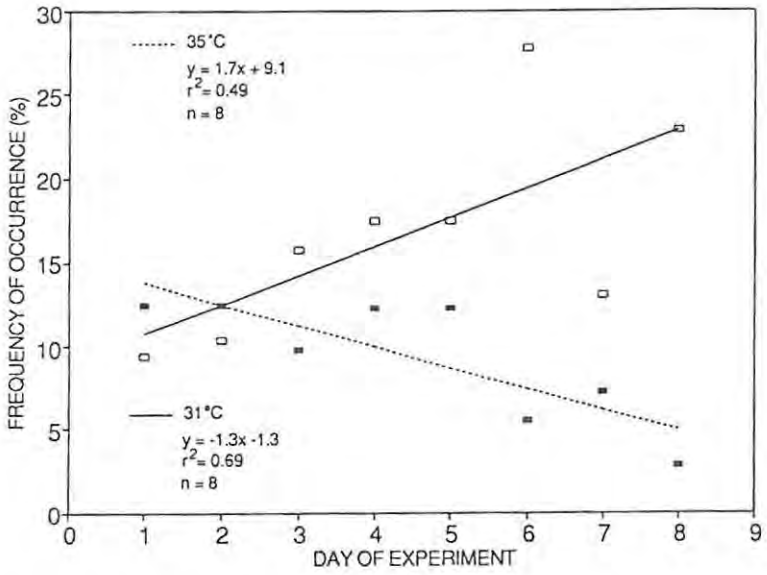

Figure 6.6. The change in the frequency of hybrid juveniles occurring at temperatures of 31 °C and 35 °C over eight days.

From these trends it appeared that the hybrids in the 31-35 °C temperature gradient were slow to gravitate to their final temperature preferendum. The transition from holding tank water at a temperature of 28 °C to the 31-35°C temperature gradient may have led to an initial overshoot phenomenon (see discussion). Once the data collected during the first 48 hours of the experiment had been excluded from the data set, a significantly lower frequency of fish were found to occur above a temperature of 34 °C (Table 6.7). Overall it was concluded that the hybrid had a wider zone of final temperature preference than *C. gariepinus*, from 28 °C to 34 °C (Fig. 6.7).

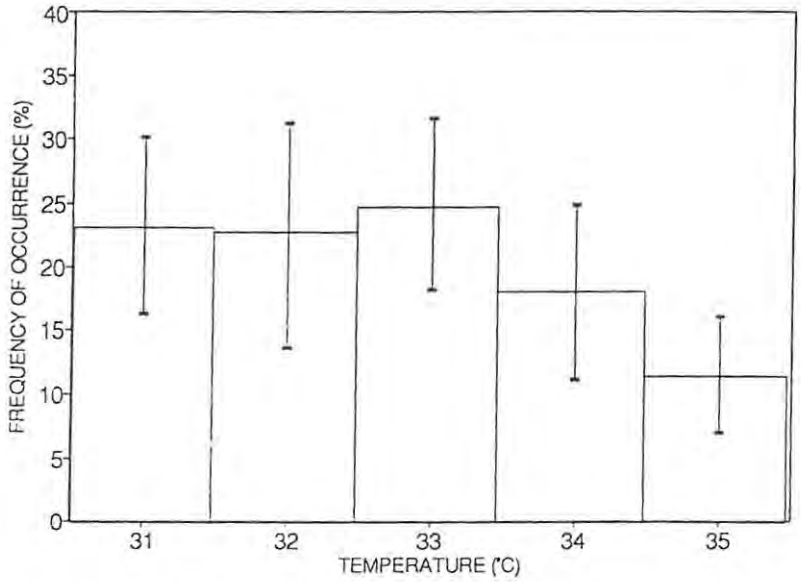

Figure 6.7. The frequency of occurrence of hybrid juveniles at temperatures within a thermal gradient, during the last six days of the second hybrid thermal preference trial.

Table 6.7. A Tukey multiple range test showing water temperatures at which hybrid juveniles occurred in similar frequencies. Homogeneous groups are linked by the same characters.

Temperature (°C)	Mean frequency (%)	Homogeneous groups
31	23.1	a
32	22.6	a
33	24.8	a
34	18.0	a b
35	11.5	b

The effect of oxygen concentration on air-breathing frequency

The oxygen consumption rate curves of the *C. gariepinus* and hybrid juveniles in the respirometer were not linear; oxygen consumption decreased as the oxygen concentration of the water decreased (Fig.

6.8). This indicated that it became increasingly more difficult for the fish to breathe aquatic oxygen as the oxygen concentration decreased.

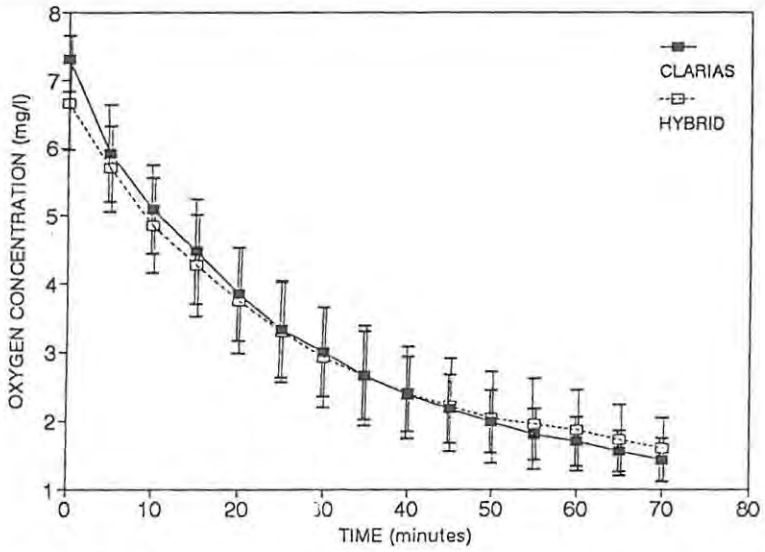

Figure 6.8. Comparison of the decrease in oxygen concentration in the respirometer over time for the hybrid and *C. gariepinus* juveniles (Vertical bars indicate standard deviation).

The individual air-breathing rate of the fish in the respirometer was highly variable, but there was a significant increase ($P < 0.05$) in the mean air-breathing frequency of the hybrids over time (Table 6.8), which was related to the change in oxygen concentration in Figure 6.9.

Table 6.8. The relationship between the air-breathing frequency of the hybrids and the decrease in oxygen concentration in the respirometer, in relation to time. Homogeneous groups are linked by the same characters.

Time (minutes)	$[O_2]$ (mg.ℓ^{-1})	Air-breaths (%)	Homogeneous groups
5	5.7 ± 0.6	2.7 ± 2.6	a b
10	4.9 ± 0.7	2.4 ± 2.2	a
15	4.3 ± 0.7	2.6 ± 3.7	a
20	3.8 ± 0.8	3.9 ± 4.2	a b c
25	3.3 ± 0.7	5.0 ± 5.4	a b c d
30	2.9 ± 0.7	6.6 ± 4.3	a b c d
35	2.7 ± 0.7	8.2 ± 4.1	a b c d
40	2.4 ± 0.7	10.4 ± 4.8	c d
45	2.2 ± 0.7	9.1 ± 4.3	b c d
50	2.1 ± 0.7	9.1 ± 2.5	b c d
55	2.0 ± 0.7	10.7 ± 3.7	d
60	1.9 ± 0.6	10.6 ± 3.4	d
65	1.7 ± 0.5	8.3 ± 2.8	a b c d
70	1.6 ± 0.5	9.7 ± 4.8	c d

± Standard deviation

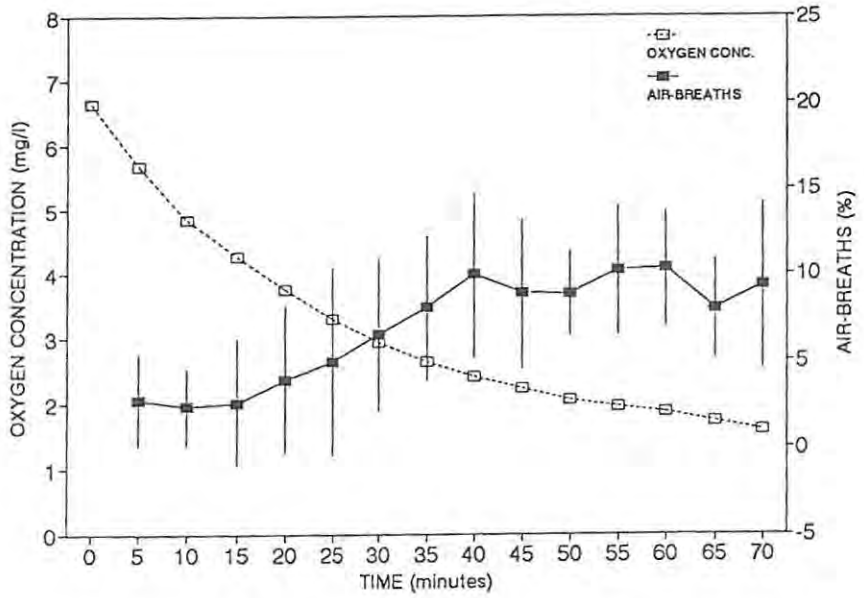

Figure 6.9. The change in the air-breathing frequency of the hybrid, and the decrease in oxygen concentration in the respirometer over time (Vertical bars indicate standard deviation).

The air-breathing frequency of the hybrids began to increase at an oxygen concentration of 3.8 ± 0.8 mg.ℓ^{-1} (50 % saturation), and stabilized at 2.2 ± 0.7 mg.ℓ^{-1} (29 % saturation). The air-breathing frequency of the hybrid increased from an average of 2.5 % to an average of 9.3 % over the duration of the experiment, which was equivalent to a change from an air-breath every 6.4 minutes to one every 1.7 minutes.

Clarias gariepinus juveniles also showed a trend toward an increased air-breathing frequency at low oxygen concentrations (Fig. 6.10), but the variations about the means were too large for this trend to be statistically significant ($P > 0.05$). Nevertheless, from Figure 6.10 it appears that the average air-breathing frequency of *C. gariepinus* increased after the oxygen concentration in the respirometer had decreased to 3.0 ± 0.7 mg.ℓ^{-1} (40 % saturation). Once the oxygen concentration decreased to 1.7 ± 0.4 mg.ℓ^{-1} (23 % saturation) the air-breathing frequency stabilized. The average percentage of air-breathing events increased from 3.3 % to 10 % as the oxygen concentration decreased; which was a change from an air-breath every 8.7 minutes to one every 2.9 minutes.

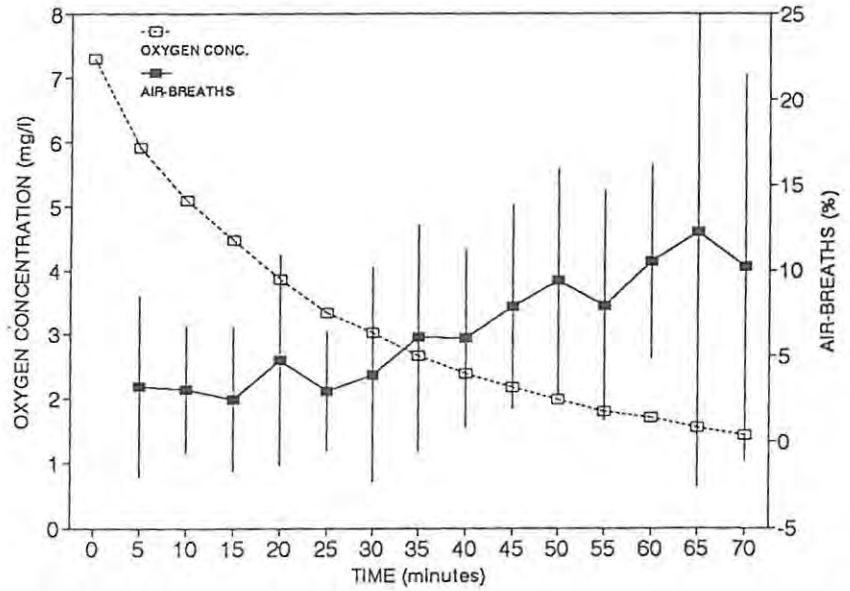

Figure 6.10. The change in the air-breathing frequency of *C. gariepinus*, and the decrease in oxygen concentration in the respirometer over time (Vertical bars indicate standard deviation).

These results indicate that although the rates of atmospheric respiration were highly variable in the fishes, they were elevated by oxygen concentrations below 4.0 mg.ℓ^{-1}.

Median lethal ammonia concentration

All of the experimental fish exposed to an NH_3 concentration of 16.2 ± 1.1 mg.ℓ^{-1} died within twenty-four hours. After 72 hours, two *C. gariepinus* juveniles died at an NH_3 concentration of 5.8 ± 1.4 mg.ℓ^{-1}. No other deaths occurred during the experiment (Table 6.9).

Table 6.9. Hybrid and *C. gariepinus* juvenile mortality at different concentrations of NH_3 over 96 hours.

[NH_3]	Species	24 Hours	48 Hours	72 Hours	96 Hours
		(%)	(%)	(%)	(%)
0.0 mg.ℓ^{-1}	*C. gariepinus*	0	0	0	0
	Hybrid	0	0	0	0
2.3 mg.ℓ^{-1}	*C. gariepinus*	0	0	0	0
	Hybrid	0	0	0	0
5.8 mg.ℓ^{-1}	*C. gariepinus*	0	0	20 ± 20	20 ± 20
	Hybrid	0	0	0	0
16.2 mg.ℓ^{-1}	*C. gariepinus*	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Hybrid	100 ± 0	100 ± 0	100 ± 0	100 ± 0

By log-probit analysis (Fig. 6.11), the 96 hour median lethal NH_3 concentration for the HL♂xCG♀ hybrid was found to be 9.1 ± 1.4 mg.ℓ^{-1} . The 96 hour LC_{50} NH_3 concentration for *C. gariepinus* was lower, at 6.5 ± 1.5 mg.ℓ^{-1} (Fig. 6.12).

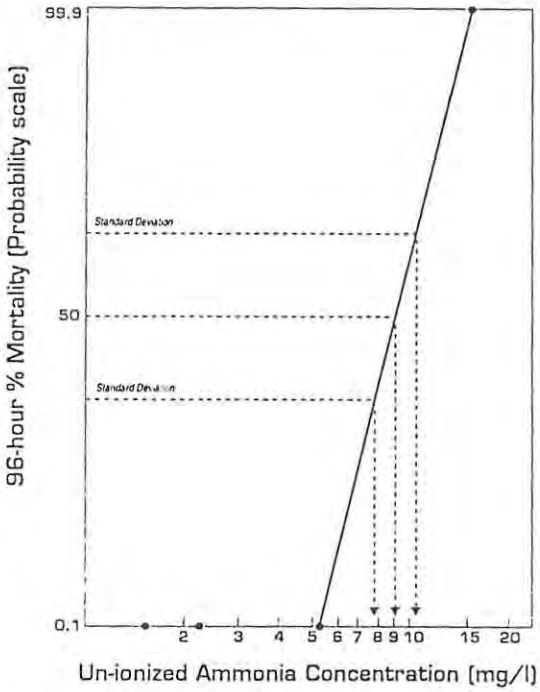

Figure 6.11. A log-probit graph showing the NH_3 concentration at which 50 % mortality of the hybrid juveniles occurred.

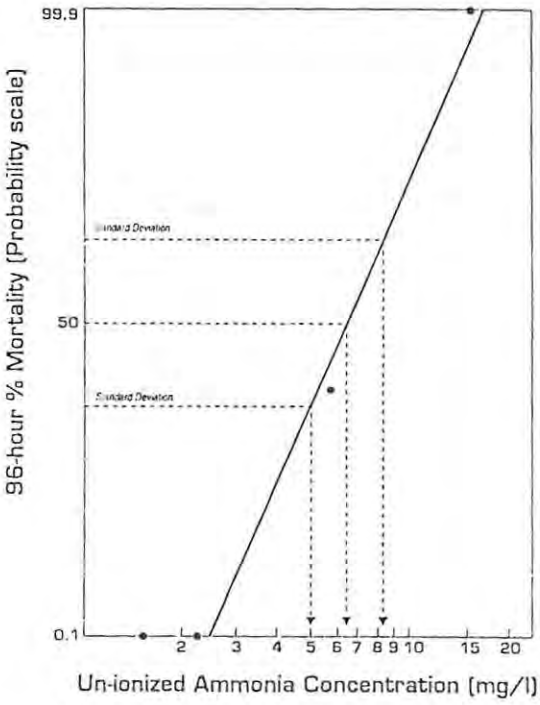

Figure 6.12. A log-probit graph showing the NH_3 concentration at which 50 % mortality of the *C. gariepinus* juveniles occurred.

The environmental conditions to which the hybrid and *C. gariepinus* juveniles were exposed during the 96 hours of the experiment are summarized in Table 6.10.

Table 6.10. The mean water quality parameters for the replicated treatments of the 96 hour NH_3 LC_{50} experiment.

Treatment	Temp. (°C)	pH	$[O_2]$ (mg.ℓ^{-1})	$[NH_3]$ (mg.ℓ^{-1})	$[NO_2]^*$ (mg.ℓ^{-1})	$[NO_3]^*$ (mg.ℓ^{-1})
Control	31.6±0.7	7.0±0.1	4.0±1.9	0.5±0.3	0.19±0.06	8.8±4.4
Low	31.4±1.0	7.0±0.1	4.1±1.9	2.3±0.6	0.08±0.02	26.4±4.4
Medium	31.5±1.1	7.0±0.1	4.2±1.8	5.8±1.4	0.12±0.04	37.4±2.2
High	31.6±0.5	7.0±0.1	4.6±2.1	16.2±1.1	n/m	n/m

* Measured after 96 hours, n/m = Not measured, ± Standard deviation

An analysis of variance showed that there was no significant differences ($P > 0.05$) in overall water temperature, pH, oxygen or nitrite concentrations for the replicated experimental treatments. However, nitrate concentration did increase with increasing ammonia concentration ($P < 0.05$), which means that some of the ammonia and nitrite in the bags was being oxidised to nitrate. At the same time ammonia was being produced by the fish as a consequence of metabolic activity. Although no NH_4Cl was added to the control bags, an average of 0.5±0.03 mg.ℓ^{-1} of NH_3 was measured in the bags over the experimental period.

Median lethal salinity

The first mortalities in the salinity LC_{50} experiment occurred after 48 hours, at the highest salinity (12.4 g.ℓ^{-1}). After 96 hours, 70±10 % of the hybrids and 80 % of the *C. gariepinus* juveniles had died at this salinity (Table 6.11). The only other mortalities occurred at a salinity of 8.2 g.ℓ^{-1}, where 10±10 % of the hybrids exposed to this salinity died after 72 hours. The surviving fish were returned to their holding tank, where they were observed for a week. However, no further mortalities occurred.

The percentage mortalities of the fish in the treatment bags after 96 hours were plotted against the salinity of the water in the bags on log-probit scales. The median lethal salt concentration for HL♂xCG♀ hybrid juveniles was found to be 11.0±2.5 g.ℓ^{-1} (Fig. 6.13), while the LC_{50} for *C. gariepinus* was 10.8±0.8 g.ℓ^{-1} (Fig. 6.14).

Table 6.11. Hybrid and *C. gariepinus* juvenile mortality at different salinities over 96 hours.

Salinity	Species	24 Hours	48 Hours	72 Hours	96 Hours
		(%)	(%)	(%)	(%)
0.6 g.ℓ^{-1}	*C. gariepinus*	0	0	0	0
	Hybrid	0	0	0	0
4.4 g.ℓ^{-1}	*C. gariepinus*	0	0	0	0
	Hybrid	0	0	0	0
8.2 g.ℓ^{-1}	*C. gariepinus*	0	0	0	0
	Hybrid	0	0	10 ± 10	10 ± 10
12.4 g.ℓ^{-1}	*C. gariepinus*	0	40 ± 20	80 ± 0	80 ± 0
	Hybrid	0	20 ± 0	70 ± 10	70 ± 10

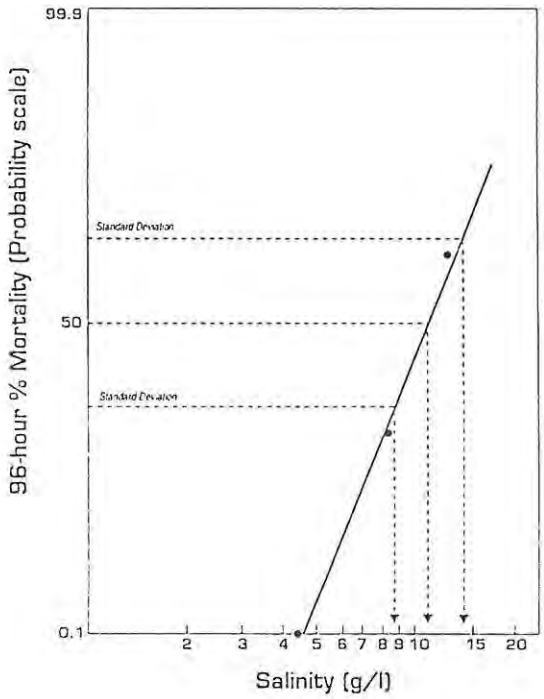

Figure 6.13. A log-probit graph showing the salinity at which 50 % mortality of the hybrid juveniles occurred.

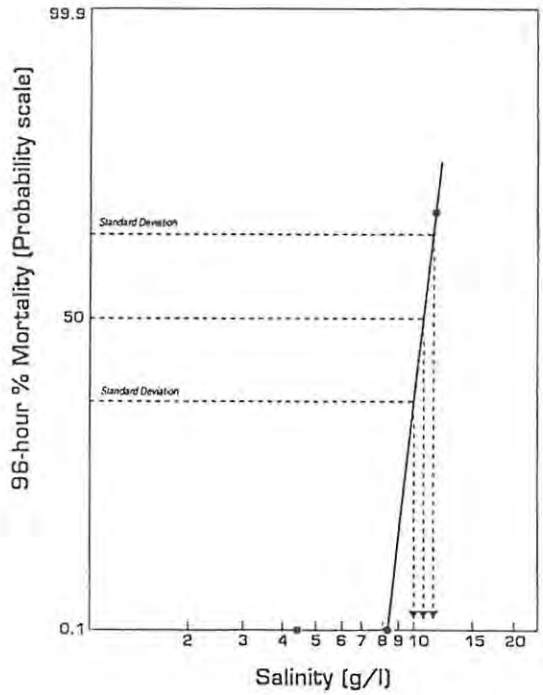

Figure 6.14. A log-probit graph showing the salinity at which 50 % mortality of the *C. gariepinus* juveniles occurred.

There were no significant differences ($P > 0.05$) in temperature, pH, oxygen, ammonia or nitrite concentrations of the water in the sealed bags. However, hardness (measured as $CaCO_3$) increased with increasing salinity (Table 6.12).

Table 6.12: The mean water quality parameters for the replicated treatments of the 96 hour salinity LC_{50} experiment.

Treatment	Salinity ($g.\ell^{-1}$)	Temp. (°C)	$[O_2]$ ($mg.\ell^{-1}$)	pH	NH_3 ($mg.\ell^{-1}$)	NO_2^- ($mg.\ell^{-1}$)	$CaCO_3$ ($mg.\ell^{-1}$)
Control	0.6 ± 0.2	27.7 ± 1.1	8.4 ± 4.8	6.6 ± 0.1	< 0.1	1.2 ± 0.2	83.8 ± 8.9
Low	4.4 ± 0.3	27.9 ± 1.1	7.8 ± 4.0	6.6 ± 0.1	< 0.1	0.9 ± 0.5	104.5 ± 28.8
Medium	8.2 ± 0.4	27.8 ± 1.1	6.2 ± 4.6	6.6 ± 0.1	< 0.1	1.2 ± 0.7	113.8 ± 28.8
High	12.4 ± 0.4	27.7 ± 1.0	8.2 ± 5.8	6.6 ± 0.1	< 0.1	1.0 ± 0.4	136.0 ± 52.4

± Standard deviation

In the instantaneous salinity tolerance experiment, neither of the two groups of fish showed signs of stress until the salinity of the water in the experimental chamber was raised above 2.0 $g.\ell^{-1}$. Henceforth, the fish became agitated each time the salinity of the water was raised, but they settled down after a while. Once the solution reached a salinity of 14 $g.\ell^{-1}$ the fish displayed characteristic

stress coloration. Their normal coloration faded to a light grey, with dark grey lips, indicating an excessive release of adrenaline (Babiker, 1984). They also showed characteristic stress behaviour, floating vertically with their heads just below the surface of the water. Once a salinity of 16 $g.\ell^{-1}$ was reached, 70 ± 10 % of the *C. gariepinus* juveniles died, while only 10 ± 10 % of the hybrids died. The surviving hybrids had a 100 % recovery rate after the experiment, but all the *C. gariepinus* juveniles died. No control fish died during the experiments.

6.4. DISCUSSION

The final zone of temperature preference between 28 °C and 30 °C for *C. gariepinus* juveniles was identical to that of the larvae and early juveniles of this species as described by and Britz and Hecht (1987). The temperature preference zone of the HL♂xCG♀ hybrid was wider, ranging from 28 °C to 34 °C.

Initially the hybrids explored the 31 °C to 35 °C temperature gradient tank without showing preferential temperature bias. In time, however, they began to enter the 34 °C and 35 °C regions of the temperature gradient less frequently. This behaviour may have resulted from their relatively low acclimation temperature (28 °C), prior to their immersion into the 31 °C to 35 °C temperature gradient. Fish are able to acclimate to different temperatures by metabolic compensation, including changes in the lipid composition of cell walls, the induction of iso-enzymes and allo-enzymes, and changes in enzyme concentration (Evans, 1990; Kelsch and Neill, 1990). Fry (1947) introduced the concept of the "scope for activity" of a fish, which is the difference between its standard or resting metabolic rate and its active metabolic rate. According to the model developed by Kelsch and Neill (1990), a fish would choose to frequent a temperature at which the scope between its standard and active metabolic activity was largest. If this temperature was not available, metabolic compensation would lead to the maximisation of the scope of metabolic activity of the fish over time (acclimation). Acclimation time depends on the change in temperature and the amount of metabolic compensation necessary by the fish, but normally ranges between 24 hours and 96 hours (Evans, 1990). The most likely explanation for the initial lack of temperature preference in the hybrids in the 31 °C to 35 °C temperature gradient was that they were moving from temperature to temperature in an attempt to optimise their scope for activity. Once the fish acclimated to the higher temperatures through metabolic compensation, they began to congregate in their zone of final temperature preference.

Kilambi and Galloway (1985) also found that the *Ctenopharyngodon idella* x *Hypothalmichthys nobilis* carp hybrids showed great variation in temperature preference, during the first nine days of exposure to a thermal gradient. Only on the tenth day did the hybrid carp congregate around their final temperature preference.

The hybrids' wider temperature preference zone may be an indication that the final temperature preferendum of *H. longifilis* is higher than that of *C. gariepinus*. It was not possible to define the final temperature preference of *H. longifilis*, but there is good circumstantial evidence that it might be higher than that of *C. gariepinus*. For example, *C. gariepinus* has colonized both temperate and tropical rivers, and has the widest latitudinal distribution of all freshwater fishes (37°00' N to 34°20' S; Skelton and Teugels, 1991; Skelton and Teugels, 1992). The distribution of *H. longifilis*, on the other hand, is restricted to the warmer tropical regions of Africa (26°00' N to 18°00' S; Teugels *et al.*, 1990; Skelton and Teugels, 1991) (see Fig. 2.1). Moreover, the fertilized eggs of *H. longifilis* can tolerate water temperatures between 22 °C and 35 °C (Legendre and Teugels, 1991), while *C. gariepinus* eggs can develop in water temperatures ranging from 19 °C to 33 °C (Bruton, 1977). Fertilized *C. gariepinus* eggs also develop faster than *H. longifilis* eggs for a given temperature. For example, *C. gariepinus* eggs hatched within 20 hours at an incubation temperature 30 °C, while *H. longifilis* eggs hatched within the same time at a temperature of 33 °C (Fig. 6.15). The rate of egg development depends on the metabolic activity of the egg cells, so the optimum temperature for metabolic activity must have been higher for *H. longifilis* eggs than for *C. gariepinus* eggs. Thus it appears that the *H. longifilis* eggs are adapted to higher water temperatures.

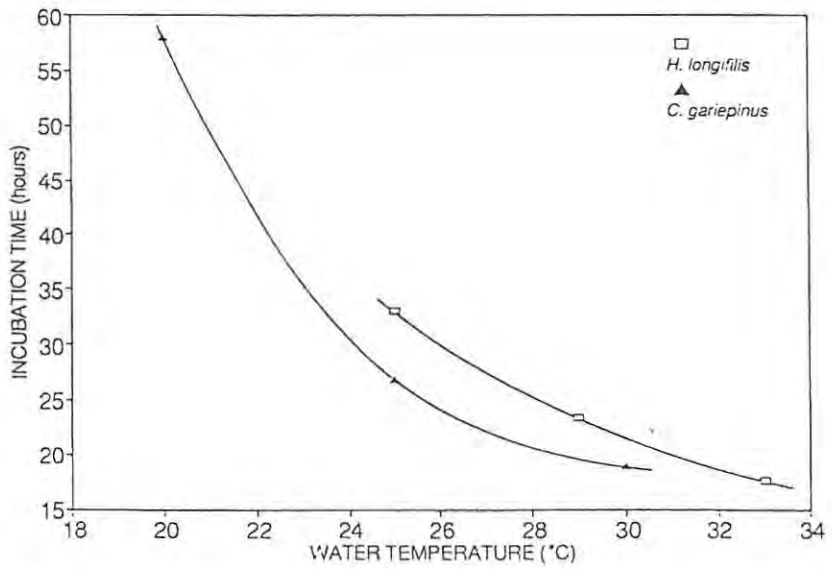

Figure 6.15. The relationship between the development rate of *C. gariepinus* and *H. longifilis* eggs and water temperature. Summarized from Hogendoorn and Vismans (1980) and Legendre and Teugels (1991).

Because the temperature preference of *H. longifilis* is not known it can only be hypothesised at this stage that the wide zone of final temperature preference of the hybrids is a result of the combination of the temperature preferenda inherited from both the *H. longifilis* and *C. gariepinus* genotypes. This

type of thermal inheritance has been reported in the stickleback hybrid (Ziuganov and Gomeluk, 1985). The stickleback hybrid had a wide temperature tolerance range, inheriting both the low temperature tolerance of *Pungitius pungitius* and the relatively high thermal tolerance of *P. platygaster*.

However, the temperature preferenda and tolerances inherited by the hybrid offspring of two species with different temperature preferenda can be manifest in other ways. For example, the sparid *Acanthopagrus schlegeli* x *Sparus sarba* hybrid only inherited the temperature tolerance of its maternal parent (Kitajima and Tsukashima, 1983). The *Salvelinus fontinalis* x *S. namaycush* trout hybrid had a final temperature preferendum of 14.6 °C, midway between that of *S. fontinalis* (17.5 °C) and *S. namaycush* (10.8 °C) (Peterson *et al.*, 1979). On the other hand, the final thermal preferendum of *Ctenopharyngodon idella* and *Hypothalmichthys nobilis* were almost identical (25.3 °C and 25.4 °C respectively), but their hybrid had a final temperature preference of 28.2 °C (Bettoli *et al.*, 1985).

Water temperature has an effect on the aquatic respiration of catfish in two principle ways. Firstly, the metabolic rate of an animal increases exponentially with its body temperature (Eckert, 1988). As the catfish are poikilothermic, their basal metabolic rates would be dependent on ambient water temperature. Because the oxygen consumption rate of an animal is an indication of its metabolic activity (Eckert, 1988), the oxygen consumption of the catfish would vary according to ambient water temperature. Secondly, the oxygen saturation point of water is inversely proportional to temperature (Morris, 1978). In other words, the higher the water temperature, the more oxygen consumed by the fishes, but there is less potential oxygen available in the water. For this reason the oxygen levels in warm water aquaculture operations need to be continuously monitored.

At a water temperature of 30 °C there was no statistical difference in the air-breathing frequency of *C. gariepinus* in poorly or well oxygenated water. The differences in the activities of the individual fish seemed to influence their mean air-breathing frequency more than the oxygen concentration of the water. Johnston *et al.* (1983) found that although the surfacing frequency of *C. gariepinus* remained unaltered when they were exposed to acute anoxia, their utilization of oxygen obtained through aerial respiration increased from 25 % to 70 %. Thus, while the surfacing behaviour of *C. gariepinus* was somewhat rhythmic (Abdel-Magid, 1971), the efficiency of the accessory respiratory organ was variable (Johnston *et al.*, 1983). If these observations were correct, then the aquaculturist would have no need to monitor and adjust the oxygen levels of the water in the fish farm, as oxygen concentration would not effect the surfacing frequency of *C. gariepinus* to any great extent.

However, there did appear to be a trend in the relationship between the air-breathing frequency of *C. gariepinus* and oxygen concentration when the data were represented graphically (Fig. 6.11). This trend was shown to be statistically significant for the hybrid (Fig. 6.10). As the trends were so similar for both species, it is likely that a relationship does exist between the air-breathing frequency of *C.*

gariepinus and oxygen concentration. However, the variation in air-breathing frequencies about the means for *C. gariepinus* was far greater than for the hybrid, and this variation probably masked any statistical relationships within the data.

The trend noted in the reaction of the two species to decreasing oxygen concentration can be described as a transition between two steady states. Air-breathing frequency remained approximately constant until a threshold oxygen concentration was reached. The air-breathing frequency then increased with decreasing oxygen content until a level was reached beyond which air-breathing frequency once again became constant, albeit at a higher frequency. The two "states" in this case were the aquatic respiration and the aerial respiration modes of the catfish.

Even at high and low concentrations of oxygen both modes of respiration were pursued, but at different rates, and most likely at different levels of efficiency (Johnston *et al.*, 1983). The aquatic respiration mode appeared to be predominant at oxygen concentrations above 3 to 4 mg.ℓ^{-1}, while the aerial mode was predominant at oxygen concentrations below 2 mg.ℓ^{-1}. Abdel-Magid (1971) also found that air-breathing in *C. gariepinus* increased when the oxygen concentration of water was between 3 to 4 mg.ℓ^{-1}, but it decreased once again as the fish acclimated to lower oxygen concentrations.

The partial pressure (PO_2) of the oxygen in the water must be higher than the partial pressure of oxygen in the blood of a fish to allow oxygen diffusion across the gills to take place (Randall, 1970). A pressure gradient of about 40 to 100 mmHg PO_2 must exist between the water and the blood, for the blood to become saturated with oxygen (Randall, 1970). Most species exhibit a threshold PO_2 value, below which oxygen consumption begins to decline as the PO_2 in the water declines. This threshold is called the critical partial pressure (Pc). Some examples of critical partial pressures of oxygen for fishes are presented in Table 6.13.

Table 6.13. Critical partial pressures of oxygen for some freshwater teleosts.

Species	Pc (mmHg)	Reference
Oncorhynchus mykiss	75-85	Burton and Heath, 1980
Salvelinus fontinalis	±80	Beamish, 1964
Lepomis macrochirus	60-100	Burton and Heath, 1980
Clarias batrachus	60-100	Hughes and Singh, 1971
Saccobranchus fossilis	50-100	Hughes and Singh, 1971
Anguilla marmoratus	30-70	Graham and Baird, 1984
Anguilla japonica	±60	Chan, 1986
Carassius carassius	±40	Burton and Heath, 1980

The air-breathing frequency of the fishes began to increase at an oxygen concentration of 3.0 $mg.\ell^{-1}$ for *C. gariepinus* and 3.8 $mg.\ell^{-1}$ for the hybrid. The PO_2 of the water in the respirometer at these concentrations was estimated following Forteath (1992). At a water temperature of 30 °C, 548 m above sea level, 3.0 $mgO_2.\ell^{-1}$ had a PO_2 of 56.4 mmHg, while 3.8 $mgO_2.\ell^{-1}$ had a PO_2 of 71.5 mmHg. Thus the critical partial pressure of oxygen for the hybrid and *C. gariepinus* was 56.4 mmHg and 71.5 mmHg respectively.

Some warm water species of fish have haemoglobin with high oxygen affinity (ie. eels and carp), which allows their blood to become saturated with oxygen at a lower PO_2 than species with haemoglobin having a lower oxygen affinity (ie. salmonids) (Forteath, 1992). The relatively low Pc of *C. gariepinus* and the hybrid (compared to the Pcs in Table 6.13) are most likely due to their blood containing haemoglobin with high oxygen affinity, as well as oxygen supplementation through air-breathing.

The hybrid had a higher Pc than *C. gariepinus*. The PO_2 at which air-breathing frequency stabilized was higher in the hybrid (41.4 mmHg) than in *C. gariepinus* (32.0 mmHg). The hybrid also had a higher average air-breathing frequency than *C. gariepinus*, at both high and low concentrations of oxygen. Opercular beat rate is an indirect measure of a fish's aquatic respiration rate (Abdel-Magid, 1971). The average opercular beat rate of the hybrid was lower than that of *C. gariepinus* (pers. obs.). This was most likely a result of the higher air-breathing frequency of the hybrid, as the opercular beat rate of the individual fish usually decreased for a short while after aerial respiration had taken place (pers. obs.; Abdel-Magid, 1971).

Although none of the above differences were statistically significant, the overall impression from these data is that the juvenile hybrids were less tolerant of low oxygen levels and more dependent on air-breathing than *C. gariepinus* juveniles. This dependence may have been inherited from the *H. longifilis* genotype. If my supposition that *H. longifilis* has a higher temperature preference than *C. gariepinus* is correct, then the oxygen saturation point of the water in which they occur would be low. The species may have adapted to lower oxygen levels by becoming more dependent on aerial respiration. Unfortunately no work has been done on the oxygen requirements and temperature preference of *H. longifilis*, or the relative efficiency of the aquatic and aerial respiratory modes of this species.

It follows that a fish with an increased air-breathing rate spends more time and energy swimming to the surface than other fish. This does not appear to have an adverse effect on the fish's growth rate. Kaiser *et al.* (1995b) studied the effect of tank design on the growth and behaviour of larval *C. gariepinus*. One set of tanks was shallow, with a large bottom surface area, while the other set was deep, with a small bottom surface area (volume was identical in both sets of tanks). The larvae in the deep tanks had to swim further to air-breathe, and spent more time swimming in the water column as

they had less surface area on which to rest. Kaiser *et al.* (1995b) found no differences in the growth rates or the behaviour of the two groups. Haylor (1992) also found that the growth rates of different groups of *C. gariepinus* larvae were the same, even though one group had to swim a distance 3.8 times that of the other groups to air-breathe. The growth rates of juvenile *C. macrocephalus* (±4.6 g) were also not influenced by the distance travelled to air-breathe, even though one group had to travel 3.3 times further per hour than the other group (Bevan and Kramer, 1987). Therefore it seems that the energy expended by the fish to air-breathe is minimal compared to its total metabolic energy budget.

From the above it seems that an increase in air-breathing rate *per se* does not significantly effect the growth rate of air-breathing catfishes. However, the need to air-breathe under ultra-high density conditions could stress the fish. The fish would have to literally force its way to the surface to air-breathe, exposing itself to possible aggressive encounters on the way, and then force its way back into the school of fish. This stress may, however, be minimal compared to the other forms of stress experienced by fish farmed at ultra-high densities. Nevertheless, until it is known to what extent an increase in aerial breathing activity effects the growth of the catfish, it is recommended that they be raised in water with an oxygen concentration of at least 3 mg.ℓ^{-1} (56.4 mmHg PO_2) for optimal growth.

It has been claimed that the air-breathing capability of clariid catfish enables them to tolerate high concentrations of ammonia, because they can decrease their rate of gill ventilation, and hence the rate at which toxins are absorbed (Kulakkattolickal and Kramer, 1988). Certainly, both the HL♂xCG♀ hybrid and *C. gariepinus* juveniles showed an exceptionally high tolerance to NH_3 when compared to most other teleosts (Table 6.14).

The 96 hour NH_3 LC_{50} for the *C. gariepinus* juveniles was almost three times higher than that reported for the larvae of this species (Britz, 1988). This was expected, as the ammonia tolerance of fishes has been shown to increase between the larval and the juvenile stage (Thurston and Russo, 1983).

Nevertheless, the NH_3 tolerance of the *C. gariepinus* juveniles was not exceptional when compared to another clariid catfish, *Clarias batrachus*. Although *C. batrachus* had a 96 hour NH_3 LC_{50} of 4.25 mg.ℓ^{-1} (Theerapongse-Krainara, 1988), its 48 hour NH_3 LC_{50} value was 15.78 mg.ℓ^{-1} (Table 6.14). The 48 hour NH_3 LC_{50} for both the hybrid and *C. gariepinus* juveniles was 9.1 mg.ℓ^{-1}.

Table 6.14. LC_{50} values for different fish species exposed to un-ionized ammonia.

Species	Time	$[NH_3]$	Reference
Oncorhynchus mykiss	8 hours	0.41 - 0.45 mg.ℓ^{-1}	Lloyd & Herbert, 1960
O. mykiss	96 hours	0.16 - 1.10 mg.ℓ^{-1}	Thurston & Russo, 1983
Salmo clarkii	96 hours	0.50 - 0.80 mg.ℓ^{-1}	Thurston *et al.*, 1978
Cynoscion nebulosus larvae	24 hours	0.28 mg.ℓ^{-1}	Daniels *et al.*, 1987
Lepomis macrochirus	--------	0.53 mg.ℓ^{-1}	Diamond *et al.*, 1993
Lates calcarifer	96 hours	0.51 mg.ℓ^{-1}	Hassan, 1992
Morone saxatilis	96 hours	1.01 mg.ℓ^{-1}	Oppenborn & Goudie, 1993
M. chrysops x *M. saxatilis*	96 hours	0.32-0.64 mg.ℓ^{-1}	Weirich, 1993
Odontesthes argentinensis	96 hours	0.80 mg.ℓ^{-1}	Ostrenky & Brugger, 1992
Pimephales promelas	96 hours	0.75 - 3.40 mg.ℓ^{-1}	Thurston *et al.*, 1983
Sparus aurata	96 hours	0.84 - 1.33 mg.ℓ^{-1}	Wajsbrot *et al.*, 1991
Menidia beryllina	96 hours	1.30 mg.ℓ^{-1}	Miller *et al.*, 1991
Galaxias maculatus	96 hours	1.60 mg.ℓ^{-1}	Richardson, 1991
Ictalurus punctatus	24 hours	0.74 - 1.91 mg.ℓ^{-1}	Sheehan & Lewis, 1986
I. punctatus	96 hours	1.60 mg.ℓ^{-1}	Colt & Tchobanoglous, 1976
Cyprinus carpio larvae	96 hours	1.74 - 1.84 mg.ℓ^{-1}	Hasan & Macintosh, 1986
Oreochromis mossambicus	96 hours	1.16 - 2.53 mg.ℓ^{-1}	Visser, 1986; Hassan, 1992
Oreochromis aurea	48 hours	2.40 mg.ℓ^{-1}	Redner & Stickney, 1979
Clarias batrachus	48 hours	15.78 mg.ℓ^{-1}	Sripumun & Somsiri, 1982
C. batrachus	96 hours	4.25 mg.ℓ^{-1}	Theerapongse-Krainara, 1988
C. gariepinus larvae	96 hours	2.30 mg.ℓ^{-1}	Britz, 1988
C. gariepinus	96 hours	6.50 mg.ℓ^{-1}	This study
HL♂xCG♀ hybrid	96 hours	9.10 mg.ℓ^{-1}	This study

Chronic ammonia toxicity occurs when fish are exposed to such high ammonia concentrations that the blood-to-water ammonia concentration gradient is reversed. Chronic ammonia toxicity can be manifest in many ways. The levels of primary stress indicators such as serum cortisol and catecholamine increase in the blood of the fish (Spotte and Anderson, 1989; Jeney *et al.*, 1992). The growth rates of fish decrease at high ammonia concentrations (Smith and Piper, 1975; Alderson, 1979). Tissue damage can occur, particularly to the gills. Epithelial cell enlargement (hyperplasia), proliferation (hypertrophy) and separation from the pillar cells (acute inflammation), lamellar detachment, blood-filled aneurysms, karyolysis and karyorrhexis of gill tissue have been observed (Smith and Piper, 1975; Burkhalter and Kaya, 1977; Cruz and Enriquez, 1981). Damage to the liver has also been recorded, including atrophied hepatocytes, cells with very dense cytoplasm, no glycogen vacuolation and pyknotic nuclei, and necrotic lesions in the hepatic tissue (Smith and Piper, 1975; Wajsbrot *et al.*, 1993). Chronic ammonia exposure has also been reported to induce capillary vessel congestion and dilation (Redner and Stickney, 1979), kidney blood vessel damage (Flis, 1968), connective tissue inflammation and hyperplasia (Thurston *et al.*, 1986), caudal damage (Marchetti, 1960) and a reduction in the oxygen carrying capacity of the blood (acidemia) (Sousa and Meade, 1977).

Kulakkat-olickal and Kramer (1988) hypothesised that clariid catfish could tolerate high concentrations of ammonia because they could lower their gill ventilation rate through air-breathing, and thus decrease the extent to which the gills were exposed to the high ammonia concentrations. However, de Villiers *et al.* (1987) and Britz (1988) found that at high concentrations of NH_3 the gill tissue of *C. gariepinus* was altered in much the same way as other species reported in the literature: epithelial cell proliferation, epithelial cell and pillar cell separation, vascular disruption and cell degeneration. Britz (1988) hypothesised that the *C. gariepinus* larval mortalities in his NH_3 LC_{50} study were caused by asphyxiation, due to gill membrane damage.

Thus it seems most likely that although gill damage did occur, the clariid catfish juveniles in this study survived asphyxiation by air-breathing. The fish at the highest NH_3 concentrations probably died from acute ammonia toxicity. Acute ammonia toxicity occurs when the passive diffusion of NH_3 into the blood is faster than the active excretion of NH_4^+ across the gill epithelia, and the ammonia concentration of the blood reaches critical levels (Wilson and Taylor, 1992). Acute toxicity results in convulsions and death, probably due to the disruption of many membrane processes through the substitution of potassium ions by NH_4^+ (Randall and Wright, 1987).

Although the gills of freshwater teleosts are generally thought to be impermeable to NH_4^+, there is evidence that some diffusion of NH_4^+ into the fish can occur when very high NH_4^+ concentration gradients exist (MacDonald and Prior, 1989; Wilson and Taylor, 1992). The NH_4^+ concentration gradients were high in the experiment. Only 0.88 % of the total ammonia in the bags existed in the NH_3 phase at the temperature and pH at which the experiment was carried out (calculated following Emerson *et al.*, 1975). Thus at a concentration of 16.2 mg.ℓ^{-1} NH_3 in the water, 1841 mg.ℓ^{-1} NH_4^+ was also present. This high concentration gradient may have allowed NH_4^+ to enter the fish, disrupting membrane processes and causing death.

The HL♂xCG♀ hybrid juveniles showed a higher tolerance to un-ionized ammonia, with an LC_{50} value of 9.1 ± 1.4 mg.ℓ^{-1} compared to 6.5 ± 1.5 mg.ℓ^{-1} for *C. gariepinus*. This increased tolerance may be related to the apparent differences in aerial and aquatic breathing rates noted for the two groups of fish, but it could also be inherited from the *H. longifilis* genotype, or result from heterosis caused by the hybridization of *C. gariepinus* and *H. longifilis*. Without knowledge of the NH_3 LC_{50} values for *H. longifilis*, it is impossible to infer the significance of the higher ammonia tolerance of the HL♂xCG♀ hybrid.

Unlike the ammonia tolerance test, there was almost no difference in the tolerance of hybrid and *C. gariepinus* juveniles to salinity over prolonged exposure times. Their median lethal salt tolerances were 10.8 ± 0.8 g.ℓ^{-1} for *C. gariepinus* and 11.0 ± 2.5 g.ℓ^{-1} for the hybrid. These salinities were slightly higher than the iso-osmotic concentration for *C. gariepinus*, which is approximately 270 ± 20 mOsm

(9.7 g.ℓ^{-1}) (Britz and Hecht, 1989; Perrott *et al.*, 1992). Most of the freshwater stenohaline teleosts reported in the literature have median lethal salinity tolerances of between 9 and 12 g.ℓ^{-1} (Table 17, in Britz, 1988) and blood osmolarities approximating 30 % of sea water (± 10 g.ℓ^{-1}) (Evans, 1993).

The salinity tolerance of the catfish appears to change as it grows. This was clearly illustrated by plotting the salinity tolerances of different sized *C. gariepinus* (Fig. 6.18), and has also been reported for other species (Natochin and Lavrova, 1974). The LC_{60} value of 10.8 g.ℓ^{-1} for the *C. gariepinus* juveniles used in the experiment compared favourably with the tolerance value predicted in Figure 6.18.

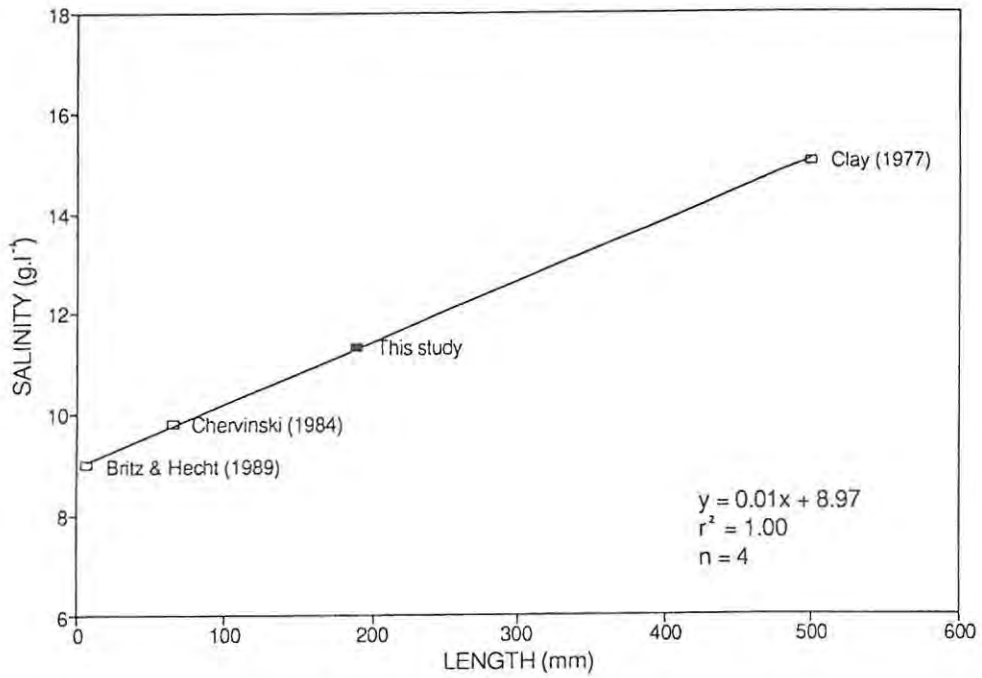

Figure 6.18. The salinity tolerance of different size *C. gariepinus*.

As the body fluids of freshwater fish have a higher osmotic concentration than that of their habitat, they need strategies to cope with excessive water entering the fish, flushing salts from the blood. Most of the water enters a fish across the gills, oral membrane and intestinal surface, as opposed to the skin (Lagler *et al.*, 1962; Motais *et al.*, 1969). Although freshwater fish drink water, they drink far less than marine fish. *Clarias gariepinus* drinks water at a rate of about 0.21 mℓ.kg^{-1}.hr^{-1}, 10 times less than the average marine teleost (Perrott *et al.*, 1992). The excess water is excreted as dilute urine (<10 % of the blood salt concentration) (Evans, 1993). The dilute urine contains creatine, creatinine, some amino acids, urea and ammonia, and some salts, particularly chlorides (Lagler *et al.*, 1962). Thus the main function of the kidneys of freshwater fish is to excrete water, while retaining sugars, salts and other vital solutes in the blood (Lagler *et al.*, 1962).

Although some re-absorbtion of salts from the urine occurs in the kidney tubules and perhaps in the bladder (Evans, 1993), active ion exchange at the gills is necessary to compensate for renal and other salt loss. The mechanisms of the exchange have not yet been fully elucidated, but seem to occur in the lamellar epithelium of the gills in freshwater stenohaline teleosts (Payan and Girard, 1984). Sodium ions are probably taken up from the water in exchange for hydrogen or ammonium ions, and chloride ions are exchanged for bicarbonate ions (Payan and Girard, 1984). To minimise salt loss across the gills by diffusion, the gill epithelial cells of freshwater stenohaline teleosts are tightly joined, and have a low permeability to ions and small organic molecules (Payan and Girard, 1984).

When the salinity of a fish's environment changes, it can tolerate the change either by osmoregulating or by osmoconforming (Lahlou *et al.*, 1969). Osmoregulating fish actively maintains their osmotic concentration within a specific range, while osmoconforming fish allow their osmotic concentration to change, in accordance with the salinity of the surrounding medium.

It seems likely that the HL♂xCG♀ hybrid and *C. gariepinus* juveniles were using a combination of both strategies to survive increased salinity. In the serial salinity increase experiment the catfish showed no behavioural changes until the salinity increased from 2 to 4 $g.\ell^{-1}$. Britz and Hecht (1989) found that the condition of *C. gariepinus* larvae deteriorated at salinities higher than 2.5 $g.\ell^{-1}$. It is plausible that *C. gariepinus* osmoregulates in salinities up to 2.5 $g.\ell^{-1}$, using a similar mechanism to *Heteropneustes fossilis*. This stenohaline, air-breathing species is related to the clariid catfishes (Burgess, 1989), and can osmoregulate in salinities of up to 5 $g.\ell^{-1}$ by increasing its urine concentration and lowering its urine output to conserve water (Goswami *et al.*, 1983).

Above 5 $g.\ell^{-1}$ *H. fossilis* becomes an osmoconformer; the osmolarity of the blood increases with increasing ambient salinity as the fish dehydrates, even though urine production is cut by 72 % at hyperosmotic levels (Goswami *et al.*, 1983). The hybrid and *C. gariepinus* juveniles probably experienced a similar phenomenon as the salinity increased above 2.5 $g.\ell^{-1}$. Osmoregulation is an energy expensive exertion. For example, even though the euryhaline rainbow trout, *Oncorhynchus mykiss*, is a good osmoregulator (Houston, 1961), the metabolic cost of osmoregulation at different salinities varied from 20 % to 27 % of the total metabolism of the fish (Rao, 1968). Increased metabolic costs due to osmoregulation could explain the loss of condition in *C. gariepinus* larvae exposed to sub-lethal salinities (Britz and Hecht, 1989). Also, an environmental stress, such as a change in salinity, induces depletion of energy reserves, indicated by a decrease in blood glucose and muscle and liver glycogen contents (Dheer *et al.*, 1986).

Dheer *et al.* (1986) exposed the air-breathing stenohaline fish *Channa punctatus* (Channidae) to 10%, 25% or 50% of its median lethal salt concentration. Interestingly, they found that while the growth rate of these fish decreased at all of these concentrations, haematological tests showed that the typical

symptoms of stress only occurred in the fish in the 25% and 50% LC_{50} tanks. It is possible that these and other fish are not stressed during osmoregulation, but that the additional energy needed to osmoregulate can reduce their growth rate. Although the evidence is circumstantial, it is likely that the best growth rates for the hybrid and *C. gariepinus* would occur at salinities less than 2.5 $g.\ell^{-1}$. Contrary to Legendre *et al.s'* (1992) findings with *H. longifilis*, the hybrid is therefore no more suitable for brackish water aquaculture than *C. gariepinus*.

To conclude, the results of the water quality parameters tested in this chapter for *C. gariepinus* and the HL♂xCG♀ hybrid are summarized in the following table (Table 6.15).

Table 6.15. Comparison of the water quality preferences and tolerances of *C. gariepinus* and the HL♂xCG♀ hybrid, established in the present study.

Water quality	*C. gariepinus*	Hybrid
Temperature	28-30 °c	28-33 °c
Oxygen	> 3.0 $mg.\ell^{-1}$	> 3.8 $mg.\ell^{-1}$
Un-ionized ammonia	6.5 ± 1.5 $mg.\ell^{-1}$	9.1 ± 1.4 $mg.\ell^{-1}$
Salinity	10.8 ± 0.8 $g.\ell^{-1}$	11.0 ± 2.5 $g.\ell^{-1}$

Most of southern Africa lies in the temperate zone. Catfish aquaculture in this region requires heated water to achieve the optimum growth rates of the fish. Both groups of fish had a temperature preference zone starting at 28 °C, which means that no extra heating costs would be necessary to farm the HL♂xCG♀ hybrids instead of pure strain *C. gariepinus*.

Extremely low levels of oxygen in the water of a fish farm would not reduce the survival rate of the catfish. However, at ultra-high densities an oxygen saturation level of below 50 % may stress the fish and cause a decline in growth rate.

The LC_{50} values established for NH_3 in this chapter were useful for comparing water quality tolerances between the two catfish groups and with other species. However, they are of little use to a fish farmer, as some fish would start dying before these NH_3 concentrations were reached. It is suggested that the highest NH_3 concentration at which no mortalities occurred be used as the maximum level for the rearing of catfish juveniles. These would be 2.3 $mg.\ell^{-1}$ NH_3 for *C. gariepinus* and 5.8 $mg.\ell^{-1}$ NH_3 for the hybrid.

Finally, while both groups of catfish can tolerate salinities of up to 8.0 $g.\ell^{-1}$ for short durations without mortality, their growth rates would most likely be inhibited in salinities of more than 2.5 $g.\ell^{-1}$.

CHAPTER 7. COMPARISON OF FILLET YIELD, PROTEIN CONTENT AND AMINO ACID PROFILE OF *CLARIAS GARIEPINUS* AND THE *HETEROBRANCHUS LONGIFILIS* ♂ X *CLARIAS GARIEPINUS* ♀ HYBRID.

7.1. INTRODUCTION

The data gathered so far clearly indicate that the HL♂xCG♀ hybrid has definite advantages over *C. gariepinus* for aquaculture in southern Africa. It was therefore decided to compare the final product of the hybrid with that of *C. gariepinus*, on the basis of the fillet yield, protein content and amino acid profile of the fish.

The fillet yield of *C. gariepinus* is comparable to that of other important aquaculture species. Abattoir records kept at a commercial catfish farm in South Africa showed that for 10 tonnes of whole *C. gariepinus* processed, 3.82 tonnes of fillets were produced (W. Uys, pers. comm.) This was an average fillet yield of 38.2 %. In comparison, the channel catfish *Ictalurus punctatus* and *Oreochromis niloticus* only had a fillet yield of 30.9 % and 25.4 % respectively (Clement and Lovell, 1994).

Hoffman and Prinsloo (1990) found that male *C. gariepinus* had a higher dressed mass yield (66.0 %) than the females (61.6 %). Dressed mass is the weight of the fish without the head, fins and viscera. They ascribed the difference in yield to the higher gonadosomatic index (GSI) of the females in comparison to the males. The *C. gariepinus* x *H. longifilis* hybrid females were found to have a very low GSI compared to mature *C. gariepinus* females (Chapter 5). The hybrid females could therefore have significantly higher fillet yields than the *C. gariepinus* females, which would improve the overall production of a catfish farm.

Hoffman *et al.* (1992) found that *C. gariepinus* had a higher wet weight protein content (18,2 %) than beef (13.6 %), lamb (15.4 %) or pork (9.1 %). The fish had a similar protein content to chicken (18.6 %), but with less than half the total fat content. The amino acid profile of *C. gariepinus* fillets has also been documented by Hoffman *et al.* (1993). However, no information is available for *H. longifilis* fillets. It is important to know whether the hybridization of *H. longifilis* with *C. gariepinus* caused any significant changes to the protein content of the fillets, particularly if the protein content of the fillets is used as a marketing strategy.

In this chapter, the fillet yield of male and female *C. gariepinus* was compared to that of the HL♂xCG♀ hybrid, and the crude protein and amino acid content of the flesh of the two groups were compared.

7.2. MATERIALS AND METHODS

Clarias gariepinus and HL♂xCG♀ hybrids of both sexes and diverse sizes were removed from the grow out tanks of the Rhodes University aquaculture facility at various times during the year (Table 7.1).

Table 7.1. The number and size range of *C. gariepinus* and HL♂xCG♀ hybrids filleted.

Species	Sex	n	Min. mass	Max. mass
C. gariepinus	male	14	367 g	1860 g
	female	19	515 g	1800 g
Hybrid	male	13	594 g	3200 g
	female	16	477 g	4520 g

The fish were placed into cold water (± 2 °C) until they were no longer active. They were dried with paper towels and weighed to 0.1 g. The fish were killed by severing their spinal cords, and were gutted. The fish were then skinned and both fillets were removed. To insure consistency the filleting was carried out by the author only. The fillets were washed and excess water was removed using absorbent paper towels, whereafter both fillets were weighed. The weight of the two fillets was expressed as a percentage of the total weight of the fish. The difference between the percent fillet yields of male and female *C. gariepinus* and HL♂xCG♀ hybrids was tested for significance ($P < 0.05$) using a one-way analysis of variance. Relationships within the data set were analyzed using a 95 % Tukey multiple range test.

Tissue samples from four *C. gariepinus* and four hybrids of approximately 600 g were analyzed for their protein content and their amino-acid profile. A 10 g sample of flesh was taken from the fillets of two males and two females from each species. For comparative purposes tissue samples were also taken from four specimens of the marine sparid *Diplodus sargus*.

7.3. RESULTS

Clarias gariepinus females had a significantly lower ($P < 0.05$) fillet yield in comparison to the other groups, over the size range tested. The average *C. gariepinus* female fillet yield was 35.1 % of the total mass of the fish, while the average fillet yield of the hybrid females was 44.4 %. There was no significant difference ($P > 0.05$) between the fillet yield of the *C. gariepinus* and hybrid males (42.8 % and 43.3 % respectively; Fig. 7.1).

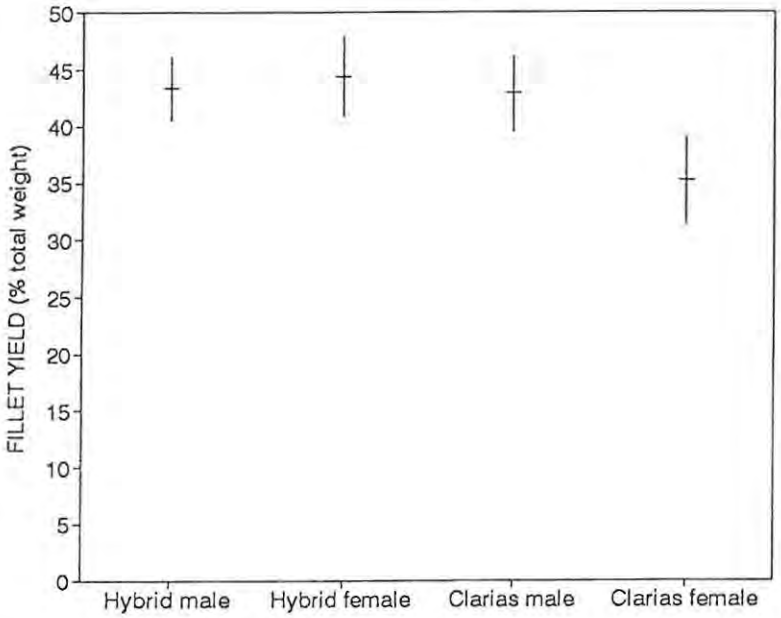

Figure 7.1. The percent fillet yield of male and female *C. gariepinus* and HL♂xCG♀ hybrids.

The average crude protein content of the samples of hybrid fillets was 18.3 %, with a moisture content of 79.7 %. The average crude protein content of the *C. gariepinus* fillets was very similar at 18.6 %, but the flesh was slightly drier, with a moisture content of 76.8 %. *Diplodus sargus* had a substantially higher protein content of 20.1 %, with a moisture content of 75.0 %. The amino acid profile of the two groups of catfish and *D. sargus* were almost identical (Table 7.2).

Table 7.2. Amino acid profile of *C. gariepinus* and HL♂xCG♀ hybrid fillets.

Amino acid	Hybrid	*C. gariepinus*	*D. sargus*
Aspartic acid	12.0 %	12.3 %	12.4 %
Threonine	4.6 %	4.5 %	4.6 %
Serine	3.5 %	3.6 %	3.7 %
Glutamic acid	19.2 %	19.6 %	19.5 %
Proline	2.9 %	2.6 %	2.7 %
Glycine	4.5 %	4.3 %	4.2 %
Alanine	5.9 %	5.9 %	6.2 %
Cystine	0.9 %	0.8 %	0.9 %
Valine	5.5 %	5.4 %	5.5 %
Methionine	0.2 %	0.1 %	0.3 %
Isoleucine	5.3 %	5.3 %	4.3 %
Leucine	8.5 %	8.5 %	8.7 %
Tyrosine	3.1 %	3.3 %	3.0 %
Phenylalanine	4.5 %	4.7 %	4.7 %
Histidine	2.8 %	2.8 %	2.7 %
Lysine	9.9 %	9.9 %	9.9 %
Arginine	6.6 %	6.4 %	6.6 %

7.4. DISCUSSION

The sex ratio of female to male hybrids has been found to be approximately 1:1 (pers. obs.; Legendre *et al.*, 1992). Assuming that a similar sex ratio was true for *C. gariepinus* raised at the Rhodes University aquaculture facility, the combined yield of fillets from male and female *C. gariepinus* was 38.9 % of the total mass of the fish produced. This figure was close to the yield of 38.2 % found under commercial conditions at Blyde River Aquaculture, which also included the incurred losses of the abattoir, such as spoiled fillets and off-cuts.

The retarded development of the ovary in the hybrid females led to a remarkable increase of 9.3 % in fillet yield compared to *C. gariepinus* females. This was not surprising as some of the *C. gariepinus* females had GSI values in excess of 18.0 %, while the GSI values of the hybrid females were seldom higher than 2.0 % (see Chapter 5). The overall fillet yield of the hybrids (male and female) was 43.9 %. Consequently 26 tonnes of *C. gariepinus* would have to have been grown and processed for every 10 tonnes of catfish fillets produced, while only 23 tonnes of the hybrid would be required to produce an equivalent mass of fillets.

The ratio of amino acids in the hybrid and *C. gariepinus* fillets were almost identical. The amino acid profile of *D. sargus* was remarkably similar to those of the two groups of catfish, although it had a higher crude protein content (20.1 %) than the catfish. The total protein content of the hybrid (18.3 %) was slightly lower than that of *C. gariepinus* (18.6 %) in this comparison, but Hoffman *et al.* (1993) found a total protein content of 18.2 % for *C. gariepinus*, based on a sample of both farm-reared and wild fish.

The protein content of the flesh of *C. gariepinus* and the hybrid compared favourably with that of other commercially important fish caught in southern Africa, as well the American channel catfish *Ictalurus punctatus* (Table 7.3).

Table 7.3. Protein content of the flesh of *C. gariepinus* and the HL♂xCG♀ hybrid, compared to other commercially important fish (Fishing Industry Research Institute, South Africa, pers. comm.; Tucker, 1985).

Species	Protein
Ictalurus punctatus	17.8 %
H. longifilis x *C. gariepinus* hybrid	18.3 %
Merluccius capensis	18.4 %
Oncorhynchus mykiss	18.5 %
Clarias gariepinus	18.6 %
Trichiurus lepturus	19.0 %
Xiphiurus capensis	19.9 %
Diplodus sargus	20.1 %
Trachurus capensis	20.2 %

In conclusion, although the hybridization of *H. longifilis* and *C. gariepinus* did not give rise to any beneficial changes in protein content or amino acid profile, the unit production cost per kilogram of hybrid fillet would be substantially lower than for *C. gariepinus*.

CHAPTER 8. DETERMINATION OF THE OPTIMUM DENSITY FOR INTENSIVE TANK CULTURE OF THE *HETEROBRANCHUS LONGIFILIS* ♂ X *CLARIAS GARIEPINUS* ♀ HYBRID.

8.1. INTRODUCTION

The rearing of fish at a high density can increase yield while optimizing water usage (Sampath, 1985; Nerrie *et al.*, 1990; Kebus *et al.*, 1992) and increasing profits (O'Sullivan and Purser, 1993). In aquaculture, the term "density" can mean either the number of fish or the total mass of fish impounded in a volume of water.

Most fish species have a threshold density. If they are held at densities higher than this threshold their growth and survival rates are impaired. One of the greatest drawbacks to tilapia farming is stunting due to overcrowding (Hulata *et al.*, 1983). Unnaturally high numbers of fish confined within a small volume of water may also lead to behavioural changes (Medland and Beamish, 1985), such as the formation of hierarchies and dominant individuals (Ten and Chua, 1978; Knight, 1987). Piper *et al.* (1982) proposed that the threshold density for rainbow trout *Oncorhynchus mykiss* increased as the fish grew. Based on this premise they derived a density index which could predict the density beyond which growth would be retarded. The index was calculated by multiplying the water volume in the tank by the average length of the fish in the tank, and dividing this product by the total mass of the fish in the tank. Thus, if the volume of water remains constant in the equation, an increase in the size (length) of the fish would allow for a proportional increase in the density (mass) of the fish.

The density index developed by Piper *et al.* (1982) was useful for predicting the effects of crowding on trout in a static body of water, but did not consider the effect that water turnover has on the density tolerance of fish. Most of the deleterious consequences of rearing fish at high densities appear to result from water quality deterioration rather than the physical and behavioural effects of overcrowding *per se*. For example, Soderberg *et al.* (1987) found that if the water requirements for respiration and waste depletion were maintained, *Salvelinus namaycush* fingerlings could be grown successfully at more than four times the density predicted by Piper *et al.*'s (1982) density index. Kebus *et al.* (1992) reported a similar result for *O. mykiss*, while Kindschi *et al.* (1991) found that *O. mykiss* could be reared at two to three times their predicted threshold density if supplemental oxygen was added to their tanks.

The rate of oxygen replenishment and waste product removal in a tank is proportional to the rate at which fresh water is added to a tank. Therefore the turnover rate of the water in a tank should also be included in a density index. Water replenishment was included in the density index proposed by Morrison and Piper (1988), which they called the "flow" index. This index was derived by dividing the total mass of fish in a tank (mg) by the product of water inflow (ℓ/min) and the average length of the

fish in the tank (mm). In other words, the faster the water turnover and the larger the fish, the heavier the mass of fish that can be kept in the tank without a decrease in production.

Fish can be cultured at different levels of intensity. The least intensive approach involves the rearing of fish at low densities in large, relatively stagnant bodies of water. This is known as extensive aquaculture, where the fish generally do not have to be fed or maintained in any way. In southern Africa, semi-intensive catfish culture implies the farming of fish in relatively small ponds (0.1 ha). The fish are usually fed a formulated diet at a predetermined rate, based on size and temperature (Uys, 1989). The average production of a semi-intensive farm is about 40 tonnes.ha^{-1} per annum (Hecht *et al.*, 1995). The densities of fish at extensive and semi-intensive farms are usually reported as tonnes of fish per hectare of water. The third approach is described as intensive culture, where fish are raised at high densities in relatively small volumes of water, with high water turnover rates. The most meaningful way to represent density in these highly intensive systems is a function of the mass of fish held in a cubic metre of water, where the water is exchanged hourly (ie. kg.m^{-3}.hr^{-1}). Examples of intensive fish farming are presented in Table 8.1. For comparative purposes, all reported density data have been converted to the kg.m^{-3}.hr^{-1} format throughout this chapter. Where only water surface areas were reported in the literature, I have assumed the water body to have an overall depth of 1.0 m. Also, hereafter the term "density" shall refer to the mass of fish in a volume of water, unless otherwise specified.

Table 8.1. Examples of densities at which various species of fish are grown under intensive aquaculture conditions.

Species	Density	Reference
Stizostedion vitreum x *S. canadense*	9.6 kg.m^{-3}.hr^{-1}	Siegwarth and Summerfelt (1990)
Oreochromis aurea	10.0 kg.m^{-3}.hr^{-1}	Henderson-Arzapalo and
O. mossambicus	33.3 kg.m^{-3}.hr^{-1}	Stickney (1982)
Cyprinus carpio	47.4 kg.m^{-3}.hr^{-1}	Brandt *et al.* (1979)
Oncorhynchus mykiss	23.5 kg.m^{-3}.hr^{-1}	Papoutsoglou *et al.* (1987)
O. tshawytscha	64.0 kg.m^{-3}. ?	Mazur and Iwama (1993)
Salmo salar	66.6 kg.m^{-3}.hr^{-1}	Kjartansson *et al.* (1988)
Ictalurus punctatus	133.1 kg.m^{-3}.hr^{-1}	Allen (1974)
Anguilla anguilla	80.0 kg.m^{-3}.hr^{-1}	Degani *et al.* (1985)
Anguilla anguilla	184.0 kg.m^{-3}.hr^{-1}	Okamoto *et al.* (1990)

While these densities are impressive, they are nowhere near as remarkable as the densities at which the clariid catfish group have been grown. In Thailand, for example, *Clarias* species were reared at densities of up to 4.5 times higher than other groups of fish on semi-intensive farms (Turner, 1990). Table 8.2 shows the densities at which clariid species have been grown in intensive systems.

Table 8.2. Examples of the densities at which clariid catfish are grown in intensive aquaculture.

Species	Density	Reference
Heterobranchus longifilis	70 $kg.m^{-3}$. ?	Kerdchuen & Legendre (1992)
Clarias fuscus	113 $kg.m^{-3}.hr^{-1}$	Diana & Fast (1989)
Clarias batrachus	266 $kg.m^{-3}.hr^{-1}$	Viveen *et al.* (1990)
Clarias gariepinus	200 $kg.m^{-3}.hr^{-1}$	Huisman & Richter (1987)
Clarias gariepinus	250 $kg.m^{-3}.hr^{-1}$	Orchid (Silver Creek, pers. comm.)
Clarias gariepinus	275 $kg.m^{-3}.hr^{-1}$	Bovendeur *et al.* (1987)
Clarias gariepinus	300 $kg.m^{-3}.hr^{-1}$	Uys (pers. comm.)

Clariid catfish can tolerate these very high densities for a number of reasons. They are air-breathers (Moussa, 1957), and they are tolerant of extremely low concentrations of dissolved oxygen and extremely high concentrations of ammonia (Chapter 6). These fish are also tolerant of poor water quality in general (Hecht and Britz, 1988; Diana and Fast, 1989; Salami *et al.*, 1993; Chapter 6). Further, *C. gariepinus* has been shown to be a social fish, hunting in extremely dense, co-operative packs (Merron, 1993). Moreover, *C. gariepinus* juveniles are highly aggressive when confined in small numbers in a large volume of water. As their numbers increase a point is reached when they no longer defend territories but invariably adopt a "rolling in" motion with little or no aggression. Reasons for this type of behaviour are presented in Hecht *et al.* (in prep.). Hecht and Appelbaum (1988) have also shown a positive correlation between increased density and decreased aggression in *C. gariepinus* larvae and early juveniles. For these reasons it seems that *C. gariepinus* is ideally suited for intensive aquaculture.

From the above, it is hypothesised that the *H. longifilis* x *C. gariepinus* hybrid would be just as suited to intensive aquaculture as *C. gariepinus*, as the *H. longifilis* parent species can also tolerate relatively high densities (Table 8.2). The faster growth rate of the HL♂xCG♀ hybrid compared to *C. gariepinus* was demonstrated in Chapter 4. It may be possible to further increase the production of the hybrid, by farming it at ultra-intensive levels. However, if the threshold density of the hybrid were exceeded, its faster growth rate would be negated. Thus, in the following study an attempt was made to determine the threshold density of the HL♂xCG♀ hybrid.

Once the threshold density of a species has been exceeded, it is likely that it will show signs of stress. According to Pickering (1981) and Wedemeyer (1981), the stress response of a fish has three levels. The primary response is a neuro-endocrine response, where adrenocorticotropic hormone (ACTH) is released from the adrenohypophysis, and catecholamines and corticosteroids are released from the interrenal glands. The secondary response results from the release of these hormones, including blood chemistry changes, tissue and histological changes, increases in nitrogen metabolism, and diuresis,

which causes electrolyte loss and osmoregulatory dysfunction. Finally, the tertiary response includes behavioural changes, retarded growth and food conversion efficiency, increased susceptibility to disease and mortality.

It would seem that the most accurate way to evaluate stress is to measure the levels of one of the primary response hormones in the blood. Plasma cortisol was recognised as potentially the most useful primary response indicator (Pickering and Stewart, 1984; Ainsworth *et al.* 1985; Wright and Giles, 1987). Unfortunately, with prolonged stress plasma cortisol levels return to normal, even though the fish is still showing tertiary stress responses (Schreck, 1981, Robertson *et al.*, 1987). Where high density was the stressor, Klinger *et al.* (1983) and Gatlin *et al.* (1986) found no relationship between density and plasma cortisol levels in *Ictalurus punctatus*. Pickering and Pottinger (1987a) found that plasma cortisol levels were influenced to a different extent by different combinations of water chemistry (pH, ammonia, oxygen *etc.*). Also, changes in plasma cortisol levels occur very rapidly in fish. For example, Ainsworth *et al.* (1985) found that handling stress would alter the plasma cortisol levels before the effects of any other stress could be measured.

Changes in the composition of the blood cells can be used to measure secondary stress responses (Pickering and Pottinger, 1987b). As a fish becomes stressed the leucocyte count increases, which can be measured as a change in the fish's haematocrit percentage.

However, the most common way to measure a stress response is at the tertiary level, where indices of growth, mortality or feed conversion are measured, and compared to a control group.

In the following series of experiments, the tertiary manifestations of stress were monitored for the hybrid using the indices of specific growth rate, feed conversion ratio, mortality rate and net yield. The experiments were designed to determine the threshold density of the hybrid by comparing predicted net yields of fish reared at different densities with the actual net yields of the fish at the densities. Once the threshold density was determined, blood samples were taken from hybrids reared at densities below and above the threshold density. These samples were tested for secondary stress responses.

8.2. MATERIALS AND METHODS

All the density experiments were carried out at the Rhodes University aquaculture facility. The system consisted of 1.5 m x 1.0 m x 1.0 m tanks contained within a 30 m x 7 m plastic "UV-dek" agricultural tunnel. The floor of the rectangular tanks sloped to one end, where the effluent water was drawn off through an upstand pipe and sleeve. The effluent from the tanks in the tunnel drained into a 120 m^3 settlement tank, from where the water passed through a four-series, 80 m^3, submerged biological filter.

The filter contained size-graded granite stone medium and was continuously aerated. Once the water had passed through the filter it flowed into a 175 m^3 retention reservoir, from where it was pumped back to the tanks. Retention time in the reservoir was approximately six hours. The entire system, including the experimental tanks, had a total volume of 350 m^3. Ground water was added to the system at a rate of approximately 18 m^3/week to compensate for evaporation and leakage.

Water from the reservoir was passed through a heat exchange system at a rate of approximately 8.0 $m^3.hr^{-1}$ during the cooler months. The combination of the "UV-dek" tunnel and the heat exchanger allowed water temperatures to be maintained at 5 to 8 °C above ambient during winter.

The volume of water in each tank was fixed, depending on the height of its upstand pipe. However, the addition of fish to the tanks resulted in water being displaced. Therefore the actual volume of water in the tank was no longer the same as the tank volume. For example, a 1.0 m^3 container holding 500 kg fish per 1.0 m^3 tank volume would in actual fact have closer to 1000 kg fish per 1.0 m^3 water volume. Thus it was necessary to calculate the volume of water displaced by the fish before the density experiments were carried out.

Water displacement by the HL♂xCG♀ hybrid

Fifty-one anaesthetized hybrid catfish ranging from 13 to 720 g were placed individually into a 10 ℓ container, filled to capacity with water at a temperature of 28 °C. The addition of the fish caused displaced water from the container to spill over a lip into a volumetric measuring cylinder. The relationship between catfish mass and displaced water volume was modelled using the least-squares method of regression analysis.

The volume of fish (V_f) in the tank could therefore be calculated by substituting their biomass for x in the linear regression equation:

$$V_f\ (m^3) = a(x) \pm b$$

where a = coefficient of x; and b = constant, for the linear regression equation.

Thus the actual volume (V_a) of water in the tank was:

$$V_a\ (m^3) = V_t - V_f$$

where V_t = tank volume (m^3).

For the purposes of these experiments, the density of the fish in a tank was considered to be the biomass of the fish (in kilograms) divided by the actual volume of water (in cubic metres) held in the tank for one hour; *viz*:

$$\text{Density} = \frac{\text{Biomass}}{V_e \times \text{turnover}} = kg.m^{-3}.hr^{-1}$$

where turnover = the time taken for 1 m^3 of water to pass through the experimental tank.

The threshold density of the HL♂xCG♀ hybrid

Two trials were carried out to determine the threshold density of the hybrid. Each of the two trials followed the same experimental procedure. In the first trial, hybrids of approximately 30.0 g were stocked into tanks containing 0.2 m^3 of water at stocking numbers of 250, 1000 and 2000 fish/m^3 of water. The water exchange rate was 1.2 $m^3.hr^{-1}$. Thirty randomly sampled fish were weighed from each tank. A one-way ANOVA was used to test that there were no significant differences ($P > 0.05$) between the average masses of the fish in the tanks at the start of the experiment. Each trial was undertaken in duplicate.

The fish were fed on 38 % protein dry pellets at 9h00 and 18h00 daily. It was noted that the feeding rate of the fish was sometimes erratic, particularly after they had been handled. For this reason they were fed by hand until they stopped eating, instead of at a calculated rate. Each tank of fish was fed from a sealed bucket containing a known quantity of food. The feed consumption of the fish could be monitored by weighing the buckets after feeding. Dead fish were removed during the feeding times, and their numbers recorded. Every fortnight the tanks were drained and the fish were counted and bulk weighed.

The performance of the fish reared in the different density tanks was compared by calculating their specific growth rates (SGR), percent mortalities (M) and feed conversion ratios (FCR). These indices were calculated as follows:

Specific growth rate:

$$SGR\ (\%) = \frac{\ln(W_1) - \ln(W_0)}{dt} \times 100$$

where W_0 & W_1 were the initial and final average mass of the fishes in the tank in grams, and dt was the number of days of the trial.

Mortality:

$$M\ (\%) = \frac{N_0 - N_1}{N_0} \times 100$$

where N_0 & N_1 were the initial and final number of fish in the tanks.

Feed conversion ratio: $$FCR = \frac{\text{kg dry feed fed}}{B_1 - B_0}$$

where B_0 & B_1 were the initial and final biomass of fish in the tanks, in kilograms.

The water temperature of the system was measured at the effluent pipe every morning. A comprehensive water quality analysis was carried out for all the experimental tanks on a monthly basis. After 100 days the progress of the first density trial was assessed. Once it became clear that the threshold density had not been attained, the trial was terminated. The tanks were cleaned, and the second trial was initiated, using larger fish, of approximately 116 g average weight.

In the second trial, the numbers and mass of fish in the tanks were recorded monthly. Also, the fish were not fed for 24 hours after they had been weighed. The experiment was terminated after 140 days, once the density of the fish in some of the tanks had exceeded 1000 $kg.m^{-3}.hr^{-1}$.

Theoretically, the density of the fish in the tanks would escalate as they grew, assuming minimal mortalities. The density of fish in the "middle density" tanks would eventually reach the initial density of the fish in the "high density" tanks. The initial density of fish in the "middle density" tanks would also eventually be replicated by the increasing density of the fish in the "low density" tanks. In this way a large range of densities could be covered using a limited number of fish and experimental tanks. For example, if the density of the fish in the "low density" tank increased from 50 to 100 $kg.m^{-3}.hr^{-1}$ during the experiment, and no signs of tertiary stress were apparent, then it could be assumed that the threshold density had not been reached. If the density of the fish in the "middle density" tank increased from 75 to 150 $kg.m^{-3}.hr^{-1}$ during the same period, but showed signs of tertiary stress, then it could be assumed that their threshold density lay somewhere between 101 and 150 $kg.m^{-3}.hr^{-1}$.

It must be noted that the terms "low density tanks", "middle density tanks" and "high density tanks" are relative, and refer to the original stocking density of fish in the tanks. Thus, while the density of the fish in, for example, the "low density tank" will increase, it will still contain a lower density of fish relative to the increasing densities of the "middle" or "high" density tanks.

Net yield (Y) was calculated by finding the difference between the initial biomass (B_0) and the final biomass (B_1) of the fish in a particular tank;

Net yield: $$Y\ (kg) = B_1 - B_0$$

The net yield of the fish reared at different, escalating densities were compared, based on the following assumptions. The first assumption was that once the threshold density of the fish had been exceeded, it would have an adverse effect on their growth and/or mortality rates, influencing their net yield. The second assumption was that the growth and mortality rates of the fish at the lowest density would be least influenced by density.

The net yield of the fish in the low density tanks were plotted against initial stocking number. This relationship was then extrapolated, to predict the net yield of the fish stocked at medium and high stocking numbers. A line was drawn through these predicted points. The observed net yield of the fish in the medium and high density tanks were then plotted against their stocking numbers. Theoretically, the observed values would be similar to the predicted values, until such time as density had a positive or negative effect on the fish in the tanks. The point at which the observed yield decreased below the expected yield was the point at which density had a negative effect on the yield of the fish. At this density the fish would theoretically have already experienced some degree of growth and survival retardation. It follows therefore that the threshold density for the fish had already been exceeded, and the optimim density at which to grow the hybrid would be slightly lower.

A one-way analysis of variance was used to test for differences ($P < 0.05$) in the SGRs, FCRs and mortality rates of fish raised at different densities. A 95 % Tukey multiple range test was used to analyse relationships within these data sets.

Secondary stress response of the HL♂xCG♀ hybrid reared at high densities

A secondary stress response analysis was carried out on the fish at the final low, middle and high densities. Blood samples were taken from 10 randomly sampled fish from each of the three densities. One millilitre of blood was drawn into a syringe from the caudal vein, through a hypodermic needle soaked in EDTA to prevent blood clotting. The blood was injected into a "vac-u-test" tube containing 15% K_3EDTA as an anti-coagulant. The blood was examined using a whole blood analyzer. Each blood sample was tested in duplicate. The blood was analyzed for red blood cell counts (RBC), haematocrit (HCT), mean cell volume (MCV) and white blood cell counts (WBC). A one-way analysis of variance was used to determine whether the blood of the fish grown above or below the threshold density showed any significant differences ($P < 0.05$). A 95 % Tukey multiple range test was used to analyse relationships within these data sets.

8.3 RESULTS

Water displacement by the HL♂xCG♀ hybrid

The relationship between fish mass and displaced volume proved to be linear and constant, throughout all fish sizes (Fig. 8.1). The volume of water displaced by the catfish in the tanks (V_f) could be related to their biomass by the following equation:

$$V_f = \frac{0.926 \times \text{biomass} + 0.0025}{1000}$$

where biomass is measured in kilograms, and displaced water is measured in cubic meters.

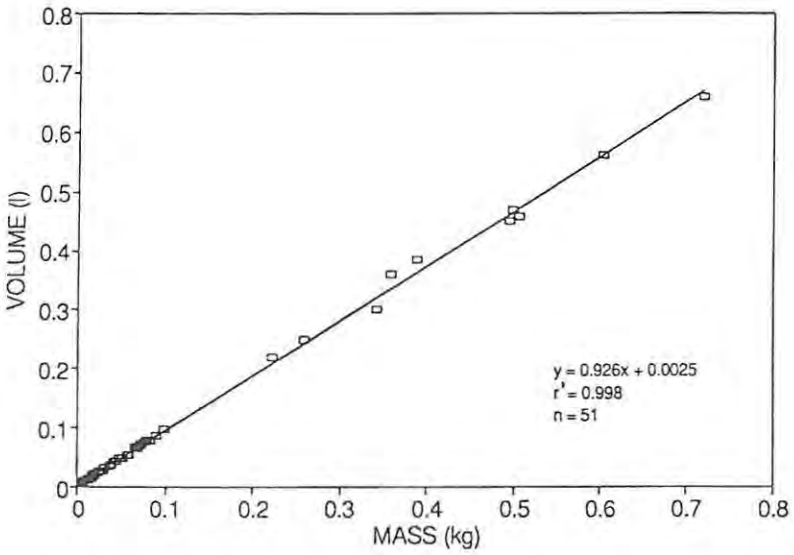

Figure 8.1. The relationship between the mass of the HL♂xCG♀ hybrid and water displacement.

The threshold density of the HL♂xCG♀ hybrid

The fish in the first threshold density trial tolerated a range of densities, from 6.2 $kg.m^{-3}.hr^{-1}$ to 123 $kg.m^{-3}.hr^{-1}$ (Table 8.3). The observed net yield of the fish reared in the middle and high density tanks was higher than the expected yield from these fish. This means that the densities attained in these tanks were not limiting, and may in fact have had a positive effect on yield (Fig. 8.2). This supposition is supported by the significantly higher ($P < 0.05$) SGRs of the fish in the middle and high density tanks, compared to the fish in the low density tanks (Table 8.4). However, the fish at the highest density had a higher mortality rate than the fish held at lower densities (Table 8.4).

Table 8.3. The mean individual mass and density of HL♂xCG♀ hybrids at the initiation and at the completion of the first threshold density trial, and their net yield after 100 days.

Stocking number (n/m^3)	250	1000	2000
Initial			
Mean individual mass (g)	29.6 ± 2.0	30.7 ± 0.8	31.6 ± 1.2
Density ($kg.m^{-3}.hr^{-1}$)	6.2 ± 4.0	26.3 ± 0.7	56.0 ± 2.3
Final			
Mean individual mass (g)	62.4 ± 2.8	77.8 ± 1.0	74.8 ± 3.5
Density ($kg.m^{-3}.hr^{-1}$)	12.9 ± 0.2	65.0 ± 1.7	123.4 ± 4.0
Net Yield ($kg.m^{-3}$)	7.9 ± 0.8	42.1 ± 2.6	67.0 ± 1.3

± Standard deviation

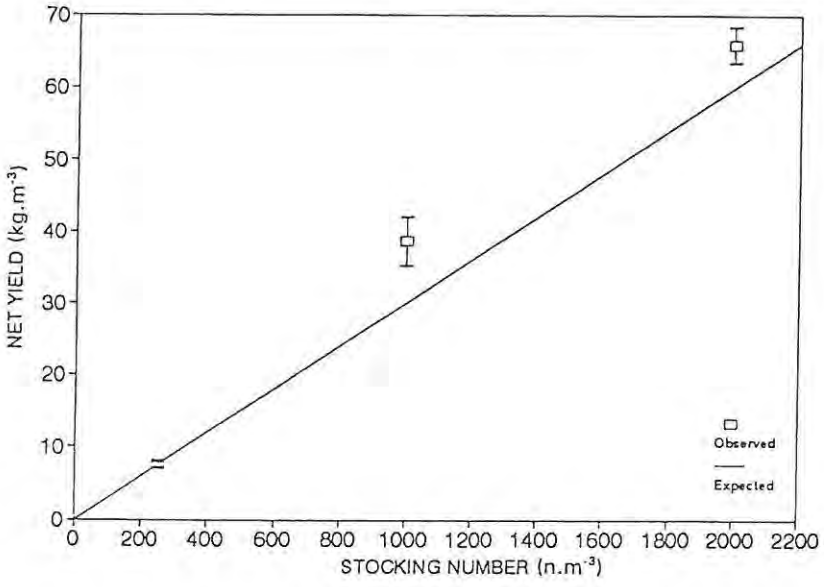

Figure 8.2. The net yield of the HL♂xCG♀ hybrid catfish at different stocking numbers, for the first density trial (Vertical bars indicate standard deviation).

Table 8.4. The specific growth rates, mortality rates and feed conversion ratios of the HL♂xCG♀ hybrid catfish raised at different densities in the first density trial.

Density	SGR (% g.day^{-1})	Mortality (%)	FCR
Low	0.68 ± 0.02[a]	6.7 ± 1.3[a]	1.85 ± 0.10[a]
Middle	0.85 ± 0.01[b]	15.7 ± 5.0[a]	1.54 ± 0.14[a]
High	0.78 ± 0.01[b]	14.5 ± 0.5[b]	1.68 ± 0.06[a]

[a] Superscript denotes homogeneous groups

The FCRs of the fish in the different treatment tanks were not significantly different ($P > 0.05$). The average FCR for the fish in this trial was 1.7 ± 0.2 (Table 8.4). The removal and weighing of the fish every fortnight probably had an effect on their FCRs. Even though the fish were fed by hand, their willingness to feed could only be ascertained after a certain amount of food had been introduced into the tanks. Also, the fish were fed until un-eaten food was left on the bottom of the tank. This un-eaten food was then flushed away by the self-cleaning action of the tanks.

At the termination of the second threshold density trial, final fish densities of 74, 407 and 965 kg.m^{-3}.hr^{-1} were attained in the low, middle and high density tanks respectively (Table 8.5).

Table 8.5. The mean individual mass and density of HL♂xCG♀ hybrids at the initiation and at the completion of the second threshold density trial, and their net yield after 140 days.

Stocking number (n/m^3)	250	1000	2000
Initial			
Mean individual mass (g)	111.0 ± 3.0	118.0 ± 10.0	121.5 ± 2.5
Density (kg.m^{-3}.hr^{-1})	23.7 ± 0.7	110.0 ± 10.5	261.3 ± 6.9
Final			
Mean individual mass (g)	362.8 ± 15.0	343.3 ± 4.4	300.0 ± 5.9
Density (kg.m^{-3}.hr^{-1})	74.4 ± 2.4	407.4 ± 10.6	965.2 ± 25.9
Net Yield (kg.m^{-3})	54.8 ± 3.3	218.5 ± 16.0	315.8 ± 2.3

The observed net yield of the fish in the middle density tanks was close to the predicted net yield (Fig. 8.3). However, the observed net yield was noticeably lower than the predicted net yield for the fish in the high density tanks. According to the assumptions made, the threshold density of the hybrids had therefore been exceeded in the high density tanks. As the highest density attained by the fish in the middle density tanks was 407 $kg.m^{-3}.hr^{-1}$, it was assumed that the threshold density of the hybrid would lie somewhere between 407 and 965 $kg.m^{-3}.hr^{-1}$.

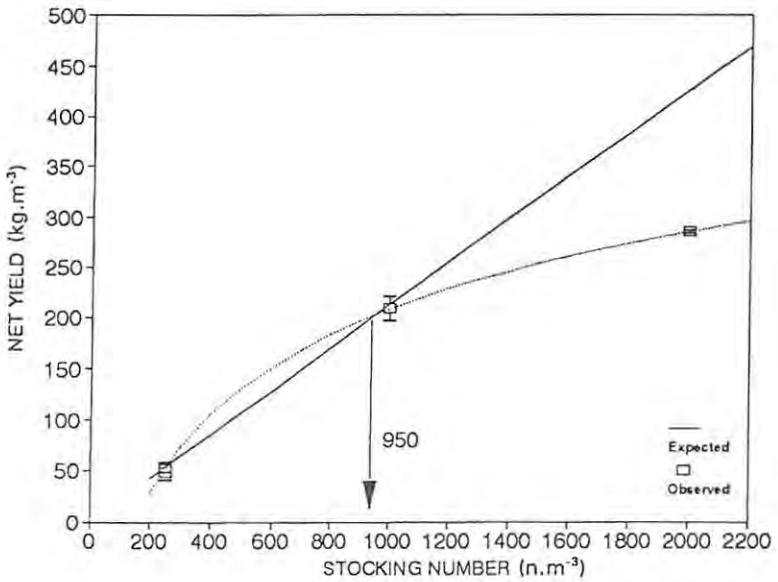

Figure 8.3. The net yield of the HL♂xCG♀ hybrid catfish at different stocking numbers, for the second density trial (Vertical bars indicate standard deviation).

By definition (see Materials and Methods), the relationship between the expected net yield of the fish in the different density tanks and the number of fish stocked into the tanks was linear. However, the relationship between the observed net yield of the fish and stocking number was found to be logarithmic in the second trial (Fig. 8.3). The point at which the observed and predicted net yield graphs intersected would be the point at which density began to have a negative effect on hybrid yield.

The expected net yield graph and the observed net yield graph intersected at a stocking number of 950 $fish/m^3$ of water (Fig. 8.3). Given that the relationship between the number of the fish in the tanks and the final density of the fish in the tanks was linear (Fig. 8.4), 950 $fish/m^3$ would have an equivalent density of 415 $kg.m^{-3}.hr^{-1}$. Thus the threshold density for the HL♂xCG♀ hybrid was 415 $kg.m^{-3}.hr^{-1}$.

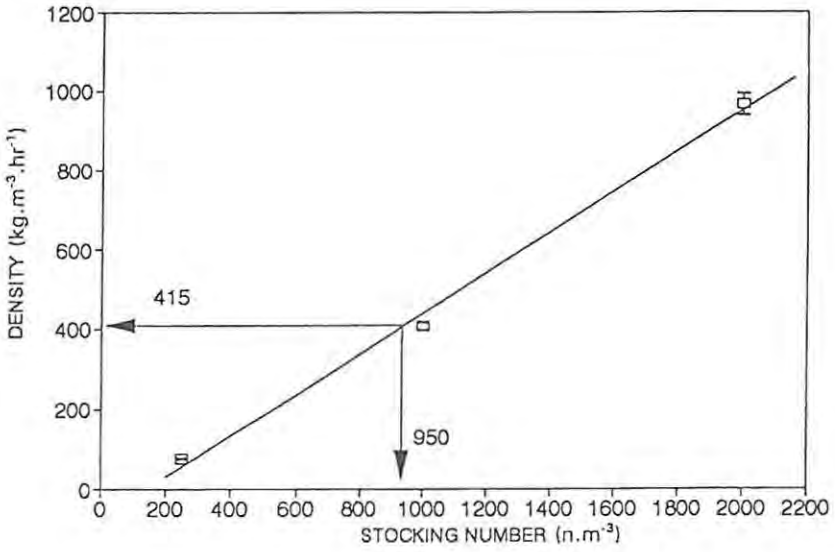

Figure 8.4. The relationship between the number of HL♂xCG♀ hybrid catfish and their final density in the second density trial.

It must be noted that even at densities in excess of 950 $kg.m^{-3}.hr^{-1}$ the hybrids continued to grow, and mortality was not significantly higher ($P > 0.05$) in the high density tanks than the low or middle density tanks (Table 8.6). Although the mean SGR was the lowest and the mean FCR was the highest for the fish in the high density tanks, these differences were not significant ($P > 0.05$).

Table 8.6. The specific growth rates, mortality rates and feed conversion ratios of the HL♂xCG♀ hybrid catfish raised at different densities in the second density trial.

Density	SGR (% $g.day^{-1}$)	Mortality (%)	FCR
Low	0.85 ± 0.05^a	13.1 ± 3.2^a	1.15 ± 0.10^a
Middle	0.77 ± 0.07^a	9.6 ± 2.9^a	1.01 ± 0.06^a
High	0.65 ± 0.01^a	17.8 ± 0.6^a	1.41 ± 0.01^a

[a] Superscript denotes homogeneous groups

The food conversion ratios during the second density trial were good, with an overall FCR of 1.17 for all the fish during the experiment. The good FCRs resulted from careful feeding management. The fish were fed until they began to lose interest in the food, instead of feeding until the food began to

accumulate on the bottom of the tank. The monthly sampling rate meant that the fish were stressed less frequently than in the first trial.

The average water temperature in the first density trial was 21.8 ± 1.1 °C. The relatively low SGRs of the fish in the first trial (Table 8.4) were probably due to these sub-optimal temperatures. The average water temperature in the second trial was higher, at 27.5 ± 1.8 °C. The pH and hardness ($CaCO_3$) of the water in the experimental system was constant for both density trials, at 7.6 ± 0.2 and 196 ± 2 ppm respectively. Oxygen, ammonia and nitrite concentrations in the tanks were influenced by density, but never reached critical levels (Table 8.7).

None of the water quality parameters measured during the two trials were limiting, but the overall water temperature was below the optimum for the hybrids. The lowest oxygen concentration recorded was 3.3 mg.ℓ^{-1}, while the highest NH_3 concentration was 0.62 mg.ℓ^{-1}. However, the water quality measurements were always taken prior to feeding. During feeding, pellet disintegration and leaching may have had an effect on the water quality in the tanks.

Table 8.7. The means of selected water quality parameters recorded in tanks containing different densities of HL♂xCG♀ hybrid catfish, at the Rhodes University aquaculture facility.

Tanks	$[DO_2]$ (mg.ℓ^{-1})	NH_3 (mg.ℓ^{-1})	Nitrites (mg.ℓ^{-1})	Nitrates (mg.ℓ^{-1})
Density trial one				
Low A	11.1 ± 1.8[a]	0.07 ± 0.01[a]	0.08 ± 0.01[a]	4.6 ± 0.1[a]
Low B	12.1 ± 2.5[a]	0.07 ± 0.01[a]	0.09 ± 0.03[a]	4.8 ± 0.1[a]
Mid A	9.8 ± 1.7[a]	0.08 ± 0.04[a]	0.08 ± 0.01[a]	4.7 ± 0.2[a]
Mid B	8.8 ± 1.6[a]	0.11 ± 0.02[a]	0.12 ± 0.02[a]	4.7 ± 0.4[a]
High A	7.3 ± 1.0[a]	0.11 ± 0.03[a]	0.13 ± 0.01[a]	5.1 ± 0.1[a]
High B	9.0 ± 2.5[a]	0.11 ± 0.02[a]	0.13 ± 0.01[a]	4.7 ± 0.3[a]
Density trial two				
Low A	7.9 ± 0.9[a]	0.10 ± 0.10[a]	0.20 ± 0.0[a]	6.1 ± 3.4[a]
Low B	7.9 ± 0.9[a]	0.10 ± 0.10[a]	0.20 ± 0.0[a]	6.1 ± 3.2[a]
Med A	6.0 ± 2.0[a]	0.20 ± 0.20[a]	0.20 ± 0.0[a]	6.2 ± 3.4[a]
Med B	6.2 ± 1.9[a]	0.20 ± 0.10[a]	0.20 ± 0.0[a]	5.8 ± 3.2[a]
High A	5.5 ± 2.1[a]	0.30 ± 0.20[a]	0.20 ± 0.0[a]	6.0 ± 3.4[a]
High B	5.4 ± 2.2[a]	0.30 ± 0.30[a]	0.20 ± 0.0[a]	6.1 ± 3.5[a]

[a] Superscript denotes homogeneous groups; ± Standard deviation

Secondary stress response of the HL♂xCG♀ hybrid reared at high densities

The white blood cell counts (WBC) of the hybrids at the lowest density of 74 $kg.m^{-3}.hr^{-1}$ were very variable. The mean WBCs of the fish from the middle (407 $kg.m^{-3}.hr^{-1}$) and highest (965 $kg.m^{-3}.hr^{-1}$) density tanks were high compared to the fish from the low density tanks. However, the difference was not significant ($P > 0.05$), due to the large variation about the mean WBC of the fish from the low density tanks (Table 8.8).

Table 8.8. Haematological indices of HL♂xCG♀ hybrids at different densities.

Haematological values	Low	Middle	High
Density ($kg.m^{-3}.hr^{-1}$)	74	407	965
White Cell Count ($10^3/m\ell$)	173.1 ± 4.8^a	202.6 ± 23.5^a	202.5 ± 15.5^a
Red Cell Count ($10^6/m\ell$)	4.1 ± 0.9^a	4.0 ± 0.4^a	4.1 ± 0.8^a
Haemoglobin (g/100 mℓ)	13.3 ± 3.5^a	16.4 ± 2.4^b	12.0 ± 1.4^a
Mean Cell Volume (μm^3)	143.7 ± 22.9^a	140.6 ± 12.2^a	159.4 ± 15.3^b
Haematocrit (%)	56.0 ± 12.3^a	55.1 ± 9.3^a	65.5 ± 11.4^a

[a] Superscript denotes homogeneous groups; ± Standard deviation

The only significant differences to be found amongst the haematological indices was that of haemoglobin and mean cell volume. Haemoglobin concentration was higher in the blood of the middle density fish than in the other two densities, while the mean cell volume was highest in the fish from the highest density tanks (Table 8.8).

Most of the haematological indices were substantially higher than those reported in the literature for *Clarias* species (Table 8.9).

Table 8.9. Haematological indices reported in the literature for clariid species.

Species	RBC ($10^6/m\ell$)	Hb (g/100 mℓ)	MCV (μm^3)	HCT (%)	Reference
C. gariepinus	2.10	5.81	137	28.90	Hattingh, 1972
C. gariepinus	1.64	5.02	200	32.64	Olandimeji *et al.*, 1988
C. gariepinus	1.39	11.64	190	26.07	Erondu *et al.*, 1993
C. isheriensis	1.55	14.56	207	31.62	Kori-Siakpere, 1985
H. longifilis	1.65	11.27	210	34.67	Erondu *et al.*, 1993
HL♂xCG♀ hybrid	1.35	8.96	180	22.00	Erondu *et al.*, 1993
HL♂xCG♀ hybrid	4.06	13.90	148	58.86	This study

This may be an indication that the fish were experiencing some degree of stress, even at densities as low as 83 $kg.m^{-3}.hr^{-1}$. However, haematological indices are highly sensitive to physiological changes in the fish, and stress is just one of the causes of these physiological changes (Fange, 1992).

8.4. DISCUSSION

The rearing of *C. gariepinus* larvae at high densities has been advocated by many authors. This is mainly because catfish larvae and early juveniles (<40 days) show reduced levels of aggression at higher densities (Hecht and Appelbaum, 1988; Kaiser *et al.*, 1995a; Kaiser *et al.*, 1995b). Although larval growth has been shown to decrease with increasing density (Appelbaum and van Damme, 1988; Kaiser *et al.*, 1995a), survival and production are maximized when there are high numbers of larvae in proportion to the bottom surface area of their tank (> 1.2 larvae/cm^2) (Kaiser *et al.*, 1995b).

The relationship between high density and decreased aggression was also manifest in larger *C. gariepinus* juveniles (±39.0 g) (Hecht *et al.* in prep.) and *H. longifilis* juveniles (±110.0 g) (Kerdchuen and Legendre, 1992), although no studies have been conducted to determine the effect of density on their growth.

Assuming that the above observations on larvae and early juveniles hold true for catfish in the grow-out phase, it could be expected that the SGRs and mortality rates of the hybrids would decrease with increasing density. However, in both density experiments there was a trend toward improved SGRs with increasing density (until the threshold density was exceeded). While the mortality rates also appeared to increase with increasing density, the only significant increase occurred at the highest density, in the first density trial.

The elevated specific growth rate of the fish at the higher densities could be due to their greater feeding activity. Hecht *et al.* (in prep.) found that at high densities, *C. gariepinus* juveniles reacted faster to the presence of food and had significantly better food consumption rates than fish at low densities. The feeding intensity of the hybrids were quite different for fish at high and low densities. Browsing fish in the low density tanks would leisurely select pellets that had settled onto the floor of the tanks. On the other hand, a fish in the high density tanks would begin thrashing about in the water as soon as it detected food, in an effort to be the first to capture the food. The thrashing action would spark a "feeding frenzy" in the tanks, where the fish would indiscriminantly attack any likely food item in the water column.

The fish at low densities may have been experiencing stress, owing to a paucity of conspecifics, and this was expressed by their loss of interest in feeding (Hecht *et al.*, in prep.). However, the most likely reason is that the "feeding frenzy" response is induced by the tactile stimulation of one fish's feeding

activity on another fish. At very high densities this response is transmitted almost instantaneously, resulting in the distinctive "explosion" of feeding activity. During this frenzy the fish are probably induced to over-eat. In the low density tanks, the fish had time to taste the food, and decide whether or not to eat it. Uneaten food eventually breaks up and is removed by the self-cleaning action of the tanks. Even though feeding was carefully monitored in the density trials, it is likely that there was more wastage of food in the tanks containing low densities of fish, than in the tanks with the higher densities.

However, at densities in excess of 950 $kg.m^{-3}.hr^{-1}$, the FCR of the fish was higher than at the lower densities. This was probably due to the intense overcrowding in the tanks. There was not enough water for the fish to manoeuvre properly to feed, or for all of the fish to reach the surface to feed. Food pellets were also crushed between the writhing bodies of the fish, or lost in the interstitial spaces between the fish. These pellets were then most likely flushed from the tanks.

While indices such as SGR, mortality rate and FCR have assisted researchers to estimate the optimum densities at which to grow larvae and early juveniles under controlled hatchery conditions, they are of little use under "real world" fish farming conditions. Net yield, on the other hand, proved to be a very useful index. It is the culmination of the interactions between SGR, FCR and mortality, and a measure of the tangible gain or loss in fish mass experienced by the farmer.

The terminal density, at which yield was zero or negative for the hybrid catfish, was not attained. Even at a density of 965 $kg.m^{-3}.hr^{-1}$ the hybrids were still growing, although more slowly than at the lower densities. The rate at which yield increased with escalating density began to abate once a density of 415 $kg.m^{-3}.hr^{-1}$ was exceeded. Thus a maximum grow-out density of approximately 400 $kg.m^{-3}.hr^{-1}$ is recommended for the hybrid. This would be the density at which net yield of the fish in the tanks is maximised, without compromising the enhanced growth potential of the HL♂xCG♀ hybrid.

The decrease in net yield at densities in excess of 400 $kg.m^{-3}.hr^{-1}$ indicated that the hybrids were experiencing some form of stress at ultra-high densities. This was supported by the higher white blood cell counts of the fish at densities of 407 $kg.m^{-3}.hr^{-1}$ and 965 $kg.m^{-3}.hr^{-1}$. However, this observation was not statistically significant due to the large variation in WBC of the fish in the low density tanks.

It could not be ascertained whether the WBCs of the hybrids in the density trials were higher than usual for fish. White blood cell counts can vary from 10^3 cells/mℓ to 10^5 cells/mℓ in different species of fish (Blaxhall, 1972). They also vary greatly within a species, depending on age, season and gonadal maturation (Puchkov, 1964). For example, the reported WBCs for *C. gariepinus* range from 3.0 x 10^3/mℓ to 7.3 x 10^5/mℓ (Olandimeji *et al.*, 1988; Erondu *et al.*, 1993).

The haematocrit percentages of the hybrids was very high (59 %) compared to that of other clariids reported in the literature (22-34 %, Table 8.9). The RBC counts were almost twice as high as the values reported in Table 8.9 for other clariid catfish, but well within the 0.1 to 12 x 10^6/mℓ range for fishes reported by Mott (1957). High RBC counts could be caused by the stimulation of erythropoiesis (new cell production) through low dissolved oxygen concentration in the water (Fange, 1992), but the oxygen levels in the experimental tanks did not reach critical levels for the hybrids, even at a density of 965 kg.m^{-3}.hr^{-1} (Table 8.7).

Although the red blood cell counts were remarkably consistent for the fish at the three densities, the mean cell volume was significantly higher at the highest density. This would explain the higher haematocrit values found in the fishes at the highest density. Red blood cell volume increases in anoxic conditions, or when there is a high concentration of carbon dioxide (CO_2) in the cells (Fuchs and Albers, 1988). This may be an indication that the fish at the highest density were air-breathing more than the fish at the other densities, as prolonged air-breathing can lead to hypercapnia (Heisler, 1993). Large volumes of water need to pass over the gills of fish for them to extract sufficient oxygen to survive. This large volume of water means that the partial pressure of CO_2 at the gill-water interface is low. The oxygen concentration of air is far higher than that of water; which means that the aerial respiration organs of fishes need only small volumes of air to extract sufficient oxygen. However, the carbon dioxide concentration in air and water is about the same, therefore the partial pressure of CO_2 increases at the air-airbreathing organ interface, leading to an increase in the CO_2 concentration in the blood of the fish (Heisler, 1993).

Haemoglobin concentration is usually closely correlated with RBC number (Fange, 1992), but the amount of haemoglobin in the fishes at a density of 407 kg.m^{-3}.hr^{-1} was significantly higher than in the other fishes. Increases in haemoglobin concentration have also been correlated to increases in fish activity (Satchell, 1991). The fish at a density of 407 kg.m^{-3}.hr^{-1} were far more active than the fish at the lower density of 74 kg.$^{-3}$.hr^{-1}. However, while densities as high as 965 kg.m^{-3}.hr^{-1} would severely limit the hybrids' opportunity to swim freely, the fish at these densities appeared to be at least as active as the fish at a density of 407 kg.m^{-3}.hr^{-1}.

Even at densities of 965 kg.m^{-3}.hr^{-1}, water quality did not appear to be limit the growth of the hybrid catfish. Oxygen concentration decreased with increasing density up to a point; but above a density of approximately 250 kg.m^{-3}.hr^{-1} it stabilized between 4.0 to 5.0 mg.ℓ^{-1}. This was probably due to the constant splashing and movement of the fishes.

Un-ionized ammonia and nitrite levels increased slightly in the higher density tanks, but did not reach chronic levels for clariid catfish. However, water quality samples were not taken during or directly after feeding. Stress due to poor water quality may have occurred at this time, as the water would turn

opaque with suspended solids, and the concentration of nitrogenous metabolites probably increased.

Satiation feeding proved to be the least wasteful method of feeding catfish. Often if the fish were stressed in some way they would not feed. When cultured fish are fed at a fixed rate, the required amount of food is usually just dumped into the ponds or tanks. Only once the food is in the water does the farmer know if the fish are feeding. Unfortunately satiation feeding is both time consuming and labour intensive. Demand feeding systems have been successfully used to feed many important aquaculture species, including tilapia, salmonids, carps and eels (Brandt *et al.*, 1979; Meriwether, 1986; Billet, 1987; Dahlmadsen and Johansen, 1989). This method of feeding catfish at high densities needs to be researched, although the continuous activity of the fish at high densities could also lead to significant food wastage.

Handling was very stressful to the fishes, and it often took up to three days before the fish began feeding again. Bulk weighing, counting and fish removal or transfer should not be done more than twice a month. Even though it is probably the most stressful way to monitor growth and mortality, the fish in the tanks must be bulk weighed and individually counted for an accurate assessment of the average mass of the fishes and biomass of the tank.

The specific growth rate, mortality rate and feed conversion ratio of the fishes in the two density trials could not be compared, due to the differences in water temperature and the initial size of the fish used in the trials. Nevertheless, during the course of the two trials hybrid catfish were reared at densities ranging from less than 10 $kg.m^{-3}.hr^{-1}$ to almost 1000 $kg.m^{-3}.hr^{-1}$. The threshold density was found to be 415 $kg.m^{-3}.hr^{-1}$, and the optimum density was set at a rounded off value of 400 $kg.m^{-3}.hr^{-1}$.

CHAPTER 9. CONCLUSION

The overall aim of this study was to assess the potential of the *H. longifilis* x *C. gariepinus* hybrid for high density, intensive aquaculture in southern Africa. From a fundamental perspective, a morphometric and meristic study of the *H. longifilis* x *C. gariepinus* cross showed that it was a pure hybrid, exhibiting characteristics of both *H. longifilis* and *C. gariepinus* (Chapter 2). This was verified by a karyological study, which showed that the hybrids had an intermediate chromosome complement (2N = 54, FN = 95) to that of *H. longifilis* (2N = 52, FN = 93) and *C. gariepinus* (2N = 56, FN = 97). In addition, the morphometric, meristic and karyological study showed that the *H. longifilis* x *C. gariepinus* hybrids produced in southern Africa were indistinguishable from those produced in West Africa (Legendre *et al.*, 1992), for the characters tested (Chapter 2). However, differences between the relative growth rates and reproductive potential of the reciprocal crosses have been recorded for the two populations of hybrids (Hecht and Lublinkhof, 1985; Hecht *et al.*, 1991; Legendre *et al.*, 1992; Chapters 3, 4 and 5). Thus, it seems that genotype-environmental interactions had an influence on gene expression in the hybrids, and it was important to limit the investigation of the aquaculture potential of the hybrid to the southern African region.

From a more practical perspective, the *H. longifilis* x *C. gariepinus* hybrid showed excellent aquaculture potential in southern Africa, when compared to *C. gariepinus*, which is the most popular siluriform species farmed in Africa. The hybrids grew at a faster rate than pure strain *C. gariepinus* in all the growth comparisons carried out in the present study (Chapter 4) and reported in the literature (Hecht and Lublinkhof, 1985; Hecht *et al.*, 1991; Legendre *et al.*, 1992), while the HL♂xCG♀ cross grew at faster rate than the CG♂xHL♀ cross for both heterosis trials (Chapter 4).

Because the HL♂xCG♀ cross had a better fertilization and hatching rate than the CG♂xHL♀ cross (Chapter 3), and grew faster than the reciprocal cross, it was chosen as the hybrid with the most potential for aquaculture in southern Africa.

This was fortuitous. While *C. gariepinus* occurs over most of the southern African region, *H. longifilis* only occurs as far south as the Zambezi river (Chapter 2). *Heterobranchus longifilis* is an exotic species to most of the southern African region, and therefore poses an environmental risk to local river and catchment systems. Consequently, it may not be farmed or kept as broodstock in the region. However, cryo-preserved *H. longifilis* sperm can be imported into southern Africa, and used for hybridization with local *C. gariepinus* females, if the resultant hybrids are sterile. Hence the HL♂xCG♀ cross would be the most practical hybrid to produce in southern Africa.

The HL♂xCG♀ hybrid was induced to reproduce and to backcross with *C. gariepinus* under controlled conditions; but its reproductive potential was severely retarded by partial gonadal, gametic and post-

zygotic sterility (Chapter 5). The probable reproductive strategy of the hybrids would, under natural conditions, require a high fecundity level. For this reason it is highly unlikely that the hybrid would be able to reproduce or backcross with *C. gariepinus* in the wild. Further research is necessary to ascertain the fecundity of the hybrid, and its natural reproductive behaviour needs to be examined before a decision can be made on the true extent of the hybrid's reproductive potential.

The HL♂xCG♀ hybrid cross grew at a significantly faster rate than *C. gariepinus*, for at least six months post hatching (Chapter 4). The magnitude of the difference in growth between the hybrid and *C. gariepinus* varied greatly in the growth comparisons. These are summarised in Table 4.5.

In production terms, the significance of the difference in growth rate between the HL♂xCG♀ hybrid and *C. gariepinus* can be explained as follows. For argument's sake, let the difference in growth between the hybrid and *C. gariepinus* be equal to the smallest difference in growth reported in Table 4.5 (i.e. 10.1 % faster growth, in total length). Assuming that *C. gariepinus* attains a mass of 600 g in one year under ideal conditions, the average total length of the fish would be 429 mmTL (calculated, $M = 0.000007TL^{3.0187}$). If the hybrids are 10.1 % larger, their total length would be 472 mm. The weight of a hybrid of this size would be 856 g (calculated, $M = 0.000009TL^{2.9775}$). All considerations being equal, if 100 tonnes of *C. gariepinus* are produced on a hypothetical fish farm in one year, then at least 142.7 tonnes of hybrids would be produced under identical conditions in one year.

One of the benefits of the partial gonadic sterility exhibited by the HL♂xCG♀ hybrid females was a significant decrease in their GSI (2 % vs. 18 % for *C. gariepinus*), resulting in a significant improvement in fillet yield (44.4 %), compared to the fillet yield of *C. gariepinus* females (35.1 %). There was no significant difference between the fillet yield of hybrid and *C. gariepinus* males (43.3 % and 42. 8 % respectively), but the 1:1 sex ratio of both species meant that the overall fillet yield of the hybrids would be 5.0 % higher than that of *C. gariepinus* (Chapter 7).

If the annual production of the hypothetical fish farm is measured in terms of fillet yield, then the HL♂xCG♀ hybrid would yield 62.6 tonnes of fillets, compared to 38.9 tonnes of fillets for *C. gariepinus*. In other words, for the same infrastructure and quantity of seedstock, production on the hypothetical fish farm would be almost double after one year, if the HL♂xCG♀ hybrid were reared instead of *C. gariepinus*.

The threshold density of the HL♂xCG♀ hybrid (415 $kg.m^{-3}.hr^{-1}$) was exceptionally high (Chapter 8). This density was 100 times higher than the density at which catfish are traditionally reared in southern Africa, on semi-intensive fish farms (40 $tonnes.ha^{-1}$). Thus, by switching from semi-intensive farming to intensive farming at high densities, the productivity per unit area of a farm cultivating hybrid catfish could be further improved. Theoretically, for a production unit (pond or tank) surface area of 1.0 ha,

40 tonnes of hybrid catfish can be produced per year through semi-intensive farming (Uys and Hecht, 1988), while 4000 tonnes of fish per year could theoretically be produced if the production unit was farmed intensively. Obviously, these figures exclude other surface area usage, such as a hatchery, office and storage buildings, access roads and other infrastructure. However, an important consideration for the intensive production unit is the maintenance of water quality through biological filtration. A rule-of-thumb estimate for effective biofiltration is that the retention time of the water in the filter should be about 30 minutes (Liao and Mayo, 1974). If the water in the intensive production unit is exchanged every 30 minutes, then 1.0 ha of biofilter unit is needed per 1.0 ha of production unit. Therefore, a combined intensive production and biofiltration unit surface area of 1.0 ha would theoretically produce about 2000 tonnes of fish.

The most important function of biological filtration is the conversion of highly toxic un-ionized ammonia to relatively non-toxic nitrate through bacterial activity (Wheaton, 1977). Most freshwater fish experience 50 % mortality in un-ionized ammonia (NH_3) concentrations of less than 1.6 mg.ℓ^{-1} (Table 6.14). The NH_3 tolerance of *C. gariepinus* was remarkable, but the NH_3 tolerance of the HL♂xCG♀ hybrid was even higher (9.1 mg.ℓ^{-1} vs. 6.5 mg.ℓ^{-1} for *C. gariepinus*, Chapter 6). The maximum recommended NH_3 concentration (where no mortalities occurred) was 5.8 mg.ℓ^{-1} for the hybrid, which was more than double that of *C. gariepinus* (2.3 mg.ℓ^{-1}). Thus the ratio of biofilter unit to production unit could be lower than anticipated for the intensive aquaculture of the HL♂xCG♀ hybrid.

The start-up costs and production costs of intensive farms are high compared to semi-intensive farms (Tucker and Robinson, 1990), due to high capital (plant and equipment) and operating costs (labour, power, artificial feed, medication, etc.) (O'Sullivan and Purser, 1993). These costs are offset by improved water quality control, in particular the ability to heat the water so that the growing season can be extended. Although the HL♂xCG♀ hybrid had a wider preferred temperature range (28 °C to 34 °C) than *C. gariepinus* (28 °C to 30 °C) (Chapter 6), both groups of fish preferred water temperatures in excess of 27 °C. In most areas of southern Africa, some level of water heating would be required to maintain water temperatures around 28 °C.

Because the water in an intensive catfish farm can be heated, and conserved through recirculation, site selection is more flexible. Traditional semi-intensive catfish farms have been limited to very specific regions in southern Africa, where the climate is warm enough for catfish growth, and where there is an ample source of water. These locations are usually far from the market, and riparian land is expensive. Site selection is further limited by the presence of brackish ground water, particularly along the warm, eastern coastal plain. If the hybrid was more euryhaline than *C. gariepinus*, then site selection for a hybrid catfish farm would be more flexible. However, the hypothesis that the HL♂xCG♀ hybrid could tolerate higher salinities than *C. gariepinus* was rejected (Chapter 6). Both groups were stenohaline, and had a 50 % mortality rate at about the same salinity (hybrid = 11.0 g.ℓ^{-1}; *C.*

gariepinus = 10.8 g.ℓ^{-1}) after 96 hours. It was recommended that the fish should not be exposed to salinities in excess of 8.2 g.ℓ^{-1} (the salinity at which the first mortality occurred), and should not be farmed in water with a salinity of more than 2.5 g.ℓ^{-1} (Britz and Hecht, 1989).

The advantage of semi-intensive aquaculture in large ponds is that the start-up and production costs are low, and the large ponds do not need to be monitored constantly for changes in water quality. The water quality parameter that needs to be monitored most closely in intensive aquaculture is oxygen concentration. However, both *C. gariepinus* and the HL♂xCG♀ hybrid can tolerate low oxygen concentrations because of their ability to air-breathe (Chapter 6). Although the frequency of air-breathing in individual fish was highly variable, both groups of fish showed an overall increase in air-breathing after the dissolved oxygen in the water decreased below a certain concentration. This oxygen concentration was 3.8 mg.ℓ^{-1} for the hybrid and 3.0 mg.ℓ^{-1} for *C. gariepinus*. It was speculated in Chapter 6 that fish reared at very high densities might be unduly stressed by the need to increase their air-breathing frequency, and it was recommended that an oxygen saturation in excess of 50 % be maintained in the tanks. Further research is necessary to elucidate whether a relationship between air-breathing frequency and stress exists in catfish reared at ultra-high densities. Even if a relationship does exist, an oxygen saturation level of 50 % was not difficult to maintain in the intensive recirculating system, even at densities of 965 kg.m^{-3}.hr^{-1} (Table 8.7). This was probably due to the high rate of water exchange, as well as the constant splashing and mixing of surface water through fish activity.

From the above summary, it appears that the culture of the *H. longifilis* ♂ x *C. gariepinus* ♀ hybrid at high densities in intensive, recirculating systems may significantly decrease the cost of producing catfish in southern Africa. At the very least, the present study has shown that catfish productivity can be enhanced, by improving the product (by way of hybridization) and by improving the means of production, by switching from semi-intensive pond culture to highly intensive recirculating systems.

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