# FACTORS AFFECTING THE MEAT QUALITY PARAMETERS OF *CLARIAS GARIEPINUS* (BURCHELL)

*by*

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I declare that the thesis hereby submitted to the University of the North for the degree of Philosophiae Doctor in Physiology in the Faculty of Natural Sciences has not previously been submitted by me for a degree at any other University, that it is my own work in design and execution, and that all material contained therein has been duly acknowledged.

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## EXECUTIVE SUMMARY

*Background and Motivation*

Fish meat quality can be defined as all those factors that a fish eater or buyer feels should be present. Thus quality will embrace intrinsic composition, degree of contamination with undesirable materials, nutritive value, degree of spoilage, damage, deterioration during processing, storage, distribution, sale and presentation to the consumer, hazards to health, satisfaction on buying and eating, aesthetic considerations, yield and profitability to the producer and middleman.

In South Africa, the African sharptooth catfish *Clarias gariepinus* industry showed a rapid increase in production in the early 1980's which has decreased to such an extent that at present, production is negligible. The reasons for this decline are market orientated. Production costs were too high for a product that did not have a high sale value. (Competition from the ornamental fish trade particularly Koi carp, also resulted in a number of farmers changing over into the production of this fish species). The catfish producers tried to market their product in the highly discerning higher income group in South Africa. Here the catfish had to compete with trout, and lately, with imported salmon products. The perception of the higher income group towards the catfish is also a negative one, *viz*, the catfish is seen as a dirty fish that tastes of mud. However, consumers from this income group, who have tasted farmed catfish are quick to change their perception.

For any marketing strategy to be successful, a sound knowledge of the product is needed. At the initiation of this study, very little data pertaining to the muscle chemical composition was available. The investigations reported in this thesis were aimed at partially addressing this scarcity.

*Objectives and Scope*

- Quantifying the chemical composition (proximate, amino acid, fatty acid and mineral contents) of the muscle of *C. gariepinus*.
- Determining the fillet yield and chemical composition of *C. gariepinus*
- Investigating the influence of genetic strain on the chemical composition of *C. gariepinus*.
- Investigating the influence of diet composition on the chemical composition of *C. gariepinus*.
- Reviewing and analysing the influence of final effluent oxidation ponds on the chemical composition

of *C. gariepinus*.

- Assessing the influence of different cooking methods on the chemical composition of *C. gariepinus* fillets.

- Establishing consumer behavioural patterns as pertaining to meat products, with special reference to *C. gariepinus*.

*Results and Conclusions*

The intrinsic factors that affect the chemical composition of fish are factors that are within the fish themselves, and are to a certain extent, genetically determined. The heterogeneity of the muscle of *C. gariepinus* was investigated in terms of proximate composition, amino acid, lipid fatty acid and muscle mineral concentrations. No distinct differences in the proximate compositions between dorsal and ventral regions were found. There was a decline in moisture and protein percentages and an increase in total lipid percentage caudally. The percentage ash showed no fixed trend. The concentration of the amino acids glycine, alanine, proline and hydroxyproline increased caudally along the musculature. A decrease in the percentage of saturated and mono-unsaturated fatty acids and an increase in the percentage poly-unsaturated fatty acids were noted caudally. The concentrations of the minerals calcium, copper, zinc and manganese showed no fixed trends caudally, whilst phosphorus, potassium and magnesium decreased and iron increased. It was postulated that these differences could be caused by the ratio of dark and light muscle differing along the musculature. It has been found in various fish species that these two muscle types differ in their chemical composition. A chemical comparison between these two muscle types was therefore performed. The light muscle had a similar moisture (78%, $p=0.3294$), significantly higher protein (19.0 vs 14.5%, $p=0.0716$) and lower total lipid (1.0 vs 5.2%, $p=0.0796$) content than the dark muscle. A comparison of the relative proportions of the fatty acids showed no difference in the amount of saturated fatty acids between the two muscle types ($\pm$34%). The dark muscle however, has a higher percentage total mono-unsaturated fatty acids (6.3% more), whilst the light muscle has a higher total percentage polyunsaturated fatty acids (4.8% more). The dark muscle had a lower $\omega3/\omega6$ ratio (0.44) than the light muscle (0.61). Of all the amino acids analyzed, only lysine was significantly lower, and hydroxyproline higher in the dark muscle. Of the minerals analyzed, the dark muscle had significantly higher concentrations of iron and zinc than the light muscle type.

During these investigations, various lipid depots were found within *C. gariepinus* and it was postulated that they may differ in their fatty acid profiles. The fatty acid profiles of the total lipids in the subcutaneous fat depot, dark and light muscle, mesenteric fat depot and liver were therefore analyzed and compared. The dark muscle had a total lipid content of 5.2 $\pm$ 3.04% compared to 1.0 $\pm$ 0.50% for the light muscle. The liver had 3.9 $\pm$

1.05% total lipid. Palmitic acid predominated in the light muscle and liver whilst oleic acid was principal in the other lipid depots. The subcutaneous and mesenteric lipid depots had high concentrations of the shorter chained fatty acids. The liver had a high proportion polyunsaturated fatty acids and the highest $\omega 3/\omega 6$ ratio (0.81).

Of primary importance to both the producer and consumer is the fillet yield that is realised. Of secondary importance, is the amount of waste produced and potential uses thereof. In *C. gariepinus* the yield is normally given in terms of the dressed round-weight or as a percentage fillet. The major waste products produced are the skin, bones (including head), gut (including liver, heart, intestines, blood, kidneys, spleen), gut fat and gonads. Two investigations were launched to determine the dressed round-weight and yield of *C. gariepinus*. In the first investigation, the drawn and dressed mass of 140-day old catfish, consisting of 306 and 384 individuals of the red (golden) and normal strains respectively, were measured and compared. The influence of sex was investigated. An attempt was made to predict the percentage drawn and percentage dressed masses from live weight by using a linear regression model, but due to high variation resulting in a poor fit ($R^2$ ranged from 0.1158 to 0.0002) these regression equations are rejected as being useful. The golden and normal male catfishes produced the highest percentage mean drawn mass (92.42%), whilst the golden females had a higher mean percentage drawn mass (89.52%) than the normal females (86.42%). The percentage dressed mass of the golden and normal males increased with increasing live mass, but decreased in golden and normal females. In the second investigation, the fillet yield and proximate chemical composition thereof, was determined for catfish from various sources (wild and farmed) and sizes. Body weight seems a reliable predictor of yield and the increase in fillet yield with increasing body weight (BWt) was not influenced by source or gender of the fish. Fish gender had no influence on the head and skin weights with increasing BWt, although the males had significantly ($p<0.05$) heavier bone and total gut weights, and females a heavier gonad weight. The fillet moisture content was influenced by the gender of the fish with increasing BWt, the females gained moisture at a significantly ($p<0.05$) higher rate ($\beta=1.0568$) than the males ($\beta=1.0012$). Gender had no influence on the other proximate chemical (protein, total lipid, ash) parameters, although the females had a faster rate of lipid deposition than the males, this was not significantly so. The calculated regression equations will enable producers and processors to predict fillet yield and proximate chemical composition at various live body weights at reasonably low costs.

The compositional development of the gonads of the golden strain was compared with that of the normal strain. Normal catfish are more fecund than the golden, especially in the smaller size classes, but around approximately 1 kg, the fecundity of the two strains is comparable. Golden catfish are able to spawn more successfully towards the end of the summer breeding season when resorption of eggs generally commences in females of the normal strain. This difference improves the aquaculture potential of the golden strain despite its lower fecundity as it means that overwintered fingerlings of this strain will have a shorter production period in the

next growth season.

A comparison was then made of the chemical composition of the muscle and gonads of sexually mature, female *C. gariepinus*. The muscle contained significantly ($p>0.0001$) more moisture, and correspondingly less protein and ash than the gonads. The total lipid did not differ significantly ($p>0.2255$) between these two organs, while of the fatty acids, palmitic and oleic acids predominated in both. The gonads had significantly higher concentrations of docosapentaenoic ($p>0.0068$) and docosahexaenoic ($p>0.0017$) acids. The amino acid profile of the gonads differed significantly from that of the muscle, with the exception of histidine, phenylalanine and lysine. The gonads had significantly higher calcium, iron, zinc and manganese and lower potassium concentrations than the muscle. The concentrations of phosphorous, magnesium and copper were similar.

A second waste product that is found in *C. gariepinus* and which may have potential uses as a by-product in human nutrition, is the mesenteric or gut fat depot. The fatty acid profiles of the mesenteric fat depot and the lipids extracted from the fillets and the fish diet of farmed catfish, were compared. No statistical differences ($p\leq0.01$) between the fatty acid profiles of the lipids from the fillet and mesenteric fat depot were noted, with the exception of eicosapentaenoic acid (C20:5ω3). Both these profiles resembled that of the lipids from the diet. The lipids from the fillet and mesenteric fat depot had 36.6 and 36.8% saturated, 37.6 and 36.9% mono-unsaturated and 19.6 and 20.6% polyunsaturated fatty acids respectively.

The endogenous factors that affect fish chemical composition are genetically controlled and are associated with the life cycle, size and sex of the fish. Four *C. gariepinus* strains were bred and raised concurrently to test whether there are any genetic influences that may contribute to the whole body chemical composition. Strain strongly influenced body total lipid, but not body protein content. Body total lipid content was associated with growth rate. The body amino acid content was not strongly influenced by strain. Strain had an influence on total body lipid fatty acid composition, although no fixed trend was established. Although strain influenced the body mineral profile, no fixed trends were manifested.

The influence of size (±360 and ±1500 g live mass) and sex on the skin and muscle chemical composition of *C. gariepinus* was investigated during a comparison of the chemical composition of gold and normal coloured strains maintained for a number of generations at the research facilities of the Aquaculture Research Unit, University of the North. The skin of the golden strain was shown to be that of an albino that contained no melanin. Size has a stronger influence than strain on the proximate, fatty acid, amino acid and mineral profiles of both the skin and muscle. For both strains, the larger sized class had statistically lower ($p>0.05$) muscle moisture and higher total lipid content than the smaller. There was no significant difference in the muscle protein content (16.7-17.6% wet mass basis) between and within the strains and size classes. The skin has

higher concentrations glycine, proline and hydroxyproline than the muscle. The major fatty acids present in both the skin and muscle were palmitic (>23%) and oleic (>28%). Of the muscle minerals, potassium was significantly ($p>0.05$) higher in the larger sized class for both strains.

The influence that size and sex may have on the muscle chemical composition was investigated and compared between farmed and wild *C. gariepinus*. The fatty acid profiles between sexes within type, were not significantly ($p\leq0.05$) different. The wild catfish had statistically higher ($p\leq0.05$) concentrations of C16:1, C17:0, C17:1 and lower concentrations of C18:1 and C18:2 than farmed catfish. *C. gariepinus* had 39.5% saturated fatty acids, 42.6% mono-unsaturated fatty acids and 17.3% polyunsaturated fatty acids. Differences in amino acid and mineral compositions of the male and female farmed catfish tested non-significant ($p\leq0.05$). However, there were amino acid differences between wild male and female (aspartic acid, glutamic acid, phenylalanine and methionine) and between farmed and wild catfish (tyrosine, valine, leucine and hydroxyproline). Lysine is well represented in the fillets (10% of total amino acids present). The levels of the minerals phosphorus, potassium, manganese and iron differed significantly between type and between sexes within type. No differences were noted for calcium, magnesium, copper and zinc.

The exogenous factors that may influence the chemical composition of *C. gariepinus* are numerous and include environmental and dietary factors. The replacement of fish meal (two sources, arbitrary designated FMA and FMB) with either, freeze dried (GVD) or oven dried (GOD) gonads of *C. gariepinus* in a starter diet for larvae of this species, was tested. No significant difference in larval growth rates were noted between the various protein sources, although fish receiving FMA had a significantly lower final body weight ($p\leq0.05$). Fish which received gonads as a protein source had a significantly higher total body lipid (GOD=28.47% Dry Mass - DM) than that receiving fish meal (FMA=13.97% DM). The whole body fatty acid composition was influenced by that of the fatty acid composition of the protein source. The hepatocytes of the fish receiving the gonads as protein source showed 100% lipid accumulation and degenerative changes, whilst those from fish meal fed fish, showed none.

In a second study on exogenic factors, the influence of various lipid sources on the muscle fatty acid composition of juvenile catfish, were tested. Juveniles were fed an artificial diet for 60 days containing no lipid (A, control), or the following lipids at 10% of the diet, sunflower oil (B, a high level of C18:1$\omega$9 & C18:2$\omega$6), cod liver oil (C, a high level of 20 and 22 C$\omega$3 fatty acids) and tallow (D, predominantly SFA & MUFA). Muscle total lipid composition was strongly influenced by diet and contained the following SFA, MUFA and PUFA percentages, and a $\omega3/\omega6$ ratio of: A - 36.33; 45.13; 15.70% & 0.52; B - 30.78; 34.54; 33.96% & 0.11; C - 33.51; 38.75; 24.59% & 1.87; D - 38.87; 46.03; 13.06% & 0.44, respectively.

During these investigations, various parasitic infections were noted, especially in the wild *C. gariepinus* specimens. The various bacterial, viral and parasitic infections noted in freshwater fish and their influence when consumed by humans, or when workers come into contact with them, are therefore reviewed. Special attention is given to those that could occur when combined in integrated aquaculture-agriculture practices with emphasis on the use of sewage waste water. The influences on human health with the consumption of fish contaminated with various chemicals and pollutants are also noted. Recommendations into controlling and minimising the potential of epidemics occurring are discussed. In South Africa, *C. gariepinus* is also found and raised in sewage purification oxidation ponds. The muscle chemical composition of catfish found in these ponds (Pietersburg), and the fish's suitability for human consumption were investigated. The fish (n=30) yielded between 41-51% fillet, which had a proximate composition of 73.76 ± 4.8087, 17.31 ± 1.4374, 7.84 ± 5.0311 and 2.82 ± 0.5278 for the percentages of moisture, protein, total lipid, and ash respectively. The muscle total lipid contained 32.85% SFA, 35.83% MUFA, 28.62% PUFA, and an $\omega3/\omega6$ ratio of 2.57. The muscle did not contain any minerals at concentrations dangerous for human consumption. It was thus concluded that catfish found in the Pietersburg sewage purification oxidation ponds are fit for human consumption.

Since it is seldom that catfish fillets are consumed raw, the effect of five cooking methods (shallow, deep fried, covered, open baked, and microwaved) on the chemical composition of *C. gariepinus* fillets was also evaluated. All five methods resulted in significant ($p<0.05$) moisture losses (58.82; 61.06; 72.38; 70.61 and 73.05% moisture, respectively) when compared to the uncooked control (77.31 %). Absorption of the frying oil resulted in significant ($p<0.01$) increases in the percentage of total lipid (3.21% control, 11.74% shallow pan, and 8.77% deep fried) of the two frying methods. The fillet lipid extract of the frying methods thus tended to have similar fatty acid compositions to that of the frying oil. No fixed pattern was discernible for the mineral composition according to the cooking methods used. However, in general the concentrating effect of moisture loss during cooking resulted in higher concentrations of the minerals (eg P and Fe) in the tissue.

The investigation was concluded with a study on consumer behavioural patterns relating to meat products, with special reference to *C. gariepinus*, in a rural (Ga-Mamphaka) and urban (Giyani) setting in Northern Province, South Africa, by means of personal interviews. The major meat type purchased was chicken (71.4% in the rural and 46.0% in the urban community). The sharptooth catfish was found to be acceptable to the majority of both urban (69.0%) and rural (57.1%) respondents. Most respondents also indicate that they would like to purchase catfish processed in a canned form. The potential market for catfish would seem to be at a price competitive with that of chicken.

## ABSTRACT

The influences of various intrinsic, endogenous and exogenous factors on the meat quality parameters of the African sharptooth catfish *Clarias gariepinus* (Burchell 1822) were investigated. Various chemical parameters, consisting mainly of muscle proximate composition, lipid fatty acid, amino acid and mineral profiles, were used to measure these influences. In a test of the muscle heterogeneity, no chemical difference were found between the dorsal and ventral regions. Differences were found between the cranial and caudal regions. These differences were as a result of a change in the ratio of dark to light muscle, the former increasing caudally. A chemical analysis of dark and light muscle showed numerous chemical differences. Various lipid depots were identified in *C. gariepinus* and the total lipid fatty acids identified. The subcutaneous and mesenteric depots had high concentrations of the shorter chained fatty acids, whilst the liver had a high proportion of polyunsaturated fatty acids and a high $\omega3:\omega6$ ratio. The genetic influence on the whole body chemical composition was tested between various strains and was found to have an influence on the lipid composition. The effect of strain on the amino acid and mineral composition was not as dominant. In a comparison between wild and farmed catfish, various differences were noted in the muscle chemical composition, these differences were mainly attributed to diet. In a latter comparison of the influence of size and strain between a golden domestic strain and a normal coloured domestic strain, size was found to have a stronger influence than strain (the fish were raised in the same environment). An investigation of the muscle chemical composition of catfish occurring naturally in final effluent oxidation ponds found the muscle to be fit for human consumption. No excessive concentrations of heavy metals were found in the muscle. When juvenile catfish were fed various lipid sources, the muscle lipid fatty acid profile was strongly influenced by the diet source. In a comparison of the drawn mass between farmed male and female of the golden and normal strains, the golden strain (both sexes) had a higher yield in comparison to the normal. For both strains, the males yielded higher drawn masses. The major cause was attributed to a difference in gonadal development. The influence of genetic type, size and sex on the fillet yield was examined. Fish size had a stronger influence on fillet yield than did type or sex. Regression equations for the prediction of yield and muscle proximate composition were calculated. The influence of season on the gonadal development was also investigated and it was found that the normal strain was sexually mature early in the breeding season, whilst the golden strain was mature in the later half of the breeding season. An investigation into the chemical composition of female gonads showed the gonads to have a good potential for utilisation as a by-produced in various animal diets. A study into the potential of replacing fish meal in the starter diet of *C. gariepinus* larvae with the gonads of the same species, showed no difference in the growth rates of the larvae receiving either gonads or fish meal. A second waste product, the mesenteric lipid depot had a fatty acid profile that made this depot an ideal supplement in human diets that are poor in energy. The changes that occur in the muscle in terms of lipid composition and mineral profile during various cooking methods were examined. Cooking tended to cause a moisture loss in the muscle, and where applicable, the fatty acid profile

resembled that of the frying oil used. Various parasitic and bacterial infections were noted during the numerous investigations, and the influence thereof on human health is reviewed. The study concludes with an examination on consumer behavioural patterns relating to meat products, with special reference to *C. gariepinus*, in a rural and urban setting in Northern Province, South Africa. The general consensus was that if the catfish was priced competitively to chicken, there would be no consumer prejudice amongst both groups studied.

## OPSOMMING

Die invloed wat verskeie intrinsieke, endogene en eksogene faktore op die vleiskwaliteitsparameters van die skerptandbaber *Clarias gariepinus* (Burchell 1922) het, is ondersoek. Verskeie chemiese parameters, wat hoofsaaklik uit spier benaderde samestelling ten opsigte van vog, proteïen, vet en as, asook vetsure, aminosure en minerale samestelling besteen, is geneem om dié invloede te bepaal. Geen verskil in die chemiese samestelling van dorsale en ventrale spierweefsel is gevind nie. Verskille het egter voorgekom tussen die kraniale en kaudale gedeeltes, hoofsaaklik as gevolg van 'n verandering in die verhouding van donker en ligte spierweefsel. Kaudaal is 'n toename in donker spierweefsel waargeneem. Betekensivolle chemiese verskille is tussen donker en ligte spierweefsel waargeneem. Verskeie vetdepots is by *Clarias gariepinus* geidentifiseer en die vetsure daarvan ontleed. 'n Kenmerk van die onderhuidse en mesenteriese vetdepots, is die relatief hoër konsentrasie kort-kettingvetsure, terwyl die lewer 'n hoë proporsie poli-onversadigde vetsure en 'n hoë verhouding $\omega 3{:}\omega 6$ het. Die invloed wat genetiese samestelling op die totale liggaams chemie het, is vir verskillende katvis varieteite getoets en is gevind om 'n invloed op vetsamestelling te hê. Die invloed op aminosuur- en minerale samestelling was nie so opvallend nie. Vergelykende studies tussen natuurlike en geproduseerde baber, het heelwat verskille in die chemiese samestelling van spierweefsel tussen die twee tipes aangedui. Hierdie verskille kan hoofsaaklik aan 'n verskil in dieët toegeskryf word. In 'n vergelykende studie om die invloed van grootte en varieteit tussen die goue en normaalgekleurde varieteite vas te stel, is gevind dat grootte 'n groter invloed as verieteit het. 'n Ondersoek na die invloed van verouderingswater in rioolsisteme op die chemiese samestelling van spierweefsel, het aangetoon dat vis wat in verouderingswater van rioolsisteme geproduseer is, geskik is vir menslike gebruik. Geen opbou van swaarmetale is in enige spierweefsel gevind nie. Vetsuursamestelling van die spierweefsel van jong vis was beïnvloed deur die tipe vet wat in die dieët ingesluit was. 'n Vergelyking in die uitslagpersentasie van kommersieël geproduseerde gene en normaal gekleurde manlike en vroulike vis, het aangetoon dat goue baber (manlik sowel as vroulik) 'n hoër uitslagpersentasie as die normaalgekleurde vis het. Hierdie tendens is hoofsaaklik toe te skryf aan 'n verskil in gonade ontwikkeling. Die invloed van genotiepe, grootte en geslag op filletmassa, is ondersoek. Visgrootte het 'n groter invloed op filletmassa as wat vis varieteit en geslag het. Regressievergelykings vir die voorspelling van opbrengs en spiersamestelling is bereken. Die invloed van klimaat (seisoene) op gonade ontwikkeling, is ook ondersoek. Dit is gevind dat normaal gekleurde baber vroeër in die seisoen geslagsrypheid bereik. Chemiese analise van die gonades, het aangetoon dat gonades 'n waardevolle neweproduk vir verskeie dierevoedsels kon bied, alhoewel geen verskil in die groei van vislarwes waargeneem is waar vismeel en gonades gebruik is nie. Die vetsuursamestelling van mesenteriese vet, maak hierdie neweproduk uiters geskik vir menslike gebruik in gemeenskappe met 'n lae energie voedselinname. Die verandering in spiervet en minerale samestelling wat by verskillende gaarmaakmetodes voorkom, is ondersoek. Wanneer vleis gekook is, is 'n verlies in spiervog waargeneem, en waar van toepassing, het die vetsuursamestelling van die gaar vleis

dié van die kookolie weerspieël. Tydens die huidige ondersoek, is verskeie parasitiese en bakteriese infeksies waargeneem en die invloed daarvan op menslike gesondheid, is bespreek. Die studie is afgesluit met 'n ondersoek na verbruikersgedragspatrone, in beide plattelandse en stedelike gebiede in die Noordlike Provinsie. Die ondersoek het aangetoon dat geen verbruikersweerstand bestaan solank vispryse vergelykbaar is met pryse vir hoendervleis nie.

## LIST OF PUBLICATIONS AND CONFERENCE PROCEEDINGS

Papers published or in press in peer-reviewed scientific journals and conference proceedings emanating from this study:

**HOFFMAN LC & PRINSLOO JF.** 1990. A comparison of the dressout percentage of the red and normal coloured strains of the African sharptooth catfish, *Clarias gariepinus* (Burchell). *SA J Food Sci Nutr* **2**:35-38.

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# Chapter 1 AN OVERVIEW OF THE CATFISH INDUSTRY AND BIOLOGICAL CHARACTERISTICS OF THE SPECIES, WITH PARTICULAR REFERENCE TO *CLARIAS GARIEPINUS*

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## GENERAL

In all communities some fish species are more highly valued than others and these preferences are very stable through time and different generations. The geographical occurrence of the fish species, the sharptooth catfish *Clarias gariepinus* in this instance, will also determine whether it is traditionally eaten or not. In the past in the northern regions of South Africa where there are a minimum number of rivers, natural water impoundments and lakes, and no easy access to the oceans, the local communities had developed a taste for the local indigenous species rather than marine fish species. This pattern has, however, changed over the past number of years as the access to commodities in the more isolated regions of the country improves. With improved roads and transport, easier access between major centres and between consumer and retail outlets, marine fish are more readily available in the traditionally landlocked areas. This has resulted in the indigenous fish species competing with marine fish species for the consumer market (Shaw, 1986).

## CLASSIFICATION

Fish are the most numerous of the vertebrates with 20 000 species known and probably many more species still unknown. Fish are usually divided into three classes: Cephalaspidomorphi, jawless fish like lampreys and slime-eels; Chondrichthyes, cartilaginous fish like sharks and rays; and Osteichthyes, bony fish, which include most of the commercially important fish. These classes represent numerous genera, which are then further subdivided into different species.

The classification into cartilaginous and bony fish (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently and vary with regard to chemical composition. Most non-taxonomists refer to these groups as teleosts (bony fish) and elasmobranchs (cartilaginous fish). Fish can also be divided into fatty and lean species, but this type of classification is based purely on some technological characteristics (Huss, 1988).

The African sharptooth catfish, *C. gariepinus*, has the following classification:

| | |
|---|---|
| **Phylum:** | Chordata |
| **Class:** | Osteichtyes |
| **Subclass:** | Actinopterygii |
| **Superorder:** | Teleostei |
| **Order:** | Siluriformes |
| **Family:** | Clariidae |

**Genus:** *Clarias*

**Subgenus:** *Clarias* Gronovius

In an in-depth systematic revision of the African species of the genus *Clarias*, Teugels (1982*a,b*, 1984), recognises six subgenera, ie: *Dinotopteroides* Fowler, *Clarias* Gronovius, *Platycephaloides* Teugels, *Clariodes* David & Poll, *Anguilloclarias* Teugels and *Brevicephaloides* Teugels. Species of the subgenus *Clarias* can easily be recognised by their head length (28.0-35.0% standard length). (Only the species of the subgenus *Dinotopteroides* show about the same head length; these however, can be distinguished by the presence of a small adipose fin). Species of the subgenus *Clarias* show a higher maximum number of gill rakers on the first branchial arch, varying between 16 and 110, depending on the body length. *C. gariepinus* (Burchell) is also known under its junior synonyms *C. lazera* Valenciennes and *C. mossambicus* Peters.

## NATURAL DISTRIBUTION AND HABITAT PREFERENCES

The African sharptooth catfish *C. gariepinus* is found in the larger East African lakes and headwaters of the Blue Nile, in Ethiopia, Somalia and the coastal rivers of Kenya. Southwards it is found along the East Coast as far as the Umtamvuma River in Natal; the upper reaches of the Kasai and Lualaba tributaries of the Zaire, the Zambia-Zaire system as well as Lakes Tanganyika and Malawi. On the West Coast, *C. gariepinus* is found from the Cuanza, Cunene and Okavango rivers in Angola, the whole Zambezi system and the Orange River in South Africa (Bell-Cross & Minshull, 1988). It is also present in the West African systems and extends into eastern Europe. According to Bruton (1988), *C. gariepinus* is the freshwater species with the widest latitudinal range in the world (about 70° latitude).

In 1981, *C. gariepinus* was introduced into China, where it is now cultured, and more recently, it has been introduced into the Philippines, Thailand and Bangladesh (Haylor, 1992).

## COMMERCIALLY CULTURED

### *History in southern Africa*

The first attempts at the experimental culture of the species in South Africa, was undertaken by Douglas Hey, at Jonkershoek, in 1941 and then thirty years later, by van der Waal (1972) at Marble Hall in the Transvaal (Hecht & Britz, 1990). Holl (1968) hatched a limited number of larvae from eggs collected from a natural spawn that occurred during the summer of 1964 after the first rains in Zimbabwe. Unfortunately, none of the larvae survived after they had reached a length of 10mm. Catfish were first successfully cultured in southern Africa in 1981 by Tom Hecht and Willem Lublinkhof on a farm in the Mazabuka district in Zambia. The

second catfish farm (Cliff Fisheries) was developed by Roy Kannemeyer in 1985 in Kimberley, this was followed soon afterwards by the establishment of Blyde River Aquaculture by Wynand Uys in the eastern Transvaal lowveld (Hecht & Britz, 1990).

*Production studies*

In 1984, Hogendoorn published a short paper where he found, from trials in fish ponds in Cameroon and in laboratory tanks in the Netherlands, that the African catfish was highly suitable for aquaculture and could replace the Nile tilapia (*Oreochromis niloticus*) in African fish farms. The latter proved unsuitable because of their high fecundity that resulted in the harvesting of too many small tilapia in over-populated ponds. In the above mentioned trials, the advantage of using *C. gariepinus* amounted to a more than 2.5-fold increase in biomass produced per pond when compared to the tilapia (Hogendoorn & Koops, 1983). The fish in this study were fed a low grade feed (1 part cottonseed cake, 1 part cows' blood and 8 parts brewers grains) which resulted in a poor overall growth and feed conversion rates for both the catfish and tilapia - after 24 weeks, the total biomass ranged from 50 to 266 $g/m^3$ for *C. gariepinus* and 16 to 115 $g/m^3$ for the tilapia. Van der Waal (1978 - South Africa) also found a low biomass production for *C. gariepinus* stocked in earthen ponds with no inorganic fertilisation (36.6 kg/ha) or with inorganic fertilisation (40.3 kg/ha). However, better results (3135 kg/ha) were found with inorganic fertilisation and additional feeding (38% protein at 5% calculated total biomass).

Huisman and Richter (1987) quote production figures (supplied by Janssen, 1983, 1984, 1985) for *C. gariepinus* grown in stagnant ponds in the Central African Republic. In a monoculture, double crop, 20 t/ha was realised whilst in polyculture with *O. niloticus*, double crop, 10 t/ha, was produced annually. In South Africa, Prinsloo and Schoonbee (1987) fed chicken offal to catfish at low rates (1.4-1.9% DM of standing crop) and still found an acceptable production of 2 t/ha over a 75 day growth period. In a second study, Prinsloo, Schoonbee and van der Walt (1989) produced a catfish yield of 9.171 t/ha over a 140 day growth period. The fish were fed a diet consisting of minced tilapia (25%), bakery-floor sweepings (37.5%) and an 18% protein formulated pelleted chicken feed (37.5%). In a latter study (Prinsloo & Schoonbee, 1992) on the utilisation of final effluent oxidation ponds, a polyculture of *Cyprinus carpio* and *C. gariepinus* yielded a production of 6151.5 kg/ha over an 100 day growth period. The fish were fed an 18% protein chicken broiler finish pellet applied at a 2% rate of the total calculated biomass during the first 28 days, thereafter at a 4% rate.

The production results mentioned, clearly show that *C. gariepinus* in either, monoculture or polyculture with other fish species, is an ideal fish species for aquacultural production. In South Africa, the phenomenal growth of the catfish industry between 1987 and 1992 has been repeatedly documented (Hecht, Uys & Britz, 1988; Hecht & Britz, 1990, 1991; Uys, 1991, Hecht, Britz & Uys, 1992). However, it has also been stated that the

biggest remaining challenge to the industry is that of market development. Uys (1993) noted that having not yet met this challenge, the industry is showing clear signs of at least a temporary regression, if not the beginning of a collapse (Table 1). At the '90 Aquaculture congress, Courtney (1991) reiterated the importance of having a marketing strategy for any aquacultural product. Courtney (1991) further noted a major reason for the failure of many an aquaculture venture lay, amongst others, in the production of a product that is the wrong size/colour/quality for the targeted market. A knowledge of the morphological and chemical composition of this fish species, and possible intrinsic variations thereon, will therefore be of great value to the industry both from a processing (Wheaton & Lawson, 1985) and marketing (Shaw, 1986) viewpoint.

**Table 1:** Production figures for sharptooth catfish in South Africa (from Uys, 1993).

| Year | Total Tonnage Produced | Number of Fingerlings Produced | Number of Active Producers |
|---|---|---|---|
| 1987 | 10 | ? | 2 |
| 1988 | 137 | 0.5 mill | 9 |
| 1989 | 203 | 2.1 mill | 15 |
| 1990 | 850 | 2.8 mill | 20 |
| 1991 | 1150 | 1.5 mill | 22 |
| 1992 | 450 | 1.2 mill | 22 |

The other important trends that have taken place in the catfish industry in South Africa, is the identification of genetic markers that differentiate between fast and slow growing catfish (Grobler, Du Preez & Van der Bank, 1992; Van der Bank, Grobler & Du Preez, 1992). Another potentially important contribution to the catfish industry is the crossing of the African sharptooth catfish *C. gariepinus* (Burchell, 1822) with the vundu *Heterobranchus longifilis* (Valenciennes, 1840), the hybrids all have a faster growth rate (Hecht & Lublinkhof, 1985; Legendre, Teugels, Cauty & Jalaberts, 1992).

## ANATOMY

One of the definitions applicable to quality (Connell, 1980, 1990) is the aesthetic appearance of the fish species (Fig 1). The consumer will more readily purchase a fish that he finds aesthetically pleasing than one that is not. Therefore, appearance is one of the first quality parameters that influences the decision of the consumer, especially the fresh fish consumer, on whether or not he will purchase the fish. A detailed description of the general anatomy and skeleton of *C. gariepinus* is given in the following paragraphs.

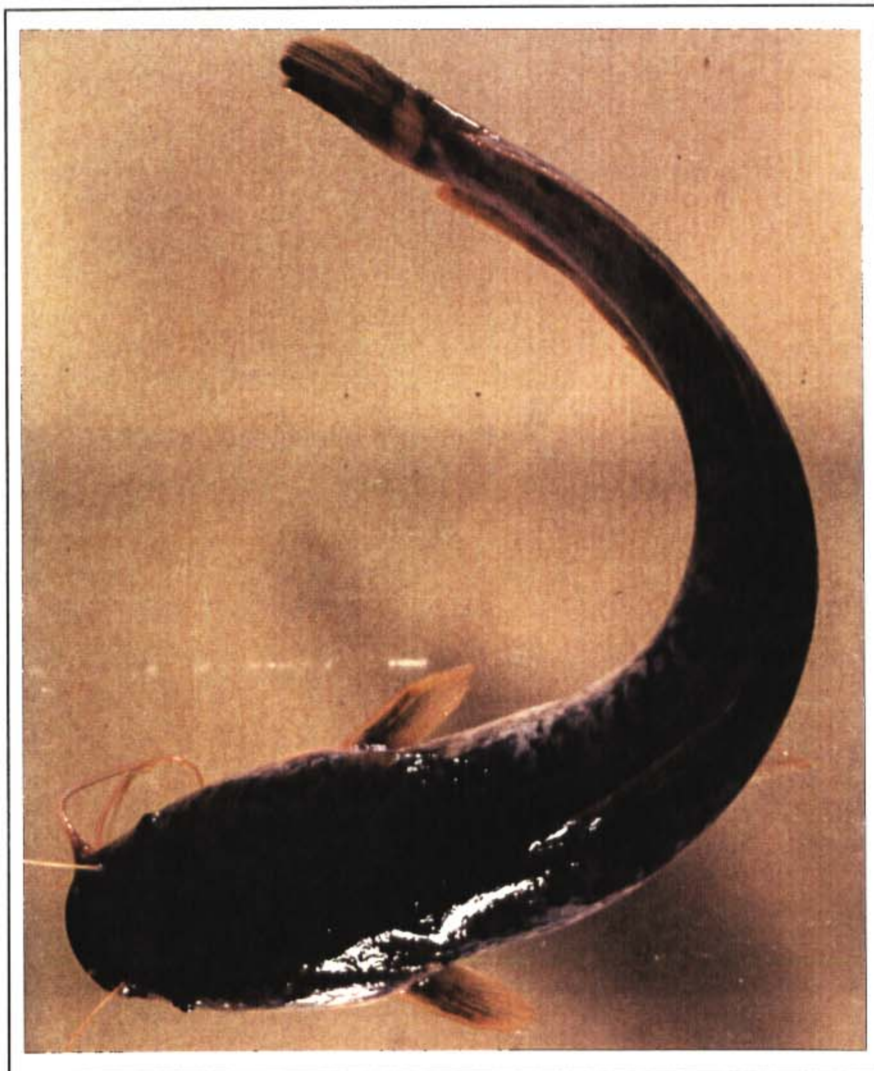

**Figure 1:** A normal coloured *Clarias gariepinus* specimen.

*External Features*

The body of *C. gariepinus* is dorso-ventrally flattened in front and laterally compressed towards the tail (Mills, 1966). Whilst the skin is tough and lacks scales, and is darkly pigmented in the dorsal and lateral parts of the body. Histological examination shows that it is the thin superficial epidermis which is pigmented, and the dermis which is tough and fibrous. The fish becomes lighter in colour, and more mottled in appearance, when exposed to bright light or a lighter coloured background/environment. This is due to the concentration of pigment within special pigment-carrying cells, the chromatophores (Figure 2).

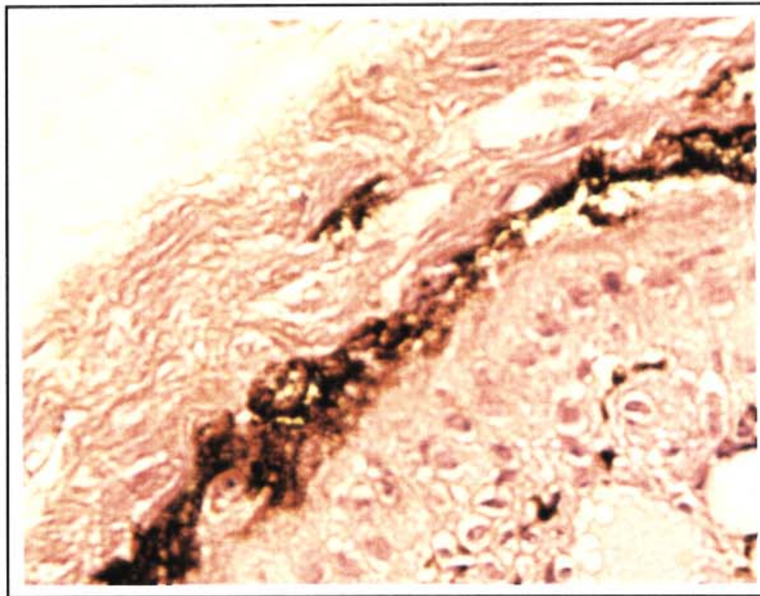

**Figure 2:** A light microscope cross section of the skin from normal coloured *Clarias gariepinus* showing the chromatophores.

A pair of large maxillary barbels arise from the lateral angles of the large terminal mouth. Inner mandibular, outer mandibular, and nasal barbels are also present in the anterior head region, and are all named according to their relationship with parts of the skull. Closely connected with each nasal barbel is a double nostril. One nasal opening occurs as a slit-like orifice immediately behind the nasal barbel, while the other is at the end of a small tubular process situated towards the tip of the snout.

The thin epidermis of the skull region appears to be without a substantial dermis owing to the development of the superficial dermal bones of the skull. The coarsely granulated surfaces of the bones are clearly definable through the epidermis.

Beneath the head, the right operculum overlaps the left, so that there is no free isthmus, or space, on the ventral surface between the two gill openings. A short distance in front of the opercular openings on the ventral surface, is a crescentic infold marking the anterior boundary of the hyoid arch. This infold permits the lower jaw to move independently of the pharyngeal floor.

Fins of fishes may be classed as either median, or paired. In *C. gariepinus* the median fins consist of a dorsal, a caudal, and an anal fin, while the paired fins consist of the pectoral and pelvic fins. The appearance of all these fins in *C. gariepinus* differs from that to be seen in most teleosts. In the first place the pelvic fins retain their primitive abdominal position, and are small. Second, a special feature is to be found in the development of strong spines in connection with the pectoral fins. When the fish moves on land these spines act as limbs. Many silurid fish, however, possess similar spines in connection with the pelvic and dorsal fins, and the use of the pectoral spines for locomotory purposes, therefore, is secondary to their protective function. A further unusual feature of the fins of *C. gariepinus* is the continuous appearance of the median fins. The dorsal fin extends along the length of the body and is only narrowly separated from the unforked caudal fin. Similarly, the anal fin extends from the caudal fin to the anus.

In both sexes of *C. gariepinus*, the urino-genital opening is situated on a papilla just behind the anus, and the male can usually be distinguished by the elongated, backwardly projecting form of this papilla. In the female the papilla takes the form of an oval eminence on which separate urinary and genital openings may sometimes be identified by means of a probe.

*The Skeleton*

Fish have a vertebral column - the backbone - and a cranium covering the brain. The adult skull of *C. gariepinus* consists almost entirely of bone. An extensive development of membrane bones in the skull of *C. gariepinus* is manifested by the presence of a strong superficial shield covering the entire head region. The shield is formed from the dermal bones of the roof of the cranium and the sides of the face, and the sutural ligaments between them are either very tight or have become ossified, thus giving added strength to the entire structure. The skull as a whole is massive in relation to the size of the body, and a number of abdominal vertebrae forming a 'complex vertebra' are fused to its base (Mills, 1966). The actual brain case is, however, small in relation to the size of the skull, and is fused to the undersurface of the dermal shield which protects the entire head.

The backbone runs from the head to the tail fin and is composed of segments called vertebrae. These vertebrae are extended dorsally to form neural spines and in the trunk region they have lateral processes that bear ribs (pleural ribs). *C. gariepinus* has approximately sixty-five vertebrae which are all cartilaginous. These bones

can be divided into eighteen abdominal vertebrae and at least forty-seven caudal vertebrae. There is no subdivision into the various regions characteristic of the vertebral column in higher vertebrates, but the first five vertebrae are, however, highly modified to form the "complex vertebrae" which is fused to the skull. The complex vertebrae encloses a bi-lobed air-sac in two lateral funnel-shaped modifications of the combined transverse processes on either side. It is possible that the attachment of the complex vertebra to the skull is a special adaptation helping to balance the large head by giving better muscle attachment, and by acting as a float through the incorporation of the air-sac.

The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes). In *C. gariepinus* the ribs consist of fourteen pairs of slender, curved bones in the lateral abdominal walls. They are loosely connected to the parapophyses of the seventh to the twentieth vertebrae. Usually in most fish species there is a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise being processed. The African sharptooth catfish, *C. gariepinus*, does not have any false ribs, thus making *Clarias* an ideal species for commercial filleting.

The dorsal fin ray has long bony spines that extend into the muscle perpendicularly and join up with the neural spines of the backbone. These spines are also known as pterygiopores or interneurals.

## MUSCLE ANATOMY AND FUNCTION

*General*

Muscular tissue forms a larger part of the mass of the fish body than it does of other vertebrates: some 40-60% of the total body mass in most fish is locomotor musculature. In part this is because economy in weight is not mandatory as it is for terrestrial and aerial forms, and in part because stringent demands are placed on the locomotor system by the density of the medium, so that a large amount of muscle is needed to generate sufficient power for rapid swimming (Bone, 1978).

The anatomy of fish muscle is very simple. Basically, there are two bundles of muscles on each side of the vertebral column and each of these bundles is further separated into an upper mass above the horizontal axial septum and a ventral mass below this septum. The muscle cells run in a longitudinal direction separated perpendicularly by sheets of connective tissue (myocommata). The muscle segments lying between the sheets of connective tissue are called myotomes. The longest muscle cells are found in the twelfth myotome counting from the head. The length of the cells, as well as the thickness of the myocommata, will increase with age. With this anatomy the fish muscle tissue contains comparatively far less connective tissue than mammalian

muscle (Huss, 1988).

The cell membrane of the muscle fibre is termed the plasmalemma (sarcolemma). External to this is the basal lamina (basement membrane) to which are attached the collagenous (reticular) fibrils of the endomysium which, in fish, is continuous to the myocomma (Bremner & Hallett, 1985).

*Organization of the myotomes*

The great majority of fishes (including *C. gariepinus*) swim using the segmented myotomal musculature. The myotomes or myomeres correspond in number with the vertebrae (over most of the vertebral coloumn), but alternate with them, so that in the midline each myomere lies opposite the back half of one vertebra and the front half of the next. Within each myomere the muscle fibres are short and tend to run roughly parallel to the long axis of the body, although some may depart by as much as 35°. Therefore most muscle fibres do not attach to skeletal parts, but instead to tough sheets of connective tissue, the myocommata (or "transverse septa"), which separates adjacent myomeres. The myocommata are anchored in the median plane to the vertebral coloumn, and to its neural and hemal spines, and to the tough median septum. Within the myocommata there may lie the segmentally arranged ribs (Lindsey, 1978).

A number of workers have attempted to provide a functional basis for the complex shape of the fish myotome, and for its internal arrangement. The attempts have been more successful in explaining the orientation of muscle fibres within the myotomes themselves; yet both presumably reflect the properties of the contractile units of the system and the properties of their insertions. The important properties in so far as myotomal design is concerned would seem to be the following:

1. Contraction takes place without volume change.
2. The muscle fibres insert onto deformable but inextensible partitions which are attached to the flexible but incompressible notochord or vertebral coloumn.
3. Deformation of the myosepta is often (but not always) restrained by intermuscular bone.
4. Flexion of the body is required only in the lateral plane.
5. During flexion, the radius of curvature will be least next to the vertical coloumn, largest just under the skin.
6. Both frequency and amplitude of flexions may vary.

The end results of the operation of the myotomal units with these properties is, of course, the lateral oscillations of the body brought about by transferring the contraction forces to the central strut. The V-folding of the myotome is probably related to the need to avoid dorsoventral flexion. The notochord lies dorsally to

accommodate the viscera below it, and if the myotomes were simple inclined blocks (the greater part of which lay below the strut), contraction would lead to ventral flexions of head and tail. With the V-shaped myotome arranged so that the arms of the V are unequal in length, and that the apex lies at the level of the notochord, solely lateral flexions are possible (Bone, 1978).

It is significant that with increase in scale, all other fishes have their myotomes arranged so that the muscle fibres of the greater part of the myotome do not run parallel to the long axis. The muscle fibres of the white or fast part of the myotome may make large angles with the long axis. These orientations are not random; Bone (1978) describes work done by Alexander (1969) where two patterns of orientation were noted in sharks and in higher teleostats. These two patterns of orientation of the fast or white fibres of the myotome were a consequence of the requirement for all fibres to contract at about the same rate, whatever their position in the myotome. In other words, although during flexion the radius of curvature of the fish as a whole will be greatest next to the vertical coloumn, and least superficially, suitable orientation of the fibres in these positions in the myotomes will enable each to contract to the same extent as the body flexes. The importance of this result is that the shape of the force/velocity curve for muscle fibres means that maximum power will be produced at a particular rate of contraction. The shape of the force/velocity curve for the myotomal muscles of any fish is not known, but it is assumed for most fishes, that the white or fast portion of the myotome will be designed for maximum power. The orientation of the muscle fibres directly reflects this requirement, for if they were not able to contract at about the same rate throughout the myotome, the power extractable from the fast portion of the myotome would be much lessened. The myotomal folding is so arranged that the muscle fibres insert into the myosepta at approximately the same angle, despite their very different orientations with respect to the long axis of the body (Bone, 1978).

*Fibre types*

Few of the muscle fibres of the myotomes of a few fish species have been studied histologically, fewer still physiologically, but the different muscle fibre types found are similar in those fish where they have been studied. Although there are great differences in general morphology between different fishes, locomotor muscle fibres are arranged in fundamentally the same way - this is probably due to the density of the medium in which fish swim (Bone, 1978).

Most fish species are faced with the conflicting demands of low speed cruise economy, and short bursts of maximum speed, this has resulted in all fishes having devised the same solution of dividing the locomotor musculature into two very different parts, each specialised for one of these functions. The muscle fibres composing each of the two contrasting parts are entirely different in design, differentiated by a whole spectrum

of histological and ultrastructural features, as well as biochemical and physiological criteria. For example, the "cruising" muscle fibres have a high content of mitochondria and high oxidative enzyme activity, as compared with the fibre type used during burst locomotion - Table 2 gives a summarisation of these differences.

**Table 2**: A comparison of fast and slow muscle fibres in fish (Bone, 1978).

| SLOW | FAST |
|---|---|
| Smaller diameter (20-50% of fast) | Larger diameter (may be more than 300 $\mu$m) |
| Well vascularized | Poorly vascularized |
| Usually abundant myoglobin, red | No myoglobin, usually white |
| Abundant large mitochondria | Few smaller mitochondria with fewer cristae |
| Oxidative enzyme systems | Enzymes of anaerobic glycolysis |
| Low activity $Ca^{2+}$-activated myofibrillar ATPase | High activity of enzyme |
| Little low molecular weight protein | Rich in low molecular weight protein |
| Stored lipid and glycogen | Glycogen stored, usually little lipid |
| Myosatellite cells abundant | Fewer myosatellite cells |
| Sarcotubular system usually less in volume than large fibres | Relatively large sarcotubular system |
| Z-lines broader than fast fibres in some cases | Z-lines usually thinner than in slow fibres |
| Distributed cholinergic innervation | Focal or distributed cholinergic innervation |
| Subjunctional folds usually absent | Subjunctional folds usually present |
| Lower resting potential than fast fibres | Higher resting potentials |
| No propagated muscle action potentials except under experimental conditions | Propagated muscle action potentials usual; may not always occur during activity of multiply innervated fibres |
| Long-lasting contractions evoked by depolarizing agents | Brief contractions evoked by depolarizing agents |

In a crude way the two main fibre types can be recognised macroscopically, even when they are found together, because in most fish, fibre types are segregated so that a region of fibres rich in myoglobin with a large vascular bed appears as a red or pink zone as compared with the adjacent pale zone of fibres of contrasting

types. The striking difference between the vascularization of the two main fibre types can be seen by the tenfold difference in the capillary to muscle fibre ratio in favour of the red fibres. In the myotomal musculature of many fish, a superficial red layer of fibres covers the main mass of white fibres. The properties of these two fibre types differ in different species, and may change with fish size. It seems that in different fish species studied, that red muscle fibres never constituted more than a quarter of the total myotomal musculature, and in most, less than 10%. If only from consideration of the power output required from the musculature at different speeds, this distribution would suggest that the red fibres were employed during cruising, the much larger mass of white fibres during bursts of speed.

The cells of the dark muscle are narrower than those of white muscle. Love (1970, 1980, 1988) gives an in-depth review of the numerous chemical differences between light and dark muscles for a large number of fish species. Although there are differences between dark and light muscles in the concentrations of the major constituents, the differences do not seem to extend to the further breakdown of such material. Thus there is a difference in protein content but apparently not in the distribution of the amino acids, a difference in the lipid content but not in the lipid fractions.

From the reviews of Love (1970, 1980, 1988), it is clear that the sampling technique used, will determine the chemical results, as sampling technique will determine the proportion of red to white muscle. Another factor to be taken into account, is the position on the body that the sample is taken - the proportion of dark muscle varies continually along the body.

In most fish there are more than two fibre types and, apparently, some overlap in function between these different types. Care must be taken when classifying muscle generally into red and white or slow and fast types. The continuous growth of fish further complicates the classification of muscle cell types as some of the fibre types could be regarded simply as stages in the development of others.

Kilarski (1990) did a histochemical study of the myotomal muscles in the roach (*Rutilius rutilius*) and found three main muscle regions: red, intermediate and white. These were distinguished on the basis of glycogen content, succinate dehydrogenase and myofibrillar ATPase activity. Except for the red fibre region, none of the described regions (white and intermediate) is homogenous. Kilarski (1990) also noted the presence of a new "tonic-like" fibre and a mosaic organization of the white fibre region. This mosaic pattern was caused by the presence of large and small fibres that differ in their histochemical characteristics (a similar mosaic pattern has also been noted in the common carp, salmon and trout - Love, 1988). It has been suggested that these small white fibres are responsible for post-larval hyperplastic growth. The precise function of the intermediate muscle fibres is obscure, although, from biochemical studies it seems that this muscle has an inherent speed of

contraction intermediate between that of the red and white muscles. It thus seems as if the intermediate muscles are recruited for a swimming speed slightly faster than slow cruising. The red muscle fibres, with their aerobic metabolism characteristics, are utilized for swimming at a cruising speed, whilst the white are utilized for fast speeds.

In most fish the slow fibres form a superficial sheet (the Seitenlinie) covering the main mass of myotomal fast fibres (Bone, 1978; Love, 1988). The latter author also notes that some very active fish species possess an additional dark muscle band near the spine. This second dark muscle band also differs chemically from the (normally) thin superficial dark muscle.

As dark muscle is more metabolically active than white muscle, it contains more intermediary metabolites, some of which contribute to its flavour. Red (dark) muscle is therefore stronger tasting than white muscle. Love (1988) notes in an earlier study that Japanese workers (who traditionally eat raw fish) found saithe to be the more tastier of different raw European fish species. Of the gadoid species (cod, haddock, etc), saithe is the most active and has the largest proportion of dark muscle.

*Histology*

Myotomal muscle fibres are often very long (in large fish, several centimetres) and insert at both ends into connective tissue sheets; fingers of connective tissue push into the ends of the muscle fibres in a complex interdigitation. At these points there are couplings between the inpocketed tubes containing collagen fibres and the sarcoplasmic reticulum (SR). These terminal couplings are also found in elasmobranch fibres, and probably in all fish fibres. It has been suggested that the couplings represent sites for calcium transfer, but no evidence has been found that proves this theory. Bone (1978) suggests that it is more probable that the couplings represent the response of the sarcoplasmic reticulum to an ingrowing portion of the sarcolemma, analogous to the SR response to the T-system. The organisation of the sarcoplasmic reticulum and the T-system is similar in most fish groups, Triads occurring at Z-line level.

Bone (1978) notes that very few quantitative studies of SR and T-systems in fish locomotor muscle have been carried out. Most of the studies done agree with studies done on other vertebrates where it is found that in slow fibres these systems concerned with activation are of lesser extent than in fast fibres.

As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, ie the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils

contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating way making the muscle look striated upon microscopic examination (Huss, 1988).

*Myofibrils*

Myofibrils are elongated intracellular contractile elements that are 1-2$\mu$ thick and are directly responsible for the already mentioned characteristic banded or striated pattern of muscle. This striation arises from the orderly alignment of the anisotropic and isotropic segments of the myofibrils. With polarised light, the discs that appear dark under the ordinary light microscope are anisotropic (birefringent) and those that appear lighter, isotropic. As a result the darker discs are known as **A** bands and the lighter discs as **I** bands. A darker line known as the **Z** line (Zwischenscheibe) divides the lighter band into two segments. A light area in the middle of the **A** band is known as the **H** band (Heller) and bisecting this band is a dark line, the **M** line (Mittelscheibe). The sarcomere or unit of muscle structure is the area between two adjacent **Z** lines (Cassens, 1971).

The basic structure of the myofibril, which is responsible for its cross-striated appearance, has been shown to be due to its overlapping arrays of two kinds of filaments. The array of thick filaments (about 110 Å diameter) is dense and anisotropic (the **A** band) while the array of thin filaments (about 50 Å diameter) is present alone in the less dense **I** band. The density of the **A** band is greatest where the two sets of filaments overlap. The **H** or lighter zone in the centre of the **A** band is the area where only thick filaments are present. In cross-section, the thick filaments of vertebrate skeletal muscle are arranged hexagonally, with the centres of the thick filaments 140 Å apart. Where the arrays overlap, each thick filament is encircled by six thin ones, and each thin filament is shared by three thick ones. Each of the thick filaments bears a large number of regularly spaced short lateral projections termed bridges. There are six longitudinal rows of projections that are staggered so that a projection occurs every 60-70 Å along the thick filaments. The rows are arranged so that they occur opposite the six thin filaments (Cassens, 1971; Swatland, 1984).

Cassens (1971) and Swatland (1984) give a good detailed description of how the different lines and bands form, and their importance in muscle dynamics.

## CHEMICAL COMPOSITION

*Principal constituents*

The chemical composition of fish varies greatly from species to species and also from individual to individual depending on age, sex, environment and season (Huss, 1988). At certain times ordinary fish appear thinner, flabbier and less lively than at others, the flesh being more watery and softer and containing less protein and

fat. Fish of this kind is said to be in poor "condition" or "out of season"; it has poor sales appeal and gives lower yields (Connell, 1980, 1990).

The principal constituents of fish and mammals are the same, Huss (1988) gives the following table as an example of the variation between them (Table 3):

**Table 3:** Principal constituents (percentage) of fish and beef muscle (Huss, 1988).

| Constituent | Fish (fillet) | | | Beef (isolated muscle) |
|---|---|---|---|---|
| | Min | Normal Variation | Max | |
| Protein | 6 | 16-21 | 28 | 20 |
| Lipid | 0.1 | 0.2-25 | 67 | 3 |
| Carbohydrate | | <0.5 | | 1 |
| Ash | 0.4 | 1.2-1.5 | 1.5 | 1 |
| Water | 28 | 66-81 | 96 | 75 |

The variations in chemical composition of fish are closely related to the feed intake. During periods of heavy feeding, the protein content of the muscle increases very slightly and then the lipid content will show a marked and rapid increase. Fish will have starvation periods for natural or physiological reasons (such as spawning or migration) or because of external factors such as shortage of food. It has been noted (Love, 1988; Connell, 1990) that in many fish species, just before spawning and during it, food reserves in the flesh, and in some species in the liver, are transferred for the development of the gonads (eggs and spawn). During spawning and for some period afterwards most fish species do not feed. As a consequence, the flesh after spawning becomes severely depleted of protein, carbohydrate and fat and the fish are accordingly "run down". Similar conditions can occur when for some reason or other, fish are not feeding or are feeding at an abnormally low level. Once fish start feeding again they normally recover their good condition.

The lipid fraction is the component that shows the greatest variation depending on availability and type of diet.

The oil composition of the lipid also varies with diet - the lipid composition in terms of fatty acid and carbon chain length, will influence among others, taste, odours and shelf life.

The proteins in fish muscle can be divided into the following three groups (Huss, 1988):

1. Structural proteins (actin, myosin, tropomyosin and actomyosin) which constitute 70-80% of the total protein content (compared with 40% in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength (>0.5 M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.5 M). This fraction constitutes 25-30% of the protein.
3. Connective tissue proteins (collagen), which constitutes approximately 3% of the protein in teleostei compared with 17% in mammals.

The isoelectric point of fish proteins is around pH 4.5-5.5. At this pH the proteins are electrically neutral and less hydrophillic than in the ionised state, which means that their water-binding capacity and solubility are at a minimum.

*Myofibrillar proteins*

Brown (1986) notes extensive work done by Connell and his associates at the Torry Research Station with cod muscle proteins, especially myosin. In general it was found that the properties of cod myosin resembled those of myosin in mammalian muscle, such as the rabbit model. The fish myosin, however, exhibits considerable instability and tends to aggregate readily. However, there are some indications (as noted by Brown, 1986) that muscle from fish in warmer waters are more stable than those from fish in colder environments.

The proteins of the myofibril account for somewhat more than half of the protein of most muscle fibres. Within the myofibril, myosin accounts for 55% to 60% of the protein content and actin accounts for about 20%. Experimentally isolated myosin molecules are just large enough to be seen by electron microscopy after they have been sprayed with atoms of metal. The two active heads of each molecule are difficult to resolve separately, so that the molecule usually has the appearance of a miniature matchstick (Swatland, 1984).

Lawrie (1974), Forrest *et al.* (1975), Junqueira and Carneiro (1983) and Swatland (1984) give descriptions of the protein molecules that occur in muscles. The authors also postulate on possible roles played by these proteins.

*Connective tissue proteins*

As already noted, the most important feature of connective tissue proteins in fish muscle is their low concentration and low stability. This is one of the main reasons that tenderness is not of particular concern with regard to most fresh fish products. It has been suggested that the total amount of stroma proteins in fish muscle is only 3 to 5% (Brown, 1986; Huss, 1988). The Japanese, who consume raw fish as sushi and sashimi, evaluate the fish meat quality by taste, odour, colour and firmness. Firmness is of particular importance because of its close relationship to the freshness of the fish muscle.

Ando, Toyohara and Sakaguchi (1992*a*) compared the firmness of muscles from different fish species and found that the density of pericellular connective tissue is related to the firmness of muscle as well as the total amount of collagen present. In the arrangement of collagen fibrils in the muscle connective tissue, a species-specific structure was recognised. In sardine muscles (showing the softest texture - 127 ± 17 g breaking strength), collagen fibrils lie at right angles to each other in a regular order. In carp muscle (150 ± 32 g breaking strength), the collagen fibrils are irregularly on thick fibres which are arranged in a regular order. These thick fibres were constructed of collagen fibrils of the same thickness. In Tiger puffer (447 ± 109 g breaking strength), the collagen fibrils in the connective tissue exist in an irregular form and form a network of collagen fibrils.

There are at least five genetically different types of collagen in connective tissues differentiated by the composition of the three helical chains which make up the molecule (Bremner & Hallett, 1985). Two different genetic forms, Types I and V, exist in fish muscle collagen, whilst Type III-like collagen is not contained in a detectable amount (Sato, Yoshinaka, Sato, Itoh & Shimizu, 1988; Sato, Yoshinaka, Itoh & Sato, 1989).

Bremner and Hallett (1985) show a range of scanning electron microscopic photos that display in fine detail how the myomeres (muscle fibres) fit into sockets on the myocomma and are attached to it by continuations of the collagenous fibrils.

Piez and Gross (1960) compared the amino acid composition of some fish collagens (from the skins) with that of mammals and found that fish collagens have less proline and hydroxyproline and more serine and threonine than mammals. Methionine is also present in greater amounts in fish. In their isolation of Type I and V collagens from carp muscle, Sato *et al.* (1988) showed Type V fraction to be richer in glutamic acid, isoleucine and hydroxy-lysine and poorer in alanine when compared to Type I. Neither Type showed any cysteine.

The importance of collagen in fish muscle lies in the role that it plays during the phenomenon termed gaping. Gaping is the term used to describe the occurrence when the connective tissues fail to hold the muscle blocks

(and fibres) together during the chill storage of fish meat (Love, Lavéty & Steel, 1969), and is of serious economic consequence, resulting in unacceptability and wastage of valuable material. In their earlier work, Love, Haq and Smith (1972) noted the importance of pH during gaping. Experiments on the resistance of isolated myocommata to mechanical rupture by pulling have shown that cod connective tissue is sensitive to small changes in pH. Strips of myocommata immersed in buffers of different pH values were slowly pulled until they broke - at the lower pH (6.2) the myocomma had only about 20% of its strength at pH 7.1 (Love, 1988). Love, Lavéty and Garcia (1972) showed, by soaking strips of myocomma first in a low-pH buffer and then in a high-pH buffer, that the weakening effect of the low pH is reversed by the high pH, and *vica versa*.

During chill storage the attachments between myomeres and the myocomma progressively deteriorate until the whole sarcolemma has deteriorated. This deterioration is caused by an enzyme(s) that shows powerful collagenolytic properties (Bremner & Hallett, 1985). Ando, Toyohara and Sakaguchi (1992*b*) in electron micrographs of rainbow trout, showed how the collagen fibres in the pericellular connective tissue disintegrated after 72 h post mortem, whilst the **Z** discs remained intact. This supports the idea that the disintegration of collagen fibres leads to loss of strength of the pericellular connective tissue, and consequently causes post mortem tenderization. The authors postulate that the disintegration of the collagen could be caused by the increase in physical strength caused by the muscle contraction during rigor mortis.

Yoshinaka, Sato, Sato and Anbe (1990) analyzed the distribution of collagen in the body of several fish species and found that collagen was contained abundantly in skin, scale, bone and fin. The total collagen in these organs ranged from 76.2% of whole body collagen for Japanese eel to 91.1% for red sea bream. However, the total collagen content in the whole body varied with species. The lowest value was 3.26% of wet body weight and 16.7% of crude protein for chub mackerel and the highest value 6.97% and 43.2%, respectively, for Japanese eel. From these results it seems reasonable to assume that collagen is one of the most abundant of proteins in fish bodies, as a whole.

*N-Containing extractives*

The N-containing extractives can be defined as the water-soluble, low molecular weight, nitrogen-containing compounds of non-protein nature. This NPN-fraction constitutes from 9 to 18% of the total nitrogen in teleosts. The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino-acids, nucleotides and purine bases (Huss, 1988). The free amino acids profile of fish muscle is influenced by many factors, including species, habitat, and position of sampling (Brown, 1986).

TMAO constitutes a characteristic and important part of the NPN-fraction in marine species where it seems to

play an important role in osmoregulation. TMAO does not appear in any freshwater fish species (Huss, 1988). TMAO can be converted to trimethylamine (TMA), which contributes to the characteristic fishy odours of fish undergoing spoilage (Brown, 1986).

Quantitatively, the main component of the NPN-fraction is creatine. In rested fish most of the creatine is phosphorylated and supplies energy for muscular contraction. The NPN-fraction also contains a high concentration of free amino acids. The relative importance of the different amino acids varies with species. Taurine, alanine, glycine and imidazole-containing amino acids seem to dominate in most fish (Huss, 1988). Of the imidazole-containing amino acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine and therefore may be involved in scombroid poisoning. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine. Two other imidazole compounds, carnosine (ß-alanyl-histidine), and anserine (ß-alanyl-1-methyl-histidine) also play an important function in the production of histamine and histamine-like products, their role in the production thereof is not clear (Brown, 1986).

The amount of nucleotides and nucleotide fragments in dead fish depends on the state of the fish (Huss, 1988; Tsuchimoto *et al.*, 1988). At the death of a fish, the normal regulation system ceases to function and the supply of oxygen and energy production stop. The cells start a new series of processes characterized by the breakdown of glycogen (glycolysis) and the degradation of energy rich compounds.

The first autolytic process in the fish muscle tissue involve the carbohydrates and the nucleotides. For a short period, the muscle cells continue the normal physiological processes but soon the production of adenosine triphosphate (ATP) stops. At low ATP levels rigor mortis develops (Swatland, 1984). ATP is further broken down by a series of dephosphorylation and deamination reactions to inosine monophosphate (IMP) which, in turn, is degraded to inosine (HxR) and the latter, to hypoxanthine (Hx) and ribose (Huss, 1988).

In Japan, considerable work has been done on the determination of freshness, and the expression thereof. A so-called K-value has been suggested for the determination thereof and this value expresses the relationship between inosine and hypoxanthine and the total amount of ATP-related compounds:

$$K(\%) = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx}$$

Very fresh fish, therefore, have low K-values, which gradually increase at a species dependent rate (Huss, 1988). The organoleptic importance of the autolytic degradation products is only partially understood, although it has been known for a long time in Japan (as noted by Huss, 1988) that IMP and other 5'-nucleotides function

as strong flavour-enhancers in quite low concentrations, and together with glutamic acid they give rise to a "meaty flavour". Inosine is said to be more or less flavourless, while hypoxanthine imparts a bitter flavour in spoiling fish. The loss of flavour in spoiling fish is, therefore, attributed to the degradation of IMP (Huss, 1988).

## Lipids

*General*

When the lipid content in the fish exceeds 1%, it functions as an energy reserve and can be classified as a fat depot. The fat depots are mostly located in the subcutaneous tissue, in the belly flap, in the collagenous tissue between the muscle fibres both in light and dark muscle, and in the head section. Huss (1988) notes that there is a large variation in the amount of lipid stored in the different locations between species and during different times within the species.

In most fish species the fat depots consist of triglycerides as in most other vertebrates. Fish lipids differ from mammalian lipids. The main difference is that they consist of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two double bonds per fatty acid molecule while the fat depots of fish contain many fatty acids with five or six double bonds (Huss, 1988).

The total number of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in lipids from freshwater fish (approximately 70%) than in lipids from marine fish (approximately 88%). Huss (1988) notes further that the composition of the lipids is not completely fixed but can vary somewhat with the feed intake.

A small portion of the lipids (less than 1% of the fish muscle) serves as essential structural parts of the cell. Typical "non-depot lipids", are the phospholipids which are phosphorus- and nitrogen-containing fats. Usually these lipids do not function as an energy reserve. However, Huss (1988) notes work done by Love, who found that in some white-fleshed fish such as cod, that have no fat depots in the muscle tissue, some of the phospholipids will be used during long starvation periods.

Japanese researchers (as noted by Huss,1988), have shown that the phospholipids are distributed unevenly in the tissues, with the dark muscle in particular being rich in these compounds. A large percentage of the fatty acids in the phospholipids are also long-chain polyunsaturated acids with five or six double bonds. In most fish species the phospholipids constitute approximately 0.5-1% of the muscle tissue.

The main sterol in fish muscle is cholesterol, found in quantities generally well under 100 mg/100 g, which are not much higher than that found in mammalian tissue (Huss, 1988).

Driedzic and Hochachka (1978) note that the free fatty acids derived from triglycerides serve as a major aerobic fuel source for energy metabolism in muscle. A good review on the fat metabolism is given by the above mentioned authors.

*Fatty acids*

Fish are capable of synthesizing palmitic acid (16:0) from acetate with the aid of fatty-acid sinthetase, the acetate being principally derived from glucose. Following this, shortening or lengthening of the chain gives either myristic (14:0) or stearic (16:0) acid, and also similar saturated or lightly unsaturated acids. Certain polyunsaturated fatty acids (PUFA) cannot, however, be synthesized by fish if the relevant precursors are not present in the feed. These precursors are termed essential fatty-acids (EFA). Fish are able to convert linoleic (C18:2$\omega$6) and linolenic (C18:3$\omega$6) acids into C20 and C22 PUFA by desaturation and elongation (Steffens, 1989; Kinsella, Lokesh & Stone, 1990).

The EFA requirement of fish differ from species to species (Watanabe, 1982). No requirement for the African catfish (*C. gariepinus*) seem to be available.

In general, fish which live in sea water have a higher demand for $\omega$3 fatty acids than fresh water fish, while cold water fish have a higher demand for $\omega$3 fatty acids than warmwater fish (Steffens, 1989). Higher $\omega$3 concentrations are needed because of the important role they play in the membrane composition (phospholipids) of fresh and marine fish species.

Phospholipids (phosphatides) are similar to neutral triglycerides, in which one of the three fatty acid molecules is replaced by phosphoric acid, which is in turn bound by an ester linkage to certain amino-alcohols. Their most important members are the lecithins and cephalins. The phospholipids play an important role for structural and functional purposes, in particular for the biophysical properties of membranes (Steffens, 1989).

The phospholipids, especially phosphatidylserine and phosphatidylglycerol, activate the membrane-bound enzyme ($Na^+/K^+$)-ATPase, which is an important component of the marine fish osmoregulatory system. These phospholipids show a significant discrimination for $K^+$ over $Na^+$ permeability. To activate ($Na^+/K^+$)-ATPase and affect membrane permeability the phospholipids must be fluid. A high proportion of the phospholipids in some natural membranes are in a fluid state. When membrane phospholipids undergo a phase transition from a fluid to a gel state, the permeability of the membrane drops considerably. The temperature of such phase

transition corresponds to the melting point of the fatty acid chain. Thus it seems that the incorporation of PUFA, especially the $\omega3$ type, which have a lower melting point than the $\omega6$ and $\omega9$ fatty acids may be related to this phenomenon that makes the membrane more permeable and more functional in sea water (Hepher, 1988).

The above reason may also explain the importance of fatty acids of the $\omega3$ type for coldwater fish, where the incorporation of low melting point fatty acids in membrane phospholipids maintains the permeability of the membrane even at low temperatures.

Docosapentaenoic acid (20:5$\omega$3) has the same growth-enhancing effect as docosahexaenoic acid (22:6$\omega$3), both these highly unsaturated $\omega3$ fatty acids have a biological or EFA efficiency higher than that of linoleic acid (18:2$\omega$6). There is an additive effect between 20:5$\omega$3 and 22:6$\omega$3 in terms of growth enhancement (Watanabe, 1982).

Where EFAs are absent from the feed, pigment loss and growth regression have been observed. Other deficiency symptoms include: greatly increased mitochondrial swelling, elevated respiration of liver homogenates, slightly reduced haemoglobin content and elevated moisture content in the muscle tissue, degeneration of the caudal fin and higher mortality rates. A typical feature is a special form of shock syndrome, which after excitation (stress), gives rise to violent swimming motion and ultimately to loss of motility, the onset of this behaviour occurring from four weeks to three months after the commencement of the deficient feed supply (Hepher, 1988; Steffens, 1989).

## VITAMINS AND MINERALS

The amount of vitamins and minerals in fish muscle tissue is species specific and can furthermore vary with season. In general Huss (1988) notes that fish meat is a good source of the B vitamins and, in the case of fatty species, also of the A and D vitamins. Some freshwater species such as the carp have a high thiaminase activity so that the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular, but also of iron and copper. Saltwater fish have a high content of iodine. The sodium content of fish meat is low, which makes it suitable for low-sodium diets.

## THIAMINASE

Brown (1986) notes the presence of an anti-thiamine factor that has long been recognized as being present in fish. The so-called Chastek's paralysis was first noted in the early 1930's in foxes fed a diet rich in raw carp. This disorder was subsequently shown to be due to thiamine deficiency. This factor is generally called

thiaminase and much of the early research done indicated that there was such an enzyme found in a variety of fish, both marine and freshwater. Concentrations are highest in viscera, but the enzyme is found in muscle tissue as well. It is heat labile, although the extent of loss of this anti-thiamine activity on heating is variable.

## INFLUENCE OF SIZE/GROWTH

*General*

Fish size is an inherent quality characteristic in that the consumer decides on what sized fish he finds visually and gastronomically more satisfying. However, there is no evidence that size is in any way related to the quality of flavour within the same species. The sized fish that the consumer wants, is not necessarily the size that delivers the highest or best yield. Normally the optimum sized fish is less than the largest. Large sizes of trout, etc, are not favoured for table use because the portion size is then too big or too expensive. For canning, specific sized fish are needed to ensure correct can-fill. Cutting or filleting machines can usually only be adapted to accept a limited size range of fish. For the machine to give maximum yield or even to work at all, it is necessary to segregate mixed batches of fish into particular size ranges suitable for particular machine settings (Connell, 1990).

With cultured fish, the role of size is more limited, as the producer, taking the preference of the consumer or processor into account, can decide at what size he wishes to harvest his crop. Normally such a crop will have a small size variation. However, the influence that size or growth has on the meat/tissue, needs elucidation so as to help the producer, processor and consumer to decide on what size is ideal.

*Tissue dimensions*

Love (1988) notes work done by a number of authors on different fish species, who noted that the number of muscle cells in the body increases as fish grow. However, as the fish reach a certain length, normally marked by the onset of maturity, there is a slower production of new fibres, as the changes associated with maturity place a drain on the protein resources of the fish.

Increasing the number of cells is insufficient to generate the increased mass of tissue found in larger fish, and the difference is made up by widening existing cells. Love (1988) notes work by a number of authors, who all found a similar trend in different fish species, so the phenomenon is probably general. In addition to becoming wider, fish muscle cells also lengthen during growth, ie, the myocommata become more widely spaced. This does not mean that there is less myocomma tissue in a given weight of musculature. Doubling the length of a fish may double the space between the myocommata, but at the same time it results in a 170%

increase in myocomma thickness (Love, 1988).

This thickening of the myocommata (connective tissue) compensates to some extent for its loss of mechanical strength in older fish. Doubling the length of cod results in connective tissue which for a given thickness has only half the strength of that of smaller fish (Love, 1988).

Although differences in composition can occur from changes with growth in the proportions of different tissues in the musculature, these differences are not permanent as tissues are dynamic, constantly breaking down and being replaced. The result of this is that if circumstances change, for example, if fish begin to migrate or starve, the composition of tissue or tissues can alter fairly rapidly (Blasco, Fernández & Gutiérrez, 1992; Tidwell, Webster & Clark, 1992). From a fish processing viewpoint, these changes can change the relative proportions of dark and white muscle (Johnstone & Goldspink, 1973). Love (1988) notes work where it was found that a swimming velocity of just under one body-length per second, maintained for 42 days, engenders an increase in the quantity of dark muscle to nearly 1.5 times that of resting fish, whereas white muscle shows a very small decrease. This is because only the dark muscle is being used at this speed and the white hardly at all. At greater speeds, the white muscle is used increasingly, so its quantity also increases, whereas any further rises in the absolute quantity of dark muscle are small compared with the dramatic initial surge.

Active swimming can also cause an increase in body length. It is common knowledge that fish caught from a flowing river tend to be longer and thinner than those from a dam or cultivated in still-waters. This is a factor that will play an important role when condition factor is used as an estimation of body composition or nutritional status.

## CHEMICAL COMPOSITION

Love (1988) gives a review on the different biochemical factors that vary in the eggs of different fish and the influence of these intrinsic egg variations on the quality of the grown fish. For example, rainbow trout eggs containing higher quantities of riboflavin will show a better hatching rate than those poorer in this vitamin. Fatty acids also play a role, for example, fish eggs low in the polyunsaturated fatty acid C22:6ω3 will also show low hatching rates. The level of this fatty acid also governs the pigmentation of the skin of certain fish. The colour or lack thereof, is a quality aspect in that it influences the perception of the product as seen by the consumer.

The mode of swimming of smaller fish (larvae) differ frequently from that of larger, mature fish, and consequently they will use different muscle types, which may influence the proportion of dark and light muscle

with the resulting change in quality.

As fish grow, their diet may change because their mouths and gullets enlarge and they are able to capture different types of prey. This change in diet will also influence the muscle chemical composition.

*Metamorphosis*

Some species of food fish migrate from fresh water to sea during development, undergoing a change of form known as metamorphosis. Love (1988) gives a review on the changes associated with metamorphosis on the more important species such as salmonids, lampreys and eels.

*Aging*

Original thought on aging in fish was that fish do not age as mammals do, but that fish, such as the plaice, increase in weight by geometrical progression until the age of puberty, as does mammals, but whereas mammals eventually decline in weight and die, the plaice continue to grow by annual increments (Love, 1988). Deaths are therefore the result of either summer or winter stress, or of aggressive behaviour.

A later view, that seems more reasonable, is that as fish age, they are increasingly unable to cope with the demands of reproduction as exhibited by the decrease in their ability to lay down lipid and protein reserves during the non-reproductive season. Love (1988) notes work done on the Kokanee salmon, that like other Pacific salmon, spawn only once, and then die. However, castrated specimens showed a prolonged life, although they did not live for ever, after about eight years, though still growing, they showed signs of senility: they lost weight, their eyes acquired a sunken appearance, and they eventually died. Aging in some fish can be manifested by exophthalmus and a type of spinal curvature called lordosis (Love, 1988).

Small species of fish tend to die earlier than larger species, and the South American 'annual fish' die after just one year, although their life span is prolonged at temperatures lower than that of their usual environment (Love, 1988). The latter may have to do with the influence of temperature on the enzymes involved in the metabolic processes.

However, the major fundamental change that is progressive and measurable, occurs in the connective tissue. In his review, Love (1988) gives an in depth study of the changes in connective tissue with aging. There is a massive invasion of the sex organs by connective tissue, which replaces germinal tissue. The myocommata thicken and the properties of the collagen also change. The myocommata break more readily under stress in

older fish (ie, the collagen is less cross-linked), but the fish more than make up for this by increased collagen thickness.

Much of the mechanical strength of the myocommata is probably supplied by aldimine intermolecular bonds which are easily destroyed by heat. Covalent intermolecular bonds however are stable to heat, and the proportion of these can be assessed by solubility data. Generally, as shown in the annual fish, the proportion of acid-soluble collagen to total collagen declines with age. Lowering the environmental temperature from 20° to 15° markedly slows the rate of insolubilisation of collagen, ie, aging is retarded (Love, 1988).

*Maturation and spawning*

The effects of maturation and spawning on the chemical composition and eating quality of fish are such that they need a more in depth study.

After fertile eggs have been shed (naturally or artificially) they develop and hatch into tiny fish. Since these cannot feed at first, (±3 days: *C. gariepinus*) the developing eggs must contain all the special substances needed by the various organs. These substances and those that supply energy are deposited in the yolk which the larvae continue to utilise after acquiring a free-swimming existence.

Love (1988), in an extensive review of the biochemical changes occurring in the gonads (mainly of marine fish species) as they mature notes the following major changes:

1. Phospholipids for the future cell membranes accumulate in the ovaries and testes of the parental fish. The vitellogenin (a complex lipophosphoprotein) circulating in the blood stream will supply calcium, phosphorous, protein and lipid. Immature and spent ovaries consist largely of connective tissue, and as protein is transferred into the developing eggs, the amino acid pattern of the whole ovary comes to resemble that of muscle and less that of collagen. This is normally noted as an increase in alanine and leucine, and a decrease in glycine, the later being concentrated in collagen. The free amino acids in the developing ovaries also consist more of the 'essential' amino acids than the 'non-essentials', which can be synthesised by fish.
2. Similarly, the mineral composition of the ovaries also change, with most of the ions originating from the body of the fish.
3. As regards the lipid composition of gonads, conflicting results have been noted (Love, 1988), however, in his earlier review, Love (1970) notes that in fourteen species, the major ovarian fatty acid is usually palmitic acid (C16:0) or oleic acid (C18:1ω9).

Maturation seems to be timed to ensure optimum survival of eggs and larvae. However, the massive enrichment of the gonads before hand does not necessarily coincide with a plentiful supply of food for the parent fish. Many fish deplete their own bodies as the climax of maturation approaches, especially in fish such as salmon, which use energy to swim upstream against the current, are busy forming eggs and sperm and starving, all at the same time. Other fish, may not feed during the winter, when the water temperatures drop below a certain level, or the water source dries up, as may happen in South Africa. During these stages of maturation and starvation, chemical changes in the muscle (fillet) are taking place, these changes will play a role in the meat quality.

Another important factor to be taken into account, is that, especially with female fish, as the gonads mature, their weight increases, resulting in a decrease in dressout percentage (yield) when compared to their male counterparts.

Love (1988) notes that older fish are not able to build up enough reserves during the feeding season, and may therefore be of an inferior quality to younger fish if slaughtered during the spawning season as more body reserves will need to be mobilised.

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# Chapter 2 MATERIALS AND METHODS

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This section provides a more detailed description of the methods used in the chemical analyses of the various parameters than that given within the various manuscripts.

## FISH SELECTED

Wild fish were seined either from the Middle Letaba Dam or from the Pietersburg Municipality sewage works. These fish were all normally coloured.

Farmed fish were either bought from a commercial farmer in the lowveld or raised in the Aquaculture Research Unit high density production system. These fish were either gold or normally coloured. The specific colour is noted in the different studies. In two of the earlier studies the golden coloured catfish is described as being "red". It was later felt that "gold" is a more appropriate description of this strain, especially from a marketing perspective.

Where applicable, the fish were freshened out (depurated) in clean running water. The individual body weights and sex of the fish are given in the specific chapters.

All fish were killed by severing the spinal column behind the skull at the end of the supraoccipital.

## MEASUREMENTS TAKEN

The live mass and total length were recorded. Thereafter, the maximum head length and head width was measured.

### Filleting Procedures

After killing, the fish were eviscerated and the viscera separated into mesenteric fat depot, gonads, liver and stomach. The skin was then cut along both sides of the dorsal and extended anal fins and around the humeral process of the skull. Pliers were used to pull the skin off in a caudal direction. Thereafter, the fillets were removed, ensuring that a minimum of musculature remained behind on the bone. The pectoral and pelvic fins were removed from the fillet, as well as any remaining rib bones. The weight of the two fillets, different components of the viscera and head were recorded separately. The fins were weighed together with the remaining bones, the latter including the dorsal, anal and caudal fins.

## SAMPLING METHODOLOGY

In the investigations (unless otherwise stated in the chapters), both whole fillets were removed, finely ground, analyzed for moisture, ash and total lipid content whilst the rest of the samples were freeze dried. These dried samples were homogenized a second time and then subsequently sampled for the different analyses.

Love (1988) suggested that ideally, white and dark muscle should be investigated separately, or if this is not possible, the entire fillet, or a carefully specified part, should be analyzed. In this study the whole fillet was analyzed since the muscle samples of some fish would have been too small if individual myocommata, or parts of the muscle only, were investigated. This investigation was also aimed at studying *C. gariepinus* as a "protein" source from a human nutritional view, and in this context, the chemical composition of the total fillet is of more value than that of a fragmented part, as it is the whole fillet that is eaten.

## CHEMICAL ANALYSIS

Unless otherwise stated, standard procedures as described in the AOAC manual were used (AOAC, 1984).

### Moisture and Ash Content

Crucibles used in the experiment were dried in an oven (50°C) for at least 24 h, thereafter they were allowed to cool in a desiccator until used. Homogenised (where applicable) sub-samples of muscle were accurately weighed into the crucibles and dried for 24 h at 70°C (higher temperatures caused changes in some of the lipids present). Thereafter the dry samples (plus crucibles) were once more cooled in a desiccator and the dry mass determined. These dry samples were then ignited in a furnace at 550-600°C for determination of ash content. When the furnace temperature had decreased to 100°C, the ash samples were cooled in a desiccator and weighed.

### Protein

The macro-kjeldahl method was used for protein determination. The measured nitrogen values were multiplied by the factor 6.25 to give an estimation of protein content. The macro-kjeldahl method consists of two steps, namely a digestion step followed by a distillation step:

*Digestion*

> 0.5 g dried sample is placed in a 800 ml digestion tube, 10 g $Na_2SO_4$ and 0.4 g Se (or 3 Kjeltabs, 3.5 g $K_2SO_4$ & 0.0035 g Se per tab) added as catalyst. After addition of 25 ml concentrated $H_2SO_4$ (98%), the digestion tubes are placed into the pre-heated (410°C) block and digested until the samples have cleared, the digestion is then extended for a further 30 min. Thereafter, the digestion tubes are removed, cooled and 350 ml distilled water added.

*Distillation*

The distillation is carried out on a Gerhardt Vapodest 1 apparatus. About 100 ml 45% NaOH solution is added to the digestion tube. From the resulting mixture, the $NH_3$ is distilled over into an ehrlenmyer flask containing 35 ml of a mixture of Boric acid (4%) and indicator solution (methyl red and methyl blue). This distillation is continued until about 200 ml distillate has distilled over, thereby ensuring that all the $NH_3$ has distilled over. The resulting distillate is titrated against a standardised 0.1 N $H_2SO_4$.

**Total Lipids**

The extraction method of Folch, Lees and Stanely (1957) as adapted by Christie (1982) was used for total lipid extraction as it yields approximately a 95-99% recovery of lipids and reads as follows:

1 g of tissue is homogenised for 1 min with 10 ml of methanol, then 20 ml of chloroform is added and the process continued for a further 2 min. The mixture is filtered and the solid residue re-suspended in chloroform-methanol (2:1 v/v, 30 ml) and homogenised for 3 min. After filtering, the solid is washed once more with chloroform (20 ml) and once with methanol (10 ml). The combined filtrates are transferred to a measuring cylinder and one quarter of the total volume of the filtrate of 0.88% potassium chloride in water is added; the mixture is shaken thoroughly and allowed to settle in a separation funnel. The lower layer is kept whilst the upper layer is removed and discarded, one quarter of the volume of the lower layer of water-methanol (1:1) is added and the washing procedure repeated. The bottom layer contains the purified lipid which can be recovered as above. This layer is transferred to a round bottom flask (pre-weighed), evaporated and the weight of the extracted lipid determined.

**Fatty Acid Methyl Esters**

The extracted total lipids (using the method of Christie, 1982) were esterified by the AOCS Official Method Ce 1b-89 (1991) as follows:

1.5 ml 0.5 N NaOH was added to a culture tube containing ±25 mg of lipid sample. The mixture was shaken, and heated at 100°C for 7 min. Thereafter it was cooled, 2 ml $BF_3$/Methyl alcohol reagent was added, the mixture shaken and heated at 100°C for 5 min. The culture tube was then cooled to 30-40°C, 1 ml of iso-octane added and the solution vortexed for 30 secs whilst still tepid. Immediately afterwards, 5 ml saturated NaCl solution was added and the mixture agitated. The iso-octane was allowed to separate and transferred to a separate tube. The methyl alcohol/water phase was separated a second time with an additional 1 ml of iso-octane. The iso-octane extracts were combined, and 1-2 $\mu$l injected

under the appropriate chromatographic conditions.

The resulting esters were then separated on a capillary column (60 m x 0.25 mm ID, supplied by Quadrex Corp, USA, Cat No 007-23A-60-0.25F) in a Hewlett-Packard 5880A gas chromatograph with a flame ionisation detector. A Hewlett-Packard 7673 automatic injector (split ratio 1:80) was used for sample injection. The run was isothermal (210°C) with helium (100 kPa) as the carrier gas. Injector and detector port temperatures were 250°C. Fatty acids were identified by comparing retention times with those of known standards (PUFA 1, Cat no 1093 and PUFA 3, Cat no 1177, Matreya Inc, USA).

**Amino Acid Analysis**

The amino acids were determined on samples that had been defatted using the methods described above for lipid determination. The method used for amino acid analysis is known as the Pico.Tag method and can be summarised as follows:

> 10-20 mg of the sample was weighed into a reaction flask and 2 ml of the acid mixture (6 N HCl and 1% phenol) added. All the air was replaced with nitrogen and a vacuum (0.01 mm Hg) then drawn. The sealed flasks were placed in an oven (110°C) for 24 h. After cooling, the mixture was made up quantitatively to 5 ml and an internal standard added. 25 $\mu$l of the 5 ml solution was placed in a hydrolysing tube and dried under vacuum until 65 millitorr. The sample was now derivatized. The pH was adjusted by adding 10 $\mu$l of solution *x* (2:2:2 - methanol:$H_2O$:tri-ethylamine) and the resulting solution dried under vacuum. 20$\mu$l of mixture *y* (7:1:1:1 - methanol:$H_2O$:TEA:PITC) was added, the solution vortexed and left to settle for 20 min at room temperature (20-25°C). Thereafter the vacuum was released and the tubes left for 2 h. The sample was then ready for HPLC analysis. The dried sample was dissolved in 200 $\mu$l of sample diluent, filtered (0.45 $\mu$m) and injected by means of an automatic injector into a reverse-phase column (steel, 3.9 mm x 15 cm, PICO.TAG COLUMN part no 88131) and read at 254 nm wavelength. Retention times were compared to that of known standards (250 pmol of Pierce H standard).

**Mineral Analysis**

Minerals were analyzed on a Varian AA 1275 atomic absorption spectrophotometer as follows:

> About 1 g sample was accurately weighed into an ashing dish. Prior to ashing, the samples were first burnt on a sand bath until smoking had ceased, which procedure removed a large proportion of the lipids. This additional step was included as samples with a high lipid content tend to "splutter" when ashed leading to a loss of sample matter. Samples were ashed at

550°C, for 30 min, whereafter the samples were cooled. 10 ml of acid mixture (6 N HCl + 6 N $HNO_3$) was added to each ashing dish to dissolve the residue. The mixture was filtered into a 100 ml volumetric flask (warm 0.1 N HCl is used to rinse the filter paper) and the volume made up with 0.1 N HCl. The resulting mixture is read at the various wavelengths for mineral content. Where applicable, dilutions are made. Standard stock solutions are used for determination of absorption curves.

## STATISTICAL ANALYSIS

The programmes of the Statistical Analysis System (SAS, 1985) used were: PROC GLM, PROC LSMEANS, PROC REG, PROC ANOVA, PROC T TEST, PROC CANDISC. The specific programmes used are mentioned in the appropriate chapters. The Harvard Graphic computer programme was used for the various graphical illustrations.

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# Chapter 3 CHEMICAL CHARACTERISTICS OF *CLARIAS GARIEPINUS* MUSCLE

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## INTRODUCTION

Muscular tissue forms a larger part of the mass of the fish body than it does of any other vertebrate: some 40-60% of the total body mass in most fish is locomotor musculature. In part this is because economy in weight is not mandatory as it is for terrestrial and aerial forms, and in part because stringent demands are placed on the locomotor system by the density of the medium, so that a large amount of muscle is needed to generate sufficient power for rapid swimming (Bone, 1978).

In round-bodied fish, as distinct from flat fish, no consistent difference has ever been found between constituents in the left and right myotomal muscles of the same fish. In a systematic study of the protein content of Atlantic cod (*Gadus morhua*), six independent laboratories found that no difference between left and right occurred, but that there was a decline in protein nitrogen from the head towards the tail (Brandes & Dietrich, 1970). The concentrations of lipids vary enormously in different parts of the body. In fat fish, there is usually a high concentration immediately under the skin and in the belly wall. Brandes and Dietrich (1970) studied the distribution of water, protein, fat and ash in different regions of the body of the Atlantic herring *(Clupea harengus)* and found the highest fat concentration, apart from in the belly wall, to be just anterior to the dorsal fin. The fat concentration tended to decrease towards the caudal region. Similarly there was an increase in the moisture and protein concentration in the same direction. The ash concentration stayed relatively constant throughout.

This variation in proximate composition along the musculature can generally be attributed to a change in the proportion of dark to light muscle. The muscle fibres of fish are often classified as either dark (red) or light (white). Dark muscle fibres generally have more abundant quantities of myoglobin, haemoglobin, mitochondria, glycogen and lipid than light fibres (Love, 1970; Bone, 1978; Carpenè, Veggetti & Mascarello, 1982; Kilarski, 1990). Dark muscles work aerobically, have relatively slower contraction speeds, and are most efficient during sustained activity (Mosse & Hudson, 1977; Bone, 1978; Bone, Kiceniuk & Jones, 1978; Mclaughlin & Kramer, 1991).

As dark muscle is metabolically more active than white muscle, it has a different chemical composition (Bone, 1978). Love (1988) notes that generally, dark muscle contains more total lipid than light muscle. This lipid contains a higher proportion polyunsaturated fatty acids, which although healthy for human consumption (Kinsella, Lokesh & Stone, 1990) will tend to become more rancid during cold storage (Love, 1988).

The spacings of the myocommata also vary along the length of the myotomal musculature. Love (1988) notes from his earlier work on cod, that the myocommata are most widely spaced at around myotome number 12 from

the cranial end, and that they become steadily more closely packed the nearer they are to the head and tail. The result of this phenomenon is that a varying proportion of myocomma collagen is found in muscle samples taken from different positions along the body. The varying chemical composition along the myotomal musculature (fillet) has implications for the processing trade in that the quality of the product may vary. For example, with varying concentrations of collagen in the sample, varying amino and fatty acids will be present which will influence, amongst others, the flavour of the product.

The position from where a sample is taken for purposes of analyses will also influence the chemical and physical results. If these factors are not taken into account, a distorted image of the product may arise.

Lipid depots represent major energy reserves in fish and occur in the body musculature (dark and light muscle type) and within the mesenteric cavity surrounding the viscera (Sheridan, 1988). The lipid level of the muscle is important to the quality of the fish. Similar to other fish species (Ando, Mori, Nakamura & Sugawara, 1993), cultured *C. gariepinus* also possesses high levels of lipid in their muscles compared with wild ones (Hoffman, Casey & Prinsloo, 1992). The excess lipid in muscle is one of the reasons why the quality of cultured fish is not necessarily favourable. It is therefore valuable for the quality improvement of cultured fish to investigate the lipid accumulation in tissues other than muscle, because dietary lipid is also transported to tissues such as mesenteric fat, subcutaneous fat, and liver (Ando *et al.*, 1993).

This section reports on the anatomical chemical heterogenity within the myotomal muscle of *C. gariepinus* and includes an analysis of the fatty acids of the dark and light muscle lipids, and the amino acids and mineral compositions of the two muscle types. The fatty acid profiles of the total lipids in the different fat depots (light and dark muscle, liver, subcutaneous fat and mesenteric fat) are also reported and discussed.

## MATERIAL AND METHODS

### *Fish*

The catfish (*C. gariepinus*) utilized were bred and maintained in the Aquaculture Research Unit's water-recirculating facilities. The fish had been maintained under identical conditions and were fed the same commercial diet (6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt: fatty acid profile of diet - see Table 1) for 18 months prior to sampling.

**Table 1:** The fatty acid profile of the commercial fish diet (% fatty acid identified/% total fatty acids present).

| Fatty Acid | % |
|---|---|
| **C14:0** | 3.1 |
| **C16:0** | 17.5 |
| **C16:1ω7** | 4.0 |
| **C18:0** | 6.7 |
| **C18:1ω9** | 26.1 |
| **C18:2ω6** | 26.9 |
| **C20:1ω9** | 1.0 |
| **C20:5ω3** | 5.2 |
| **C22:0** | 0.5 |
| **C22:1ω9** | 1.3 |
| **C22:5ω3** | 0.6 |
| **C22:6ω3** | 3.1 |

*Preparation of fish muscle samples to test for muscle heterogeneity*

Three female catfish with live weights and total lengths of 3665.2 g, 743 mm; 2632.2 g, 710 mm; 2155.0 g, 650 mm respectively, were analysed chemically. The fish were slaughtered and filleted as described in Chapter 2. Thereafter, the right fillets were finely cut, homogenised and subsamples analyzed for the different chemical parameters. The left fillets were cut into portions according to Figure 1, each of these portions were finely ground and subsamples analyzed. Subsamples A, B, C, and E, F, G were separated from each other by cutting the fillet perpendicular to- and along the midline. The separation between A/B and E/F was at the end of myotome 15 (perpendicular with the pelvic fins), between B/C and F/G at the end of myotome 27, and between C/D and G/D at the end of myotome 39. Subsample D ended at the tail junction. Subsample E contained the empty gut cavity with its peritoneum.

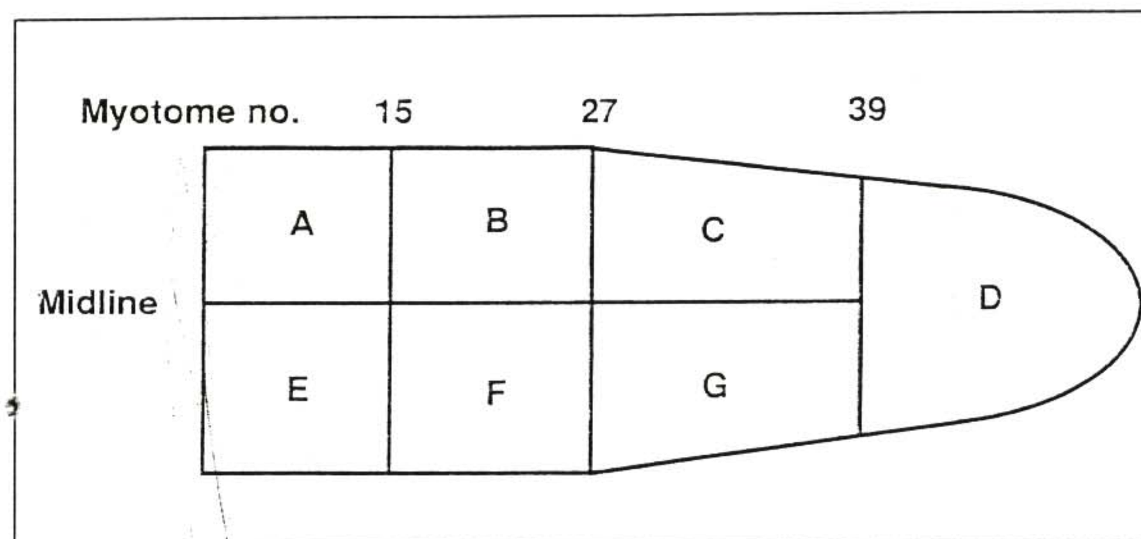


**Figure 1:** A schematic presentation of a catfish fillet showing the position and lettering of the different sub-samples analysed.

*Comparison of dark and light muscle*

In this investigation, the muscle of four catfish (2463.2 ± 471.1 g live mass) were analyzed individually for chemical composition. Immediately after pithing, the fish were skinned and the subcutaneous dark muscle on the lateral plane, posterior to the head and pectoral fin, in the region of the head kidney, removed. Light myotomal muscle was dissected from the same position on the body, but closer to the spinal column.

Differences between muscle types for the different chemical variables were tested by means of paired comparisons. A $t$-statistic was computed for the testing of the null hypothesis.

*Measurements of myotome widths*

Three catfish of varying size (306.0 g, 36.4 mm; 1773.7 g, 570.0 mm; 2646.0 g, 720.0 mm live mass and total length, respectively) were killed and skinned. The widths of individual myotomes were measured on the surface of the fillets along the midline, starting cranially and moving caudally.

*The fatty acid composition of various lipid depots*

The fish used were the same four specimens examined during the comparison of the dark and light muscle types. The subcutaneous fat depot behind the pectoral fin, the subcutaneous dark muscle on the lateral plane, posterior to the head in the region of the head kidney, and the light myotomal muscle directly below the dark

muscle, but closer to the backbone were dissected and the fatty acids of the total lipids analysed. A sub sample of the liver and mesenterium (gut) fat depots were also removed for analysis.

Differences between fat depots for the different fatty acids were tested by means of paired comparisons. A paired-t statistic was computed for testing the null hypothesis, that is, that the mean of the differences between two fat depots equals zero.

*Chemical analysis*

The methodologies of the different chemical analysis performed are as described in Chapter 2. The chemical parameters tested were: proximate composition, amino acids, total lipid fatty acid profiles and mineral composition.

**RESULTS**

*Fish muscle heterogeneity*

The proximate composition of the different subsamples from the different positions (Fig 1) of the three fish are shown in Figure 2a, b and c. The data given in the boxes to the left of the schematic fillets represent the results of the analysis of the subsample from the right myotomal musculature. Although there are variations between the proximate composition of the myotomal musculature of the three fish, the same trends are shown between the different portions within the left fillet. The general trend is a decrease in the percentage moisture and protein, from the head to the tail. The percentage fat shows the opposite, a lower concentration in the cranial region, and a higher concentration in the caudal end of the myotomal musculature. The ash content shows no fixed trend dorsally, ventrally, cranially or caudally. No distinct difference is noted between the dorsal and ventral regions in terms of moisture, protein or fat.

The amino acid, fatty acid and mineral compositions of the right and left fillet subsamples are presented in Tables 2, 3 and 4 respectively. There is a decrease in the percentage of saturated and monounsaturated fatty acids and an increase in the percentage of polyunsaturated fatty acids towards the posterior. Of special note is the high concentration of 3,6,9,12,15,18-docosahexaenoic acid (C22:6ω3) in the caudal segment (Segment D - Table 3).

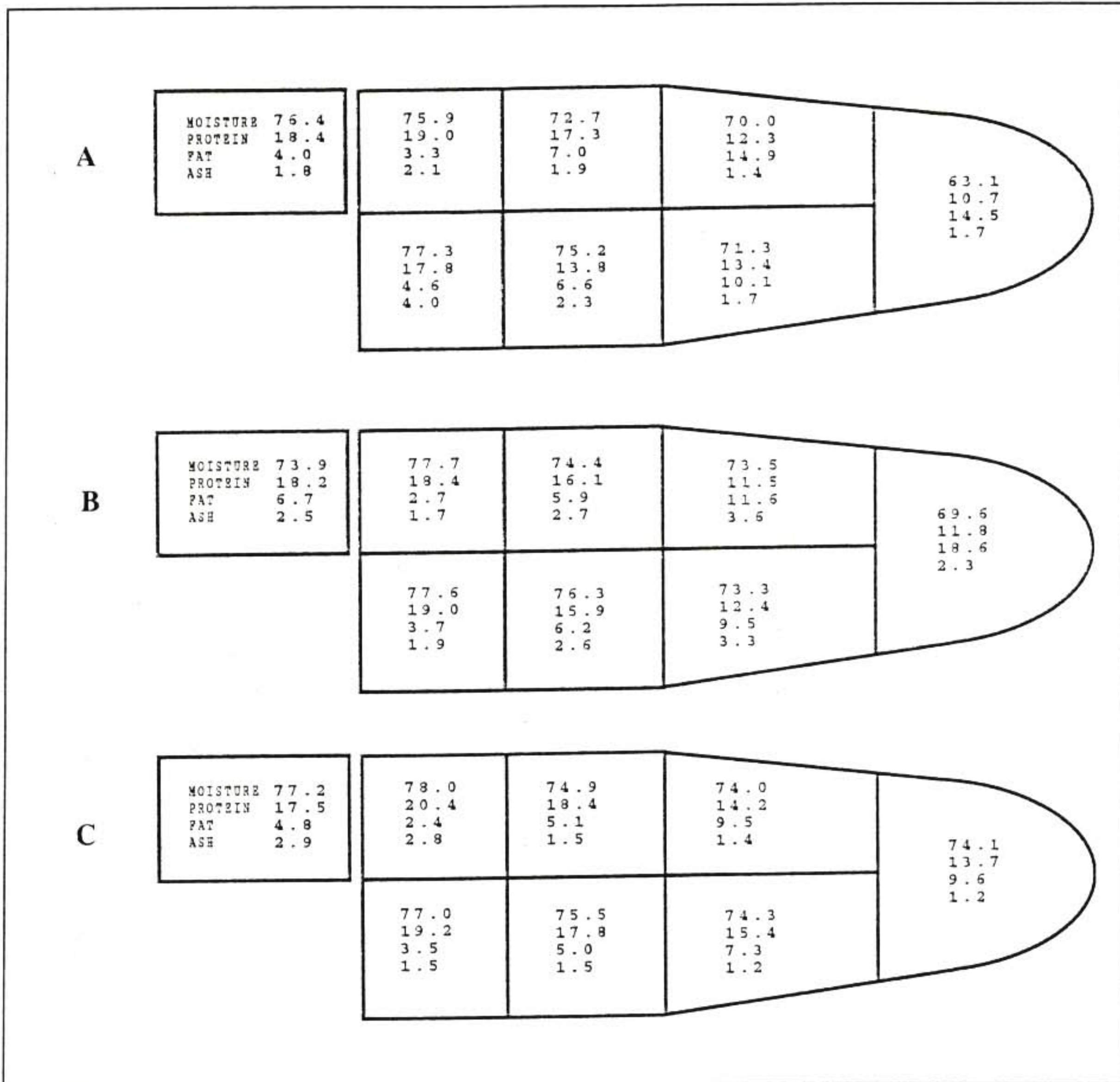


**Figure 2:** Schematic fillets from three fish showing the proximate composition of the muscle (A = 3665.2 g live mass; B = 2632.2 g live mass; C = 2155.0 g live mass).

**Table 2:** Amino acid concentrations in different parts of the musculature of *Clarias gariepinus* (g/100 g fat free dry mass).

| Amino Acid | Right Fillet | Position on musculature | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dorsal | | | | Ventral | | | |
| | | A | B | C | D | E | F | G | D |
| **Asp** | 8.2 | 7.9 | 9.0 | 8.7 | 8.0 | 9.1 | 9.1 | 8.7 | 8.0 |
| **Glut** | 12.2 | 11.9 | 13.4 | 13.1 | 12.2 | 13.5 | 13.6 | 13.0 | 12.2 |
| **Ser** | 3.9 | 3.6 | 4.2 | 4.1 | 3.9 | 4.4 | 4.1 | 4.0 | 3.9 |
| **Gly** | 4.7 | 3.9 | 4.9 | 4.9 | 6.0 | 6.2 | 5.2 | 5.1 | 6.0 |
| **His** | 1.8 | 1.7 | 2.0 | 1.9 | 1.8 | 2.0 | 2.0 | 1.4 | 1.8 |
| **Arg** | 4.9 | 4.4 | 5.5 | 5.6 | 5.5 | 5.8 | 5.7 | 5.6 | 5.5 |
| **Thre** | 4.0 | 3.9 | 4.5 | 4.3 | 4.1 | 4.6 | 4.5 | 4.4 | 4.1 |
| **Ala** | 4.7 | 4.3 | 5.1 | 5.0 | 5.0 | 5.5 | 5.2 | 5.2 | 5.0 |
| **Pro** | 3.1 | 2.6 | 3.3 | 3.3 | 3.8 | 3.9 | 3.4 | 3.5 | 3.8 |
| **Tyr** | 2.7 | 2.5 | 3.2 | 3.2 | 2.9 | 3.2 | 3.2 | 3.2 | 2.9 |
| **Val** | 3.7 | 3.2 | 4.2 | 4.2 | 4.0 | 4.2 | 4.5 | 4.5 | 4.0 |
| **Meth** | 2.0 | 2.1 | 2.6 | 2.3 | 2.5 | 2.6 | 2.7 | 2.7 | 2.5 |
| **Iso** | 3.6 | 3.1 | 4.3 | 4.4 | 4.1 | 4.2 | 4.6 | 4.6 | 4.1 |
| **Leu** | 5.9 | 5.4 | 7.5 | 7.5 | 6.8 | 7.4 | 7.6 | 7.7 | 6.8 |
| **Phe-ala** | 3.1 | 2.8 | 4.2 | 4.1 | 3.9 | 4.2 | 4.1 | 4.3 | 3.9 |
| **Lys** | 5.9 | 5.6 | 10.0 | 10.0 | 9.5 | 9.8 | 9.3 | 10.0 | 9.5 |
| **Hydro** | 1.2 | 0.4 | 0.6 | 0.7 | 1.3 | 0.8 | 0.7 | 0.7 | 1.3 |

**Table 3:** Percentage of fatty acids from the lipids in different parts of the musculature of *Clarias gariepinus*.

| Fatty Acid | Right Fillet | Position on musculature | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dorsal | | | | Ventral | | | |
| | | A | B | C | D | E | F | G | D |
| **14:0** | 1.5 | 1.4 | 1.5 | 1.8 | 1.2 | 1.4 | 1.5 | 1.7 | 1.2 |
| **16:0** | 23.9 | 24.0 | 23.5 | 22.5 | 20.4 | 23.3 | 23.6 | 22.8 | 20.4 |
| **16:1ω7** | 7.1 | 6.1 | 6.6 | 6.8 | 6.1 | 7.0 | 6.7 | 7.1 | 6.1 |
| **18:0** | 6.7 | 7.3 | 6.9 | 6.6 | 7.8 | 6.5 | 7.0 | 6.7 | 7.8 |
| **18:1ω9** | 25.9 | 23.4 | 24.2 | 23.6 | 20.7 | 25.2 | 24.6 | 23.9 | 20.7 |
| **18:2ω6** | 16.8 | 14.6 | 16.5 | 16.3 | 14.3 | 16.7 | 16.4 | 16.4 | 14.3 |
| **18:3ω6** | 1.6 | 1.5 | 1.4 | 1.3 | 1.1 | 1.6 | 1.5 | 1.4 | 1.1 |
| **20:1ω9** | 1.2 | 1.0 | 1.1 | 1.2 | 1.0 | 0.9 | 1.0 | 1.1 | 1.0 |
| **20:5ω3** | 0.9 | 1.4 | 1.2 | 1.2 | 2.1 | 1.0 | 1.1 | 1.1 | 2.1 |
| **22:0** | 0.1 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **22:1ω11** | 1.2 | 1.9 | 1.6 | 1.5 | 2.4 | 1.4 | 1.5 | 1.4 | 2.4 |
| **22:1ω9** | 1.2 | 2.3 | 1.8 | 2.0 | 4.4 | 1.4 | 1.5 | 1.7 | 4.4 |
| **22:5ω3** | 0.9 | 1.2 | 1.1 | 1.0 | 1.4 | 1.0 | 1.0 | 1.0 | 1.4 |
| **22:6ω3** | 3.3 | 6.2 | 4.6 | 4.4 | 8.4 | 3.9 | 4.1 | 4.2 | 8.4 |
| **Unidentified** | 7.7 | 7.5 | 7.9 | 9.6 | 8.5 | 8.5 | 8.3 | 9.3 | 8.5 |
| **SFA** | 32.2 | 32.9 | 32.0 | 31.1 | 29.6 | 31.4 | 32.3 | 31.4 | 29.6 |
| **MUFA** | 36.6 | 34.7 | 35.3 | 35.1 | 34.6 | 35.9 | 35.3 | 35.2 | 34.6 |
| **PUFA** | 23.5 | 24.9 | 24.8 | 24.2 | 27.3 | 24.2 | 24.1 | 24.1 | 27.3 |

**Table 4:** Mean mineral concentrations in different parts of the musculature of *Clarias gariepinus* (values expressed on a dry mass basis, SD in parenthesis).

| Mineral | Right Fillet | Position on musculature | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dorsal | | | | Ventral | | | |
| | | A | B | C | D | E | F | G | D |
| P % | 1.2 (0.110) | 1.3 (0.267) | 1.1 (0.092) | 1.0 (0.190) | 1.0 (0.127) | 1.2 (0.074) | 1.2 (0.091) | 1.2 (0.156) | 1.0 (0.127) |
| Ca % | 0.1 (0.017) | 0.1 (0.013) | 0.1 (0.015) | 0.1 (0.077) | 0.1 (0.026) | 0.1 (0.014) | 0.1 (0.028) | 0.1 (0.005) | 0.1 (0.026) |
| K % | 1.5 (0.231) | 1.8 (0.137) | 1.5 (0.086) | 1.1 (0.156) | 0.9 (0.150) | 1.6 (0.063) | 1.5 (0.089) | 1.3 (0.087) | 0.9 (0.150) |
| Mg % | 0.2 (0.003) | 0.2 (0.004) | 0.2 (0.011) | 0.1 (0.011) | 0.1 (0.008) | 0.2 (0.006) | 0.2 (0.009) | 0.1 (0.008) | 0.1 (0.008) |
| Fe ppm | 26.9 (5.387) | 26.1 (3.445) | 30.7 (1.893) | 29.0 (3.880) | 33.1 (3.655) | 24.5 (2.722) | 29.2 (4.330) | 30.2 (3.834) | 33.1 (3.655) |
| Cu ppm | 2.8 (0.815) | 3.2 (1.477) | 3.2 (0.393) | 2.9 (1.068) | 2.9 (1.689) | 2.9 (1.600) | 2.9 (0.814) | 2.0 (0.412) | 2.9 (1.689) |
| Zn ppm | 29.0 (3.369) | 29.0 (0.763) | 28.0 (0.898) | 28.3 (2.228) | 40.6 (5.288) | 25.6 (1.749) | 25.2 (2.489) | 28.2 (1.663) | 40.6 (5.288) |
| Mn ppm | 1.6 (0.886) | 1.3 (0.443) | 0.3 (0.434) | 0.6 (0.440) | 0.6 (0.436) | 1.0 (0.762) | 0.6 (0.438) | 0.6 (0.439) | 0.6 (0.436) |

All values for the proximate composition of the right fillet, lie between the maximum and minimum values of the subsamples from the left fillet (Fig 2). Similarly, the values obtained for amino acids, fatty acids and minerals (Table 2, 3 and 4, respectively) were between the extreme values for that specific parameter. These results indicate that the sampling methodology employed gave representative values of the muscle chemical composition.

*Comparison of dark and light muscle*

The light muscle in all instances had a similar moisture (78%, $p \geq 0.3294$) and a significantly higher protein (19.0%, $p \geq 0.0716$) content than the dark muscle (14.5%, Table 5). Correspondingly, a higher total lipid (5.2% vs 1.0%, $p \geq 0.0796$) content was found in the dark muscle whilst the ash content did not differ greatly (3%, $p \geq 0.4082$).

**Table 5:** The mean proximate composition of light and dark muscle from the African sharptooth catfish, *Clarias gariepinus* (n=4).

| Percentage Composition | Muscle Type | | Mean Diffs (SE) | Prob > \| T \| |
|---|---|---|---|---|
| | Dark (±SD) | Light (±SD) | | |
| **Moisture** | 78.7 (1.779) | 78.5 (1.962) | 0.2 (0.170) | 0.3294 |
| **Protein** | 14.5 (1.552) | 19.0 (1.882) | -4.5 (1.618) | 0.0716 |
| **Total Lipid** | 5.2 (3.039) | 1.0 (0.502) | 4.2 (1.589) | 0.0796 |
| **Ash** | 3.4 (0.886) | 3.2 (1.338) | 0.2 (1.589) | 0.4082 |

A comparison of the relative proportions of the fatty acids of the lipids identified are shown in Table 6. There is no difference in the amount of saturated fatty acids (SFA) between the two muscle types ($\pm$34 %, Table 6). The dark muscle however has a higher percentage total mono-unsaturated fatty acids (MUFA, 6.3% more), whilst the light muscle has a higher total percentage polyunsaturated fatty acids (PUFA, 4.8% more). The dark muscle had statistically significantly higher concentrations of palmitoleic (C16:1$\omega$7 - $p \geq 0.0015$ : 0.9% more), oleic (C18:1$\omega$9 - $p \geq 0.0357$ : 5.6% more) and linolenic acids (C18:3$\omega$3 - $p \geq 0.0513$ : 0.5% more), but statistically significantly lower concentrations arachidonic (C20:4$\omega$6 - $p \geq 0.0514$ : 1.2% less), eicosapentaenoic (C20:5$\omega$3 - $p \geq 0.0092$ : 0.8% less), gondoic (C22:1$\omega$9 - $p \geq 0.0467$ : 0.2% less), docosapentaenoic (C22:5$\omega$3 - $p \geq 0.0044$ : 0.4% less) and docosahexaenoic (C22:6$\omega$3 - $p \geq 0.0252$ : 3.5% less) acids. The dark muscle had a lower $\omega 3/\omega 6$ ratio (0.44) than the light muscle (0.61).

Of all the amino acids tested, only lysine and hydroxyproline showed significant differences between the dark and light muscle types (Table 7). The concentration of lysine was lower in the dark muscle, whilst that of hydroxyproline was higher.

Of the minerals analyzed (Table 8), there was no significant difference in the amounts of phosphorus, copper and manganese in the dark and light muscles. The dark muscle type had significantly higher concentrations of iron (71.0 vs 29.0 mg/kg respectively) and zinc (91.2 vs 36.6 mg/kg respectively) than the white muscle type. The levels of all the other minerals were higher in the light muscle type.

The widths of the individual myotomes are indicated in Figure 3. There is no definite increase in myotome width towards myotome number 12 for the African sharptooth catfish in contrast to the cod as reported by Love (1988). It would seem as if the African sharptooth catfish has the widest myotomes directly behind the head (myotome number 6), thereafter, there is a slight decrease in myotome width in the caudal direction - reaching minimum widths between myotomes 20 to 45. There is then a gradual increase in width towards myotome number 50. This maximum is not as high as that experienced near the head. Close to the tail end (myotome number $\pm$56), there is a sudden decrease in myotome width. The myotome widths all increase with increasing fish size.

*The fatty acid composition of various lipid depots*

As already noted (Table 5), the dark muscle contained 5.2 $\pm$ 3.04% total lipid compared to the 1.0 $\pm$ 0.50% of the light muscle, the latter containing 4.4% more protein than the dark (moisture and ash content between the two muscle types were similar). The liver had a mean lipid content of 3.9 $\pm$ 1.05% (n=4) on a wet mass basis.

**Table 6:** Fatty acid profile of the lipids of light and dark muscle types of the African sharptooth catfish. *Clarias gariepinus* (% fatty acid identified/% total fatty acid present, n=4).

| Methylated Fatty acid | Dark (±SD) | Light (±SD) | Mean Diffs (SE) | Prob > \| T \| |
|---|---|---|---|---|
| **14:0** | 1.8 (0.477) | 1.7 (0.257) | 0.1 (0.232) | 0.8200 |
| **16:0** | 22.8 (0.778) | 23.0 (0.130) | -0.2 (0.382) | 0.5844 |
| **16:1ω7** | 5.2 (0.264) | 4.3 (0.207) | 0.9 (0.037) | 0.0015 |
| **18:0** | 7.7 (0.460) | 7.4 (0.370) | 0.3 (0.335) | 0.4024 |
| **18:1ω9** | 26.4 (1.933) | 20.8 (1.867) | 5.6 (1.077) | 0.0357 |
| **18:2ω6** | 18.2 (1.012) | 17.9 (1.308) | 0.3 (0.559) | 0.6837 |
| **18:3ω3** | 2.0 (0.964) | 1.5 (0.789) | 0.5 (0.107) | 0.0513 |
| **18:3ω4** | 0.9 (0.459) | 0.8 (0.210) | 0.1 (0.150) | 0.4300 |
| **20:1ω9** | 0.3 (0.064) | 0.2 (0.132) | 0.1 (0.099) | 0.2064 |
| **20:3ω3** | 0.0 (0.035) | 0.1 (0.197) | -0.1 (0.124) | 0.4919 |
| **20:3ω6** | 0.7 (0.035) | 0.6 (0.235) | 0.1 (0.156) | 0.9546 |
| **20:4ω3** | 0.4 (0.091) | 0.2 (0.085) | 0.2 (0.013) | 0.0047 |
| **20:4ω6** | 1.7 (0.694) | 2.9 (0.433) | -1.2 (0.279) | 0.0514 |
| **20:5ω3** | 1.2 (0.087) | 2.0 (0.190) | -0.8 (0.085) | 0.0092 |
| **22:0** | 1.8 (0.470) | 2.6 (0.803) | -0.8 (0.477) | 0.2108 |
| **22:1ω9** | 0.2 (0.053) | 0.4 (0.051) | -0.2 (0.043) | 0.0467 |
| **22:1ω11** | 0.1 (0.075) | 0.2 (0.183) | -0.1 (0.081) | 0.2419 |
| **22:5ω3** | 1.1 (0.211) | 1.5 (0.170) | -0.4 (0.031) | 0.0044 |
| **22:6ω3** | 4.3 (1.026) | 7.8 (0.689) | -3.5 (0.557) | 0.0252 |
| **24:0** | 0.3 (0.190) | 0.1 (0.248) | 0.2 (0.073) | 0.2516 |
| **SFA** | 34.4 | 34.8 | -0.4 | |
| **MUFA** | 32.2 | 25.9 | 6.3 | |
| **PUFA** | 30.5 | 35.3 | -4.8 | |
| **Ratio ω3/ω6** | 0.44 | 0.61 | | |

Where SFA = Saturated Fatty Acids, MUFA = Mono-unsaturated Fatty Acids, PUFA = Poly-unsaturated Fatty Acids

In Table 9 the mean fatty acid profiles of the five fat depots are displayed, while Table 10 gives the probability values relating to comparisons of the means of the different fat depots. In the light muscle and liver the predominant fatty acid was palmitic (C16:0) acid whereas oleic (C18:1ω9) acid was the major fatty acid in the other three depots. The light muscle and liver had similar fatty acid profiles with only two of the identified fatty acids differing between them, namely, myristic (C14:0) and linoleic (C18:2ω6) acids, both being more abundant in the light muscle. The levels of a large number of the fatty acids identified differed significantly when comparing the light muscle lipid with that of the subcutaneous and gut fat depots. A trend that manifests itself in respect of these differences, is higher concentrations of the shorter chained fatty acids (<20 carbons, saturated as well as unsaturated) in the subcutaneous and gut fat depots, whilst the white muscle had higher concentrations of the longer chained fatty acids (>20 carbons, saturated and unsaturated). However, more fatty acid levels differed significantly between the light muscle and subcutaneous fat depot (12 fatty acids - Table 10) than between the light muscle and gut (mesenterium) fat depot (9 fatty acids). The dark muscle also had a similar mean lipid fatty acid composition to that of the liver. The levels of myristic (C14:0), linoleic (C18:2ω6) and arachidonic (C20:4ω6) acids only differed significantly between these two fat depots. The first two fatty acids were present in higher concentrations in the dark muscle, whilst the latter was present in higher concentrations in the liver.

The liver had significantly higher concentrations of the longer chained fatty acids (especially unsaturated) than the subcutaneous and gut fat depots. The latter two fat depots had very similar fatty acid compositions, with only myristic (C14:0) and palmitic (C16:0) acids being present in a higher concentration in the subcutaneous depot.

The light muscle and liver had similar proportions saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids (Table 9), although the type of fatty acids making up these classes differed as manifested by the ω3/ω6 fatty acid ratio, the liver having the higher ratio. Of all the different fat depots, the liver had the highest ω3/ω6 (0.81) ratio with the subcutaneous (0.30) and gut fat depots (0.34) having the lowest. These latter mentioned two lipid depots had the lowest concentrations of PUFAs, but the highest relative MUFA contents. The SFA concentrations did not differ greatly between the five different lipid depots.

**Table 7:** The mean amino acid content of light and dark muscle of the African sharptooth catfish, *Clarias gariepinus* (g/100 g fat free dry mass, n=4).

| Amino Acid | Dark (±SD) | Light (±SD) | Mean Diffs (SE) | Prob > \| T \| |
|---|---|---|---|---|
| Asp | 9.2 (0.131) | 10.2 (0.744) | -1.0 (0.430) | 0.1028 |
| Glut | 13.7 (0.116) | 14.6 (1.438) | -0.9 (0.722) | 0.3090 |
| Ser | 4.5 (0.100) | 4.5 (0.355) | 0.0 (0.215) | 0.8473 |
| Gly | 5.2 (0.375) | 4.5 (0.298) | 0.7 (0.280) | 0.0973 |
| His | 2.3 (0.123) | 2.3 (0.193) | 0.0 (0.139) | 0.9736 |
| Arg | 5.8 (0.213) | 5.8 (0.479) | 0.0 (0.311) | 0.9705 |
| Thre | 4.7 (0.094) | 5.0 (0.437) | -0.3 (0.210) | 0.2958 |
| Ala | 5.4 (0.142) | 5.6 (0.391) | -0.2 (0.258) | 0.5305 |
| Pro | 3.9 (0.245) | 3.3 (0.249) | 0.5 (0.232) | 0.1152 |
| Tyr | 3.3 (0.065) | 3.4 (0.245) | -0.1 (0.136) | 0.3718 |
| Val | 4.4 (0.256) | 4.7 (0.569) | -0.3 (0.161) | 0.2435 |
| Meth | 2.6 (0.128) | 2.8 (0.176) | -0.2 (0.117) | 0.1896 |
| Iso | 4.2 (0.204) | 4.4 (0.450) | -0.2 (0.133) | 0.2958 |
| Leu | 7.4 (0.167) | 7.9 (0.804) | -0.5 (0.399) | 0.2637 |
| Phe-ala | 4.0 (0.146) | 4.3 (0.420) | -0.3 (0.144) | 0.1446 |
| Lys | 8.0 (0.478) | 9.5 (0.815) | -1.5 (0.233) | 0.0088 |
| Hydro | 0.8 (0.144) | 0.4 (0.119) | 0.4 (0.086) | 0.0285 |

**Table 8:** The mineral profile of dark and light coloured muscle of the African sharptooth catfish, *Clarias gariepinus* (values expressed on a dry mass basis, n=4).

| **Mineral** | **Dark (±SD)** | **Light (±SD)** | **Mean Diffs (SE)** | **Prob > \| T \|** |
|---|---|---|---|---|
| **P g/kg** | 8.3 (1.804) | 9.2 (0.000) | -0.9 (1.041) | 0.4647 |
| **Ca g/kg** | 30.4 (1.617) | 42.7 (4.498) | -12.3 (1.690) | 0.0184 |
| **K g/kg** | 12.7 (1.365) | 16.0 (3.180) | -3.3 (1.099) | 0.0970 |
| **Mg g/kg** | 1.6 (0.000) | 2.1 (0.058) | -0.5 (0.033) | 0.0039 |
| **Fe mg/kg** | 71.0 (9.498) | 29.0 (3.663) | 42.0 (6.530) | 0.0234 |
| **Cu mg/kg** | 3.1 (1.203) | 2.6 (0.636) | 0.5 (0.722) | 0.6222 |
| **Zn mg/kg** | 91.2 (23.161) | 36.6 (3.773) | 54.6 (11.730) | 0.0432 |
| **Mn mg/kg** | 1.5 (0.006) | 1.5 (0.859) | 0.0 (0.499) | 0.9920 |

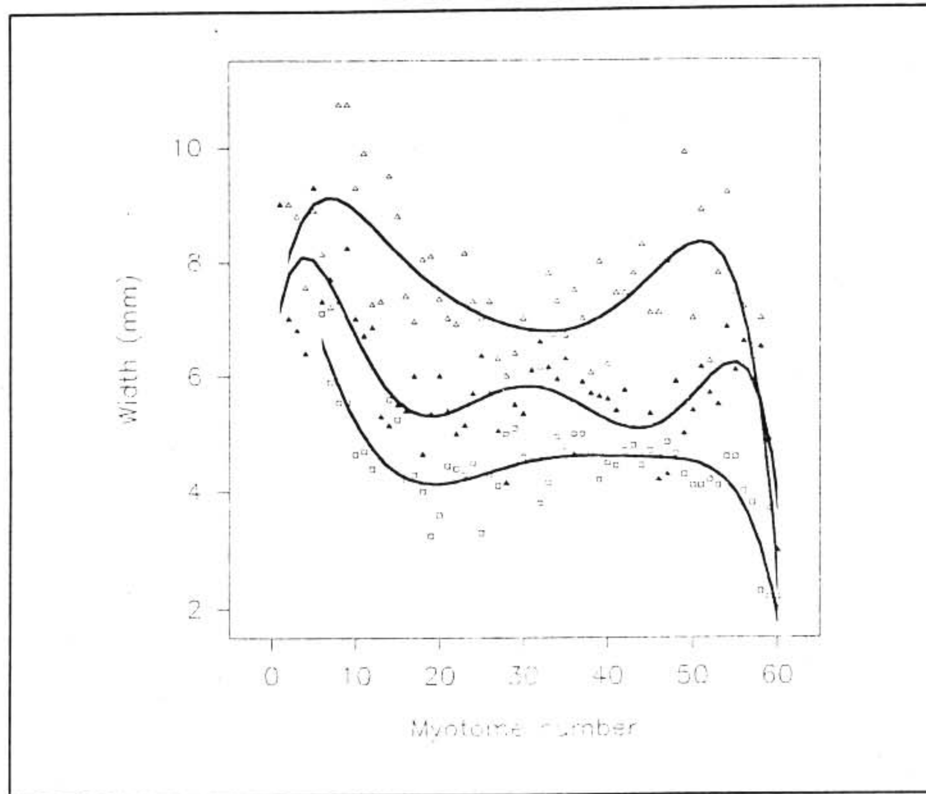


**Figure 3:** Myotomal widths of different sized fish measured on the surface of the musculature starting cranially and moving caudally (□ = 306.0 g live mass, 36.4 cm total length; ▲ = 1773.7 g live mass, 57.0 cm total length; △ = 2646.0 g live mass, 720.0 cm total length).

**Table 9:** Fatty acid profile of the lipids of the different fat depots of the African sharptooth catfish, *Clarias gariepinus* (fatty acids identified as % of total fatty acids present, n=4; ±SD in parenthesis)

| | Light Muscle | Dark Muscle | Liver | Subcutaneous Fat | Gut Fat |
|---|---|---|---|---|---|
| **14:0** | 1.7 (0.257) | 1.8 (0.477) | 1.3 (0.245) | 1.5 (0.185) | 1.2 (0.091) |
| **16:0** | 23.0 (0.130) | 22.8 (0.778) | 21.9 (1.730) | 23.9 (0.535) | 23.1 (0.542) |
| **16:1ω7** | 4.3 (0.207) | 5.2 (0.264) | 5.4 (1.543) | 6.2 (0.327) | 5.3 (0.670) |
| **18:0** | 7.4 (0.370) | 7.7 (0.460) | 8.5 (1.832) | 6.2 (0.659) | 5.9 (0.361) |
| **18:1ω9** | 20.8 (1.867) | 26.4 (1.933) | 21.3 (7.517) | 29.5 (1.579) | 28.1 (2.444) |
| **18:2ω6** | 17.9 (1.308) | 18.2 (1.012) | 13.6 (2.366) | 20.0 (1.593) | 21.7 (2.294) |
| **18:3ω3** | 1.5 (0.789) | 2.0 (0.964) | 1.4 (0.635) | 2.5 (1.314) | 2.5 (0.890) |
| **18:3ω4** | 0.6 (0.210) | 0.7 (0.459) | 0.8 (0.270) | 1.0 (0.146) | 1.1 (0.067) |
| **20:1ω9** | 0.2 (0.132) | 0.3 (0.064) | 0.1 (0.161) | 0.6 (0.409) | 0.6 (0.084) |
| **20:3ω6** | 0.6 (0.235) | 0.7 (0.035) | 0.9 (0.503) | 0.4 (0.239) | 0.6 (0.140) |
| **20:4ω6** | 2.9 (0.433) | 1.7 (0.694) | 4.4 (1.724) | 0.6 (0.047) | 0.5 (0.015) |
| **20:5ω3** | 2.0 (0.190) | 1.2 (0.087) | 2.4 (0.824) | 0.8 (0.261) | 0.6 (0.252) |
| **22:0** | 2.6 (0.803) | 1.8 (0.470) | 2.9 (0.792) | 1.2 (0.219) | 1.3 (0.297) |
| **22:1ω9** | 0.4 (0.051) | 0.2 (0.053) | 0.4 (0.131) | 0.6 (0.409) | 0.2 (0.085) |
| **22:1ω11** | 0.2 (0.182) | 0.1 (0.075) | 0.2 (0.172) | 0.2 (0.083) | 0.2 (0.266) |
| **22:5ω3** | 1.5 (0.170) | 1.1 (0.211) | 1.8 (0.722) | 0.9 (0.252) | 1.4 (0.954) |
| **22:6ω3** | 7.8 (0.689) | 4.3 (1.026) | 9.7 (5.617) | 2.2 (0.547) | 3.3 (2.213) |
| **24:0** | 0.1 (0.248) | 0.3 (0.190) | 0.6 (0.710) | 0.2 (0.084) | 0.2 (0.191) |
| **SFA** | 34.8 | 34.4 | 35.2 | 33.0 | 31.7 |
| **MUFA** | 25.9 | 32.2 | 27.4 | 37.1 | 34.4 |
| **PUFA** | 34.8 | 29.9 | 35.0 | 28.4 | 31.7 |
| **Ratio ω3/ω6** | 0.60 | 0.42 | 0.81 | 0.30 | 0.34 |

Where SFA = Saturated Fatty Acids, MUFA = Mono-unsaturated Fatty Acids, PUFA = Poly-unsaturated Fatty Acids

**ble 10**: P values, based on Student's *t* value, for testing the null hypothesis that the difference between two means equals zero, for the total lipid fatty acid composition of different fat pots in *Clarias gariepinus* (n=4)

| ty Acid | Light = Dark | Light = Liver | Light = Subcut fat | Light = Gut fat | Dark = Liver | Dark = Subcut fat | Dark = Gut fat | Liver = Subcut fat | Liver = Gut fat | Subcut fat = Gut fat |
|---|---|---|---|---|---|---|---|---|---|---|
| **4:0** | 0.8200 | 0.0722 | 0.4235 | 0.1265 | 0.0607 | 0.4741 | 0.2137 | 0.4679 | 0.8240 | 0.0434 |
| **6:0** | 0.5844 | 0.3687 | 0.0907 | 0.8574 | 0.3157 | 0.1697 | 0.4592 | 0.2329 | 0.3571 | 0.0618 |
| **6:1ω7** | 0.0015 | 0.2696 | 0.0221 | 0.0823 | 0.7999 | 0.0913 | 0.8570 | 0.5411 | 0.8187 | 0.1814 |
| **8:0** | 0.4024 | 0.3883 | 0.0685 | 0.0274 | 0.4142 | 0.1336 | 0.0589 | 0.2344 | 0.1690 | 0.2282 |
| **8:1ω9** | 0.0357 | 0.9127 | 0.0274 | 0.0355 | 0.2684 | 0.0412 | 0.0378 | 0.1429 | 0.1483 | 0.1993 |
| **8:2ω6** | 0.6837 | 0.0745 | 0.0063 | 0.0776 | 0.0313 | 0.1181 | 0.0426 | 0.0395 | 0.0003 | 0.2875 |
| **8:3ω3** | 0.0513 | 0.3020 | 0.0911 | 0.1307 | 0.1123 | 0.1364 | 0.3271 | 0.1174 | 0.1296 | 0.9954 |
| **8:3ω4** | 0.4300 | 0.4631 | 0.0306 | 0.0212 | 0.7251 | 0.0102 | 0.0728 | 0.5733 | 0.2581 | 0.4598 |
| **0:1ω9** | 0.2064 | 0.7418 | 0.1579 | 0.1634 | 0.1157 | 0.3435 | 0.6095 | 0.2385 | 0.2066 | 0.3965 |
| **0:3ω6** | 0.9546 | 0.5232 | 0.0426 | 0.4226 | 0.4608 | 0.1660 | 0.4583 | 0.2999 | 0.2783 | 0.3454 |
| **0:4ω6** | 0.0514 | 0.2679 | 0.0808 | 0.1600 | 0.0384 | 0.1613 | 0.4238 | 0.0183 | 0.1743 | 0.5764 |
| **0:5ω3** | 0.0092 | 0.5462 | 0.0195 | 0.0060 | 0.1137 | 0.1906 | 0.0701 | 0.1122 | 0.0961 | 0.1332 |
| **2:0** | 0.2108 | 0.7671 | 0.1229 | 0.1734 | 0.1013 | 0.1937 | 0.3667 | 0.0426 | 0.0589 | 0.1843 |
| **2:1ω9** | 0.0467 | 0.6729 | 0.0475 | 0.0660 | 0.2714 | 0.2031 | 0.3379 | 0.0261 | 0.0130 | 0.2355 |
| **2:1ω11** | 0.2419 | 0.1946 | 0.5432 | 0.0831 | 0.3157 | 0.0942 | 0.2827 | 1.0000 | 0.2543 | 0.4436 |
| **2:5ω3** | 0.0044 | 0.5262 | 0.0052 | 0.8082 | 0.1528 | 0.0375 | 0.5196 | 0.0844 | 0.3612 | 0.3373 |
| **2:6ω3** | 0.0252 | 0.6243 | 0.0080 | 0.0820 | 0.1954 | 0.0234 | 0.3392 | 0.1263 | 0.0860 | 0.3730 |
| **4:0** | 0.2516 | 0.5426 | 0.5725 | 0.9039 | 0.6465 | 0.3438 | 0.3291 | 0.9891 | 0.4238 | 0.3225 |

## DISCUSSION

The trend shown in the proximate composition of the myotomal musculature (decrease in moisture and protein, increase in total fat towards the caudal region - Figure 2a, b, c) supports the data of Manthey, Hilge and Rehbein (1988) for the African catfish and European catfish (*Silurus glanis*), who noted a similar trend in the fat distribution. These results are in direct contrast to that found for the Atlantic herring, *Clupea harengus* (Brandes & Dietrich, 1970) and silver salmon, *Oncorhynchus kisutch* (Karrick & Thurston, 1964), but similar to that found for the Atlantic cod, *Gadus morhua* (Brandes & Dietrich, 1970). The channel catfish (*Ictalurus punctatus*), however, showed a uniform fat distribution over the whole length of the musculature (Manthey *et al.*, 1988).

This variation in the proximate composition along the musculature can largely be attributed to the change in the proportion of light (white) and dark (red) muscle. In a study of sixteen marine species (Mosse & Hudson, 1977), the proportion of red muscle increased caudally. For the bluntnose minnow (*Pimephales notatus* Rafinesque), the proportion of red muscle (expressed as a percentage of the total muscle in cross-section) increased from 1.13 % to 3.50 % in a caudal direction (Gill, Weatherley & Bhesania, 1982). In 84 species examined by Greer-Walker and Pull (1975), red muscle fibres never constituted more than a quarter of the total myotomal musculature, and in most, less than a tenth. It has been reported in a number of fish species, that red muscle contains more fat than white muscle (Johnston, Ward & Goldspink, 1975; Mosse & Hudson, 1977; Bone, 1978; Gill *et al.*, 1982; Uno, Morishita & Takahashi, 1987). In rainbow trout (*Salmo gairdneri*), the total fat in the white muscle amounted to 2% but varied in red muscle from 10 to 12% (Kiessling, Johansson & Storebakken, 1989).

There is also an increase in the number of myotomes per unit area towards the caudal region (Figure 3). This tendency leads to an increase in the number of myocommata present, per unit weight. Love (1988) notes that the myocommata do not necessarily increase in thickness in the caudal region. Red muscles are also smaller in diameter than white muscles, which implies that there will be more cell walls per volume of tissue in red muscle compared to white muscles (Johnston *et al.*, 1975; Bone, 1978). The latter two factors lead to an increase in the percentage of collagen present caudally. The increase in percentages of red muscle and collagen, and decrease in percent white muscle along the myotomal muscle towards the caudal region, will amongst others, influence the amino acid, fatty acid and mineral profiles.

The red muscle is normally used for slow aerobic contractions (cruising travel mode) whilst the white muscle is used for strong fast anaerobic contractions (rapid, short bursts - Bone, 1978). The African catfish, swims with a slow side-to-side undulating movement called subcarangiform (Lindsey, 1978) where it uses mainly the tail region. However, upon receiving an external stimulation, *Clarias* swims rapidly by utilizing all its musculature. It has been noted that the more flexible the part of the body, the higher the collagen composition of that part (Sato, Yoshinaka, Sato & Shimizu, 1986).

The increasing concentrations of the amino acids glycine, alanine, proline and hydroxyproline posteriorly (Table 2), indicate an increase in the collagen content (posteriorly) because the amino acid composition of collagen is unique. Glycine (one molecule occurring in every three amino acids), alanine (one in nine) and proline plus hydroxyproline (two in nine) account for two-thirds of all amino acids present in collagen (Love, 1988). In a study of the collagen of cod (Love, Yamaguchi, Créac'h & Lavéty, 1976), glycine had a concentration of 24.4, alanine 9.7, proline 10.5 and hydroxyproline 6.6 (concentrations expressed as % of protein). In the present investigation, the increase in percentage collagen per mass is further shown by the high concentration of these four last mentioned amino acids in region E, the region that contained the gut peritonium.

In the musculature of *C. gariepinus*, there is a decrease in total percentage of saturated and monounsaturated fatty acids and an increase in the percentage polyunsaturated fatty acids moving caudally (position A has 32.9%, 36.6%, 23.5% and position D, 29.6%, 34.6%, 27.3% SFA, MUFA and PUFA respectively), whilst no noticeable difference is noted between the dorsal and ventral portions (Table 3). Kinsella, Shimp, Mai and Weihrauch (1977) found the fatty acid composition of the lipids of trout to be similar between the ventral and dorsal sections. Uno *et al.* (1987) also noted a similar fatty acid composition between the dorsal and ventral muscle of cultured red sea bream. Kinsella *et al.* (1977) found an increase in the concentration docosahexaenoic acid (C22:6ω3) in a posterior direction for both lake trout and salmon. Kiessling *et al.* (1989) found that the levels of saturated fatty acids were similar in white and red muscle of the rainbow trout, whereas levels of mono-unsaturated fatty acids were higher and polyunsaturated fatty acids lower in red than in the white muscle of the trout studied. A similar trend was noted for cultured red sea bream viz: equal saturated fatty acid content in the dark and white muscle but higher monounsaturated and lower polyunsaturated fatty acid contents in the dark muscle (Uno *et al.*, 1987). In a study on the lipids of the muscle of white sucker (*Catostomus commersoni*), Mai and Kinsella (1979) also found that the white muscle contained higher concentrations of polyunsaturated fatty acids.

The major differences in the individual fatty acids identified have similar trends in the dorsal and ventral segments, namely, a decrease in palmitic (C16:0 - 24.0% in position A and 20.4% in position D) and oleic (C18:1ω9 - 23.4% in position A and 20.7% in position D) and an increase in cetoleic (C22:1ω11 - 1.9% in position A and 2.4% in position D) and docosahexaenoic (C22:6ω3 - 6.2% in position A and 8.4% in position D) acids, moving caudally. The latter two fatty acids showing a remarkable increase in their concentration in the tail segment. Mai and Kinsella (1979) found that dark (red) muscle had a higher phospholipid concentration than the white (light) muscle. Love (1988) notes that phospholipids, that make up an integral part of microscopic structures such as cell walls and organelles like mitochondria, are rich in docosahexaenoic acid (C22:6ω3). This may explain the high concentration thereof in the tail segment as it is known that red muscle cells are smaller than white muscle cells, and therefore contain more cell wall per volume, and also contain more mitochondria (Johnston & Maitland, 1980; Johnston, 1982).

The high concentration of docosahexaenoic acid (C22:6ω3) in the tail could have important implications for the

processing industry, since oxidation of this fatty acid is largely responsible for the development of off-flavour and off-odour during cold-storage (Love, 1988).

Few data pertaining to the change in mineral composition along the myotomal musculature of fish could be found. From Table 4, the percentage phosphorus decreases in a caudal direction (dorsally and ventrally - 1.3% in position A and 1.0% in position D), whereas the percentage calcium shows no fixed trend (0.1% in both positions A and D). In a review of the chemical constituents of red and white muscle types, Love (1970) notes two species (*Gadus morhua* - Atlantic cod and *Lateolabrax japonicus* - sea bass) that contain less inorganic phosphate in red than white muscle. No consistent difference in the calcium concentration of dark and white muscle has been noted (Love, 1970). The percentages of potassium and magnesium also decrease in the caudal direction (for *C. gariepinus* muscle), both in the dorsal and ventral segments (1.8% and 0.2% in position A and 0.9% and 0.1% in region D, respectively). Potassium (Love, 1970) is often found to decrease in the muscle from head to tail, probably as a reflection of the decrease in cell size towards the tail and the increase in the proportion of connective tissue, which is poor in potassium. Potassium was found to be less concentrated in the dark muscle of the Atlantic halibut (*Hippoglossus hippoglossus* - Love, 1970). Phosphorus, magnesium and potassium are all intracellular ions, and as the proportion of small sized cells increases (red muscle cells), the amount of extracellular fluid relative to the intracellular fluid also increases (Love, 1988). The iron concentration increases in the caudal direction (in both the dorsal and ventral segments - 26.1ppm in position A and 33.1ppm in position D), which is consistent with the fact that dark muscle contains more haem pigments (for most fish species - Love, 1970). No trend is noted for the copper, zinc and manganese concentrations, with the exception of a high zinc concentration in the tail (segment D) - this is in agreement with Love (1970) who notes that in most fish, zinc has a higher concentration in dark than in light muscle.

Immediately after killing, the dark muscle in *C. gariepinus* is dark red in colour and is located just under the skin along each side of the body and near the spine. The light muscle is light pink in colour and decreases caudally from 93% (behind the head) to 91% (at the tail) of the total musculature (Fig 4). In a study of sixteen marine species, the proportion of dark muscle was found to increase caudally (Mosse & Hudson, 1977). The relative quantity of dark muscle present at any position of the musculature varies from species to species. In an extensive study (Nahhas, Jones & Goldspink, 1982) on the proportion of dark muscle in 42 families (84 species), the percentage of dark muscle was found to vary from 0 to 26.1% (sample site was one third of the fish length from the tail), the proportion dark muscle present being determined by the swimming activity of the fish. Generally, dark muscle fibres respire aerobically and light fibres anaerobically; dark fibres are used for sustained swimming whilst light fibres are used for faster swimming, particularly, burst swimming (Nahhas *et al.*, 1982). *C. gariepinus*, swims with a slow side-to-side undulating movement called subcarangiform which explains the caudal increase in dark muscle experienced in this species.

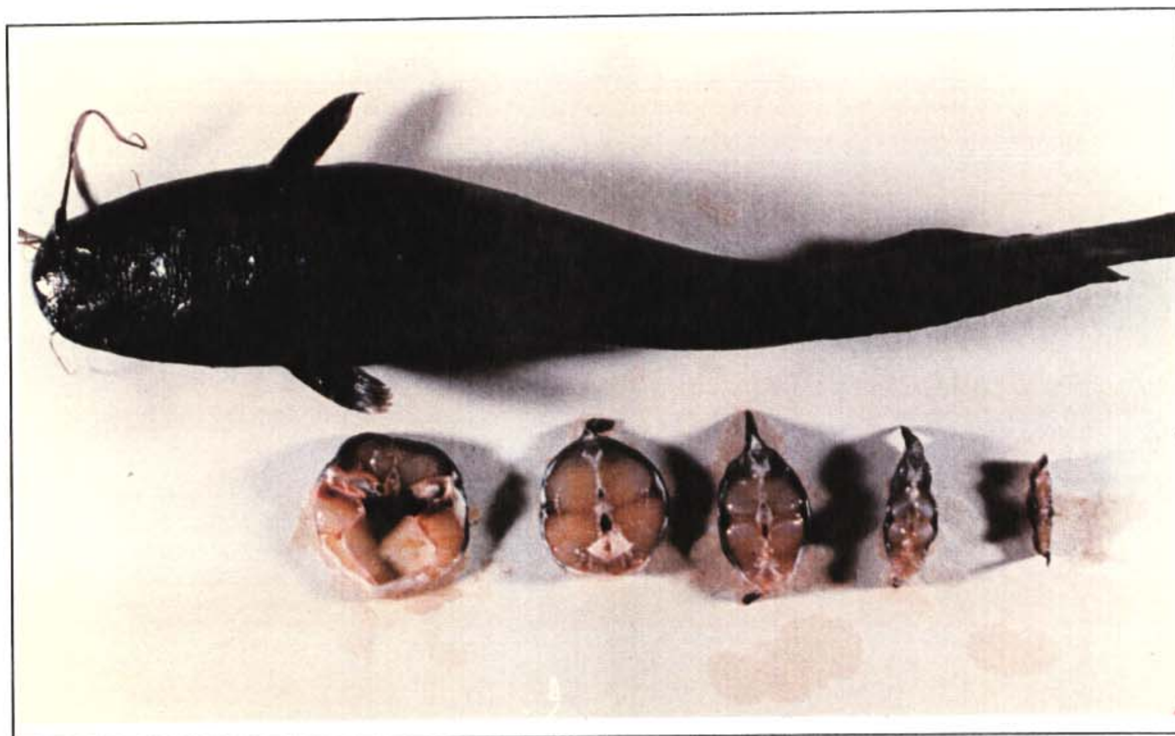

**Figure 4:** Cross-sections of the musculature of *Clarias gariepinus* showing the caudal increase in dark muscle.

Love (1970, 1980, 1988) notes that there are major differences in the chemical composition of these two types of muscle. Generally, dark muscle contains less moisture, protein and ash and more lipid than light muscle. Lipid content in fish is highly variable, depending on factors such a diet, season, environmental temperature, age, sex and weight of fish (Huss, 1988). The lipid content of the total flesh of *C. gariepinus* has been found to vary between 0.9 to 21.7% (unpublished results).

The higher percentage of lipid (and lower percentage of protein) in dark muscle compared to light muscle (5.2 vs 1.00%) is analogous to results reported for a number of different fish species; however, the quantity of lipid is lower. In rainbow trout (*Salmo gairdneri*), the lipid in the light muscle amounted to 2% but varied in dark muscle from 10 to 12% (Kiessling, Åsgård, Storebakken, Johansson & Kiessling, 1991). In sockeye salmon (*Oncorhynchus nerka*), the dark flesh contained over eight times more lipid than did the light (Porter, Kramer & Kennish, 1992). Total lipid content reported for the white sucker (*Catostomus commersoni*), where the dark muscle had 6.2% lipid compared to the 1.4% of the light muscle (Mai & kinsella, 1979) is similar to that found in the present investigation (Table 5).

The investigation into the lipid composition of white sucker (*Catostomus commersoni* - Mai & Kinsella, 1979) realised similar differences to that of *C. gariepinus*, namely, a higher percentage palmitoleic (C16:1ω7) and lower percentages palmitic (C16:0) and docosahexaenoic acids (22:6ω3) in the dark muscle. The higher percentage of PUFA in the light

muscle of *C. gariepinus* is in direct contrast to that noted in the sockeye salmon (*Oncorhynchus nerka* - Porter *et al.*, 1992) but similar to that of white sucker (*Catostomus commersoni* - Mai & Kinsella, 1979) and rainbow trout (*Oncorhynchus mykiss* - Watanabe, 1982). In the latter study, the dark muscle of the rainbow trout had a lower percentage SFA, a higher percentage MUFA and lower PUFA than the light muscle. In the present investigation (Table 6), there was a similar concentration of SFA between the two muscle types, and also a higher MUFA and lower PUFA in the dark muscle. However, the rainbow trout had higher concentrations C22:6ω3 in both muscle types (17.5 and 12.4% in light and dark muscle, respectively, water temperature 12°C) than did the African sharptooth catfish (7.8 and 4.3%, respectively, water temperature >20°C).

The environmental temperature plays an important role in the degree of saturation of the fatty acids. Generally, the lower the environmental temperature, the higher the degree of unsaturation in body lipid. The reason for this increase can probably be found at several processes in the lipid metabolism (Mai & Kinsella, 1979; Watanabe, 1982; Stickney & Hardy, 1989), for example, membrane fluidity (Bell, Henderson & Sargent, 1986) and enzyme activity (Kiessling & Kiessling, 1993). *C. gariepinus* normally prefers warm waters (28-30°C) which may explain why the muscle of this species contains lower concentrations of the PUFAs, especially C22:6ω3 (fish in the present investigation were kept at an environmental temperature varying between 24-30°C). The lower concentration of longer chained PUFAs could be diet-related (Watanabe, 1982; Stickney & Hardy, 1989).

With respect to the difference in amino acid composition between dark and light muscle (Table 7), Love (1988) quotes earlier work which claims that dark muscle contains higher concentrations of glycine, leucine, phenylalanine and proline. In the present investigation, of these four amino acids, only glycine was present in significantly ($p>0.0973$) higher concentrations, and leucine and phenylalanine were, in fact, lower in the dark muscle. The significantly ($p>0.0285$) higher hydroxyproline concentration in the dark muscle could be attributed to a higher concentration of collagen per unit muscle mass. Dark muscle cells are narrower than light muscle cells (Kiessling & Kiessling, 1993) which results in a higher concentration of investing collagenous tubules being present in a given weight of dark muscle tissue when compared to light muscle (Higgins, 1990).

In a review of the mineral constituents of red and white muscle types, Love (1970) notes two species (*Gadus morhua* - Atlantic cod and *Lateolabrax japonicus* - sea bass) that contain less inorganic phosphate in red than white muscle - a result consistent with the present investigation, although the difference between dark and light muscle (0.9 g/kg dry mass) was not statistically significant ($p>0.4647$). No consistent difference in the calcium concentration of dark and white muscle has been noted (Love, 1970). In this investigation the light muscle had significantly ($p>0.0184$) more calcium (12.3 g/kg more - Table 8). The percentages potassium and magnesium also differ significantly between dark and light muscle types, the levels of both minerals being lower in the dark muscle (Table 8). The concentration of potassium was often found to be lower in dark muscle cells, probably as a reflection of the smaller cell size and the increase in the proportion of connective tissue (per weight of tissue), which is poor in potassium (Love, 1970).

Potassium was found to be less concentrated in the dark muscle of the Atlantic halibut (*Hippoglossus hippoglossus* - Love, 1970). The iron concentration in the dark muscle was significantly ($p>0.0234$) higher in the dark muscle, which is consistent with the fact that dark muscle contains more haem pigments (for most fish species - Love, 1970). No significant difference is noted for the copper and manganese concentrations between the two muscle types. There is a significantly ($p>0.0432$) higher zinc concentration in the dark muscle - this is in agreement with Love (1970) who notes that in most fish zinc has a higher concentration in dark than in light muscle.

In our laboratory, we have observed that fat (>5% wet mass muscle total lipid) *C. gariepinus* specimens of both the normal and golden coloured strains, have a thin, subcutaneous fat layer (Fig 5). This subcutaneous fat layer is slightly thicker on the ventral abdominal wall (Fig 6) and thickens into a localised subcutaneous fat depot on the midline between the operculum and the pectoral fin (Fig 7). A large ventral mesenteric fat depot is also usually present, lying between the gonads and the intestines (Fig 8 - Hoffman, Casey & Prinsloo, 1993). In very fat specimens, a second fat depot occurs around the spleen in the mesenteric cavity. Fat deposition in the kidneys, in particular in the kidney gland posterior to the head, on the lateral body-line has also been observed. This gland is sometimes greatly enlarged in very fat specimens. In the golden coloured strain, small sub-epithelial haemorrhages were observed over these enlarged glands. These haemorrhages are similar to those described by Roberts (1970) in marine Plaice (*Pleuronectes platessa*). One or both kidney glands may be swollen.

The most general and significant use of lipids is as energy reserves (Sheridan, 1988). A secondary use of lipids are for organ development (gonads and testes) during maturation (Love, 1988). Similar to most teleostean species (reviewed by Love, 1988), *C. gariepinus* also stores more lipid in the dark muscle than light muscle. However, whether the dark muscle or the liver serves as the major lipid reservoir in *C. gariepinus* is not clear and the physiological usefulness of the different depots can be revealed only from studies on the activity of the different enzymes (Sheridan, 1988).

An interesting phenomenon in the present investigation into the fatty acid profiles of the various lipid depots, was the high concentrations of the oleic ($C18{:}1\omega9$) and linoleic ($C18{:}2\omega6$) acids present in the subcutaneous and mesenteric fat depots. Both of these fatty acids were present in high concentrations in the diet (26.1 and 26.9% respectively) fed the fish (Table 1), indicating that similar to other fish species (Watanabe, 1982), the chemical body composition of the African sharptooth catfish *C. gariepinus* could also possibly be manipulated by the diet fed.

## CONCLUSION

The differences in the proximate composition, fatty acid and mineral profiles, and to a lesser extent, amino acid profiles between dark and light muscle types, support the results of the earlier study into the muscle heterogeneity of

the African sharptooth catfish. As mentioned, there is a decline in moisture and protein percentages and an increase in total lipid percentage caudally in the musculature of the catfish (Fig 2). Similarly, a decrease in the percentages SFA and MUFA and an increase in the percentage PUFA were noted caudally (Table 3). The concentration of the amino acids glycine, alanine, proline and hydroxyproline increased caudally along the musculature (Table 2). The concentrations of the minerals calcium, copper, zinc and manganese showed no fixed trends caudally, whilst phosphorus, potassium and magnesium decreased and iron increased (Table 4). Most of these trends were correctly attributed to a difference in the proportion of dark and light muscle, the proportion of the former increasing caudally. The only major incongruity between these two investigations, is the trends manifested by the fatty acids, in particular, the PUFAs, that increased in concentration caudally (Table 3). This increase was previously attributed to dark muscle having a higher concentration PUFA, which, from the present investigation, does not seem to be true. A possible explanation for this phenomena could lie in the relative proportions of the different lipid classes namely, phospholipids, cholesterol, free fatty acids, diglycerides, triglycerides and cholesterol esters present (Love, 1988). The fatty acid profiles of these lipid classes differ and warrant further investigation in terms of their concentration and composition in *C. gariepinus* muscle.

The results of the chemical analysis of the right fillet and different parts of the left fillet, as well as that of the two muscle types, support the finding of Love (1988) who suggests that ideally, white and dark muscle should be investigated separately, failing which the entire fillet, or a carefully specified part thereof, should be analyzed. In these earlier investigations into the muscle chemical composition (Hoffman & Prinsloo, 1990; Hoffman *et al.*, 1993), samples of minced and homogenised whole fillets were analyzed when the investigations were launched to study *Clarias gariepinus* as a "protein" source from a nutritional viewpoint. In this context, the composition of the total fillet is of more value than that of the part; as often, the whole fillet is consumed.

**Figure 5:** A *Clarias gariepinus* fillet showing a well developed subcutaneous fat layer.

**Figure 6:** A thick subcutaneous layer on the ventral abdominal wall of *Clarias gariepinus*.

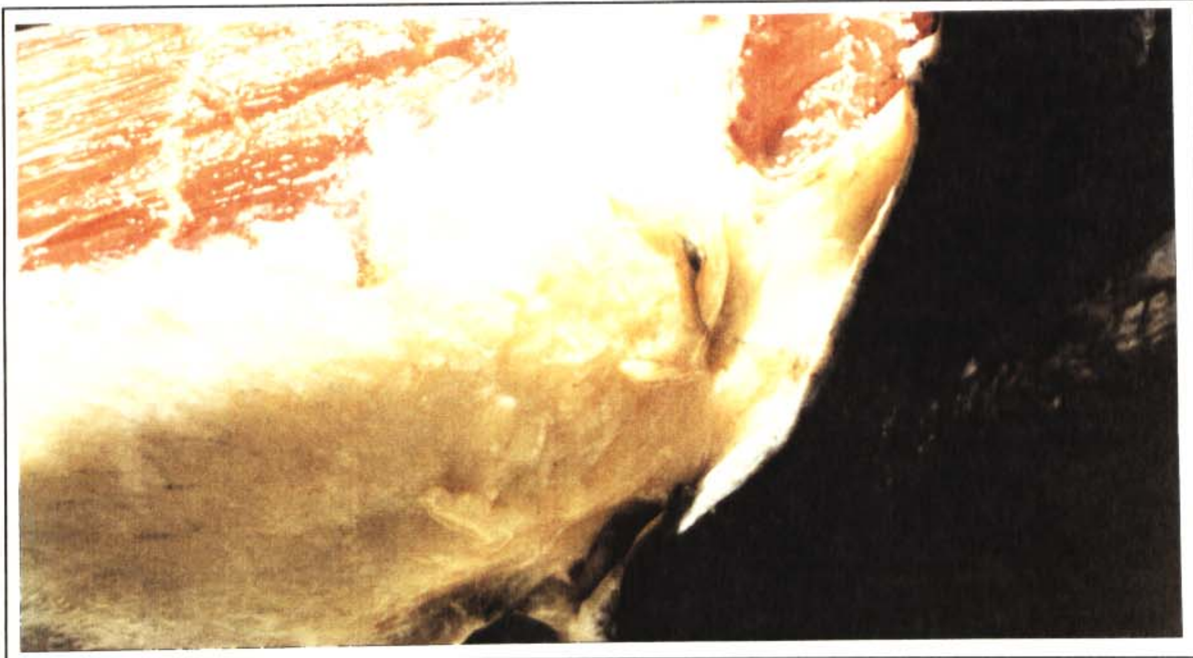

**Figure 7:** A thick subcutaneous fat depot on the midline between the operculum and pectoral fin.

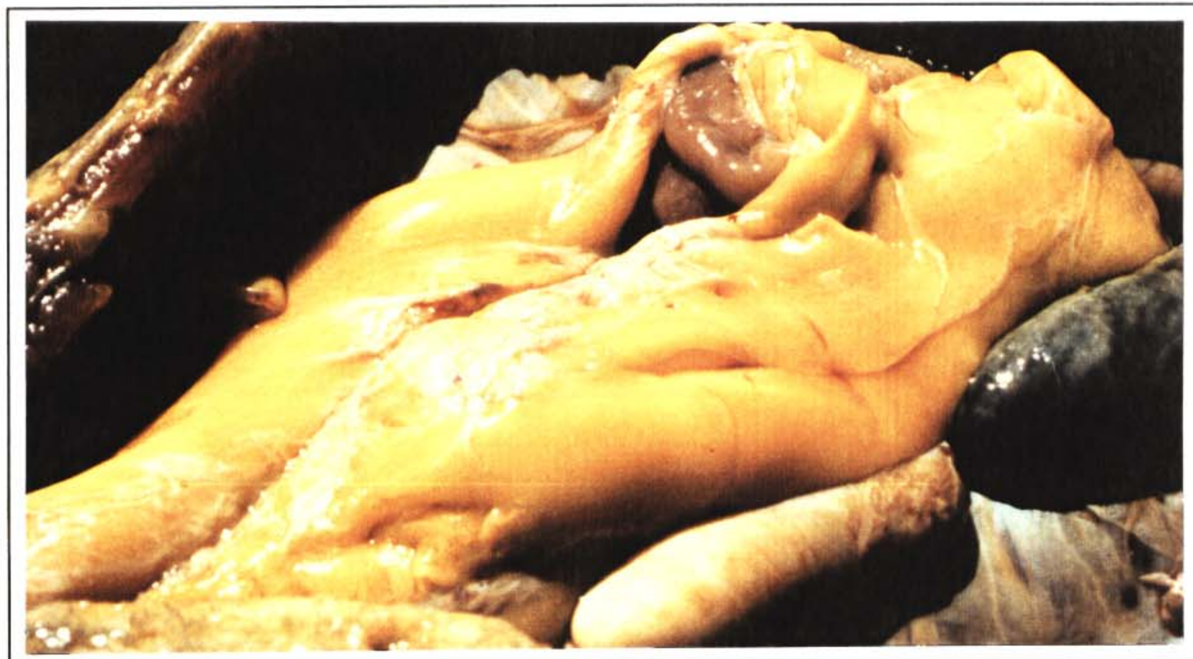

**Figure 8:** A large mesenteric lipid depot found in *Clarias gariepinus*.

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# Chapter 4 YIELD CHARACTERISTICS OF *CLARIAS GARIEPINUS*

CONTENTS

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**Material and Methods** **4.4**

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**INTRODUCTION**

At present, in South Africa, farmed and wild African sharptooth catfish *C. gariepinus*, are of a similar genotype with one merely having been captured and bred under controlled conditions (possibly for a number of generations in the older production farms). No scientific selection has been applied to develop a carcass with specific characteristics such as dressing yield. Fundamental to such selection, is an identification and analysis of carcass characteristics or components of dressing percentage and a better understanding of their relationships with live measurements so as to allow a more efficient selection (Dunham, Joyce, Bondari & Malvestuto, 1985).

As is the case with other livestock, the environment influences genetic expression of growth rate, maturity and carcass characteristics, in both male and female fish (Shearer, 1994). At present, the major criteria used for an acceptable marketable fish is based only on weight, 600-800 g live weight. No differentiation is made between sexes or physiological maturity. The body conformation (and dressout percentage) of a catfish could change with age, size and degree of sexual maturity. As the season progresses, gonads of females who are sexually mature, will start developing (Prinsloo, Schoonbee & Hoffman, 1990). This will not only influence the catfish yield, but also the muscle chemical composition, as it is well documented that as gonads develop in fish, various chemicals may be mobilised from various organs (including the muscle) to the gonads (Love, 1970, 1980, 1988).

The fecundity of *C. gariepinus* from various parts of southern Africa has been studied by a number of research workers (Groenewald, 1957; Pott, 1969; Mulder, 1971; Van der Waal, 1972; Gaigher, 1977; Bruton, 1979). Egg production increases from approximately 7 000 at 350 mm total length (TL) to about 10 000 at TL between 600 and 700 mm. Bruton (1979) revealed that the lowest gonadosomatic index (GSI) may occur between April and May in the coastal areas of Natal. An improvement in the GSI occurs as from May reaching a peak in the period September to December. From this it is clear that any attempt to breed this fish between March and May would encounter conditions of egg resorption which would not be conductive to its artificial spawning. Spawning trials which were undertaken during March were reasonably successful but already posed problems in obtaining a high percentage of viable eggs.

In 1984, a few specimens of a gold-coloured mutant of the African sharptooth catfish were obtained from an induced spawning experiment in the hatchery of the Turfloop Fish Breeding and Research Station in Northern Transvaal (Prinsloo, Schoonbee & Theron, 1989). These fingerlings were grown to sexual maturity and were then spawned. The resulting F2 generation fish were reared and their pond production potential studied (Prinsloo, Schoonbee & Van der Walt, 1989). One-year-old female fish of the gold strain were observed to possess consistently smaller gonads than fish of the same age of the normal strain, suggesting an inferior fecundity of the gold strain. In the earlier investigations into the aquaculture potential of this mutant, the term

red-coloured was used to describe this strain, subsequently changed to gold strain or golden coloured catfish. The reasons are that it was felt that gold would be a better description, especially as pertaining to a marketing strategy (Hoffman & Prinsloo, 1990).

At present, catfish gonads are classified as a waste product. With the increasing production of *C. gariepinus*, waste disposal could become a problem, and alternative uses for this waste need to be investigated. Catfish roe or gonads may replace fish meal in starter diets for larvae (Garatun-Tjeldstφ, Opstad & Huse 1989) or, with the proper processing, become a valuable product for human consumption (similar to caviare). Traditionally caviare is the pickled roe of sturgeon, but lately the roe from other large fish species have been utilised for this expensive delicacy.

With the successful establishment of the gold strain of *C. gariepinus*, the question arose as to which strain delivers the better production. Preliminary results indicate that the golden strain has a somewhat inferior growth to that of the normal strain (Prinsloo *et al.,* 1989*b*), although its attractive colour at a marketable size may offset this disadvantage, due to the high price per unit mass that it may fetch. In a preliminary investigation, the dressed yield between the two strains is compared, and the influence of sex thereon, determined.

In a more comprehensive study, the carcass fillet yield values and the fillet proximate chemical composition of both male and female *C. gariepinus* from numerous wild and farmed populations is reported. Regression equations to predict the yield from live weight are also calculated. The relationship between the various proximate chemical (moisture, crude protein, total lipid and ash) constituents of the fillets and the influence of increasing body weight thereon, are displayed. The calculated equations and knowledge of the inter-relationships between the various chemical parameters will be of value to both the producers and processors in their management programmes.

In view of the marketing potential of the gold strain in aquaculture (Hoffman & Prinsloo, 1990), it was decided to examine the fecundity of sexually mature specimens for comparison with the fecundity of the normal strain of the same age. For purposes of comparison, it was decided to use one-year-old fish of both strains, which have reached maturity, but have not yet spawned.

As fish gonads develop, the composition of the muscle and gonads change as some constituents are removed from the muscle to the gonads (Love, 1988). An analysis of the gonads and muscle will therefore be of value to both human nutritionists and fish producers.

Although *C. gariepinus* fillets may have a low fat content (2.4%), a mesenteric fat depot is frequently observed. This depot also occurs in commercially farmed catfish and varies between 0.0-5.0% of the body weight (0-16 g fat depot; 35-600 g body weight - unpublished data). At present this depot is classified by the trade as a waste product. It is known (Sheridan, 1988) that the major lipid storage sites in fish are mesenteric fat, muscle and liver. The mesenteric fat depot noted in *C. gariepinus* melts readily upon heating and can be used as an oil base for frying purposes. A comparison of the fatty acid profile of this depot with that of lipids from the fillet and diet, will help dieticians in the formulation of balanced diets for human nutrition.

## MATERIAL AND METHODS

*Catfish utilised*

*Yield of Turfloop normal and golden strains*

The spawning methods and growth and production studies of the fish used are described in detail by Prinsloo *et al.* (1989). The two strains (gold and normal - Fig 1) were stocked together in equal numbers in two experimental earthen ponds at Turfloop Fish Breeding Station in Lebowa, at a total density of 15 000 fish per hectare. Feed used consisted of a mixture of minced tilapia (25%), bakery floor-sweepings (37.5%) and an 18% protein formulated pelleted chicken feed (37.5%). Feed was applied for six days a week in quantities, calculated as a dry mass, at 4% of the estimated total fish mass in the ponds. The feed was adjusted fortnightly on estimates of fish growth, done on samples of fish seined from the ponds, the sample size varying between 15% and 25% of the total number of fish initially stocked in each pond.

**Figure 1:** Normal and gold coloured strains of African sharptooth catfish, *Clarias gariepinus*.

After seining the production ponds at the end of the production period (140 days), 384 normal and 306 gold-

coloured catfish were randomly selected and fasted for three days in concrete reservoirs supplied with a throughflow of fresh water. Thereafter the fish were sexed and their live mass (LM) recorded (Fig 2). The fish were then slaughtered and drawn, which involves the removal of the viscera only, and this drawn mass (DGM) was recorded (Fig 3). The dressed mass (DM), which involves the additional removal of head and fins of the drawn fish was then noted (Fig 4). This differs from the procedures followed by Smith and De Beer, who had the skin removed as well in their definition (Smith & De Beer, 1988).

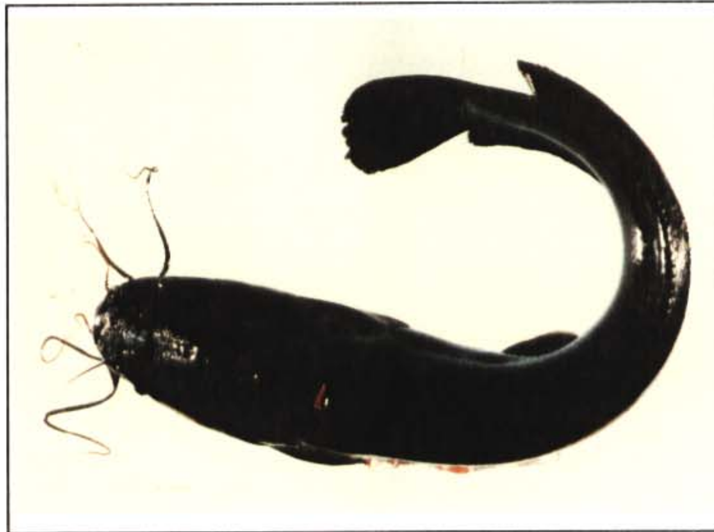


**Figure 2:** A normal coloured catfish whereupon live weight was determined.

**Figure 3:** Drawn mass - a normal coloured catfish with viscera removed.

**Figure 4:** Dressed mass - a normal coloured catfish with viscera and head removed.

The differences between the two strains and sexes in terms of different variables were tested by means of Duncan's multiple range test (SAS, 1985).

*Catfish utilised in fillet yield investigation*

Catfish were obtained from numerous sources as depicted in Table 1. The catfish designated Normal Turfloop are fish that are normal coloured and of the so-called Turfloop strain. These fish have been maintained for a number of generations in the Aquaculture Research Unit's water recirculating system. The Normal Turfloop strain has been selected (indirectly) over a number of generations (actual number unclear) for traits such as fast growth, and to a lesser extent, yield. The latter included selection of fish with a smaller (not measured) head. The fast growth trait was normally selected through the identification of the fast growers of a generation as the new brood stock. These catfish were of the same genetic strain, but not the same generation, as that described in the initial comparison between the Turfloop normal and golden strains. The fish received commercially prepared diets. The catfish from the Pietersburg Oxidation ponds are fish that occur wild in the Pietersburg final effluent oxidation ponds and are of various ages. These fish mainly fed on other wild fish (*C. gariepinus* and *Oreochromis mossambicus*) and zooplankton. The fish analysed from a commercial farmer in the Hoedspruit region had a similar selection history to that of the Turfloop normal strains, although the two strains were not related. These fish also received a commercially prepared diet. The wild fish from the Middle Letaba Dam were of various ages and occured naturally. Their diet was similar to that of the Pietersburg Oxidation pond fish in that they mainly ate other wild fish species.

Individual body length, body weight, head weight, skin weight, viscera weight (gonad weight, liver weight, gut

fat weight, gut weight), fillet weight and total bone weight were measured to the nearest 1 mm or 0.1 gram. The method of slaughtering and dressing has been described previously (Chapter 2) and consists of the manual removal of the viscera and skin, followed by filleting. To minimise the variation in dressing methodology, the same person filleted all the fish.

*Proximate analysis*

After filleting, freshly minced muscle (red, white and intermediate muscle types) sub-samples were analyzed for moisture content (60°C, 24 h), nitrogen (protein = N x 6.25) and ash (AOAC, 1984). Another sub-sample was analyzed for total lipid (using chloroform/methanol as solvent) according to the method of Folch, Lees and Stanley (1957) as adapted by Christie (1982). These methods are described fully in Chapter 2.

The various parameters were fitted to regression equations with body weight as the independent variable. Linear regression equations gave good fits (as shown by the $R^2$ values), and the influence of sex on the group as a whole, was tested by comparing the regression lines as described in Snedecor and Cochran (1980). Due to the limitation of sample sizes within fish source, comparison of the various parameters between sources were not tested.

In the comparison of the fillet chemical composition with increasing live body weight, the data was tested allometrically by transforming the data to a log function and comparing it with the log body weight (as suggested by Shearer, 1994), thus equalising error variances.

*Comparison of fecundity between normal and gold coloured strains*

The fish used in this investigation into the influence of strain on the fecundity of catfish, came from the same experiment as described for the yield investigation (Turfloop normal and golden strains). Detail of the experimental layout of the production investigation is given by Prinsloo *et al.* (1989). In total, 193 normal-coloured and 156 gold-coloured females were randomly selected for fecundity (GSI) determinations.

The gonads of 10 randomly selected females of each strain were removed and preserved in Gilson's fluid to loosen the eggs (Humason, 1979). Fecundity of each female was determined according to Bagenal (1966).

Results were statistically evaluated using Duncan's multiple range test (SAS Basics, 1985).

*Chemical composition of gonads*

*Fish* - The muscle and gonads of five female fish (1702.0 ± 162.0 g mean body weight) fed a commercial pelleted diet (6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt: for fatty

acid profile of diet - see Table 2) were investigated. All females were ready for spawning, with their gonads at maturity stage 4 (Nikolsky, 1963); the gonads had achieved their maximum mass, but eggs were not extruded when light pressure was applied to the abdomen.

The methods employed for the chemical analysis are described in detail in Chapter 2. The proximate composition, amino and fatty acids and mineral profiles of the muscle and gonads were determined. Differences between the muscle and the gonads for the different chemical parameters were tested by means of paired comparisons (students *t*-test).

*Chemical evaluation of mesenteric lipid depot*

The same fish utilised in the investigation into the chemical composition of the gonads were used. The fatty acids were determined using the same methodologies as described in Chapter 2.

*Statistical evaluation*

Linear regressions ($y = a + bx$) were used for the determination of the percentages drawn (%DGM) and dressed masses (%DM), fillet yield, as well as the mass of the other carcass components (head mass, skin mass, bone mass, total gut mass and gonad mass) with increasing live weight. For the fitting of the various proximate parameters (moisture, protein, lipid and ash) to body weight, log-log regression equations were used ($\log y = \log\alpha + \beta\log x$).

Table 1: Weights of African catfish *Clarias gariepinus* utilised in the investigation into the fillet yield.

| Code | Total | | | | Female | | | | Male | | | | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean Weight | Min Weight | Max Weight | N | Mean Weight | Min Weight | Max Weight | N | Mean Weight | Min Weight | Max Weight | |
| A | 60 | 235.7 ±133.38 | 35.9 | 574.8 | 21 | 164.3 ±85.31 | 35.9 | 330.2 | 39 | 274.2 ±139.47 | 53.5 | 574.8 | Normal Turfloop |
| B | 3 | 2817.5 ±771.96 | 2155.0 | 3665.2 | 3 | 2817.5 ±771.96 | 2155.0 | 3665.2 | | | | | Normal Turfloop |
| C | 5 | 1702.0 ±181.07 | 1501.1 | 1912.1 | 5 | 1702.0 ±181.07 | 1501.1 | 1912.1 | | | | | Normal Turfloop |
| D | 30 | 521.1 ±108.00 | 328.3 | 739.1 | 9 | 518.9 ±107.12 | 375.9 | 658.2 | 21 | 522.1 ±111.0 | 328.3 | 739.1 | Normal Turfloop |
| O | 30 | 2922.5 ±1254.0 | 998.9 | 6650.0 | 19 | 2628.6 ±1062.66 | 998.9 | 4390.0 | 11 | 3429.9 ±1442.33 | 1644.2 | 6650.0 | Piertersburg Oxidation ponds |
| P | 30 | 652.4 ±116.88 | 520.0 | 912.1 | 15 | 632.5 ±113.25 | 520.0 | 904.8 | 15 | 672.2 ±120.95 | 554.5 | 912.1 | Commercial farmer, Hoedspruit. |
| W | 41 | 1186.4 ±406.10 | 666.6 | 2685.7 | 22 | 1005.59 ±232.27 | 666.6 | 1684.0 | 19 | 1395.8 ±466.16 | 919.1 | 2685.7 | Wild, Middle Letaba Dam |
| Total | 199 | 1018.23 ±1067.11 | 35.90 | 6650.0 | 94 | 1134.4 ±1052.64 | 35.9 | 4390.0 | 105 | 914.2 ±1074.22 | 53.5 | 6650.0 | |

**Table 2:** The fatty acid profile of the commercial diet fed fish (fatty acid identified as % of fatty acids present).

| Fatty Acid | % |
|---|---|
| **C14:0** | 3.05 |
| **C16:0** | 17.52 |
| **C16:1ω7** | 3.99 |
| **C18:0** | 6.67 |
| **C18:1ω9** | 26.05 |
| **C18:2ω6** | 26.91 |
| **C20:1ω9** | 0.96 |
| **C20:5ω3** | 5.23 |
| **C22:0** | 0.48 |
| **C22:1ω9** | 1.29 |
| **C22:5ω3** | 0.62 |
| **C22:6ω3** | 3.13 |

## RESULTS

*Yield of Turfloop normal and golden strains*

The results of the basic data for the male and female catfish of the Turfloop normal and golden strains in terms of live mass (LM), drawn mass (DGM), dressed mass (DM), percentage drawn mass (%DGM) and percentage dressed mass (%DM) are summarised in Table 3. The latter two variables are expressed as a percentage of live mass.

**Table 3:** Characteristics of male and female catfish of the Turfloop normal and golden strains.

| Variable | Mean | SD | Min | Max | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | **Turfloop normal female** (n=193) | | | | **Gold female** (n=156) | | | |
| LM (kg) | 0.636 | 0.179 | 0.298 | 1.349 | 0.513 | 0.107 | 0.266 | 0.995 |
| DGM (kg) | 0.547 | 0.146 | 0.275 | 1.126 | 0.459 | 0.093 | 0.245 | 0.819 |
| Gonads (g) | 4.875 | 3.956 | 0.098 | 15.916 | 2.482 | 1.904 | 0.080 | 7.850 |
| DM (kg) | 0.390 | 0.108 | 0.193 | 0.817 | 0.333 | 0.068 | 0.175 | 0.577 |
| %DGM | 86.413 | 4.313 | 74.902 | 93.934 | 89.522 | 2.703 | 82.664 | 94.563 |
| %DM | 61.588 | 3.634 | 51.828 | 68.298 | 65.029 | 2.582 | 57.664 | 70.273 |
| GSI | 7.205 | 5.256 | 0.239 | 20.839 | 4.720 | 3.343 | 0.137 | 13.157 |
| | **Turfloop normal male** (n=191) | | | | **Gold male** (n=150) | | | |
| LM (kg) | 0.678 | 0.202 | 0.279 | 1.283 | 0.585 | 0.151 | 0.308 | 1.143 |
| DGM (kg) | 0.626 | 0.184 | 0.261 | 1.157 | 0.541 | 0.138 | 0.286 | 1.045 |
| Gonads (g) | 0.162 | 0.110 | 0.010 | 0.624 | 0.112 | 0.062 | 0.000 | 0.314 |
| DM (kg) | 0.448 | 0.134 | 0.182 | 0.865 | 0.395 | 0.105 | 0.204 | 0.807 |
| %DGM | 92.419 | 2.116 | 81.196 | 98.824 | 92.424 | 1.301 | 89.295 | 95.368 |
| %DM | 65.991 | 1.919 | 58.110 | 69.954 | 67.358 | 1.536 | 61.880 | 70.604 |
| GSI | 0.226 | 0.114 | 0.017 | 0.680 | 0.187 | 0.081 | 0.044 | 0.513 |

Where LM = live mass; DGM drawn mass; DM = dressed mass; %DGM = percentage drawn mass; %DM = percentage dressed mass; GSI = gonadal somatic index

Duncan's multiple range test ($\alpha = 0.05$) for the variables shown in Table 3 are summarised in Table 4. Means with the same letter are not significantly different.

**Table 4:** Duncan's multiple range test for the different fish carcass variables, between the means of the sexes (male and female), within the gold and normal strains of *C. gariepinus* ($\alpha$ = 0.05, degrees freedom = 686).

| Variable | Normal Male | Normal Female | Gold Male | Gold Female | MSE |
|---|---|---|---|---|---|
| | | | Means* | | |
| Live Mass (kg) | 0.678[a] | 0.636[b] | 0.585[c] | 0.513[d] | 0.0279 |
| Drawn Mass (kg) | 0.626[a] | 0.547[b] | 0.541[b] | 0.459[c] | 0.0216 |
| Dress Mass (kg) | 0.448[a] | 0.390[b] | 0.395[b] | 0.333[c] | 0.0216 |
| %DGM | 92.419[a] | 86.418[b] | 92.423[a] | 89.522[c] | 8.4644 |
| %DM | 65.991[a] | 61.588[b] | 67.358[c] | 65.030[d] | 6.7357 |

* means in the same row with the same letter are not significantly different.

All fish used in this investigation were of the same age when slaughtered. The normal male catfish are on average heavier (678 g LM) than the normal females (636 g LM). The mean mass of the gold strain, of both sexes, is less than that of the normal strain, with the gold male having a higher mean live mass (585 g LM) than the gold female (513 g LM). These differences in mean LM are significant (Table 4).

As expected, the decrease in mean drawn mass (DGM) is of the same order as that of mean LM, normal male (626 g), normal female (547 g), gold male (541 g) and gold female (459 g) (Table 3). Surprisingly, the mean drawn mass of the normal females and gold males, does not differ significantly at the 5% level (Table 4), although there is a significant difference between the latter and the means of both the normal males and gold females.

The mean DM of the gold males and normal females do not differ significantly ($\alpha$ = 0.05), but the gold males have a higher (395 g) mean DM than the normal females (390 g). However, the normal males still have the highest mean DM (448 g) and the gold females the lowest (333 g). The latter two mean DMs differ significantly at the 5% level from each other and from the gold males and normal females (Table 4).

When the drawn mass and dressed mass are expressed as percentage of live mass, the order of rank changes to gold males having the highest mean percentages, followed by normal males, the gold females, whilst the normal females have the lowest mean percentages of drawn and dressed mass (Table 3).

Mean percentage drawn mass (%DGM) does not differ significantly between the gold (92.42%) and normal males (92.42%) but differs significantly from the gold (89.52%) and normal females (86.44%). There is, however, a significant difference between the mean %DGM of the females (Table 4). Although the order in respect of mean percentage dress mass (%DM) does not differ from that of the mean percentage drawn mass (%DGM) (Table 4), there is a significant difference ($\alpha = 0.05$) between the mean %DM in both sexes and both strains as shown by Duncan's multiple range test.

The linear regression equations ($y = a + bx$) for the prediction of percentage drawn mass (%DGM) and percentage dressed mass (%DM) are given in Table 5 in respect of both sexes within the two strains. The regression relationships are illustrated in Figures 5 and 6. As can be seen from Table 5 (Fig 5), the percentage drawn mass (%DGM) decreases in both sexes of both strains with increasing live mass (LM), with the rate of decrease, shown by the regression coefficient, differing between the sexes and strains. The gold male decreases the least, with a decreasing rate of 1.4% DGM per kilogram increase in LM compared to 1.75% in the case of the normal male. In both strains of females, %DGM decreases at a faster rate per unit increase in live mass when compared to the males. The normal females also decrease at a faster rate (8.17% per kg LM) than the gold females (5.5% per kg LM) (Table 5).

Both normal and gold males show an increase in percentage dressed mass (%DM) with increasing live mass (LM), compared to the decrease shown by the females (Fig 6). The gold males increase at a faster rate (2.29% per kg LM) than the normal males (0.13% per kg LM), whilst the normal females decrease at a slightly faster rate (4.56% per kg LM) than the gold females (4.21% per kg LM) (Table 5).

Although these results may look highly significant (especially from a commercial view), care should be taken in the interpretation of the results (as described above) because of the poor fit of the regression equations to the data (as shown by the low $R^2$ values depicted in Table 5). This poor fit may be due to the large variation in live mass (Table 3) and the influence that sexual development may have on the dress-out percentage.

**Table 5:** Linear regression equations for the prediction of percentage drawn mass (%DGM) and percentage dressed mass (%DM) of both sexes for normal and gold strains of *Clarias gariepinus*, with live mass (LM) in kg as independent variable

| Strain | Sex | Parameter Estimate | | CV | $R^2$ |
|---|---|---|---|---|---|
| | | Intercept | Regression coefficient | | |
| **Percentage drawn Mass (%DGM)** | | | | | |
| Normal | Male | 93.60 | -1.75 | 2.26 | 0.0278 |
| Normal | Female | 91.61 | -8.17 | 4.71 | 0.1158 |
| Gold | Male | 93.06 | -1.14 | 1.40 | 0.0174 |
| Gold | Female | 92.34 | -5.50 | 2.96 | 0.0476 |
| **Perecentage Dressed Mass (%DM)** | | | | | |
| Normal | Male | 65.90 | 0.13 | 2.91 | 0.0002 |
| Normal | Female | 64.49 | -4.56 | 5.76 | 0.0507 |
| Gold | Male | 66.02 | 2.29 | 2.23 | 0.0508 |
| Gold | Female | 67.19 | -4.21 | 3.92 | 0.0306 |

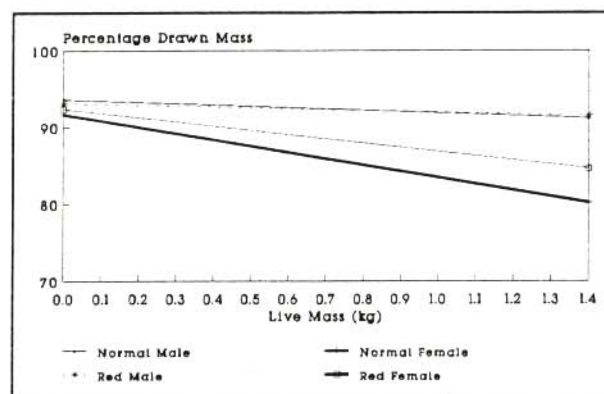



**Figure 5:** A comparison of the percentage drawn mass between males and females of the gold (red) and normal coloured strains of *C. gariepinus* with increasing live mass.

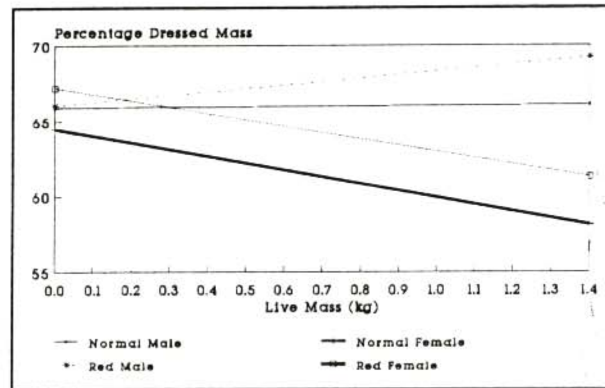



**Figure 6:** A comparison of the percentage dressed mass between males and females of the gold (red) and normal coloured strains of *C. gariepinus* with increasing live mass.

*Yield of total catfish population*

As can be seen in Table 1, the body weight of the fish sampled varied from 35.9 g to 6650.0 g. In the whole population, 94 female (body weight ranging from 35.9 to 4390.0 g) and 105 male (body weight ranging from 53.5 to 6650.0 g) catfish were sampled.

In Table 6 linear regression equations ($y = a + bx$) for the prediction of yield (mass of fillet, g) are given for the various groups of catfish investigated. The regression equations for the pooled males and pooled females are also included. Due to the small sample sizes of groups B and C, their linear regression equations were not calculated, but data from these two groups were used in the calculation of the global pooled and global pooled male and global pooled female equations. The linear regression equations for the fillet yield of the various groups of catfish were very similar. Statistical comparison (Table 7) of the linear regression equations for females and males showed no significant differences ($p > 0.05$) between the slopes ($\beta_{females} = 0.4697$; $\beta_{males} = 0.4715$) whilst the intercepts differed significantly at the 5% level, but not at the 1% ($\alpha_{females} = -20.7377$; $\alpha_{males} = -7.7711$).

The lighter fish (both sexes) tend to have a lower percentage yield which stabilises at about 47% (Fig 7). However, there is a linear relationship ($R^2 = 0.9934$) for the whole pooled population between fillet weight with increasing body weight as depictor, as shown by the regression equation ($R^2 = 0.9934$, $n = 198$):

$$\text{Fillet yield (g)} = -13.2828 + 0.4699 \times \text{BWt (g)}$$

BWt = Body Weight (Table 6).

The other carcass components that contribute to the total body weight can be grouped under head mass, skin mass, bone mass, total gut mass and gonad mass. The liver, gut, and mesenteric lipid depot, are all classified under total gut mass. In aquacultural terms, the gonad weight is normally expressed as the Gonadosomatic Index (GSI) and is calculated as (gonad weight)/(total body weight)*100. In Table 8 the various linear regression equations, with body weight as independent variable, for the pooled female and male, and the population as a whole, are given for these carcass components. Figures 8-13 depict the relationships of these components with increasing body weight. Sex has no influence on the head and skin weight, although the males have significantly ($p<0.05$) heavier bone and total gut weights, whilst the females have a heavier gonad weight. On further analysis of the components of the total gut, the males show a heavier mesenteric fat depot (Fig 14) than the females.

**Table 6:** Linear regression equations for the prediction of fillet yield (g) of African catfish *C. gariepinus*.

| Code | Total | | | Female | | | Male | | |
|---|---|---|---|---|---|---|---|---|---|
| | α | ß | $R^2$ | α | ß | $R^2$ | α | ß | $R^2$ |
| A | -11.9700 | 0.4972 | 0.9824 | -12.9921 | 0.4763 | 0.9861 | -6.8793 | 0.4874 | 0.9809 |
| D | -29.7242 | 0.5347 | 0.9809 | -13.4184 | 0.4976 | 0.9705 | -35.4983 | 0.5482 | 0.9879 |
| O | -24.3997 | 0.4784 | 0.9835 | -3.8772 | 0.4704 | 0.9784 | -49.0700 | 0.4859 | 0.9854 |
| P | -48.2691 | 0.5078 | 0.8020 | -88.7301 | 0.5405 | 0.8310 | 29.4828 | 0.4216 | 0.9709 |
| W | -20.4509 | 0.4573 | 0.9753 | 28.6505 | 0.4081 | 0.9288 | -38.4845 | 0.4706 | 0.9807 |
| Pooled | -13.2828 | 0.4699 | 0.9934 | -20.7377 | 0.4697 | 0.9916 | -7.7711 | 0.4715 | 0.9953 |

**Table 7:** Comparison of regression lines describing the fillet yield of female and male African catfish *C. gariepinus*.

| | | | | | | | Deviations from Regression | | |
|---|---|---|---|---|---|---|---|---|---|
| | | df | $\Sigma x^2$ | $\Sigma xy$ | $\Sigma y^2$ | Reg Coef | df | SS | MS |
| | Within | | | | | | | | |
| 1 | Female | 93 | 103047982.1 | 48399910.6 | 22925939.9 | 0.4697 | 92 | 193312.8 | 2101.23 |
| 2 | Male | 104 | 120011562.7 | 56585786.4 | 26806820.3 | 0.4715 | 103 | 126464.2 | 1227.81 |
| 3 | | | | | | | 195 | 319777.0 | 1639.88 |
| 4 | Pooled, *W* | 197 | 223059544.8 | 104985697.0 | 49732760.2 | 0.4707 | 196 | 319960.6 | 1632.45 |
| 5 | | | Difference between slopes | | | | 1 | 183.5 | 183.56 |
| 6 | Between *B* | 1 | 2406107.0 | 970286.6 | 391277.7 | | | | |
| 7 | *W* + *B* | 198 | 225465651.8 | 105955983.5 | 50124037.9 | | 197 | 330775.0 | |
| 8 | | | Between adjusted means | | | | 1 | 10814.4 | 10814.44 |

Comparison of slopes: $F=0.11$ ($df = 1,195$) not significant $p > 0.05$

Comparison of elevations: $F=6.62$ ($df = 1,196$) $0.01 < p < 0.05$

**Table 8:** Linear regression equations of the relationships between body weight and body components of female and male African catfish *C. gariepinus*.

| Carcass component | Pooled Population | | | Pooled Female | | | Pooled Male | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\alpha$ | ß | $R^2$ | $\alpha$ | ß | $R^2$ | $\alpha$ | ß | $R^2$ |
| Head | -10.0109 | 0.2646 | 0.9778 | -8.2892 | 0.2587 | 0.9736 | -10.7004 | 0.2712 | 0.9815 |
| Bone | 5.8308 | 0.1229 | 0.9845 | 4.5698* | 0.1186* | 0.9827 | 6.8231* | 0.1283* | 0.9906 |
| Skin | -2.8142 | 0.0556 | 0.9817 | -3.5204 | 0.0556 | 0.9835 | -2.2959 | 0.0557 | 0.9801 |
| Total Gut | 4.3051 | 0.0464 | 0.7353 | 5.2836* | 0.0398* | 0.7997 | 3.9238* | 0.0531* | 0.7447 |
| GSI | 4.5964 | -0.0007 | 0.0195 | 9.8080* | -0.0021* | 0.1195 | 0.8622* | -0.0001* | 0.0339 |

$\alpha$ and ß (between sexes) indicated with * differ significantly at the 5% level.

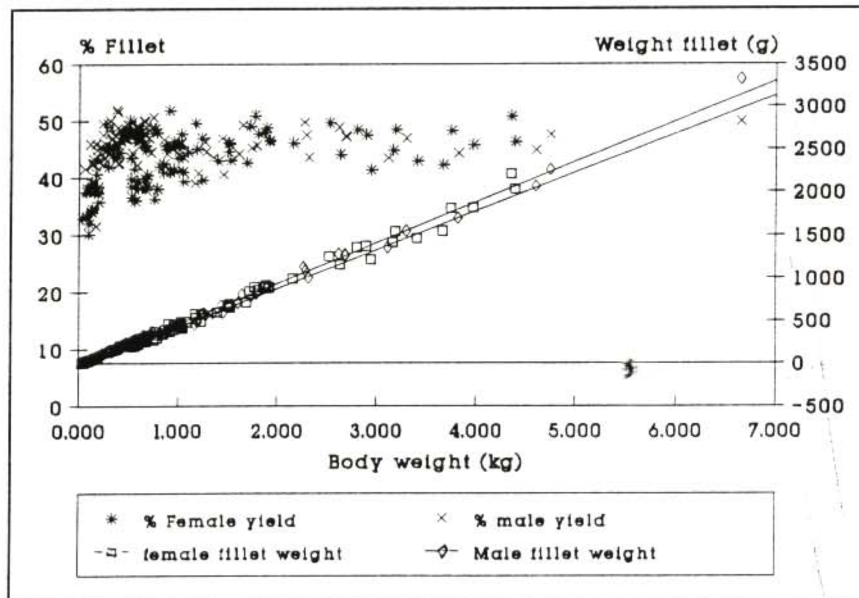



**Figure 7:** The fillet yield of female and male *C. gariepinus* with increasing body weight.

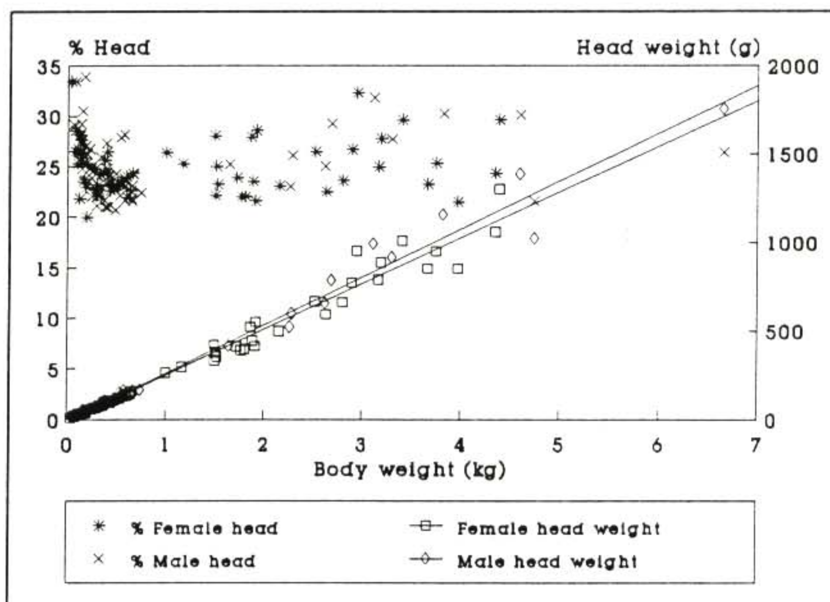



**Figure 8:** The relationship between male and female *C. gariepinus* head mass with increasing body weight.

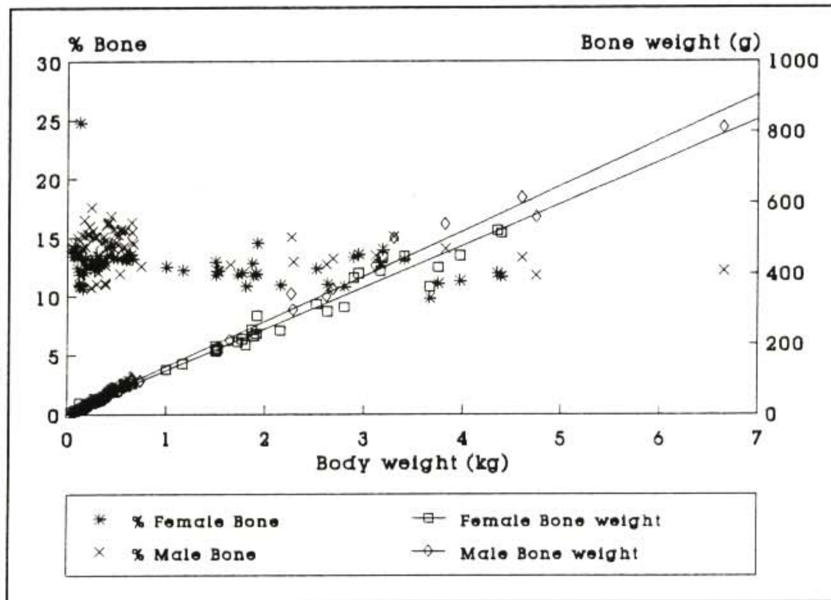



**Figure 9:** The relationship between male and female *C. gariepinus* bone mass with increasing body weight.

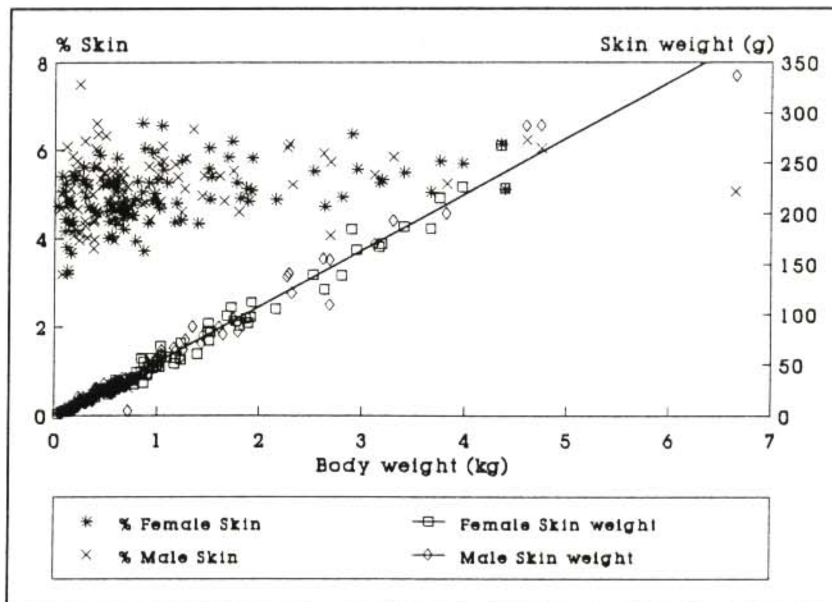



**Figure 10:** The relationship between male and female *C. gariepinus* skin mass with increasing body weight.

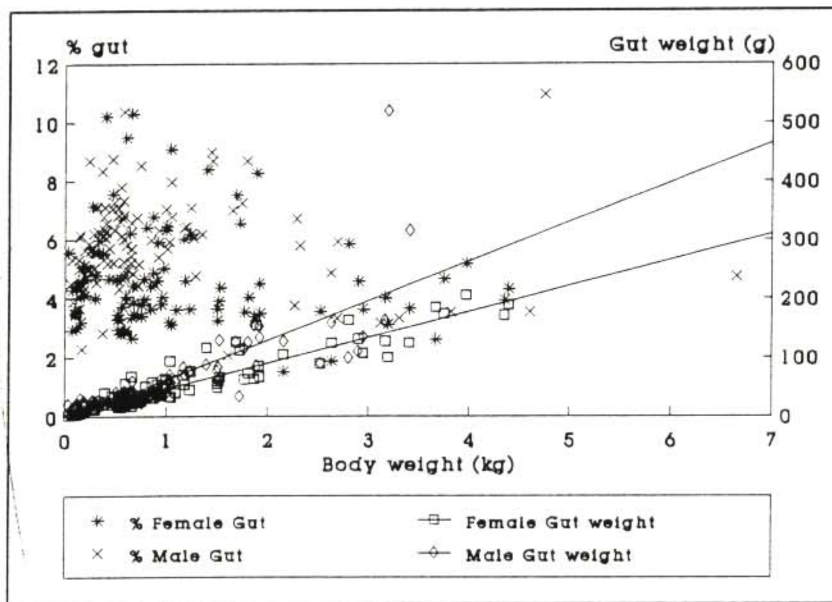



**Figure 11:** The relationship between male and female *C. gariepinus* gut mass with increasing body weight.

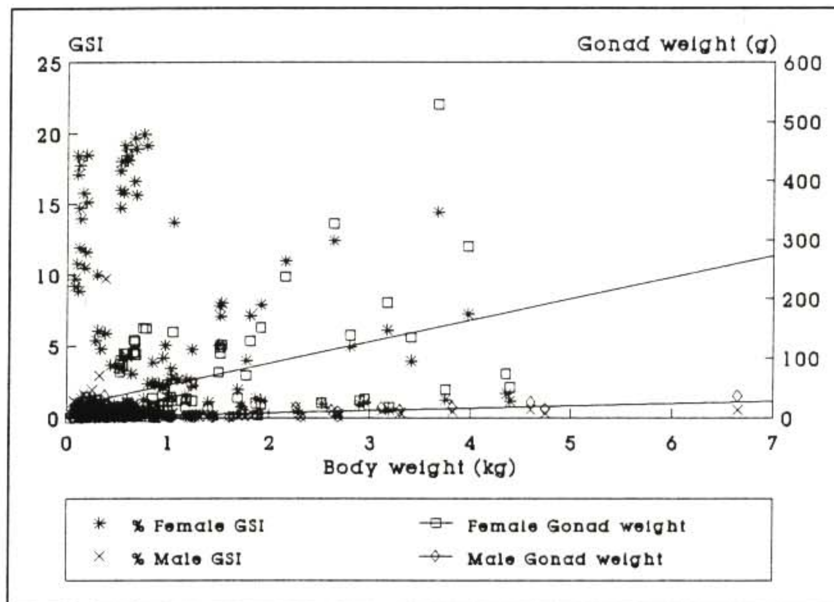



**Figure 12:** The relationship between male and female *C. gariepinus* gonad mass with increasing body weight.

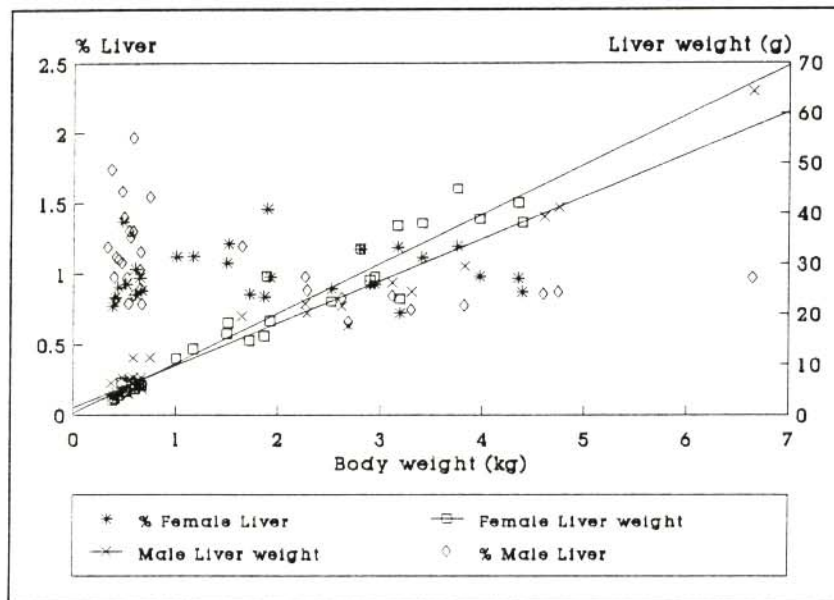



**Figure 13:** The relationship between male and female *C. gariepinus* liver mass with increasing body weight.

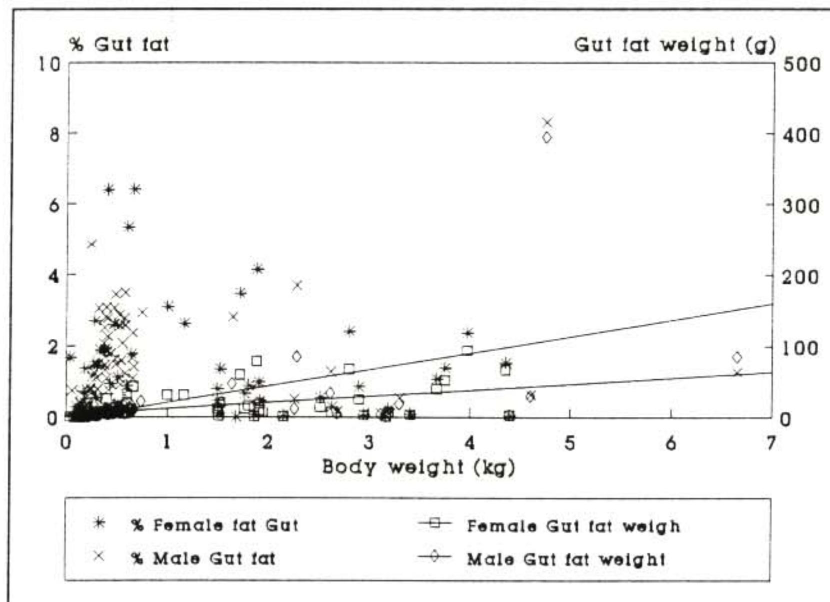



**Figure 14:** The relationship between male and female *C. gariepinus* mesenteric fat depot mass with increasing body weight.

*Chemical composition*

The relationships of the percentage and logarithmic mass of the various proximate composition parameters (moisture, protein = N x 6.25, total lipid and ash) with increasing body weight for the two sexes (data pooled), are summarised in Figs 15-22. The regression equations ($\log y = \alpha + \beta \log x$) for the prediction of the proximate composition on the transformed data are given in Table 9. The total mass of the chemical component was calculated by multiplying the analyzed mass (as a proportion) by the fillet mass. The fillet moisture content (when expressed as a logarithmic function) was influenced by the sex of the fish with increasing body weight (log), the females ($\beta = 1.0568$) gained moisture significantly ($p < 0.05$) faster than the males ($\beta = 1.0012$). Sex had no influence on the rate of increase ($p > 0.05$) of the other proximate chemical parameters with increasing body weight, although the intercepts may have differed significantly at the 5% level (Table 9).

The condition factor (total length/body weight x 100) is sometimes used as an indication of the nutritional condition of fish and as such it should rise in step with fat and protein (and a little carbohydrate) reserves. In Table 10, the Pearson's correlation coefficients between the condition factor and the proximate composition parameters (percentage) are given. The condition factor had the highest correlation with the percentage ash (0.4422). The correlation coefficients with percentages total lipid (0.3960) and moisture (-0.3786) is somewhat lower, whilst that with percentage protein is negligible (-0.0055). Percentages moisture and lipid had a high correlation (-0.9091), as the moisture in the fillet increases, the total lipid content decreases (Fig 23).

**Table 9:** Log-log linear regression equations for female and male African catfish *C. gariepinus*, from various sources for the prediction of fillet proximate chemical composition.

| Chemical parameter | Pooled | | | Female | | | Male | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\log\alpha$ | $\log\beta$ | $R^2$ | $\log\alpha$ | $\log\beta$ | $R^2$ | $\log\alpha$ | $\log\beta$ | $R^2$ |
| Moisture | -0.5397 | 1.0260 | 0.9919 | -0.6477* | 1.0568* | 0.9948 | -0.4532* | 1.0012* | 0.9938 |
| Protein | -1.1014 | 1.0052 | 0.9814 | -1.1821* | 1.0267 | 0.9847 | -1.0432* | 0.9903 | 0.9817 |
| Total lipid | -3.5780 | 1.5672 | 0.8240 | -3.6898 | 1.6122 | 0.8609 | -3.4307 | 1.5085 | 0.7785 |
| Ash | -2.4496 | 1.1476 | 0.9280 | -2.6285* | 1.2056 | 0.9425 | -2.2755* | 1.0889 | 0.9145 |

$\alpha$ and $\beta$ (between sexes) indicated with * differ significantly at the 5% level.

**Table 10:** Pearson's correlation coefficients between the condition factor and proximate chemical parameters (%) for African catfish *C. gariepinus*.

| **Parameters** | **Condition factor** | **Moisture (%)** | **Protein (%)** | **Total Lipid (%)** |
|---|---|---|---|---|
| **Moisture (%)** | -0.3786 | | | |
| **Protein (%)** | -0.0055 | 0.0057 | | |
| **Total lipid (%)** | 0.3960 | -0.9091 | -0.3031 | |
| **Ash (%)** | 0.4422 | -0.6290 | 0.0592 | 0.6108 |

*Comparison of fecundity between normal and golden coloured strains*

Results on fish and gonad mass, total number of eggs and GSI of 10 randomly selected fish of both strains are summarized in Table 11. Calculations of GSI for the various mass groups, ranging from less than 499 g to more than 900 g, are listed in Table 11. Comparative diagrams illustrating the latter results, are reflected in Figure 24.

Table 11 shows that live mass, gonadal mass, GSI, total number of eggs, and total number of eggs per unit live mass differed significantly between the females of the two strains. The normal-coloured strain showed a coefficient of variation (*CV)* twice as large as that shown by the gold-coloured strain for body mass (Table 11). The gold strain showed a larger *CV* for gonad mass and total number of eggs. However, the mean number of eggs per unit gonad mass did not differ significantly.

A comparison of results obtained for GSI for the two strains (Table 12) showed a statistically significant difference in the parameter between the two strains for the 500-699 and 700-899 g mass groups. No significant test for the live mass groups of both strains above 900 g were performed because of the absence of gold females in this mass group. The GSI values for the different mass groups showed a high *CV* in both strains. However, the normal-coloured strains showed the highest *CV* for GSI. From Figure 24 the following deductions could be made. In the smaller sized fish, (Fig 24A), the GSI of the gold strain was initially higher. Both strains showed an increase in GSI with increasing live mass, the tempo of increase in GSI being larger in the normal strain.

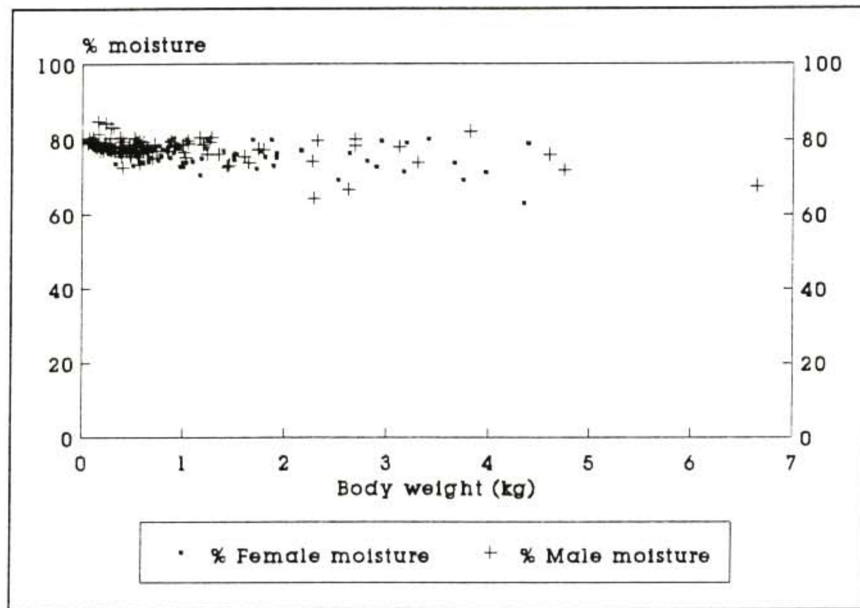



**Figure 15:** The relationship between the percentage moisture of male and female *C. gariepinus* with increasing body weight.

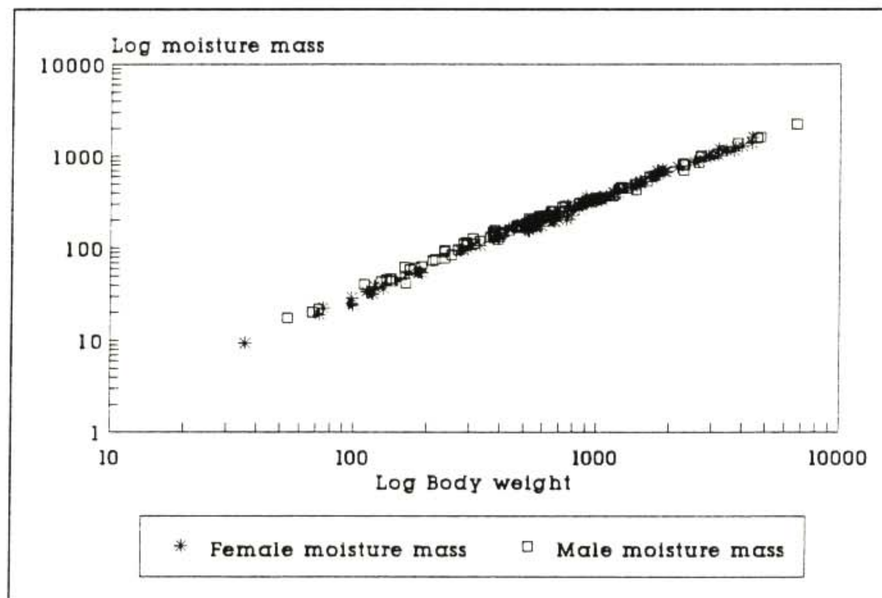



**Figure 16:** The relationship between the log of the moisture mass of male and female *C. gariepinus* with increasing log body weight.

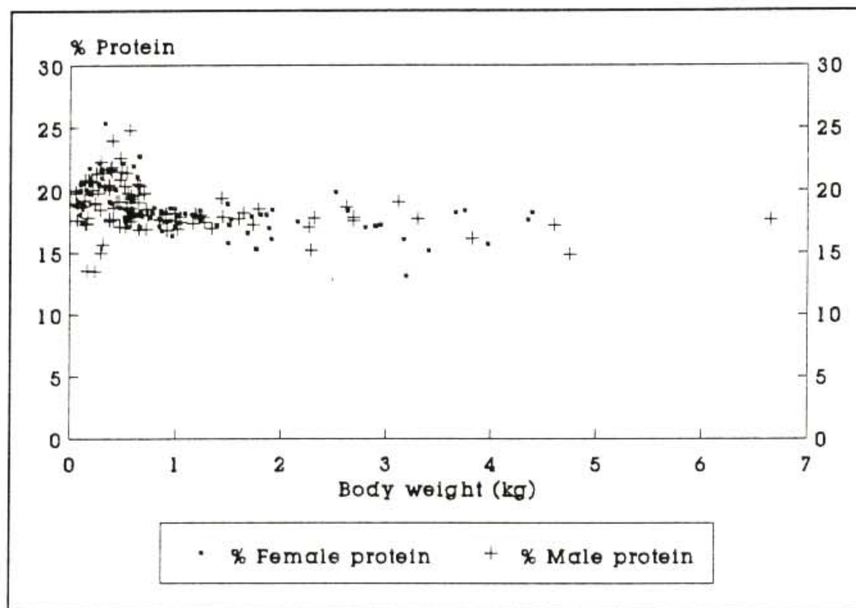



**Figure 17:** The relationship between the percentage protein of male and female *C. gariepinus* with increasing body weight.

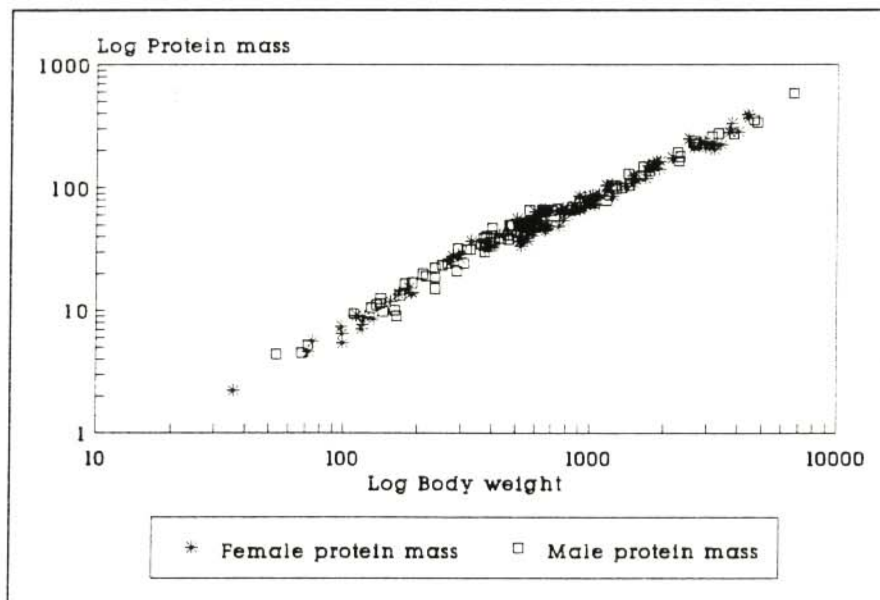



**Figure 18:** The relationship between the log of the protein mass of male and female *C. gariepinus* with increasing log body weight.

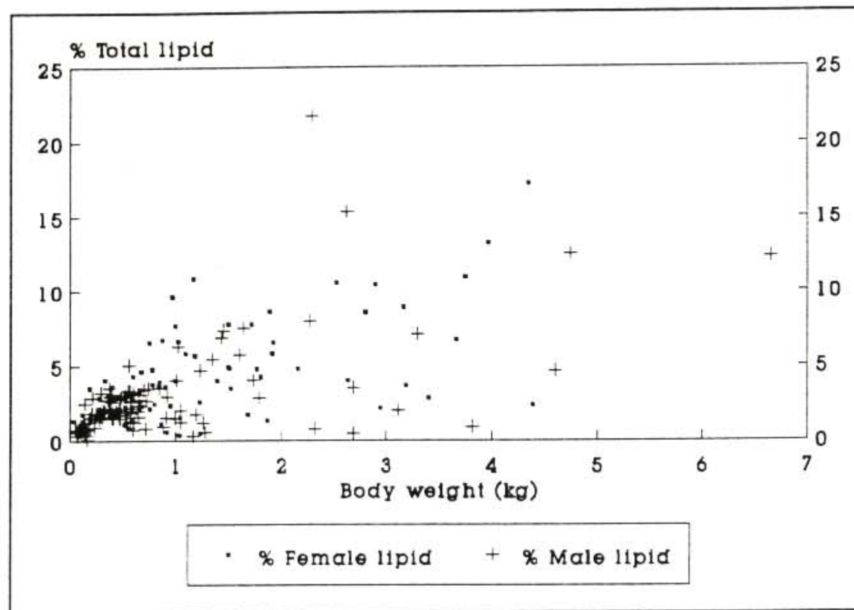



**Figure 19:** The relationship between the percentage total lipid of male and female *C. gariepinus* with increasing body weight.

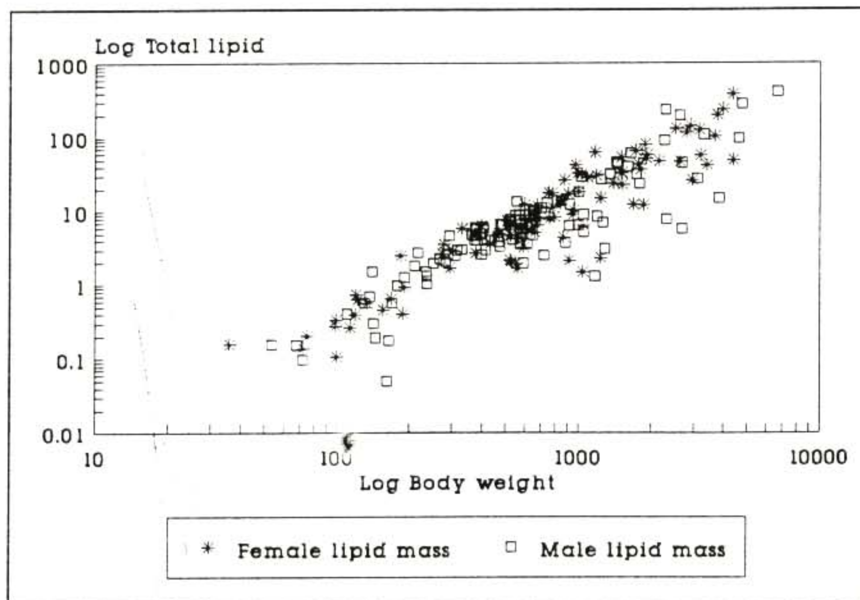



**Figure 20:** The relationship between the log of the total lipid mass of male and female *C. gariepinus* with increasing log body weight.

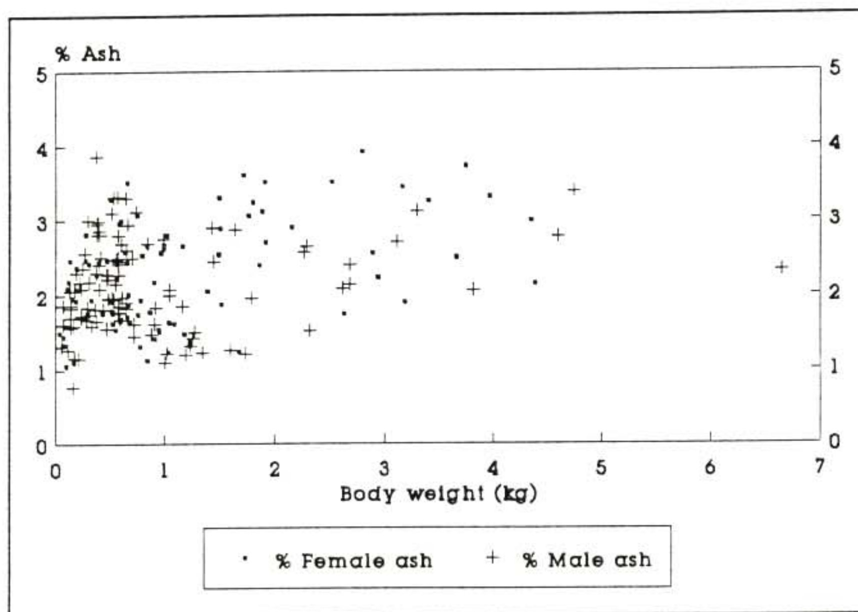



**Figure 21:** The relationship between the percentage ash of male and female *C. gariepinus* with increasing body weight.

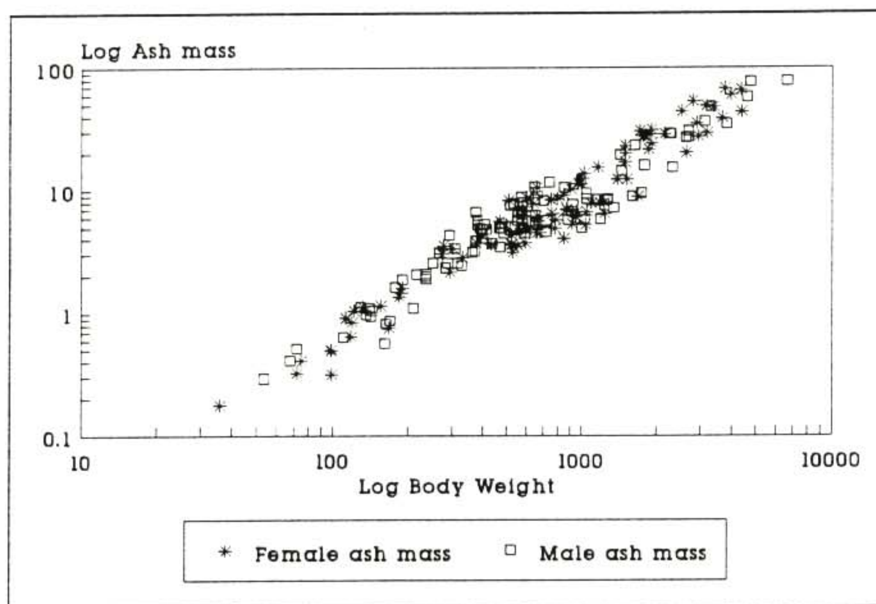



**Figure 22:** The relationship between the log of the ash mass of male and female *C. gariepinus* with increasing log body weight.

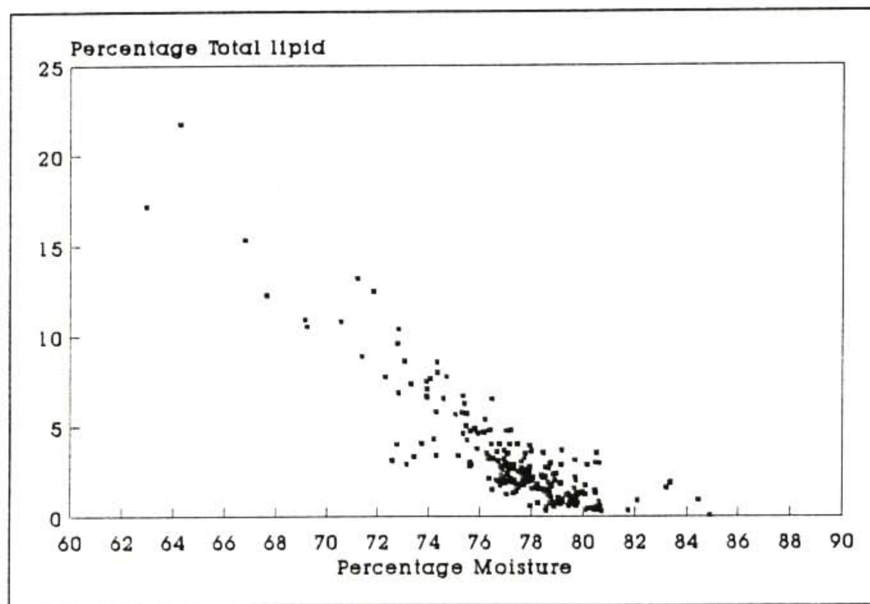
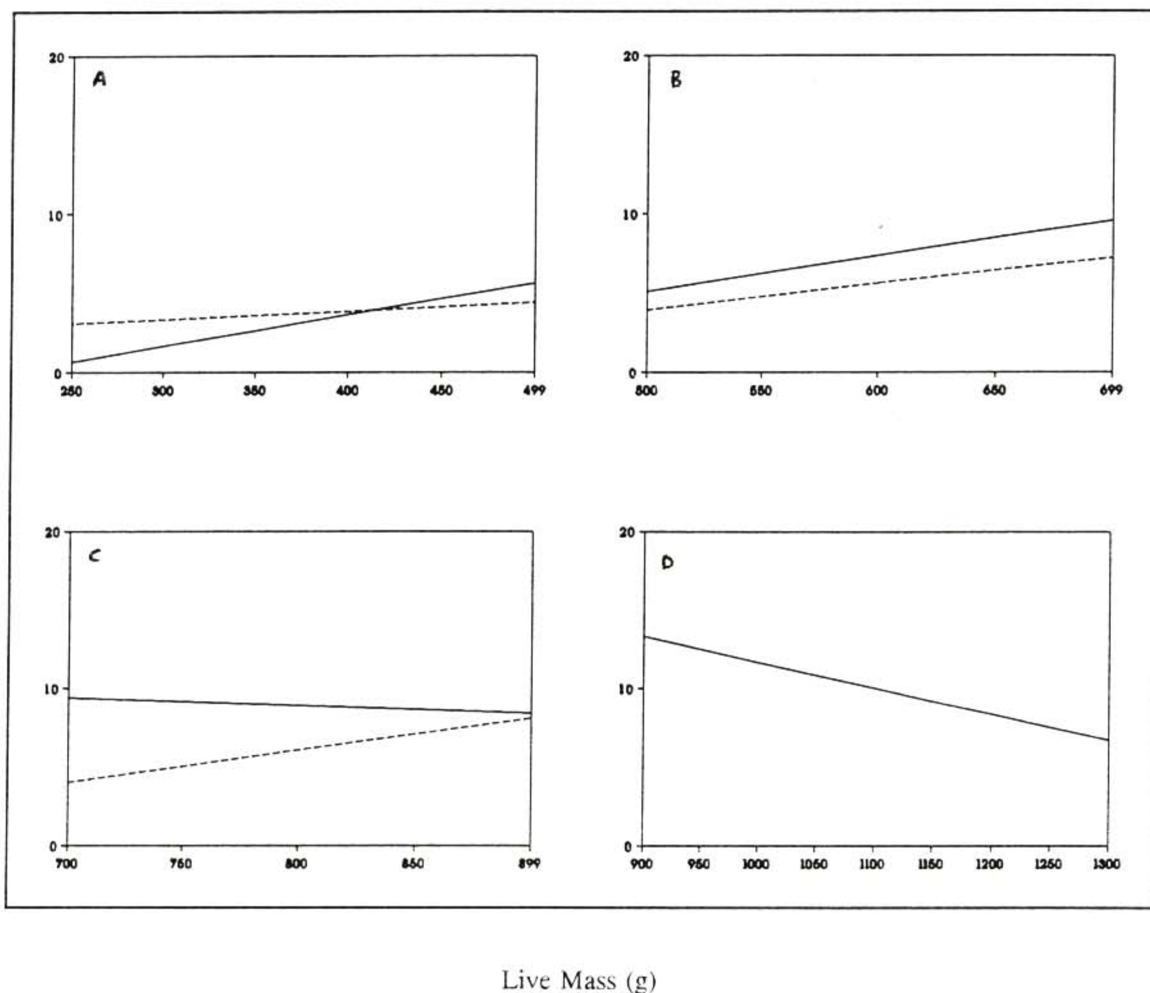



**Figure 23:** The relationship between fillet percentage moisture and percentage total lipid for *C. gariepinus*.




Live Mass (g)

**Figure 24:** A comparison of the GSI values between females of different mass classes of the gold (------) and normal (——) strains of *C. gariepinus* (A=250-499 g; B=500-699 g; C=700-899 g; D=900-1300 g) in autumn, towards the end of the spawning cycle.

**Table 11:** Mean values for body mass, gonad mass, total number of eggs and GSI of the normal and gold-coloured strains of *C. gariepinus* based on the analysis of 10 randomly selected one-year-old specimens of each.

| | Mean | Minimum value | Maximum value | Standard deviation | Coefficient of variation |
|---|---|---|---|---|---|
| **Normal-coloured strain** | | | | | |
| Body Mass (BM) (g) | 669.6* | 442.0 | 925.0 | 164.25 | 24.5 |
| Gonad Mass (GM) (g) | 52.4* | 30.6 | 80.2 | 17.15 | 32.7 |
| Total number of eggs | 47940.0* | 25070.0 | 77245.0 | 13592.00 | 28.4 |
| GSI | 7.8* | 5.2 | 10.3 | 1.63 | 20.9 |
| Number of eggs per BM | 73.9* | 45.7 | 105.7 | 21.60 | 29.2 |
| Number of eggs per GM | 953.9 | 574.1 | 1235.9 | 230.02 | 24.1 |
| **Gold-coloured strain** | | | | | |
| Body Mass (BM) (g) | 539.2* | 433.0 | 656.0 | 68.91 | 12.8 |
| Gonad mass (GM) (g) | 21.5* | 5.1 | 43.8 | 13.60 | 63.3 |
| Total number of eggs | 18793.5* | 4305.0 | 38326.0 | 12049.50 | 64.1 |
| GSI | 3.9* | 1.2 | 8.4 | 2.43 | 62.0 |
| Number of eggs per BM | 34.7* | 7.0 | 73.4 | 22.11 | 63.8 |
| Number of eggs per GM | 884.1 | 597.9 | 1128.4 | 156.36 | 17.7 |

* Differences statistically different at the 95% level

At a mass of approximately 450 g and heavier, the values for GSI of the normal strain have already overtaken those of the gold strain so that in fish heavier than 500 g (Fig 24B), the GSI values of the normal strain were consistently better than those of the gold strain. In fish larger than 700 g (Fig 24C), the gold strain showed a progressive improvement in tempo of change in GSI within size in contrast to the normal strain, of which the GSI gradually declined to reach an almost similar status to that of the gold strain at about 900 g. The normal strain (Fig 24D) showed a decrease in the tempo of change in GSI for fish heavier than 900 g.

**Table 12:** GSI of different mass groups of both the gold and normal-coloured strains of one-year-old sharptooth catfish *C. gariepinus*.

| Strain | Mass group | Parameter | *n* | Mean | Minimum | Maximum | Coefficient of variation |
|---|---|---|---|---|---|---|---|
| Normal-coloured | <499 | Live Mass | 41 | 446 | 298 | 497 | 12.1 |
| | | GSI | | 4.567 | 0.239 | 20.839 | 114.8 |
| | 500-699 | Live Mass | 95 | 586 | 500 | 695 | 9.9 |
| | | GSI | | 7.045* | 0.276 | 17.479 | 73.3 |
| | 700-899 | Live Mass | 43 | 787 | 700 | 886 | 7.3 |
| | | GSI | | 8.982* | 0.370 | 16.737 | 51.4 |
| | >900 | Live Mass | 14 | 1 068 | 912 | 1 349 | 14.4 |
| | | GSI | | 10.564 | 0.804 | 17.453 | 39.8 |
| Gold-coloured | <499 | Live Mass | 83 | 438 | 266 | 499 | 9.9 |
| | | GSI | | 4.342 | 0.313 | 11.476 | 76.2 |
| | 500-699 | Live Mass | 64 | 570 | 501 | 698 | 9.9 |
| | | GSI | | 5.124* | 0.137 | 13.157 | 66.3 |
| | 700-899 | Live Mass | 8 | 774 | 726 | 840 | 5.5 |
| | | GSI | | 5.536* | 0.904 | 10.455 | 60.6 |
| | >900 | Live Mass | 1 | 955 | | | |
| | | GSI | | 3.757 | | | |

* Differences statistically different at the 95% level

*Chemical comparison of gonads*

Table 13 shows the mean proximate composition of the muscle and gonads of the fish analyzed. The muscle contained a significantly higher ($p>0.0001$) percentage moisture than the gonads, whilst the latter had correspondingly significantly higher percentages protein ($p=0.0006$) and ash ($p=0.0008$). Although the gonads had a higher mean percentage total lipid (6.97%), it did not differ significantly ($p=0.2255$) from that of the muscle (5.91%).

The fatty acids (expressed as a percentage of total fatty acids present) of the lipids extracted from the muscle and gonads are shown in Table 14. Of the 13 fatty acids identified in the muscle and gonads, only palmitic (C16:0), stearic (C18:0), gondoic (C20:1ω9) and cetolic (C22:1ω11) acids did not differ significantly between the muscle and gonads. Of importance is the statistically significantly higher concentrations of docosapentaenoic acid (C22:5ω3) and docosahexaenoic acid (C22:6ω3) in the gonads (Table 14). The muscle had 36.52% saturated, 37.44% monounsaturated and 18.45% polyunsaturated fatty acids compared to 35.7; 31.33 and 20.97% respectively, for the gonads.

The amino acid profiles of the muscle and gonads are shown in Table 15. All the amino acids tested for, with the exception of histidine, phenylalanine and lysine, differed significantly between the muscle and gonads. Similarly, all the minerals tested for (Table 16), with the exception of phosphorus, magnesium and copper, differed significantly between the muscle and gonads.

*Mesenteric lipid composition*

The fish used for this investigation had a dressout percentage [(fillet mass ÷ body mass) x 100] of 46.8 ± 2.6% and a mesenteric fat depot of 0.9 ± 0.3% of the total body mass. This lipid depot had a mean weight of 14.8 ± 5.0g.

The fatty acid profile (expressed as a percentage of total fatty acids identified) of the lipids of the fillets, mesenteric fat depot and the lipids of the commercial feed are given in Table 17. It is clear that there is no statistical significant difference ($p\leq0.01$) between the fatty acid profiles of the fish fillets and the mesenteric fat depots, with the exception of C20:5ω3 and C20:0, the latter not being present in the mesenteric fat depot. The fatty acid profiles of the lipids of the fish fillets, mesenteric depot and the diets are also similar.

**Table 13:** A statistical comparison of the mean proximate composition of the muscle and gonads of *C. gariepinus* females (n=5).

| | Muscle | | Gonads | | Paired Comparison | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SE | Prob> \| T \| |
| **Moisture** | 75.74 | 0.9039 | 63.40 | 1.5143 | 12.34 | 0.7015 | 0.0001 |
| **Protein** | 17.43 | 1.1030 | 26.12 | 1.8164 | -8.69 | 0.8858 | 0.0006 |
| **Lipid** | 5.91 | 1.3865 | 6.97 | 0.8006 | -1.06 | 0.6967 | 0.2255 |
| **Ash** | 3.00 | 0.6456 | 6.13 | 0.3208 | -3.13 | 0.7594 | 0.0008 |

**Table 14:** A statistical comparison of the mean (n=5) fatty acid profiles of the lipids from the muscle and gonads of *C. gariepinus* females (fatty acids identified expressed as a percentage of total fatty acids).

| Fatty Acid | Muscle | | Gonads | | Paired Comparison | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SE | Prob> \| T \| |
| **C14:0** | 1.70 | 0.0988 | 1.05 | 0.2157 | 0.65 | 0.0923 | 0.0021 |
| **C16:0** | 26.25 | 2.1312 | 25.89 | 1.0210 | 0.36 | 0.9327 | 0.7250 |
| **C16:1ω7** | 6.14 | 0.7869 | 4.08 | 0.5192 | 2.06 | 0.3931 | 0.0063 |
| **C18:0** | 8.23 | 0.6476 | 8.68 | 0.1954 | -0.45 | 0.3099 | 0.2218 |
| **C18:1ω9** | 29.56 | 1.2303 | 25.85 | 2.2870 | 3.71 | 0.6382 | 0.0044 |
| **C18:2ω6** | 13.43 | 1.2301 | 7.21 | 1.8558 | 6.22 | 1.3429 | 0.0098 |
| **C20:0** | 0.27 | 0.1512 | 0.08 | 0.0634 | 0.19 | 0.0668 | 0.0466 |
| **C20:1ω9** | 1.31 | 0.3043 | 1.37 | 0.3006 | -0.06 | 0.1225 | 0.6395 |
| **C20:5ω3** | 1.15 | 0.3029 | 1.74 | 0.2303 | -0.59 | 0.1385 | 0.0128 |
| **C22:0** | 0.07 | 0.0274 | nd | | | | |
| **C22:1ω11** | 0.43 | 0.6150 | 0.03 | 0.0626 | 0.40 | 0.2821 | 0.2234 |
| **C22:5ω3** | 0.85 | 0.2086 | 1.21 | 0.1346 | -0.36 | 0.0711 | 0.0068 |
| **C22:6ω3** | 3.02 | 0.7647 | 10.81 | 2.5587 | -7.79 | 1.0351 | 0.0017 |

**Table 15:** A statistical comparison between the mean (n=5) amino acid profiles of the muscle and mature gonads of *C. gariepinus* females (amino acids expressed as g/100g lipid free dry mass).

| Amino Acid | Muscle | | Gonads | | Paired Comparison | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SE | Prob > \| T \| |
| Aspartic acid | 8.65 | 0.1283 | 6.64 | 0.1557 | 2.01 | 0.0836 | 0.0001 |
| Glutamic acid | 13.76 | 0.1283 | 9.52 | 0.1982 | 4.24 | 0.1180 | 0.0001 |
| Serine | 4.00 | 0.1228 | 5.44 | 0.1350 | -1.44 | 0.0894 | 0.0001 |
| Glycine | 4.05 | 0.1227 | 2.77 | 0.2203 | 1.28 | 0.0827 | 0.0001 |
| Histidine | 1.99 | 0.0594 | 2.07 | 0.1137 | -0.08 | 0.0572 | 0.2344 |
| Arginine | 5.45 | 0.0597 | 5.15 | 0.1686 | 0.30 | 0.0604 | 0.0082 |
| Threonine | 4.42 | 0.0856 | 3.80 | 0.1901 | 0.62 | 0.1166 | 0.0061 |
| Alanine | 5.24 | 0.4480 | 5.83 | 0.1236 | -0.59 | 0.2424 | 0.0717 |
| Proline | 3.04 | 0.0841 | 3.79 | 0.1236 | -0.75 | 0.0404 | 0.0001 |
| Tyrosine | 3.20 | 0.1076 | 2.78 | 0.0422 | 0.42 | 0.0487 | 0.0011 |
| Valine | 3.92 | 0.1089 | 4.88 | 0.2056 | -0.96 | 0.1329 | 0.0020 |
| Methionine | 2.65 | 0.1358 | 2.31 | 0.0611 | 0.34 | 0.0695 | 0.0075 |
| Isoleucine | 3.57 | 0.1842 | 4.85 | 0.2528 | -1.28 | 0.1853 | 0.0023 |
| Leucine | 6.54 | 0.3794 | 7.70 | 0.2709 | -1.16 | 0.2193 | 0.0061 |
| Phenylalanine | 3.23 | 0.2928 | 3.47 | 0.1053 | -0.24 | 0.1469 | 0.1894 |
| Lysine | 6.50 | 0.8398 | 6.82 | 0.4436 | -0.32 | 0.4504 | 0.5117 |
| Hydroxyproline | 0.23 | 0.0415 | 0.08 | 0.0390 | 0.15 | 0.0217 | 0.0023 |

**Table 16:** A statistical comparison of the mean (n=5) mineral profiles of the muscle and gonads of *C. gariepinus* females (DM).

| Mineral | Muscle | | Gonads | | Paired Comparison | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SE | Prob> \| T \| |
| P % | 0.99 | 0.0404 | 1.01 | 0.0277 | -0.02 | 0.0215 | 0.4504 |
| Ca % | 0.10 | 0.0195 | 0.23 | 0.0876 | -0.13 | 0.0429 | 0.0388 |
| K % | 1.48 | 0.2433 | 0.84 | 0.3638 | 0.64 | 0.2369 | 0.0535 |
| Mg % | 0.17 | 0.0212 | 0.20 | 0.0719 | -0.03 | 0.0379 | 0.4457 |
| Fe ppm | 34.52 | 4.2267 | 180.20 | 84.4327 | -145.68 | 36.6828 | 0.0165 |
| Cu ppm | 3.49 | 1.3994 | 4.15 | 2.5383 | -0.66 | 1.5939 | 0.7009 |
| Zn ppm | 24.13 | 1.6247 | 58.09 | 21.4993 | -33.96 | 10.0902 | 0.0282 |
| Mn ppm | 1.59 | 0.8811 | 9.90 | 6.2354 | -8.31 | 2.4973 | 0.0291 |

In this investigation, a high oleic acid (C18:1ω9) concentration was noted in both the fillets and mesenteric fat depot (29.6 and 29.1%, respectively), however, the concentration of linoleic acid (C18:2ω6), whilst relatively high in the fish (13.4 and 14.7%, respectively), was lower than that found in the diet (26.9%). Linolenic acid (C18:3ω3) was not found in the commercial diet, and only trace concentrations were found in the fish. Eicosapentaenoic acid (C20:5ω3) followed a similar trend as linoleic acid, being higher in the lipids of the diet (5.2%) than in the lipids of the fillet (1.2%) or fat depot (1.7%). Trace concentrations of docosapentaenoic acid (C22:5ω3) were found in the diet and the fish, whilst the diet, fillet and fat depot had similar concentrations of docosahexaenoic acid (C22:6ω3; 3.1, 3.0 and 2.4%, respectively).

The lipids of the fillet and mesenteric fat depot had 36.6 and 36.8% saturated, 37.6 and 36.9% mono-unsaturated and 19.6 and 20.6% polyunsaturated fatty acids respectively.

**Table 17:** The fatty acid profiles of the lipids of the diet, fillet and mesenteric fat depot of *C. gariepinus* (fatty acids expressed as a percentage of total fatty acids identified, SD in parenthesis).

| Fatty acid | Diet | Fillet | Fat depot |
|---|---|---|---|
| C14:0 | 3.1 | 1.7 (0.099) | 1.8 |
| C16:0 | 25.9 | 26.3 (2.131) | 27.0 (1.796) |
| C16:1ω7 | 34.0 | 6.1 (0.787) | 5.9 (0.872) |
| C18:0 | 6.7 | 8.2 (0.648) | 7.8 (0.982) |
| C18:1ω9 | 26.1 | 29.6 (1.230) | 29.1 (3.117) |
| C18:2ω6 | 26.9 | 13.4 (1.230) | 29.1 (3.117) |
| C18:3ω3 | nd | 0.1 (0.672) | 1.0 (0.101) |
| C20:0 | nd | 0.3 (0.151) | nd |
| C20:1ω9 | 1.0 | 1.3 (0.304) | 1.5 (0.265) |
| C20:5ω3 | 5.2 | 1.2* (0.303) | 1.7* (0.126) |
| C22:0 | 0.5 | 0.1 (0.027) | 0.2 (0.217) |
| C22:1ω11 | 1.3 | 0.4 (0.615) | 0.4 (0.100) |
| C22:5ω3 | 0.6 | 0.9 (0.209) | 0.8 (0.111) |
| C22:6ω3 | 3.1 | 3.0 (0.765) | 2.4 (0.392) |

nd = not detected
* significantly different ($p \leq 0.01$)

## DISCUSSION

The gold and normal strains of the catfish, *Clarias gariepinus*, were spawned together and were therefore of the same age at slaughter. Hence a comparison of their means is justifiable, but this comparison should only be used as an indication of potential trends, as the young juveniles were overwintered in different ponds and at different densities, which led to a difference in mean live mass of the gold (81.5 g) and normal (121.7 g) strains at the start of the experimental phase (Prinsloo *et al.*, 1989). The difference in the growth and development of the two strains, as shown in the final mean live mass (Table 3) could, therefore have been influenced to a certain extent by this difference in initial mean live mass.

An interesting trend, shown in Figure 5 is the difference in the gradient of the regression equations for percentage drawn mass (%DGM) between the sexes; the females show a sharper decline in %DGM compared to that of the males (Table 5). This should only be seen as a trend as the regression equations gave poor fits (Table 5) and would most probably have given better results if the data had been transformed. The drawn mass consists of the live mass (LM) of the fish minus the mass of the viscera. Gonads are included in this classification of viscera. Prinsloo *et al.* (1990) have shown that with an increase in live mass, there is an identical increase in female gonadal development. It has been shown that the mass of the gonads of the female increases at a faster rate than that of the males. Making use of the same fish as those in this study, they (*idem*) found a statistical ($\alpha = 0.05$) difference in Gonadal Somatic Index (GSI) between the males and females as well as between females of the gold and normal strains. It therefore appears that the difference in percentage drawn mass (%DGM) between the sexes is largely due to a difference in gonadal mass, whilst the difference in %DGM between the gold and normal females is most probably due to a difference in tempo of development of gonads.

It is expected that this trend of females showing a sharper decline in percentage drawn mass compared to the males could be transposed to the percentage dressed mass (%DM), as this entails the additional decapitation of the drawn fish carcass. The females indeed show a decrease in %DM with increasing LM (Fig 6), the rate of decrease being virtually the same for both the gold and normal strains, namely 4%. The only noticeable difference seems to be at the intercept, where the gold females have a 3% higher percentage dressed mass than the normal females. This phenomenon could mainly be ascribed to the difference in GSI between the two strains. Surprisingly, the males show a slight increase in %DM with increasing LM. This seems to indicate that for a unit increase in live mass, the proportional development of the carcass composition differs between the males and females, with more mass going towards 'meat' production in the male. The main factor contributing to the difference in drawn mass (DGM) and dressed mass (DM) is the removal of the head. The negative tempo of change in carcass dressout per unit increase in live mass (LM) decreases between percentage drawn mass (%DGM) and percentage dressed mass (%DM) (Table 5).

It would, therefore, appear that a larger proportion of the mass increase per unit live mass increase goes towards an increase in dressed mass and less towards head mass, as the fish develops. This could indicate that the head is an earlier developing organ in the overall carcass composition of the catfish. This trend will, however, have to be quantified as it could have an important influence on determining when the fish is at an economical marketable weight.

In the case of the American channel catfish, *Ictalurus punctatus*, it is reported that at an age of 13 months the male catfishes were 43% heavier (600 g) than the females (420 g). The percentage head mass was 6% more in males whilst the percentage viscera was about 7% more in females. As the sums of the waste products in both were nearly equal, the dressing percentages in these young fish were very close between the two sexes (males 64.2%, females 64.5%). In 22 month old fish with a similar mean body mass (males 580 g, females 424 g) to the 13-month old fish, the dressing percentages of the males was slightly higher (68.5%) than that of the females (67.2%), although the males were 37% heavier than the females. The percentage viscera mass (males 10.0%, females 10.8%) as well as the percentage head mass (males 21.6%, females 22.3%) remained nearly the same between the sexes (Dunham *et al.*, 1985).

On average, the catfish used in this study seem to have a higher dress-out percentage (Table 3) compared to the 55-60% reported by Smith and De Beer (1988). A possible explanation could lie in a difference in slaughter procedures. From the results of this study it would seem that as a meat producer (%DM), the male sharptooth catfish is superior to the female. If the male also shows a superior daily growth gain and feed conversion ratio, monosexing could be recommended.

The body weight range of the catfish sampled in the investigation on the fillet yield (Table 1), adequately covers that utilised commercially by various fish farmers. The mean percentage fillet of the African sharptooth catfish, *C. gariepinus* in the present investigation lies around 42.9% for the females and 45.9% for the males. However, the percentage fillet (yield) of the catfish with increasing body weight, is not strongly influenced by the sex of the fish (Tables 6 & 7, Fig 7). It has been found that the percentage of edible portion in food-size channel catfish (*I. punctatus*) is also similar for each sex because males typically have larger heads, but females have a larger percentage of viscera (Dunham *et al.*, 1985; Simco *et al.*, 1989). For example, during a comparison of male and female channel catfish, Robinson and Robinette (1993) found that the females in both first (600 g BWt) and second year (1200 g BWt) groups, had a heavier mesenteric lipid depot, but similar yields (within the age group) to that of the males. *C. gariepinus* differs from *I. punctatus* in that there is no difference in the head weight between sexes (Fig 8), but rather that males have a heavier bone (Fig 9) and total gut weight (Fig 11). The latter is caused by the heavier mesenteric lipid depot displayed by the male catfish (Fig 14). A possible explanation for this phenomenon lies in the fact that most of the fish were investigated during the summer, the

period when *C. gariepinus* breeds and the females all have well developed gonads (Prinsloo *et al.*, 1990) which may result in a lack of space within the abdomen for any lipid depots. The females may also have utilised some of the mesenteric lipid for gonad development (Love, 1988).

Fish strain has been found to influence body traits, including body conformation and yield in channel catfish *I. punctatus* (Heaton, Boggess & Worthington, 1973; Dunham & Smitherman, 1983), rainbow trout *Salmo gairdneri* (Smith, Kincaid, Regenstein & Rumsey, 1988; Gjerde, 1989) and Atlantic salmon *Salmo salar* (Gjerde & Gjedrem, 1984). The influence of strain on the yield and body conformation of the African catfish *C. gariepinus* needs to be investigated in more depth.

From the data of Clement and Lovell (1994), it would seem as if *I. punctatus* has a lower fillet yield than *C. gariepinus*. In the mentioned study, pond raised channel catfish (610 g BWt) had a fillet yield of 30.2%, compared to 47.0% for female and 47.7% for male African sharptooth catfish, of a similar body weight (coded **D**, Table 1). The African catfish also has a higher percentage fillet yield than bluefish *Pomatomus saltatrix* (37.5%), croaker *Micropogon undulatus* (22.2%), flounder *Pseudopleuronectes americanus* (40.0%), sea bass *Centropriates striatus* (26.9%), grey sea trout *Cynoscion nobilis* (35.1%), spot *Leiostomus xanthurus* (32.8%) and tilapia *O. niloticus* (25.4% - Anthony *et al.*, 1983; Clement & Lovell, 1994). The fillet yield of cultured white surgeon *Acipenser transmontanus* (570-3360 g BWt) is very similar to that of *C. gariepinus* and varies between 32-50% (Price, Hung, Conte & Strange, 1989).

The linear equations for the prediction of the fillet yield from live mass (Table 6) will enable producers to predict the expected yield of a pond by weighing a sub-sample of fish from that pond. The producer will then be able to plan his marketing and processing strategies from this data without having to go to the expense of having to slaughter fish so as to obtain the same information. Another important facet of being able to predict the fillet yield of the fish without having to slaughter the fish is in broodstock selection. Selecting of broodstock can be done on the fish themselves and not on siblings, thus enabling the producer to make faster progress in the trait selected.

Degani (1988) studied the body composition of smaller *C. gariepinus* (30 to 200 g) and found a decrease in percentage moisture and an increase in percentage protein with increasing body weight. The percentages of relative fat and ash were constant. In his review, Shearer (1994) notes that in young fish the percentage of whole body protein increases for a period as a result of both recruitment of new muscle cells and an increase in the diameter of existing cells. Thereafter, the fish reach what is known as the point of chemical maturity, and the percentage protein becomes relatively constant. From the results of this investigation (Fig 17), the constant percentage protein would indicate that *C. gariepinus* had already reached the point of chemical

maturity. The large variation in the total lipid content (Fig 19) of the catfish muscle, can be directly attributed to dietary influences (Love, 1970, 1980, 1988; Reinitz, 1983; Henken, Boon, Cattel & Lobée, 1987; Li & Lovell, 1992; Nettleton & Exler, 1992; Sheridan, 1994; Shearer, Åsgård, Andorsdöttir & Aas, 1994)

The chemical composition data was tested by transforming the data to a log function and comparing it with the log body weight (Table 9). Over the live mass range tested, the body moisture content showed a slight increase ($\beta = 1.0260$) for the pooled sample. The increase in body protein content with increasing live weight ($\beta = 1.0052$) was less than that of the moisture content. The body lipid accretion is highly positively curvilinear, ie, fat accretion is at 1.5672 times the rate of live weight increment. Although the $R^2$ value was not as high as that of protein and water, 0.82 still indicates a good fit as it accounts for 82% of the variation in lipid, a chemical component that shows a high variation in animals because of its metabolic nature.

Shearer (1994) on re-analysing data from various sources allometrically (for example the results of Reinitz, 1983; on rainbow trout), found that size and not diet or strain/type influenced the actual chemical composition of the fish.

The high inverse relationship between the moisture and total lipid contents ($r=0.9091$) of the fillets, is similar to that for other species such as rainbow trout ($r=0.877$ - Reinitz, 1983), striped bass and hybrid striped bass (Brown & Murphy, 1991). The low correlation of condition factor with the actual chemical composition of the muscle of *C. gariepinus* (Table 10) implies that determining this ratio as an indication of the chemical composition of the fish, will only be of limited value to producers.

The regression equations from Table 9 will enable producers and processors to determine the potential chemical composition of fish at relatively low costs. As noted by Shearer (1994), accurate weight-composition tables could save considerable time and expense over direct measurement of proximate composition. The results in this investigation is a beginning at the compilation of such tables.

Judging from the results obtained on the GSI of the two strains of *C. gariepinus*, the gold variety clearly appears to be inferior in egg production, particularly amongst the smaller sized females (Tables 11 & 12, Fig 24). However, a correction in this condition begins to occur as both fish grow larger so that at a mass of approximately 1 kg, the relative gonad mass and fecundity would be similar in the two strains. Based on the numbers of eggs produced, the lower fecundity (Table 11) of the gold strain need not be an obstacle in the large-scale spawning of this strain. It was noted during breeding experiments that a relatively large percentage of the ova of the normal-coloured females, but not of the gold strain, was already in a resorption phase in autumn. This phenomenon of resorption of eggs of *C. gariepinus* at the end of summer was observed and

described by Holl (1968); Cambray, Hahndiek and Hahndiek (1977); Gaigher (1977); Bruton (1979) and Clay (1979). The present results confirmed this tendency for the normal variety. The fact that the gold strain may be able to spawn more successfully in autumn, must be considered a superior characteristic which would enable the late summer production of this strain. The availability of suitably sized juveniles at the onset of spring will enable farmers to commence quite early with the growing out of catfish for marketing purposes. This could be applied particularly to the lowveld areas where these juveniles could be overwintered in outside nursery ponds.

The proximate composition of the muscle (Table 13) of the *C. gariepinus* utilised in the gonad composition investigation, differs from that of a previous study (Hoffman, Casey & Prinsloo, 1992), where the following fillet composition for wild females were noted: 78.4 ± 1.1% moisture, 18.3 ± 0.8% protein, 2.0 ± 0.6% lipid and 0.4 ± 0.1% ash. The females from the previous investigation were caught in the wild as they were moving towards their spawning grounds, (light pressure on the abdomen caused the eggs to extrude freely), and are therefore on a different physiological basis. Love (1988) notes that as fish gonads develop, the muscle chemical composition changes, with lipids and carbohydrates being mobilised before protein. The higher percentage lipid in the muscle from the present study may also be due to the nutritional status of the fish, as the fish from the present investigation were fed *ad lib* in the laboratory.

Care is needed in interpreting concentrations of chemical components made on a fresh weight basis, especially in the ovaries, as the latter take up a considerable volume of water just prior to spawning (Love 1970). This can result in an apparent decrease of some components. Results of various studies on the proximate composition of the gonads (Love 1970; Lu, Ma, Williams & Chung, 1979; Eliassen & Vahl 1982*a,b*) show an increase in the protein concentration as the ovaries develop. Simultaneously, a decrease in the mineral content (ash) is found, whilst the water concentration remains reasonably constant.

Comparison between the muscle and gonads on a dry mass basis, show no difference between protein concentrations (71.6 and 71.3%, respectively), but a higher lipid concentration in the muscle (23.7 vs 19.1%, respectively) and higher ash in the gonads (2.9 vs 6.2%, respectively).

The two fatty acids palmitic (16:0) and oleic (18:1) predominate in both the muscle and gonads (Table 14), the latter however, being significantly ($p > 0.0044$) lower (±4%) in the gonads compared to the muscle. In a study of the lipids of the edible portions of six New Zealand freshwater fish species, Vlieg and Body (1988) also found these two fatty acids to predominate. However, *C. gariepinus* has higher muscle concentrations of linoleic (18:2ω6) and lower concentrations of gondoic (20:1ω9) acids than the New Zealand freshwater fish species.

The fatty acid profile of the muscle is similar to that found in the previous study on the whole fillet of farmed *C. gariepinus* (Hoffman *et al.*, 1992), but differed from that of Chetty *et al.*, (1989). The latter found lower concentrations of linoleic and linolenic acids and higher concentrations of the polyunsaturated fatty acids, docosapentaenoic and docosahexaenoic acids. This difference may be attributed to diet (Stickney & Andrews 1971; Greene & Selivonchick 1989; Morishita, *et al.*, 1989), as the present study was conducted on cultivated fish, whilst that of Chetty and co-workers was done on wild fish, whose diet differs.

Love (1988) notes that in 14 marine fish species, the major ovarian fatty acid is usually palmitic or oleic. The composition of six New Zealand marine fish species' ova, support this observation (Vlieg & Body, 1988). The latter authors also recorded high concentrations of docosahexaenoic (C22:6ω3 - ±20%) acid, but in the present investigation, 10.8% docosahexaenoic acid was noted. Docosahexaenoic acid is deemed to be an essential fatty acid necessary for the successful hatching of eggs (Watanabe, 1982; Love, 1988) and the survival and growth of fish larvae (Koven, Kissil & Tandler, 1989; Lemm & Lemarie, 1991). Watanabe (1982) discussed the enhancing effect on fish growth of the highly unsaturated fatty acids, and also notes that warm water fish (such as *C. gariepinus*) need less essential fatty acids than cold water fish, probably due to the role that they play, as phospholipids, in osmo-regulation (Steffens, 1989). This may explain why *C. gariepinus* gonads have less highly unsaturated fatty acids than the species noted above.

The amino acid profile of the muscle in the present investigation (Table 15) is similar to that of the wild females in the earlier study (Hoffman *et al.*, 1992), with the exception of a lower lysine concentration in the present investigation (6.5 ± 0.8 vs 10.3 ± 1.3 g/100 g lipid free dry mass). The muscle of *C. gariepinus* has similar amino acid concentrations to that of the European common carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss* and the Atlantic salmon *Salmo salar* (Hepher, 1988) with the exception of histidine, isoleucine, phenylalanine and valine, which tend to be lower for the African sharptooth catfish.

The amino acid concentration of the gonads from the present investigation (Table 15), is similar to that of the American channel catfish, *Ictalurus punctatus,* with the exception of threonine, the latter having a higher concentration (6.0 vs 3.8 g/100 g lipid free dry mass - Hepher, 1988). Mullet roe, which is often regarded as a delicacy (Lu *et al.*, 1979), has a similar amino acid profile to that of *C. gariepinus* with the exception of higher concentrations of proline (10.39), isoleucine (4.95), leucine (7.95), phenylalanine (4.78) and lysine (9.85). These, with the exclusion of proline, are all essential amino acids.

In the comparison of the amino acid profiles between the muscle and gonads (Table 15), all the amino acids differed significantly in their mean concentration with the exception of histidine, phenylalanine and lysine. Love (1988) notes in general, that as gonads of fish mature and protein is transferred into the maturing eggs, the

amino acid pattern of the whole ovary comes to resemble more that of the muscle and less that of collagen, the major component of immature gonads. This phenomenon was observed in chum salmon (Love, 1988) as a relative increase in alanine and leucine, and a decrease in glycine, the latter being more concentrated in connective tissue. The present investigation supports this phenomenon in that the gonads had significantly higher concentrations of alanine and leucine, and significantly lower concentrations of glycine than the muscle. The low concentration of collagen in the gonads is further sustained by the significantly lower hydroxyproline concentration of the gonads (0.08 ± 0.0390 g/100 g lipid free dry mass) compared to the muscle (0.23 ± 0.0415 g/100 g lipid free dry mass). Hydroxyproline is not exclusive to collagen, but its occurrence elsewhere is rare enough for it to be used as a measure of the amount of collagen present in a tissue (Love 1988).

The muscle mineral concentrations of the present investigation (Table 16) do not differ greatly from that of the previous investigation, and is lower than that reported for other fish species (Hoffman *et al.*, 1992). With the exception of potassium, the gonads contain similar (phosphorous, magnesium, copper) or significantly higher concentrations of calcium, iron, zinc and manganese, when compared to the muscles. Fletcher and King (1978*a,b*) studied the seasonal dynamics of metals in sockeye salmon and winter flounder and found that approximately 30% of the zinc, copper and magnesium and 60% of the calcium incorporated into the gonads did not come directly from food. The copper, calcium and magnesium could all have been absorbed from seawater, whilst zinc was assumed to have come from storage areas in the fish other than the liver. Fletcher and King found (1978*a*) that the salmon accumulated zinc, calcium, magnesium and phosphate in the ovary. Since the maturing salmon were kept in freshwater with no feed, they assumed that almost all of these ions originated in body tissues other than the liver. Julshamn and Braekkan (1976) as quoted by Love (1988) found that copper, sodium and manganese in the ovaries of Atlantic cod increase during maturation, while potassium, calcium, magnesium, iron, zinc and cobalt decline.

The results of this investigation into the chemical composition of muscle and gonads show that a deeper study of the transfer of different chemical constituents, especially the fatty acids of the lipids and minerals from the muscle, liver, diet or environment (water) into the gonads of the African sharptooth catfish, *C. gariepinus* during their maturation is needed. Simultaneously, the depletion of these constituents from the muscle (fillet), and the possible influence it may have on human nutrition will be elucidated.

The possibility of using *C. gariepinus* gonads as a protein substitute in larvae nutrition also needs investigation. The high concentration of the essential polyunsaturated fatty acid, docosahexaenoic acid (10.81%), seems to indicate that *C. gariepinus* gonads may be a good source of this fatty acid in larval nutrition.

Further investigations are needed into the possibility of changing the fatty acid profile (especially the ω-3 fatty

acids) of the African catfish, *C. gariepinus*, by utilizing different dietary components, as was the case for some other fish species (Csengeri *et al.*, 1978; Bautista & de la Cruz, 1988; Cai & Curtis, 1990; Viola & Arieli, 1990).

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# Chapter 5 THE INFLUENCE OF VARIOUS GENETIC STRAINS ON THE BODY CHEMICAL COMPOSITION OF *CLARIAS GARIEPINUS*

CONTENTS

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## INTRODUCTION

Since the earliest production studies of the African sharptooth catfish *Clarias gariepinus* (Van der Waal, 1978), considerable progress has been made in artificial propogation (Schoonbee, Hecht, Polling & Saayman, 1980; Hecht, Saayman & Polling, 1982; Polling, van der Waal & Schoonbee, 1987), rearing (Hecht, 1982; Uys & Hecht, 1985; Polling, Schoonbee, Prinsloo & Wiid, 1988) and commercial production of this species (Bok & Jongbloed, 1984; Prinsloo, Schoonbee & Van der Walt, 1989) in South Africa. The successful transfer of scientific knowledge to the farming community has resulted in the South African sharptooth catfish industry experiencing a phenomenal growth, from 10 tons in 1987 to an estimated 1 000 tons for the 1991/92 season (Hecht & Britz, 1990; Uys, 1991).

During this growth, a number of catfish lines or strains have been developed by both commercial producers and research institutions. Some of these strains have been shown to contain differing genetic allele frequencies in terms of growth rate and feed conversion ratio (Grobler, Du Ptreez & Van der Bank, 1992; Van der Bank, Grobler & Du Preez, 1992; Van der Walt, Van der Bank & Steyn, 1993*a*). From these studies, it is apparent that the faster growth rate is experienced during the early developmental stage of *C. gariepinus* juveniles, and that the fish will maintain this advantage in body size until they reached a marketable size of ±600 g.

One of the questions that arose from these studies, is whether there are any chemical differences in the muscle composition of different African sharptooth catfish strains? The chemical composition of fish muscle is a quality characteristic, and is largely determined by endogenous and exogenous factors that operate simultaneously. According to Shearer (1994), the endogenous factors are genetically controlled and are associated with the life cycle, size and sex of the fish. The exogenous factors are numerous, and can broadly be classified as either being of an environmental and/or of a dietary nature.

Very little information pertaining to the chemical body composition of this species is available. The revival of interest in the fatty acid content of fish has been based chiefly on the fact that marine fish oils are known to be rich in n-3 polyunsaturated fatty acids (Singh & Chandra, 1988; Ackman, 1989; Burr, 1989; Chetty *et al.*, 1989; Ota, Sasaki, Abe & Takagi, 1990). Kinsella, Lokesh and Stone (1990) extensively reviewed the role that dietary n-3 polyunsaturated fatty acids have in human nutrition and the possible mechanisms which involve fatty acids in human metabolism.

It is well documented that the fatty acid profile of a fish, within narrowly defined physiological levels, is influenced by its diet (Stickney & Andrews, 1971; Haumann, 1989; Morishita *et al.*, 1989; Greene & Selivonchick, 1990; Ota *et al.*, 1990; Tidwell & Robinette, 1990). According to this view, *C. gariepinus* feeding mainly on fish, crustaceans, insects and vascular plants in the wild (Van Senus, 1989), should differ

in its fatty acid composition from farmed catfish receiving a balanced high protein diet.

Another facet of quality, as defined by the fresh fish consumer, is the aesthetic appearance and freshness of the fish (Huss, 1988; Connell, 1990). These two parameters are subjective and can therefore be influenced by consumers in respect of their likes, dislikes, prejudices, etc. The African sharptooth catfish (*C. gariepinus*), with its mottled appearance (Mills, 1966) can very easily lead to a prejudice forming with the consumer against the purchasing of this fish as a fresh dressed product. With the establishment of the so called "golden" strain at the Turfloop Fish Breeding Station by the research team of the University of the North and Lebowa Nature Conservation (Prinsloo, Schoonbee & Theron, 1989), the way has been opened for the marketing of the "golden catfish" as an alternative to the normal coloured, in the fresh fish market. Tucker and Robinson (1990) noted that certain processed fish products such as whole dressed fish are more attractive to consumers, when produced from albino channel catfish (*Ictalurus punctatus*), than normally coloured fish.

A similar growth potential (Prinsloo & Schoonbee, 1989; Prinsloo, Schoonbee & Theron, 1989) and a higher dressout percentage (Hoffman & Prinsloo, 1990) is an added incentive for the large scale production of the golden strain. The question can be posed whether the difference between the normal and golden coloured strains are only superficial, or whether these differences extend to the chemical composition of the myotomal muscle?

Human dietary protein requirements are partially based on the needs for the eight essential amino acids that are not synthesized by the body (Irwin & Hegsted, 1971). Since the classical nitrogen balance studies concerned with establishing the quantitative requirements of those amino acids in human adults (Rose, 1957), various other investigations supplementing these results have been conducted. Young and Bier (1987) considered that the requirements for at least some, and perhaps for most of the essential amino acids, as determined by Rose (1957) in healthy adults, may have been significantly underestimated.

Irrespective of what the amino acid requirements of adults or growing children may be, knowledge of the quantitative composition of all food sources is a prerequisite to aid the nutritionist in diet formulation and nutrient labeling (Weibrauch *et al.*, 1977). Similarly, a knowledge of the mineral composition of different food sources is an aid to the nutritionalist to ensure formulation of balanced diets (Linder, 1985).

Since the African sharptooth catfish *C. gariepinus* is emerging as an additional protein source in Africa (Hecht & Britz, 1990), knowledge of its body composition in terms of the fatty acid, amino acid and mineral concentrations will be of great use to nutritionists, fishery biologists and food scientists.

In this paper a number of investigations into the influence that genotype may have on the body chemical

composition of the African sharptooth catfish, *Clarias gariepinus* is examined. In the first investigation, the whole body chemical composition of four different strains of *C. gariepinus* juveniles that were raised together under identical environmental conditions, were examined. In the second investigation, the chemical composition of the muscle and skin of a normal and golden coloured strains were evaluated and compared. In the third investigation, a comparison was made between the muscle chemical composition of commercially farmed and wild catfish. The chemical parameters tested were whole body proximate composition, amino and fatty acid profiles and mineral content.

## MATERIALS AND METHODS

*Comparison of whole body chemical composition from juvenile catfish originating from four different genetic strains*

*Parental Stock*

The broodstock were identified as Domestic (D), Netherlands (N), Wild (W) and Golden (G) catfish. The domestic strain was normal coloured and had been maintained at the Rand Afrikaans University; the selection of this strain is discussed in Van der Walt *et al.* (1993*a*) and was the strain designated **E** in their investigation. These broodstock fish had been selected for traits such as growth rate and body conformation. The origins of the normal coloured Netherland strain is discussed in Van der Walt *et al.* (1993*b*). The normal coloured wild strain was netted in the Olifants River in the Kruger National Park. With the exception of the wild strain, the other three strains were tagged and maintained in the same environment, receiving the same diet for at least three months prior to breeding. The wild strain were maintained with the three other strains for three weeks before being bred.

With the exception of the Netherlands strain, the broodstock bred consisted of three fecund females and two males of the same strain, respectively. Due to a shortage of Netherland males, the Netherland females were crossbred with two Domestic (D) males.

*Larvae*

Larvae of the four *C. gariepinus* strains were artificially spawned and reared concurrently, using standard hatchery techniques (Schoonbee *et al.*, 1980; Prinsloo, Hoffman & Theron, 1993). The fertilised eggs and hatched larvae of the four strains were maintained in separate containers connected to a common water recirculating system, care being taken to ensure that the larval density between the strains were as equal as possible. Batches of 200 larvae (in triplicate) from each strain were randomly selected and placed into 60 l aquaria, 24 days after hatching. These 12 aquaria were connected to a common water recirculating system.

*Sampling*

At the beginning of each week, three groups of 20 apparently healthy fish were randomly sampled from each aquarium and the total body mass determined. Mean mass was calculated for the computation of feeding rate. The number of dead fish per aquarium was recorded daily and removed. The experiment was terminated on day 56.

*Feeding regime*

Initially, the feeding regime of Schippers *et al.* (1992) was followed: satiation feeding of larvae during first 20 days with natural occuring plankton. From day 21 to 28, plankton was gradually replaced with a 52% trout fry (No 2) commercial diet (supplied by Upstream Industries, Greenside, RSA). From day 29 until day 56, the fish were fed at a level of 8% live weight per day (Hogendoorn *et al.*, 1983) continuously over an 18 h period, using automatic feeders.

*Water quality*

A flow rate between 1.0-1.2 l/min/aquarium was maintained. Water temperature was constant at 29 ± 0.5 °C. Three times a week, 6 $m^3$ (50%) of the total recirculating water volume was replaced. Weekly, selected water quality parameters were tested according to APHA (1980) and were well below the accepted maximum limits for *C. gariepinus* larvae (Viveen *et al.*, 1985).

*Chemical analysis*

The fish diet was analysed for proximate composition, mineral and fatty acid profiles. At the end of the experiment, ten fish from each tank were pooled (±60 g - as the individual fish were too small for separate analysis) and sub-samples analysed for proximate, amino acid, fatty acid and mineral profiles. The methodologies were as described in Chapter 2.

*Statistical Analysis*

An analysis of variance was done on all the parameters with genetic strain as predicted variable. A least square mean (LSMean) was calculated for each genetic strain and exceedance probabilities were calculated for pairwise differences between strains.

*Chemical comparison of golden and normal coloured catfish*

*Histological Study*

A normal and a golden coloured fish were slaughtered and a portion of skin and underlying muscle posterior to the supraoccipital plate removed, and fixed in 10% buffered formalin, dehydrated with ethanol and embedded in paraffin in a vacuum oven. Thereafter, five micrometer sections were stained with Delafield haematoxylin

and counter stained with eosin (Humason, 1979) to investigate the presence or absence of melanophores.

*Chemical Study*

*Fish used*

Five young fish (10 months old) of both golden (mean live mass ± SD = 359.9 ± 36.3 g) and normal (366.7 ± 32.5 g) strains, that were bred and raised together under identical conditions (commercial diet - 6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt) were pithed and dressed using standard procedures (Hoffman, Casey & Prinsloo, 1993). A second group of larger fish consisting of six golden (1469.3 ± 312.8 g) and six normal (1809.6 ± 321.3 g) specimens, aged between 2-3 years, and that had been maintained together in the Aquaculture Research Unit's water recirculating facilities for at least a year were also analysed. These fish differed in age, but not their nutritional status (same as for small sized fish).

*Chemical analysis*

The myotomal muscles and skin of both strains and mass classes (of each fish) were analyzed in triplicate for proximate composition, amino acid profile, fatty acid and mineral profiles.

*Statistical Analysis*

A linear model was fitted to the data with strain and size class as predictor. PROC GLM of the SAS package (SAS, 1985) was used and an analysis of variance was carried out. A mean and standard error was calculated for each strain and size class and a matrix of exceedence probabilities were calculated to test for pairwise differences between strain and size classes. The FISHER LSD test was used for testing pairwise differences since it was recently shown (Saville, 1990) that this test is optimal.

*Identification Codes Used*

A three letter code was used to identify the different samples analyzed. The first letter in the code depicts the skin colour of the two strains, **N** for the normal and **G** for the golden strains. The second letter depicts the tissue analyzed, **M** for muscle and **S** for skin, whilst the third letter depicts the size of the fish, **L** for large and **S** for small. As example, the muscle of the large fish of the golden strain will have the following code GML.

*Muscle chemical comparison of commercialy produced and wild catfish*

*Fish*

Eight month old male (n=15) and female (n=14) farm raised *C. gariepinus* were purchased from a commercial farm situated in the Lowveld, Transvaal, South Africa, during February 1991, at the height of summer. Catfish with a mass of approximately 600 g were selected as this sized fish is considered ideal for processing by the meat trade. Concurrently, wild males (n=19) and wild females (n=22) (1-2 years old), were collected with gill

nets from the Middle Letaba Dam, also situated in the Lowveld. The farmed catfish were fed a commercial 38% crude protein diet, whilst the wild catfish diet evidently consisted mainly of fish, crustaceans, insects and vascular plants (Van Senus, 1989).

*Processing*

Fish were transported live to the Mobil Research Aquarium at the University of the North, Sovenga. All specimens were freshened out in running dechlorinated tap water for two days, thereby facilitating the emptying and removal of stomach contents. Fish were then slaughtered and dressed, which consists of removing the gonads, gut and skin, followed by filleting (Hoffman & Prinsloo, 1990).

*Chemical analysis*

The myotomal muscles of both strains were analyzed in triplicate for proximate composition, amino acid profile, fatty acid and mineral profiles.

*Endoparasitic Infections*

The wild fish were all heavily infested with larval *Contracaecum* spp. nematodes (Mashego & Saayman, 1981; Van As & Basson, 1984). These parasites were found in the mesenterium of both wild male and female *C. gariepinus*. Farmed fish were not visibly infected.

*Statistical analysis*

Different procedures of the Statistical Analysis System (SAS) were used to analyse data. Differences between the two types and sexes in respect of variables were tested by means of Duncan's multiple range test (SAS, 1985).

## RESULTS

The chemical composition of the commercial diet fed to the larvae is indicated in Table 1.

The specific growth curves of the four *C. gariepinus* strains are shown in Figure 1. The final mean body (g $\pm$SD) of the strains were as follows: Domestic 3.433 $\pm$ 1.770; Golden 5.433 $\pm$ 1.944; Netherlands 5.173 $\pm$ 2.299 and Wild 6.093 $\pm$ 2.876. A LSMeans analysis showed these final masses to differ highly significantly from each other ($p=0.0001$). The exception was the difference between strains G and N, which only differed statistically at the 10% level ($p=0.0721$). The percentage survival between the four strains varied (Golden 93%, Domestic 86%, Netherlands and Wild 77%, respectively).

**Table 1:** The proximate, mineral and fatty acid composition of the commercial diet fed to *Clarias gariepinus* larvae.

| | Conc % | Mineral | Conc | Fatty Acid | Prop Conc |
|---|---|---|---|---|---|
| **Moisture** | 7.21 | **P %** | 2.28 | **C14:0** | 4.05 |
| **Protein** | 50.21 | **Ca %** | 2.83 | **C16:0** | 18.48 |
| **Lipid** | 7.36 | **Mg %** | 0.31 | **C16:1ω7** | 4.98 |
| **Ash** | 12.54 | **K %** | 0.30 | **C16:2ω4** | 0.37 |
| **Fibre** | 1.19 | **Zn ppm** | 19.29 | **C18:0** | 4.51 |
| | | **Fe ppm** | 125.78 | **C18:1ω7** | 0.14 |
| | | **Mn ppm** | 65.00 | **C18:1ω9** | 19.52 |
| | | **Cu ppm** | 1.31 | **C18:2ω4** | 0.16 |
| | | | | **C18:2ω6** | 13.29 |
| | | | | **C18:3ω3** | 4.64 |
| | | | | **C18:3ω4** | 0.17 |
| | | | | **C18:4ω1** | 0.12 |
| | | | | **C18:4ω3** | 1.07 |
| | | | | **C20:0** | 0.27 |
| | | | | **C20:1ω9** | 0.18 |
| | | | | **C20:3ω3** | 0.45 |
| | | | | **C20:4ω6** | 7.33 |
| | | | | **C20:5ω3** | 7.76 |
| | | | | **C22:0** | 0.24 |
| | | | | **C22:1ω9** | 0.35 |
| | | | | **C22:1ω11** | 0.73 |
| | | | | **C22:5ω3** | 1.09 |
| | | | | **C22:6ω3** | 8.42 |

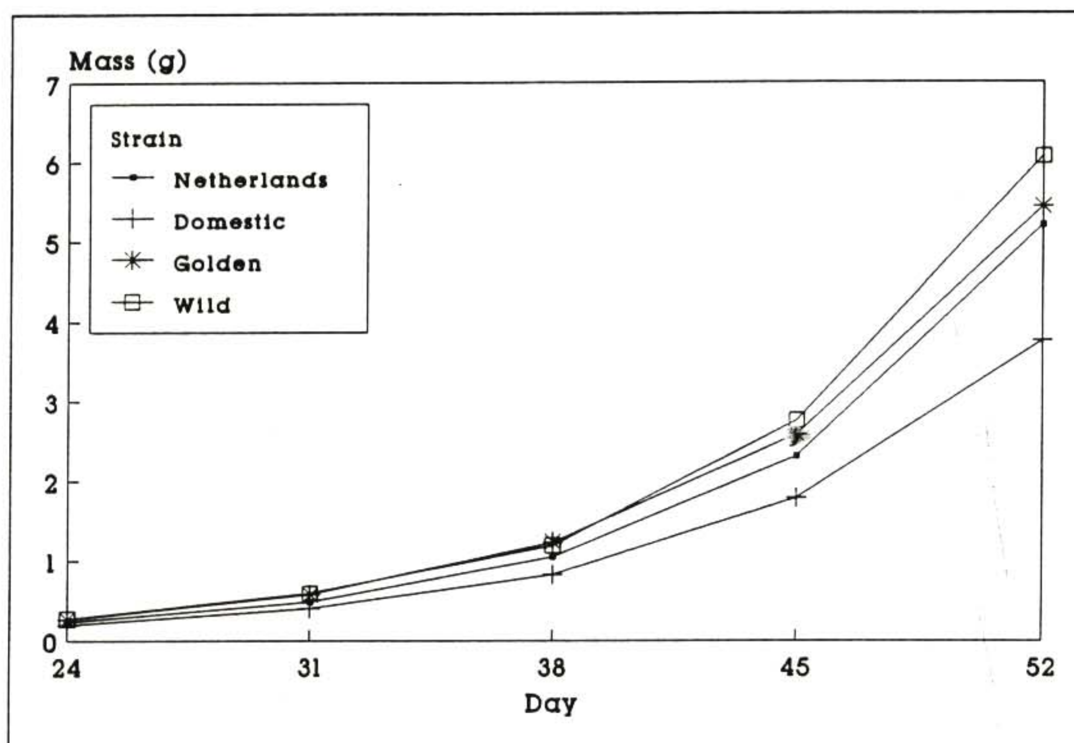


**Figure 1:** The growth curves of *Clarias gariepinus* larvae from four different genetic strains.

The statistical analysis of the LSMeans of the proximate composition of the four larvae strains are presented in Tables 2a and 2b. The golden catfish had the highest moisture content (81.13%) whilst that of the other strains did not differ significantly from one another. None of the strains differed statistically in their mean percentage protein, with the wild strain having the highest (13.10%) and the golden strain the lowest (12.30%). The domestic strain had the highest percentage total lipid (3.30%) which differed from that of the golden (2.36%, $p=0.0132$) and the wild (2.74%, $p=0.0958$), but not from that of the Netherlands (3.22%, $p=0.7943$). The ash content did not differ significantly between any of the four strains and varied between 2.25% for the wild and 1.99% for the Netherlands strains.

**Table 2a:** The mean proximate composition of four *C. gariepinus* strains (N=3, g/100 g wet mass).

| | LSMean | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | **Domestic** | **Gold** | **Neth** | **Wild** | |
| **Moisture** | 80,25 | 81,13 | 79,82 | 79,88 | 0,3646 |
| **Protein** | 12,87 | 12,30 | 12,49 | 13,10 | 0,3352 |
| **Lipid** | 3,30 | 2,36 | 3,22 | 2,74 | 0,2098 |
| **Ash** | 2,09 | 2,07 | 1,99 | 2,25 | 0,1415 |

**Table 2b:** Probability values calculated for pairwise differences between four *C. gariepinus* strains for the proximate chemical parameters.

| | $p>\mid T\mid$ HO: $LSMean_i=LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | **D = G** | **D = N** | **D = W** | **G = N** | **G = W** | **N = W** |
| **Moisture** | 0,1275 | 0,4248 | 0,4896 | 0,0350 | 0,0416 | 0,9152 |
| **Protein** | 0,2661 | 0,4459 | 0,6357 | 0,7040 | 0,1299 | 0,2318 |
| **Lipid** | 0,0132 | 0,7943 | 0,0958 | 0,0199 | 0,2361 | 0,1443 |
| **Ash** | 0,9229 | 0,6085 | 0,4656 | 0,6764 | 0,4117 | 0,2301 |

Where D = domestic strain
G = gold strain
N = Netherlands strain
W = wild strain

The statistical analysis of the whole body amino acids identified are indicated in Table 3a and 3b. There were no statistical differences in the concentrations of any of the amino acids between domestic and golden strains

and between Netherlands and wild strains. However, between the other strain groupings, aspartic acid, glutamic acid, serine, arganine, threonine, alanine, isoleucine, leucine, phenylalanine and lysine concentrations differed significantly.

The fatty acid profiles of the total body lipid for the four genetic strains are shown in Table 4a and b. With the exception of C18:0, C18:2ω4, C18:2ω6, C18:3ω3, C20:0, C20:2ω6, C20:3ω3 and C20:3ω6, the fatty acids of the total lipids differed significantly between various of the strain groupings. However, these differences, between groupings, showed no fixed trend. Statistical differences in the percentages of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), and in the ratio of ω3 to ω6 fatty acids were noted between the domestic and golden strains, and the Netherlands and golden strains. The Netherland strain had the highest concentration of SFA (33.40%) whilst the domestic strain had the highest MUFA concentration (29.00%). The golden strain had the highest concentration of PUFA (37.84%) and ω3/ω6 ratio (2.20).

The statistical analysis of the LSMeans of the mineral profiles determined from the whole body samples of the four genetic strains are summarised in Table 5a and b. There were no statistically significant differences in the concentrations of phosphorus and calcium. The potassium concentration was similar for all strain combinations with the exception of that between the domestic and wild strains. The golden strain had a significantly lower magnesium and higher manganese concentration than the other three strains. The concentrations of these two minerals did not differ significantly between the domestic, Netherlands and wild strains. The domestic strain had a low zinc concentration (9.82 ppm DM) which only differed statistically from that of the Netherlands and wild strains. The Netherlands strain had an iron concentration similar to the wild, but statistically higher than that of the domestic and golden strains. The domestic strain had a significantly lower copper concentration than the other three strains, whose concentrations were similar.

**Table 3a:** The mean amino acid whole body composition of four *C. gariepinus* strains (N=3, g/100g fat free dry mass).

| | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | Domestic | Gold | Neth | Wild | |
| **Aspartic acid** | 6.78 | 7.11 | 5.86 | 6.02 | 0.2249 |
| **Glutamic acid** | 12.39 | 12.21 | 11.56 | 11.93 | 0.2975 |
| **Serine** | 4.26 | 4.31 | 4.05 | 4.10 | 0.0519 |
| **Glycine** | 5.67 | 5.61 | 5.83 | 5.17 | 0.3194 |
| **Histidine** | 1.92 | 1.94 | 1.87 | 1.93 | 0.0442 |
| **Arginine** | 5.80 | 5.72 | 5.44 | 5.53 | 0.0953 |
| **Threonine** | 4.29 | 4.34 | 3.92 | 4.02 | 0.0671 |
| **Alanine** | 5.84 | 5.64 | 6.03 | 6.07 | 0.1391 |
| **Proline** | 4.02 | 4.01 | 4.07 | 3.93 | 0.1735 |
| **Tyrosine** | 2.94 | 2.99 | 2.88 | 2.95 | 0.0562 |
| **Valine** | 3.50 | 3.70 | 3.49 | 3.45 | 0.0957 |
| **Methionine** | 2.10 | 2.07 | 2.00 | 2.04 | 0.0459 |
| **Isoleucine** | 3.17 | 3.30 | 3.02 | 3.04 | 0.0819 |
| **Leucine** | 5.87 | 6.01 | 5.65 | 5.72 | 0.1201 |
| **Phenylalanine** | 3.33 | 3.37 | 3.12 | 3.11 | 0.0430 |
| **Lysine** | 7.46 | 7.24 | 6.53 | 6.51 | 0.1878 |
| **Hydroxyproline** | 1.28 | 1.24 | 1.28 | 1.38 | 0.1299 |

**Table 3b:** Probability values calculated for pairwise differences between four *C. gariepinus* strains for the amino acid composition.

| | $p>|T|$ HO: $LSMean_i=LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | D = G | D = N | D = W | G = N | G = W | N = W |
| **Aspartic acid** | 0.3298 | 0.0201 | 0.0453 | 0.0044 | 0.0093 | 0.6145 |
| **Glutamic acid** | 0.6801 | 0.0840 | 0.3127 | 0.1610 | 0.5341 | 0.3968 |
| **Serine** | 0.5713 | 0.0184 | 0.0529 | 0.0076 | 0.0211 | 0.5150 |
| **Glycine** | 0.8976 | 0.7271 | 0.3006 | 0.6343 | 0.3586 | 0.1802 |
| **Histidine** | 0.7571 | 0.5077 | 0.8768 | 0.3405 | 0.8768 | 0.4183 |
| **Arginine** | 0.5854 | 0.0295 | 0.0803 | 0.0716 | 0.1897 | 0.5385 |
| **Threonine** | 0.6360 | 0.0045 | 0.0216 | 0.0023 | 0.0103 | 0.3226 |
| **Alanine** | 0.3389 | 0.3623 | 0.2823 | 0.0826 | 0.0619 | 0.8567 |
| **Proline** | 0.9895 | 0.8436 | 0.4763 | 0.8333 | 0.4841 | 0.3694 |
| **Tyrosine** | 0.5472 | 0.4965 | 0.8710 | 0.2168 | 0.6571 | 0.4045 |
| **Valine** | 0.1844 | 0.9492 | 0.7215 | 0.1654 | 0.1060 | 0.7752 |
| **Methionine** | 0.7286 | 0.1749 | 0.3825 | 0.2915 | 0.5878 | 0.5878 |
| **Isoleucine** | 0.2718 | 0.2508 | 0.2941 | 0.0420 | 0.0502 | 0.9112 |
| **Leucine** | 0.4231 | 0.2249 | 0.4129 | 0.0629 | 0.1260 | 0.6636 |
| **Phenylalanine** | 0.5293 | 0.0080 | 0.0068 | 0.0031 | 0.0027 | 0.9154 |
| **Lysine** | 0.4382 | 0.0081 | 0.0074 | 0.0277 | 0.0251 | 0.9515 |
| **Hydroxyproline** | 0.8467 | 1.0000 | 0.5774 | 0.8467 | 0.4576 | 0.5774 |

**Table 4a**: The mean whole body total lipid fatty acid composition of four *C. gariepinus* strains (N=3, % of total fatty acids measured).

| | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | Domestic | Gold | Neth | Wild | |
| C14:0 | 2.60 | 2.44 | 2.72 | 2.54 | 0.0607 |
| C16:0 | 21.64 | 21.06 | 22.27 | 21.85 | 0.1825 |
| C16:1ω7 | 4.62 | 4.26 | 4.51 | 4.20 | 0.1051 |
| C16:2ω4 | 1.11 | 1.42 | 1.25 | 1.23 | 0.0697 |
| C18:0 | 7.25 | 7.15 | 7.26 | 7.25 | 0.0855 |
| C18:1ω7 | 0.14 | 0.13 | 0.14 | 0.34 | 0.0449 |
| C18:1ω9 | 23.11 | 21.19 | 22.24 | 21.90 | 0.3827 |
| C18:2ω4 | 0.14 | 0.14 | 0.14 | 0.15 | 0.0094 |
| C18:2ω6 | 9.70 | 9.89 | 9.93 | 10.16 | 0.1822 |
| C18:3ω3 | 3.75 | 3.58 | 3.55 | 3.65 | 0.1547 |
| C18:3ω4 | 0.20 | 0.23 | 0.21 | 0.20 | 0.0058 |
| C18:4ω1 | 0.25 | 0.15 | 0.13 | 0.13 | 0.0281 |
| C18:4ω3 | 0.63 | 0.50 | 0.57 | 0.56 | 0.0235 |
| C20:0 | 0.19 | 0.22 | 0.20 | 0.23 | 0.0183 |
| C20:1ω9 | 0.45 | 0.42 | 0.34 | 0.38 | 0.0229 |
| C20:2ω6 | 0.08 | 0.04 | 0.06 | 0.04 | 0.0239 |
| C20:3ω3 | 1.06 | 1.69 | 1.00 | 1.02 | 0.2820 |
| C20:3ω6 | 0.35 | 0.10 | 0.33 | 0.09 | 0.1671 |
| C20:4ω3 | 0.45 | 0.39 | 0.39 | 0.39 | 0.0061 |
| C20:4ω6 | 1.31 | 1.21 | 1.38 | 1.88 | 0.2063 |
| C20:5ω3 | 4.00 | 4.15 | 4.31 | 4.13 | 0.0674 |
| C22:0 | 1.04 | 1.09 | 0.93 | 0.86 | 0.0617 |
| C22:1ω9 | 0.21 | 0.26 | 0.22 | 0.26 | 0.0151 |
| C22:1ω11 | 0.47 | 0.54 | 0.52 | 0.57 | 0.0246 |
| C22:5ω3 | 1.42 | 1.50 | 1.35 | 1.45 | 0.0353 |
| C22:6ω3 | 10.76 | 12.89 | 10.82 | 11.60 | 0.3833 |
| C24:0 | nd | 0.10 | 0.07 | 0.13 | 0.0101 |
| SFA | 32.72 | 32.03 | 33.40 | 32.86 | 0.2059 |
| MUFA | 29.00 | 26.81 | 27.96 | 27.65 | 0.4317 |
| PUFA | 34.99 | 37.84 | 35.30 | 36.55 | 0.5229 |
| ω3/ω6 | 1.92 | 2.20 | 1.87 | 1.88 | 0.0964 |

**where nd = not detected**

**Table 4b:** Probablility values calculated for pairwise differences between four *C. gariepinus* strains for the body fatty acid composition.

| | $p>$ \| T \| HO: $\text{LSMean}_i = \text{LSMean}_j$ | | | | | |
|---|---|---|---|---|---|---|
| | **D = G** | **D = N** | **D = W** | **G = N** | **G = W** | **N = W** |
| **C14:0** | 0.0937 | 0.1890 | 0.5046 | 0.0103 | 0.2634 | 0.0653 |
| **C16:0** | 0.0570 | 0.0397 | 0.4255 | 0.0016 | 0.0155 | 0.1450 |
| **C16:1ω7** | 0.0403 | 0.4806 | 0.0216 | 0.1268 | 0.6971 | 0.0682 |
| **C16:2ω4** | 0.0144 | 0.1840 | 0.2706 | 0.1361 | 0.0901 | 0.7936 |
| **C18:0** | 0.4178 | 0.9787 | 1.0000 | 0.4036 | 0.4178 | 0.9787 |
| **C18:1ω7** | 0.9594 | 1.0000 | 0.0126 | 0.9594 | 0.0116 | 0.0126 |
| **C18:1ω9** | 0.0075 | 0.1439 | 0.0553 | 0.0900 | 0.2279 | 0.5512 |
| **C18:2ω4** | 0.9137 | 0.5920 | 0.2565 | 0.6309 | 0.2495 | 0.4758 |
| **C18:2ω6** | 0.4819 | 0.3980 | 0.1165 | 0.8805 | 0.3366 | 0.4113 |
| **C18:3ω3** | 0.4511 | 0.3874 | 0.6598 | 0.9060 | 0.7461 | 0.6598 |
| **C18:3ω4** | 0.0020 | 0.2555 | 0.6938 | 0.0114 | 0.0035 | 0.4379 |
| **C18:4ω1** | 0.0277 | 0.0146 | 0.0146 | 0.6858 | 0.6858 | 1.0000 |
| **C18:4ω3** | 0.0039 | 0.0933 | 0.0585 | 0.0684 | 0.1088 | 0.7713 |
| **C20:0** | 0.1950 | 0.7098 | 0.1615 | 0.3338 | 0.9009 | 0.2806 |
| **C20:1ω9** | 0.4332 | 0.0108 | 0.0622 | 0.0384 | 0.2168 | 0.2894 |
| **C20:2ω6** | 0.2818 | 0.4785 | 0.2428 | 0.6088 | 1.0000 | 0.5683 |
| **C20:3ω3** | 0.1528 | 0.8841 | 0.9354 | 0.1218 | 0.1348 | 0.9483 |
| **C20:3ω6** | 0.3210 | 0.9455 | 0.3033 | 0.3524 | 0.9673 | 0.3333 |
| **C20:4ω3** | 0.0007 | 0.0014 | 0.0009 | 0.6875 | 0.8396 | 0.5847 |
| **C20:4ω6** | 0.7242 | 0.8164 | 0.0896 | 0.5616 | 0.0508 | 0.1294 |
| **C20:5ω3** | 0.1544 | 0.0111 | 0.1996 | 0.1251 | 0.8656 | 0.0958 |
| **C22:0** | 0.5826 | 0.2304 | 0.0732 | 0.0983 | 0.0300 | 0.4670 |
| **C22:1ω9** | 0.0472 | 0.8798 | 0.0768 | 0.0603 | 0.7828 | 0.0978 |
| **C22:1ω11** | 0.0785 | 0.1878 | 0.0177 | 0.5804 | 0.3652 | 0.1631 |
| **C22:5ω3** | 0.1475 | 0.2399 | 0.4833 | 0.0207 | 0.4104 | 0.0800 |
| **C22:6ω3** | 0.0044 | 0.9241 | 0.1613 | 0.0051 | 0.0450 | 0.1865 |
| **C24:0** | nt | nt | nt | 0.3273 | 0.1414 | 0.0674 |
| **SFA** | 0.0453 | 0.0477 | 0.6557 | 0.0015 | 0.0211 | 0.1025 |
| **MUFA** | 0.0071 | 0.1269 | 0.0570 | 0.0956 | 0.2078 | 0.6180 |
| **PUFA** | 0.0048 | 0.6829 | 0.0674 | 0.0088 | 0.1184 | 0.1294 |
| **ω3/ω6** | 0.0767 | 0.7165 | 0.7499 | 0.0427 | 0.0459 | 0.9642 |

where nt = not tested as fatty acid not detected in strain D

**Table 5a:** The mean mineral whole body composition of four *C. gariepinus* strains (N=3, dry mass).

| | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | **Domestic** | **Gold** | **Neth** | **Wild** | |
| **P %** | 1.63 | 1.45 | 1.21 | 1.65 | 0.2249 |
| **Ca %** | 1.89 | 2.11 | 2.02 | 1.95 | 0.1287 |
| **Mg %** | 0.16 | 0.11 | 0.15 | 0.16 | 0.0145 |
| **K %** | 1.22 | 1.35 | 1.47 | 1.56 | 0.1202 |
| **Zn ppm** | 9.82 | 13.39 | 14.96 | 15.10 | 1.8220 |
| **Fe ppm** | 50.72 | 63.00 | 94.00 | 75.67 | 8.5098 |
| **Mn ppm** | 5.97 | 13.56 | 6.09 | 6.42 | 2.6514 |
| **Cu ppm** | 1.23 | 2.14 | 2.86 | 2.14 | 0.3311 |

**Table 5b:** Probability values calculated for paiwise differences between four *C. gariepinus* strains for the mineral composition.

| | $p>$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | **D = G** | **D = N** | **D = W** | **G = N** | **G = W** | **N = W** |
| **P %** | 0.6006 | 0.2299 | 0.9352 | 0.4721 | 0.5470 | 0.2039 |
| **Ca %** | 0.2628 | 0.4998 | 0.7770 | 0.6319 | 0.3886 | 0.6899 |
| **Mg %** | 0.0372 | 0.8506 | 0.9249 | 0.0504 | 0.0433 | 0.9249 |
| **K %** | 0.4653 | 0.1730 | 0.0788 | 0.4865 | 0.2476 | 0.6187 |
| **Zn ppm** | 0.2029 | 0.0813 | 0.0744 | 0.5610 | 0.5252 | 0.9553 |
| **Fe ppm** | 0.3372 | 0.0070 | 0.0719 | 0.0328 | 0.3233 | 0.1662 |
| **Mn ppm** | 0.0776 | 0.9760 | 0.9085 | 0.0814 | 0.0932 | 0.9324 |
| **Cu ppm** | 0.0872 | 0.0084 | 0.0872 | 0.1654 | 1.0000 | 0.1654 |

*Comparison of golden an normal strains*

*Histology of the catfish skin*

The microscopic study of the skin shows the normal coloured strain to possess melanin whilst the golden strain has none (Fig 2 & 3). There are also pronounced differences in the appearance and colour of the two types of dressed catfish. The dermal membranes and flesh of the skinned golden fish are almost white whilst the normal coloured have typical light grey membranes and darker flesh.

*Chemical Analysis*

*Proximate composition*

*Muscle*

In Table 6*a* the data pertaining to the proximate composition of the muscle of the four groups is displayed, with the statistical comparison of these means represented in Table 6*b*. The differences between the proximate composition means were more pronounced between size classes than between strains within size class. For both strains, the larger size classes (GML & NML) had statistically significant lower moisture and higher total lipid contents than the smaller size classes (NMS & GMS). Within size classes, the golden strain muscle (GML & GMS) had lower moisture and higher lipid contents when compared to the normal strain (NML & NMS), these differences in the larger sized class (GML=NML) was statistically significant but not in the smaller size class (GMS=NMS). No significant difference in the muscle protein content between and within the two strains and two size classes was noted (Tables 6*a* & 6*b*). The percentage protein (on a wet mass basis) varied from 16.7% for NMS to 17.6% for GML. The ash content of the muscle from NML differed significantly from that of NMS, but was similar to that of GML and GMS (Tables 6*a* & 6*b*).

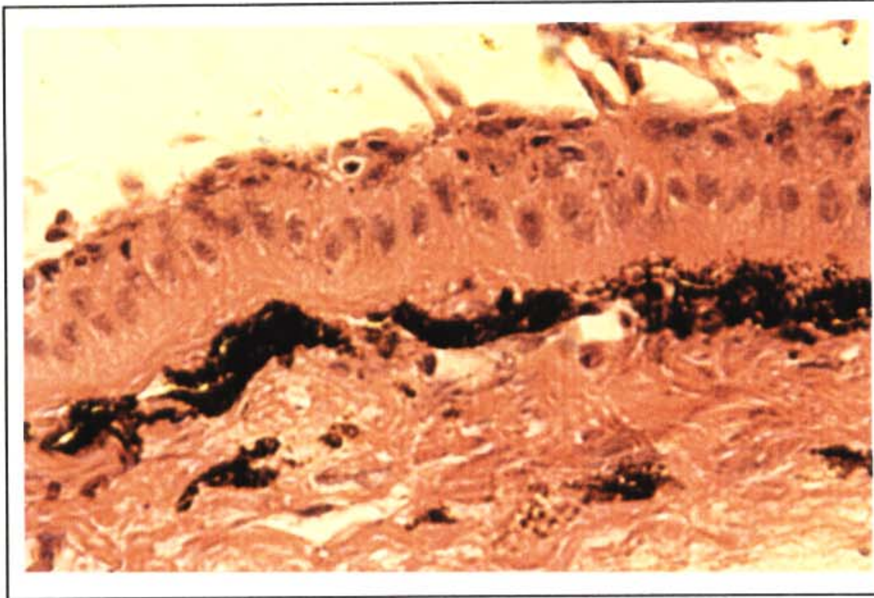

**Figure 1:** A cross-section of a normal coloured *Clarias gariepinus* specimen. Note the prescence of melanin in the chromatophores.

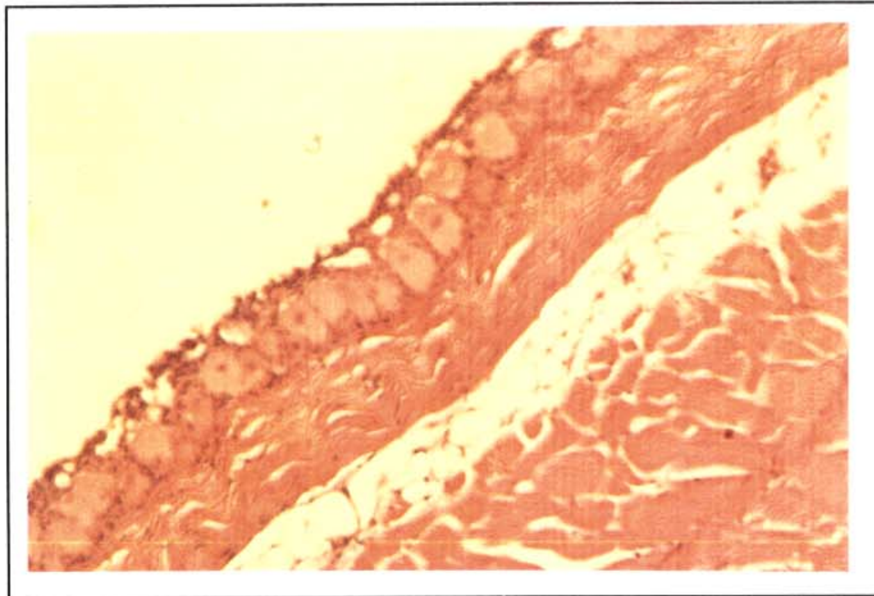

**Figure 2:** A cross-section of a golden coloured *Clarias gariepinus* specimen. Note the abscence of melanin.

**Table 6*a*:** The mean proximate composition of *C. gariepinus* muscle (g/100 g wet mass).

| Parameter | LSMean | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GML<br>n=6 | GMS<br>n=5 | NML<br>n=6 | NMS<br>n=5 | |
| Moisture | 74.5 | 77.7 | 75.9 | 78.5 | 0.7237 |
| Protein | 17.6 | 17.3 | 16.9 | 16.7 | 0.6152 |
| Lipid | 6.9 | 3.3 | 5.00 | 2.7 | 0.7095 |
| Ash | 1.1 | 1.4 | 1.2 | 2.2 | 0.0754 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 6*b*:** Probability values calculated for pairwise differences between two strains of *C. gariepinus* for muscle proximate composition.

| Parameter | $p>\|T\|$ HO: $LSMean_i=LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | GML=GMS | GML=NML | GML=NMS | GMS=NML | GMS=NMS | NML=NMS |
| Moisture | 0.0045 | 0.1734 | 0.0007 | 0.0943 | 0.4928 | 0.0200 |
| Protein | 0.7477 | 0.4013 | 0.2967 | 0.6293 | 0.4858 | 0.8043 |
| Lipid | 0.0055 | 0.0629 | 0.0003 | 0.1177 | 0.5964 | 0.0378 |
| Ash | 0.0369 | 0.4681 | 0.0001 | 0.1493 | 0.0001 | 0.0001 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

*Skin*

The proximate composition and statistical comparison of the skin of the two strains and size classes are displayed in Tables 7*a* and 7*b*, respectively. In both size classes, the golden strain (GSL & GSS) had the higher moisture content, although only that difference in the large size class was statistically significant (GSL=NSL, p=0.0141 - Table 7*b*). For the skin protein content, the differences in means attributed to size was greater than that attributed to strain, the differences in the former being more significant than in the latter (the protein content between the two strains within the large size class tested non-significant). The total lipid content of the skin did not differ significantly between strains and within size classes. There were significant differences in the skin ash content between the two size classes, the small size (NSS & GSS) had higher percentages ash than the larger sized fish (NSL & GSL). No fixed trend in ash content between strains within size classes was manifested, although all the differences between the means were statistically significant.

*Muscle versus Skin*

In general, the muscle samples had significantly higher moisture, lower protein and higher ash contents than the skin. The lipid contents of the muscle and skin showed no fixed trends except that the large sized group tended to have a higher lipid content in the muscle than the skin (Tables 6*a* & 7*a*).

*Amino acid composition*

*Muscle*

The mean amino acid profiles and statistical analysis of the differences between the means of the myotomal muscles of the strains and size classes of the catfish are shown in Tables 8*a* and 8*b*, respectively. For all the amino acids tested, low variations within the classes were noted (manifested by low standard errors of the LSMeans). Of the amino acids tested, only glycine, histidine, proline and hydroxyproline did not differ between strains and size classes, and within strains for the different size classes. The amino acids that differed significantly between the strains and size classes showed no fixed trends.

*Skin*

The mean amino acid content of the skin from the two strains and size classes are represented in Table 9*a*, whilst Table 9*b* displays the statistical results for the analysis of the difference of the means. The mean concentrations of alanine and methionine did not differ statistically between and within strains and size classes. The mean concentrations of glycine, proline and hydroxyproline were all higher in the larger sized groups for both strains. The concentrations of the other amino acids showed no fixed trend.

**Table 7*a*:** The mean proximate composition of *C. gariepinus* skin (g/100 g wet mass).

| Parameter | LSMean | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GSL n=6 | GSS n=5 | NSL n=6 | NSS n=5 | |
| **Moisture** | 73.2 | 73.1 | 70.5 | 72.1 | 0.7237 |
| **Protein** | 23.4 | 20.0 | 24.2 | 22.2 | 0.6152 |
| **Lipid** | 3.9 | 4.6 | 3.6 | 2.6 | 0.7095 |
| **Ash** | 0.4 | 0.7 | 0.3 | 1.3 | 0.7054 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

**Table 7*b*:** Probability values calculated for paiwise diefferences between two strains of *C. gariepinus* skin proximate composition.

| Parameter | $p >$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | GSL=GSS | GSL=NSL | GSL=NSS | GSS=NSL | GSS=NSS | NSL=NSS |
| **Moisture** | 0.9621 | 0.0141 | 0.3968 | 0.0465 | 0.4875 | 0.2166 |
| **Protein** | 0.0030 | 0.3797 | 0.2582 | 0.0004 | 0.0837 | 0.0694 |
| **Lipid** | 0.6070 | 0.7431 | 0.3036 | 0.4357 | 0.1848 | 0.4436 |
| **Ash** | 0.0496 | 0.3636 | 0.0001 | 0.0087 | 0.0007 | 0.0001 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

*Muscle versus Skin*

The muscle had higher concentrations aspartic, glutamic acids, histidine, threonine, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine than the skin. The other identified amino acids were all present in lower concentrations in the muscle of both size classes and strains. Of importance were the higher glycine ($>15\%$ vs $\pm 4\%$), proline ($>9\%$ vs $\pm 3\%$) and hydroxyproline ($>6\%$ vs $<0.6\%$), and lower isoleucine ($<2\%$ vs $>4\%$), leucine ($<3.5\%$ vs $>6\%$) and lysine ($<4\%$ vs $>7\%$) concentrations in the skin when compared to the muscle of both size classes and strains.

*Fatty acids*

*Muscle*

The mean fatty acid profiles of the lipids and the statistical comparisons thereof, from the muscle of both strains and size classes are shown in Tables 10*a* and 10*b*. The following fatty acids were present in concentrations below 1% in the muscles of both sizes and strains and were therefore not included in Table 10*a*: C16:2$\omega$4, C18:1$\omega$7, C18:3$\omega$4, C18:4$\omega$1, C20:0, C20:1$\omega$9, C20:2$\omega$6, C20:3$\omega$6, C20:4$\omega$3, C22:1$\omega$9, C22:1$\omega$11 and C24:0. Size had a stronger influence on the fatty acid profiles than did strain as manifested by the number of fatty acids differing in concentrations between size classes and between strains. In the large size class (GML & NML), only linoleic acid differed significantly, GML having the higher concentration (18.3 vs 15.2%). In the smaller size class, only myristic and eicosapentaenoic acids showed significant differences between strains. Size had a stronger influence in the golden strain (6 fatty acids differing significantly between GML and GMS) than in the normal coloured strain (only 2 fatty acids differing significantly between NML & NMS).

*Skin*

The mean fatty acid profiles of the skin lipids and the statistical comparisons thereof, for both coloured strains and size classes are shown in Tables 11*a* and 11*b*. The same fatty acids that were present in concentrations below 1% in the muscles of both sizes and strains, were also present in trace quantities in the skin and are therefore not included in Tables 11*a* and 11*b*. Fish size had a stronger influence on the mean fatty acid profile of the fish skin than did the strain. In the large size class (GSL & NSL), only palmitoleic, arachidonic and docosapentaenoic acids differed statistically significantly between the strains. In the smaller mass size (GSS & NSS), only stearic acid differed significantly. However, within the golden strain, six fatty acids differed between the two size classes. For the normal strain, five fatty acids differed between the size classes. Only palmitic and linoleic acids differed in both strains in the comparison of size within a strain. For both strains, the differences were similar, lower palmitic and higher linoleic acid concentrations in the larger size class.

**Table 8a:** The mean amino acid composition of *C. gariepinus* muscle (g/100 g fat free dry mass)

| Amino acid | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GML n=6 | GMS n=5 | NML n=6 | NMS n=5 | |
| Aspartic acid | 9.75 | 9.53 | 7.26 | 10.23 | 0.1618 |
| Glutamic acid | 14.89 | 14.59 | 13.38 | 15.91 | 0.2406 |
| Serine | 4.50 | 4.33 | 4.29 | 4.65 | 0.0842 |
| Glycine | 4.67 | 4.06 | 4.51 | 4.30 | 0.2805 |
| Histidine | 2.23 | 2.18 | 2.16 | 2.28 | 0.0397 |
| Arginine | 6.07 | 5.73 | 5.79 | 6.16 | 0.1151 |
| Threonine | 5.00 | 4.76 | 4.70 | 5.20 | 0.0909 |
| Alanine | 5.62 | 5.57 | 6.49 | 5.88 | 0.1181 |
| Proline | 3.61 | 3.16 | 3.42 | 3.33 | 0.1581 |
| Tyrosine | 3.54 | 3.44 | 3.18 | 3.69 | 0.0610 |
| Valine | 4.71 | 4.48 | 4.14 | 4.88 | 0.0594 |
| Methionine | 2.71 | 2.69 | 2.31 | 3.12 | 0.0634 |
| Isoleucine | 4.46 | 4.39 | 3.60 | 4.64 | 0.0722 |
| Leucine | 7.64 | 7.46 | 6.17 | 7.96 | 0.1171 |
| Phenylalanine | 4.10 | 4.10 | 3.12 | 4.26 | 0.0776 |
| Lysine | 9.48 | 8.76 | 6.69 | 9.04 | 0.2097 |
| Hydroxyproline | 0.57 | 0.30 | 0.48 | 0.34 | 0.1635 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 8*b*:** Probability values calculated for pairwise differences between amino acids of the muscle of two *C. gariepinus* strains.

| Amino acid | $p>\|T\|$ HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | **GML=GMS** | **GML=NML** | **GML=NMS** | **GMS=NML** | **GMS=NMS** | **NML=NMS** |
| **Aspartic acid** | 0.4497 | 0.0001 | 0.0971 | 0.0001 | 0.0404 | 0.0001 |
| **Glutamic acid** | 0.4899 | 0.0001 | 0.0202 | 0.0070 | 0.0107 | 0.0001 |
| **Serine** | 0.2724 | 0.0965 | 0.2921 | 0.7772 | 0.0680 | 0.0196 |
| **Glycine** | 0.2164 | 0.6777 | 0.4549 | 0.3643 | 0.6637 | 0.6814 |
| **Histidine** | 0.4311 | 0.2344 | 0.4885 | 0.8478 | 0.2044 | 0.1015 |
| **Arginine** | 0.0996 | 0.1046 | 0.6434 | 0.7406 | 0.0706 | 0.0769 |
| **Threonine** | 0.1418 | 0.0266 | 0.2041 | 0.6908 | 0.0216 | 0.0034 |
| **Alanine** | 0.8212 | 0.0001 | 0.2144 | 0.0001 | 0.2050 | 0.0055 |
| **Proline** | 0.1093 | 0.4028 | 0.3155 | 0.3447 | 0.5880 | 0.7449 |
| **Tyrosine** | 0.3291 | 0.0003 | 0.1717 | 0.0475 | 0.0001 | 0.0001 |
| **Valine** | 0.0365 | 0.0001 | 0.1039 | 0.0029 | 0.0023 | 0.0001 |
| **Methionine** | 0.8924 | 0.0002 | 0.0008 | 0.0019 | 0.0022 | 0.0001 |
| **Isoleucine** | 0.5894 | 0.0001 | 0.1655 | 0.0001 | 0.0992 | 0.0001 |
| **Leucine** | 0.3827 | 0.0001 | 0.1263 | 0.0001 | 0.0420 | 0.0001 |
| **Phenylalanine** | 0.9704 | 0.0001 | 0.2203 | 0.0001 | 0.2723 | 0.0001 |
| **Lysine** | 0.0556 | 0.0001 | 0.2328 | 0.0001 | 0.5051 | 0.0001 |
| **Hydroxyproline** | 0.3459 | 0.7001 | 0.4271 | 0.5266 | 0.8956 | 0.6291 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 9a:** The mean amino acid composition of *C. gariepinus* skin (g/100 g fat free dry mass).

| Amino acid | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GSL n=6 | GSS n=5 | NSL n=6 | NSS n=5 | |
| Aspartic acid | 5.91 | 5.13 | 5.97 | 5.80 | 0.1618 |
| Glutamic acid | 10.15 | 9.04 | 10.10 | 9.81 | 0.2406 |
| Serine | 5.16 | 4.66 | 5.11 | 5.21 | 0.0842 |
| Glycine | 19.15 | 15.22 | 19.18 | 16.19 | 0.2805 |
| Histidine | 1.08 | 1.24 | 1.07 | 1.39 | 0.0397 |
| Arginine | 7.17 | 6.63 | 7.33 | 7.24 | 0.1151 |
| Threonine | 3.53 | 3.23 | 3.51 | 3.54 | 0.0909 |
| Alanine | 7.99 | 7.75 | 8.03 | 7.84 | 0.1181 |
| Proline | 10.67 | 8.93 | 10.65 | 9.25 | 0.1581 |
| Tyrosine | 1.38 | 1.47 | 1.37 | 1.70 | 0.0610 |
| Valine | 2.56 | 2.41 | 2.73 | 2.65 | 0.0594 |
| Methionine | 1.53 | 1.35 | 1.50 | 1.42 | 0.0634 |
| Isoleucine | 1.69 | 1.72 | 1.88 | 1.94 | 0.0722 |
| Leucine | 3.24 | 2.87 | 3.53 | 3.30 | 0.1171 |
| Phenylalanine | 1.99 | 1.79 | 2.32 | 1.88 | 0.0776 |
| Lysine | 3.45 | 3.01 | 4.20 | 3.34 | 0.2097 |
| Hydroxyproline | 7.55 | 6.51 | 7.39 | 6.85 | 0.1635 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

**Table 9*b*:** Probability values calculated for pairwise differences between the amino acids of the skins of two *C. gariepinus* strains.

| **Amino acid** | $p>$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | **GSL=GSS** | **GSL=NSL** | **GSL=NSS** | **GSS=NSL** | **GSS=NSS** | **NSL=NSS** |
| **Aspartic acid** | 0.0119 | 0.8280 | 0.7052 | 0.0061 | 0.0481 | 0.5649 |
| **Glutamic acid** | 0.0158 | 0.9013 | 0.4346 | 0.0167 | 0.1227 | 0.4826 |
| **Serine** | 0.0028 | 0.7056 | 0.7227 | 0.0048 | 0.0030 | 0.4915 |
| **Glycine** | 0.0001 | 0.9399 | 0.0001 | 0.0001 | 0.0963 | 0.0001 |
| **Histidine** | 0.0385 | 0.8621 | 0.0002 | 0.0237 | 0.0642 | 0.0001 |
| **Arginine** | 0.0133 | 0.3679 | 0.7559 | 0.0016 | 0.0133 | 0.6493 |
| **Threonine** | 0.0743 | 0.8813 | 0.9450 | 0.0849 | 0.0963 | 0.8422 |
| **Alanine** | 0.2593 | 0.8064 | 0.4746 | 0.1723 | 0.7061 | 0.3447 |
| **Proline** | 0.0001 | 0.8459 | 0.0001 | 0.0001 | 0.3155 | 0.0001 |
| **Tyrosine** | 0.3900 | 0.9244 | 0.0070 | 0.4192 | 0.0782 | 0.0067 |
| **Valine** | 0.1792 | 0.6200 | 0.4049 | 0.0046 | 0.0568 | 0.4347 |
| **Methionine** | 0.1176 | 0.7655 | 0.3265 | 0.1695 | 0.5854 | 0.4456 |
| **Isoleucine** | 0.8621 | 0.0968 | 0.0642 | 0.2073 | 0.1283 | 0.6072 |
| **Leucine** | 0.0876 | 0.1024 | 0.7575 | 0.0029 | 0.0732 | 0.2702 |
| **Phenylalanine** | 0.1569 | 0.0082 | 0.4131 | 0.0005 | 0.5809 | 0.0027 |
| **Lysine** | 0.2476 | 0.0244 | 0.7783 | 0.0028 | 0.4292 | 0.0253 |
| **Hydroxyproline** | 0.0015 | 0.5343 | 0.0240 | 0.0043 | 0.3123 | 0.0641 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

*Muscle versus Skin*

The predominant fatty acids in both muscle and skin of both strains and size classes were C16:0, C16:1ω7, C18:0 C18:1ω9, C18:2ω6 C18:3ω3, C20:4ω6, C22:0 and C22:6ω3. None of the fatty acids showed any fixed trends when their mean concentrations were compared between muscle and skin.

**Table 10*a*:** The mean fatty acid composition of *C. gariepinus* muscle (% of total fatty acids measured)

| Fatty acid | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GML n=6 | GMS n=5 | NML n=6 | NMS n=5 | |
| C14:0 | 1.1 | 1.0 | 1.2 | 1.3 | 0.0714 |
| C16:0 | 23.8 | 24.8 | 24.6 | 24.7 | 0.4792 |
| C16:1ω7 | 5.1 | 5.9 | 5.6 | 5.4 | 0.3136 |
| C18:0 | 7.0 | 7.7 | 7.3 | 7.8 | 0.2460 |
| C18:1ω9 | 28.9 | 32.3 | 30.9 | 29.6 | 1.2375 |
| C18:2ω6 | 18.3 | 12.4 | 15.2 | 13.4 | 0.7743 |
| C18:3ω3 | 2.4 | 2.2 | 2.2 | 1.9 | 0.1102 |
| C20:4ω6 | 1.6 | 1.6 | 1.5 | 2.0 | 0.3761 |
| C20:5ω3 | 0.8 | 0.5 | 0.7 | 0.6 | 0.0555 |
| C22:0 | 1.7 | 1.9 | 1.5 | 2.3 | 0.1629 |
| C22:5ω3 | 0.8 | 0.6 | 0.7 | 0.7 | 0.0727 |
| C22:6ω3 | 3.4 | 3.1 | 3.3 | 4.3 | 0.4507 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 10*b*:** Probability values calulated for pairwise differences of fatty acids of the muscle of two *C. gariepinus* strains.

| **Fatty acid** | **$p > \mid T \mid$ HO: LSMean$_i$=LSMean$_j$** | | | | | |
|---|---|---|---|---|---|---|
| | **GML=GMS** | **GML=NML** | **GML=NMS** | **GMS=NML** | **GMS=NMS** | **NML=NMS** |
| **C14:0** | 0.5055 | 0.2250 | 0.0878 | 0.0733 | 0.0263 | 0.5646 |
| **C16:0** | 0.1511 | 0.2748 | 0.2197 | 0.6830 | 0.8349 | 0.8484 |
| **C16:1ω7** | 0.0937 | 0.2515 | 0.5042 | 0.5432 | 0.3210 | 0.6640 |
| **C18:0** | 0.0452 | 0.3383 | 0.0259 | 0.2548 | 0.8108 | 0.1678 |
| **C18:1ω9** | 0.0763 | 0.2538 | 0.7242 | 0.4739 | 0.1672 | 0.4574 |
| **C18:2ω6** | 0.0001 | 0.0069 | 0.0002 | 0.0236 | 0.4325 | 0.1310 |
| **C18:3ω3** | 0.4350 | 0.4728 | 0.0183 | 0.9227 | 0.1137 | 0.0819 |
| **C20:4ω6** | 0.9348 | 0.7396 | 0.5703 | 0.8140 | 0.5344 | 0.3785 |
| **C20:5ω3** | 0.0003 | 0.3060 | 0.0265 | 0.0037 | 0.0951 | 0.1911 |
| **C22:0** | 0.3688 | 0.5015 | 0.0179 | 0.1288 | 0.1382 | 0.0036 |
| **C22:5ω3** | 0.0425 | 0.1420 | 0.2656 | 0.5018 | 0.3542 | 0.7641 |
| **C22:6ω3** | 0.7394 | 0.9814 | 0.1697 | 0.7563 | 0.1055 | 0.1632 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 11*a*:** The mean fatty acid composition of *C. gariepinus* skin (% of total fatty acids measured)

| Fatty acid | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GSL n=6 | GSS n=5 | NSL n=6 | NSS n=5 | |
| **C14:0** | 1.0 | 1.1 | 1.0 | 1.2 | 0.0714 |
| **C16:0** | 23.1 | 25.6 | 23.9 | 25.4 | 0.4792 |
| **C16:1ω7** | 4.7 | 6.1 | 5.6 | 5.4 | 0.3136 |
| **C18:0** | 7.6 | 7.4 | 7.4 | 8.7 | 0.2460 |
| **C18:1ω9** | 28.6 | 31.3 | 30.5 | 29.7 | 1.2375 |
| **C18:2ω6** | 16.1 | 13.5 | 15.1 | 12.1 | 0.7743 |
| **C18:3ω3** | 2.3 | 2.3 | 2.4 | 2.0 | 0.1102 |
| **C20:4ω6** | 2.9 | 2.1 | 1.9 | 3.0 | 0.3761 |
| **C20:5ω3** | 0.7 | 0.4 | 0.7 | 0.5 | 0.0555 |
| **C22:0** | 2.0 | 1.7 | 1.7 | 2.1 | 0.1629 |
| **C22:5ω3** | 0.9 | 0.6 | 0.7 | 0.6 | 0.0727 |
| **C22:6ω3** | 4.2 | 2.6 | 3.7 | 3.3 | 0.4507 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

**Table 11*b*:** Probability values calculated for pairwise differences between the fatty acids of the skin of two *C. gariepinus* strains.

| Fatty acid | $p > \mid T \mid$ HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | GSL=GSS | GSL=NSL | GSL=NSS | GSS=NSL | GSS=NSS | NSL=NSS |
| **C14:0** | 0.3547 | 0.9254 | 0.0815 | 0.3013 | 0.4469 | 0.0614 |
| **C16:0** | 0.0055 | 0.2599 | 0.0110 | 0.0437 | 0.8012 | 0.0801 |
| **C16:1ω7** | 0.0174 | 0.0681 | 0.2807 | 0.3368 | 0.2154 | 0.6313 |
| **C18:0** | 0.6711 | 0.6965 | 0.0109 | 0.9166 | 0.0086 | 0.0038 |
| **C18:1ω9** | 0.2299 | 0.2937 | 0.6161 | 0.7288 | 0.5253 | 0.6978 |
| **C18:2ω6** | 0.0695 | 0.4130 | 0.0071 | 0.2274 | 0.3763 | 0.0304 |
| **C18:3ω3** | 0.7225 | 0.3237 | 0.1926 | 0.6284 | 0.1407 | 0.0328 |
| **C20:4ω6** | 0.2163 | 0.0791 | 0.9678 | 0.8037 | 0.2527 | 0.1205 |
| **C20:5ω3** | 0.0166 | 0.8283 | 0.1644 | 0.0212 | 0.3292 | 0.2089 |
| **C22:0** | 0.2243 | 0.1541 | 0.7716 | 0.9766 | 0.1802 | 0.1304 |
| **C22:5ω3** | 0.0061 | 0.0577 | 0.0123 | 0.1870 | 0.8027 | 0.2981 |
| **C22:6ω3** | 0.0543 | 0.4431 | 0.2784 | 0.1712 | 0.4284 | 0.6388 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

*Mineral concentrations*

*Muscle*

The mean mineral profile of the muscles for strains and size classes are shown in Table 12*a* and the statistical analysis thereof, in Table 12*b*. In the comparison of strain within size class, potassium content was significantly lower in the muscle of the normal strain, in both size classes, whilst the magnesium, manganese and copper

contents were similar in the larger sized class, but significantly lower in NMS when compared to GMS. The other minerals did not differ between the strains within a size class. When comparing the mineral content between sizes within a strain, potassium content was lower in the larger size class in both the golden and normal coloured strains. Manganese and copper also varied in the golden strain (GML=GMS), but did not vary in the normal strain, where phosphorus varied (NML=NMS).

*Skin*

The mean mineral content of the skin is shown in Table 13*a* whilst Table 13*b* shows the statistical analysis of the differences between the means within, and between strains and size classes. No statistically significant differences were found between the size classes within a strain, with the exception of copper, which was higher in NSL than in NSS. In a comparison of the mean mineral composition of strain within a size class, no trend was manifested for the two size classes. In the larger sized class, statistical differences were noted for calcium and zinc concentrations, whilst only manganese showed a statistical difference in the smaller sized class.

**Table 12*a*:** The mean mineral composition of *C. gariepinus* muscle (dry mass)

| Mineral | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GML n=6 | GMS n=5 | NML n=6 | NMS n=5 | |
| P mg/g | 9.7 | 9.8 | 9.7 | 10.5 | 0.0284 |
| Ca mg/g | 1.2 | 1.4 | 1.0 | 1.6 | 0.0451 |
| Mg mg/g | 2.1 | 2.6 | 2.1 | 1.9 | 0.0203 |
| K mg/g | 14.7 | 16.4 | 13.1 | 13.7 | 0.0624 |
| Zn μg/g | 34.6 | 38.8 | 42.3 | 25.0 | 7.7544 |
| Fe μg/g | 28.4 | 25.0 | 28.2 | 27.9 | 8.8791 |
| Mn μg/g | 2.2 | 4.5 | 1.6 | 1.2 | 0.3755 |
| Cu μg/g | 2.1 | 4.8 | 1.8 | 1.8 | 0.3165 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

**Table 12*b*:** Probability values calculated for pairwise differences of the mineral composition of the muscle of two *C. gariepinus* strains.

| Mineral | $p>$ \| T \| HO: $LSMean_i=LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | GML=GMS | GML=NML | GML=NMS | GMS=NML | GMS=NMS | NML=NMS |
| P | 0.7717 | 0.9672 | 0.0795 | 0.8018 | 0.1556 | 0.0859 |
| Ca | 0.7395 | 0.7131 | 0.5859 | 0.4959 | 0.8381 | 0.3752 |
| Mg | 0.1131 | 0.8581 | 0.6391 | 0.0811 | 0.0526 | 0.7649 |
| K | 0.0729 | 0.0727 | 0.0001 | 0.0010 | 0.0235 | 0.0001 |
| Zn | 0.7156 | 0.4883 | 0.4122 | 0.7655 | 0.2597 | 0.1436 |
| Fe | 0.8009 | 0.9875 | 0.9716 | 0.8124 | 0.8358 | 0.9834 |
| Mn | 0.0003 | 0.2763 | 0.0696 | 0.0001 | 0.0001 | 0.4175 |
| Cu | 0.0001 | 0.4985 | 0.4233 | 0.0001 | 0.0001 | 0.8752 |

Where GML=gold muscle large, GMS=gold muscle small, NML=normal muscle large and NMS=normal muscle small.

*Muscle versus Skin*

The muscle phosphorus, magnesium and potassium contents were higher than that of the skin for both strains and size classes. The concentrations of the latter two minerals were always higher in the larger size class for both tissues. The calcium, iron, copper and manganese content of the different tissues showed no fixed trend between and within strains and size classes. The iron and zinc contents showed high variations within the sample classes as manifested by the large standard errors of the LSMean (Tables 7*a* & 8*a*). These high coefficients of variation explain why mean values that seem different, do not differ significantly (for example Table 7*b*: NML=NMS = 0.1436 when the Zn values for NML and NMS are 42.3 and 25.0 $\mu$g/g DM, respectively).

**Table 13*a*:** The mean mineral composition of *C. gariepinus* skin (dry mass)

| Mineral | LSMeans | | | | Standard Error of Mean |
|---|---|---|---|---|---|
| | GSL n=6 | GSS n=5 | NSL n=6 | NSS n=5 | |
| P mg/g | 5.3 | 5.8 | 5.5 | 5.5 | 0.0284 |
| Ca mg/g | 2.5 | 1.2 | 1.3 | 1.3 | 0.0451 |
| Mg mg/g | 1.0 | 0.8 | 1.0 | 0.8 | 0.0203 |
| K mg/g | 1.7 | 2.4 | 1.7 | 3.1 | 0.0624 |
| Zn μg/g | 37.7 | 52.3 | 57.1 | 69.4 | 7.7544 |
| Fe μg/g | 44.7 | 19.3 | 55.0 | 28.5 | 8.8791 |
| Mn μg/g | 1.2 | 1.1 | 1.8 | 2.5 | 0.3755 |
| Cu μg/g | 3.0 | 3.5 | 3.6 | 2.6 | 0.3165 |

**Table 13*b*:** Probability values calculated for pairwise differences of the skin mineral composition of two *C. gariepinus* strains.

| Mineral | $p >$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|
| | GSL=GSS | GSL=NSL | GSL=NSS | GSS=NSL | GSS=NSS | NSL=NSS |
| P | 0.2720 | 0.4858 | 0.5688 | 0.5916 | 0.6420 | 1.0000 |
| Ca | 0.1167 | 0.0729 | 0.1555 | 0.9225 | 0.8921 | 0.9528 |
| Mg | 0.6281 | 0.9770 | 0.5824 | 0.6116 | 0.9544 | 0.5664 |
| K | 0.5209 | 0.9955 | 0.2030 | 0.5180 | 0.5770 | 0.2014 |
| Zn | 0.2849 | 0.0864 | 0.0248 | 0.7237 | 0.2805 | 0.3691 |
| Fe | 0.1080 | 0.3591 | 0.2990 | 0.3781 | 0.6083 | 0.7691 |
| Mn | 0.8015 | 0.3008 | 0.0575 | 0.2743 | 0.0631 | 0.2748 |
| Cu | 0.2456 | 0.1840 | 0.5469 | 0.9413 | 0.1305 | 0.0955 |

Where GSL=gold skin large, GSS=gold skin small, NSL=normal skin large and NSS=normal skin small.

*Comparison of farmed and wild catfish*

*Proximate analysis*

Results of basic proximate analysis of the farmed and wild males and females, respectively, as well as pooled data, are given in Table 14. Mean body mass of the selected farmed males (606.0 g) and females (586.8 g) did not differ significantly when tested by Duncan's multiple range test ($p \leq 0.05$). The mean body mass of wild males (1 096.4 g) and females (865.7 g) differed significantly from each other and from that of farmed males and females.

The calculated mean percentage protein in the fillets of the four groups, on a fat free, dry mass basis were 91.5% for farmed males, 92.0% for farmed females, 92.2% for wild males and 93.7% in wild females, the differences being non-significant. The mean percentages fat in the fillets on a dry mass basis, of the four groups were 11.4% for farmed males, 10.0% for farmed females, 13.1% for wild males and 9.4% for wild females, these differences also being non-significant (Table 14). The large coefficient of variation in percentage fat (Table 14) indicates that a wide range of total body fat is experienced for *C. gariepinus*.

*Fatty acids*

The mean percentages fatty acids for the four groups and the pooled data are shown in Table 15. Concentrations are expressed as a percentage of area for all fatty acids identified. Sex did not significantly influence the fatty acid profile within type. Fatty acids with uneven numbers of carbon atoms were present in small quantities (<2.0%), with wild fish (male and female) having statistically significantly higher concentrations of C17:0 and C17:1 than farmed catfish.

**Table 14:** Fillet proximate composition of male and female farmed and wild African catfish, *C. gariepinus*.

| Variable | | Mean | Standard Deviation | Lowest Value | Highest Value |
|---|---|---|---|---|---|
| **Farmed male** | | | | | |
| BM (g) | 5 | 606.0 | 57.038 | 554.5 | 700.5 |
| % Moisture | 5 | 77.0 | 1.937 | 75.1 | 79.4 |
| % Protein (DM) | 5 | 81.1 | 5.951 | 73.4 | 88.2 |
| % Fat (DM) | 5 | 11.4 | 6.792 | 3.4 | 20.5 |
| % Ash (DM) | 5 | 2.2 | 0.371 | 1.7 | 2.5 |
| **Farmed female** | | | | | |
| BM (g) | 5 | 586.8 | 95.362 | 520.0 | 751.6 |
| % Moisture | 5 | 78.0 | 2.393 | 74.5 | 80.6 |
| % Protein (DM) | 5 | 82.8 | 7.209 | 70.3 | 88.1 |
| % Fat (DM) | 5 | 10.0 | 9.034 | 4.3 | 25.7 |
| % Ash (DM) | 5 | 2.2 | 0.751 | 1.5 | 3.1 |
| **Wild male** | | | | | |
| BM (g) | 5 | 1096.4 | 162.379 | 919.1 | 1 278.5 |
| % Moisture | 5 | 78.2 | 2.117 | 75.4 | 80.6 |
| % Protein (DM) | 5 | 80.2 | 8.677 | 68.6 | 90.2 |
| % Fat (DM) | 5 | 13.1 | 9.078 | 2.8 | 25.5 |
| % Ash (DM) | 5 | 1.4 | 0.285 | 1.1 | 1.8 |
| **Wild female** | | | | | |
| BM (g) | 5 | 865.7 | 145.053 | 666.6 | 1045.6 |
| % Moisture | 5 | 78.4 | 1.137 | 77.5 | 79.7 |
| % Protein (DM) | 5 | 84.9 | 3.538 | 81.8 | 90.8 |
| % Fat (DM) | 5 | 9.4 | 2.690 | 5.5 | 12.0 |
| % Ash (DM) | 5 | 1.9 | 0.398 | 1.5 | 2.5 |
| **Pooled** | | | | | |
| BM (g) | 20 | 788.7 | 242.010 | 520.00 | 1278.5 |
| % Moisture | 20 | 77.9 | 1.879 | 74.50 | 80.6 |
| % Protein (DM) | 20 | 82.2 | 6.354 | 68.64 | 90.8 |
| % Fat (DM) | 20 | 11.0 | 6.920 | 2.80 | 25.7 |
| % Ash (DM) | 20 | 1.9 | 0.556 | 1.10 | 3.1 |

where BM = body mass, DM = dry mass

**Table 15:** Results of Duncan's multiple range test for means of male and female farmed and wild *C. gariepinus* fillet fatty acid contents expressed as percentage of total identified fatty acids (±SD in parenthesis).

| Fatty acid | Farmed male | Farmed female | Wild male | Wild female | MSE | POOLED |
|---|---|---|---|---|---|---|
| | Means* | | | | | |
| 14:0 | 2.5[a] (2.396) | 2.0[a] (2.780) | 2.8[a] (2.674) | 5.3[a] (3.610) | 8.412 | 3.1 (2.977) |
| 15:0 | 0.2[a] (0.198) | 0.0[a] (0.080) | 0.4[a] (0.472) | 0.4[a] (0.397) | 0.107 | 0.3 (0.344) |
| 16:0 | 24.8[a] (2.114) | 24.2[a] (1.055) | 24.2[a] (2.153) | 23.6[a] (1.296) | 3.005 | 24.2 (1.651) |
| 16:1ω7 | 5.3[a] (0.938) | 5.4[a] (0.711) | 12.6[b] (2.810) | 12.8[b] (2.164) | 3.490 | 9.0 (4.125) |
| 17:0 | 0.2[a] (0.384) | 0.0[a] (0.000) | 1.4[b] (1.262) | 1.8[b] (1.037) | 0.704 | 0.8 (1.100) |
| 17:1 | 0.1[a] (0.092) | 0.0[a] (0.000) | 1.1[b] (0.176) | 1.3[b] (0.167) | 0.132 | 0.6 (0.674) |
| 18:0 | 9.0[a] (1.744) | 8.4[a] (0.686) | 8.7[a] (1.759) | 8.1[a] (0.948) | 1.876 | 8.5 (1.297) |
| 18:1ω9 | 33.5[a] (1.121) | 32.6[a] (1.766) | 29.8[b] (2.901) | 27.5[b] (1.589) | 3.830 | 30.8 (3.017) |
| 18:2ω6 | 19.3[a] (2.088) | 21.0[a] (3.883) | 8.4[b] (3.258) | 8.3[b] (1.159) | 7.849 | 14.3 (6.604) |
| 18:3ω3 | 2.5[a] (0.659) | 3.0[a] (0.828) | 3.2[a] (1.935) | 3.4[a] (1.955) | 2.171 | 3.0 (1.392) |
| 20:0 | 0.6[a] (0.540) | 0.9[a] (0.562) | 0.4[a] (0.378) | 0.8[a] (0.198) | 0.197 | 0.7 (0.448) |
| 20:1 | 0.4[a] (0.407) | 0.8[a] (0.577) | 0.6[a] (0.610) | 1.3[a] (1.109) | 0.525 | 0.8 (0.738) |
| 22:0 | 0.9[a] (0.255) | 1.3[a] (0.445) | 2.7[b] (1.574) | 2.8[b] (0.427) | 0.731 | 1.9 (1.161) |
| 22:1ω11 | 0.8[a] (0.164) | 0.6[a] (0.223) | 2.1[b] (1.381) | 2.1[b] (0.707) | 0.621 | 1.4 (1.032) |

* means with the same letter are not significantly different ($p<0.05$).

Wild catfish also had significantly higher levels of palmitoleic acid (C16:1), but significantly lower levels of oleic (C18:1) and linoleic (C18:2) fatty acids when compared to farmed catfish (Table 15). The percentage difference of linoleic acid was $\pm 12\%$. Wild catfish also had a statistically significantly higher percentage of behenic acid (C22:0). The means of the other fatty acids tested non-significant.

*Amino acids*

For all amino acids tested (Table 16), the differences between farmed male and female catfish tested non-significant. However, wild males and females differed statistically ($p<0.05$) in respect of the following amino acids: aspartic acid, glutamic acid, phenylalanine and methionine. Although significant, the calculated differences between these means were all less than 1%.

Ignoring the influence of sex, a comparison between farmed and wild catfish showed only tyrosine, valine, leucine and hydroxyproline to differ significantly ($p<0.05$). The percentages for these specific amino acids for farmed and wild sharptooth catfish were tyrosine, 3.01 and 2.89; valine, 3.89 and 3.80; leucine, 6.57 and 6.25; hydroxyproline, 0.38 and 0.48, respectively.

*Mineral composition*

The mineral composition of farmed and wild male and female *C. gariepinus* are summarised in Table 17. Mean concentrations of iron showed the highest variation of all the minerals (Mean Square Error = 627.575 ppm). The iron concentration of the wild males (84.4 ppm) and females (80.6 ppm) did not differ statistically significantly from one another and from that of the farmed male (106.8 ppm) and females (70.0 ppm), although the latter two differed statistically significantly from each other. Zinc showed the second highest variation (MSE = 30.150 ppm) of the analysed minerals, but did not differ significantly between types and between sex within type. Calcium, magnesium and copper showed no significant difference between sex within type. The farmed males (1.126 mg/g) had significantly higher phosphorous values than the farmed females (0.946 mg/g), wild males (0.958 mg/g) and females (0.868 mg/g). The wild catfish did not differ significantly from one another.

Although the sexes within type did not differ significantly from one another for potassium (Table 17), there was a significant difference between the farmed females (1.642 mg/g) and wild males (1.884 mg/g), but not between farmed males (1.836 mg/g) and wild females (1.718 mg/g). For manganese, a significant difference between farmed males (3.4 ppm) and wild males (2.0 ppm) was found, but no significant difference between the respective females, or between the sexes within types was noted.

**Table 16:** Results of the Duncan's multiple range test for the means of different amino acids of male and female farmed and wild *C. gariepinus* fillets (g/100 g fat free DM, ±SD in parenthesis)

| Amino acid | Farmed male | Farmed female | Wild male | Wild female | MSE | POOLED |
|---|---|---|---|---|---|---|
| | Means* | | | | | |
| Asp | 8.4$^{ab}$ (0.161) | 8.3$^{b}$ (0.114) | 8.3$^{b}$ (0.150) | 8.6$^{a}$ (0.219) | 0.027 | 8.4 (0.195) |
| Glu | 13.2$^{ab}$ (0.428) | 13.3$^{ab}$ (0.111) | 13.0$^{b}$ (0.250) | 13.4$^{a}$ (0.347) | 0.0951 | 3.2 (0.330) |
| Ser | 3.6$^{a}$ (0.092) | 3.5$^{a}$ (0.068) | 3.5$^{a}$ (0.055) | 3.5$^{a}$ (0.061) | 0.005 | 3.5 (0.075) |
| Gly | 4.3$^{a}$ (0.297) | 4.4$^{a}$ (0.165) | 4.1$^{a}$ (0.128) | 4.3$^{a}$ (0.229) | 0.046 | 4.3 (0.220) |
| His | 1.9$^{a}$ (0.049) | 1.9$^{a}$ (0.026) | 1.8$^{b}$ (0.056) | 1.9$^{ab}$ (0.100) | 0.004 | 1.9 (0.071) |
| Arg | 5.9$^{a}$ (0.198) | 6.1$^{a}$ (0.112) | 5.9$^{a}$ (0.151) | 6.0$^{a}$ (0.205) | 0.029 | 6.0 (0.171) |
| Tre | 4.0$^{a}$ (0.108) | 3.9$^{a}$ (0.053) | 3.9$^{a}$ (0.051) | 4.0$^{a}$ (0.074) | 0.006 | 4.0 (0.075) |
| Ala | 5.2$^{a}$ (5.216) | 5.2$^{a}$ (0.114) | 5.2$^{a}$ (0.123) | 5.3$^{a}$ (0.152) | 0.021 | 5.2 (0.143) |
| Pro | 3.0$^{a}$ (0.110) | 3.0$^{a}$ (0.077) | 3.1$^{a}$ (0.168) | 3.1$^{a}$ (0.129) | 0.012 | 3.0 (0.126) |
| Tyr | 3.0$^{a}$ (3.016) | 3.0$^{a}$ (0.034) | 2.8$^{b}$ (0.181) | 3.0$^{ab}$ (0.142) | 0.015 | 3.0 (0.142) |
| Val | 3.9$^{a}$ (0.104) | 3.9$^{a}$ (0.041) | 3.7$^{b}$ (0.138) | 3.9$^{ab}$ (0.079) | 0.009 | 3.8 (0.111) |
| Iso | 3.6$^{a}$ (0.111) | 3.6$^{a}$ (0.090) | 3.4$^{a}$ (0.263) | 3.6$^{a}$ (0.191) | 0.031 | 3.6 (0.190) |
| Leu | 6.6$^{a}$ (0.196) | 6.6$^{a}$ (0.073) | 6.1$^{b}$ (0.387) | 6.4$^{ab}$ (0.275) | 0.067 | 6.4 (0.312) |
| Phe | 3.5$^{a}$ (0.069) | 3.4$^{a}$ (0.080) | 3.1$^{b}$ (0.303) | 3.4$^{a}$ (0.296) | 0.048 | 3.3 (0.255) |
| Lys | 10.1$^{a}$ (0.458) | 9.9$^{a}$ (0.374) | 9.9$^{a}$ (0.851) | 10.3$^{a}$ (1.255) | 0.6621 | 0.1 (0.760) |
| Met | 2.6$^{ab}$ (0.111) | 2.6$^{ab}$ (0.068) | 2.5$^{b}$ (0.174) | 2.7$^{a}$ (0.120) | 0.015 | 2.6 (0.137) |
| Hyd-Pro | 0.4$^{a}$ (0.117) | 0.4$^{a}$ (0.079) | 0.5$^{a}$ (0.079) | 0.5$^{a}$ (0.199) | 0.010 | 0.4 (0.109) |

* means with the same letter are not significantly different ($p<0.05$)

**Table 17:** Results of the Duncan's multiple range test for mineral content of male and female farmed and wild *C. gariepinus* fillets (means expressed on a DM basis, ±SD in parenthesis).

| Mineral | Farmed male | Farmed female | Wild male | Wild female | MSE | POOLED |
|---|---|---|---|---|---|---|
| | Means* | | | | | |
| Ca mg/g | 0.192[a] | 0.182[a] | 0.152[a] | 0.150[a] | 0.001 | 0.169 |
| | (0.026) | (0.050) | (0.016) | (0.037) | | (0.037) |
| P mg/g | 1.126[a] | 0.946[b] | 0.958[b] | 0.868[b] | 0.007 | 0.975 |
| | (0.080) | (0.109) | (0.054) | (0.088) | | (0.124) |
| Mg mg/g | 0.217[a] | 0.203[a] | 0.221[a] | 0.223[a] | 0.000 | 0.216 |
| | (0.007) | (0.019) | (0.015) | (0.015) | | (0.016) |
| K mg/g | 1.836[ab] | 1.642[b] | 1.884[a] | 1.718[ab] | 0.024 | 1.770 |
| | (0.109) | (0.181) | (0.144) | (0.173) | | (0.172) |
| Mn ppm | 3.4[a] | 2.8[ab] | 2.0[b] | 2.4[ab] | 0.575 | 2.650 |
| | (0.548) | (0.837) | (0.000) | (1.140) | | (0.875) |
| Fe ppm | 106.8[a] | 70.0[b] | 84.4[ab] | 80.6[ab] | 627.575 | 85.450 |
| | (46.976) | (4.062) | (15.726) | (6.309) | | (26.791) |
| Cu ppm | 5.6[a] | 5.0[a] | 5.6[a] | 4.6[a] | 9.850 | 5.200 |
| | (1.140) | (0.707) | (5.320) | (3.050) | | (2.913) |
| Zn ppm | 30.4[a] | 28.8[a] | 31.6[a] | 32.6[a] | 30.150 | 30.850 |
| | (6.804) | (4.658) | (5.595) | (4.615) | | (5.244) |

* means with the same letter are not significantly different ($p<0.05$).

## DISCUSSION

The observation that the wild strain grew better than any of the other strains (Fig 1), is surprising as the other three strains had, over a number of generations, all been selected for, amongst others, fast growth rate. Although the wild strain had the highest mean final mass, it also had the highest variation (SD=2.876). Similarly, the Netherland strain also showed a SD higher than that for the domestic and golden strains. This high variation in the mean final mass of the wild strain is expected as the wild strain has had no artifical selection applied to it for any parameter and should therefore show a high degree of heterozygosity (Van der Walt *et al.*, 1993*b*). Although the Netherland strain has been selected for high growth rate (Van der Walt, *et al.*, 1993*b*), it was crossbred with domestic males (also selected for superior growth rate - Van der Walt *et al.*, 1993*a*) who were not genetically linked, resulting in offspring that should show zero inbreeding (Falconer, 1981). This outbreeding would explain the high standard deviation in the mean final body mass of this strain (Falconer, 1981). The small variation in body mass of the golden and domestic strains is a direct result of the selection history of these two strains (Van der Walt *et al.*, 1993*a*). At present, the golden and wild strains have not been analysed electrophoretically, to see whether the allele (glucose-6-phosphate isomerase), associated with a fast growth rate (Van der Walt *et al.*, 1993*a*), is present. The poor growth rate expressed by the domestic strain (which has been identified as possessing glucose-6-phosphate isomerase - Van der Walt, *et al.* 1993*a*), is disappointing, but may be explained by differing environmental conditions in the present investigation to that of Van der Walt and co-workers (1993*a*). These authors (Van der Walt *et al.* 1993*a*) note that a fish from a given locality, selected for rapid growth according to a specific biochemical genotype, might not grow according to its expected performance in a different environment. The results of Reinitz (1977) support this observation. Reinitz (1977) reported a strong genotype-environmental interaction in rainbow trout, where it was found that transferrin phenotypes associated with increased mass, differed between the localities where the fish were raised. A number of investigations on the growth rates of different rainbow trout strains at different temperatures and diets (Ayles, Bernard & Hendzel, 1979; Smith *et al.*, 1988; Wangila & Dick, 1988), has also shown that genotype does influence the growth rate in this species.

The occurrence that the faster growing strains (wild and golden in this investigation) show body total lipid concentrations lower than the other strains (Table 2*a*) has also been reported for rainbow trout (*Salmo gairdneri* - Ayles *et al.*, 1979; Smith *et al.*, 1988). Gjerde (1989) however, found no correlation between growth rate and body composition in rainbow trout. Gjerde and Schaeffer (1989) showed body lipid content to have a heritability of 0.47, whilst that of moisture and protein was 0.33 and 0.03, respectively, in rainbow trout. Although a number of authors (Austreng and Refstie, 1979; Gjerde, 1989; Gjerde and Schaeffer, 1989) also noted a genetic influence on the body protein content of rainbow trout, Shearer (1994) on re-evaluation of their data, concluded that the authors did not take size as a covariant when analysing the data and that size (and diet), rather than

strain, influences the body protein composition of fish. The present investigation supports the conclusion of Shearer (1994), in that no significant difference in body protein content was noted between the four strains who were all similar sized.

Interpretation of the relevance of the statistically different concentrations of the various amino acids between the four strains are difficult to make, firstly because these dissimilarities are relatively small in terms of mass differences, and secondly, no literature (with the exception of our own study - Table 8) pertaining to the influence of strain on the body amino acid composition of fish could be found. In the study on the comparison of normal and gold coloured catfish (Table 8), similar results to the present investigation were noted. In the latter study (Table 8), the golden strain (similar genetic pool to golden strain in present investigation) was compared to a normal coloured domesticated catfish strain (not genetically related to any of the three normal coloured strains in the present investigation). The same amino acids as noted in Table 3a and b, as well as tyrosine, valine and methionine showed statistically significant differences between the two strains (Table 8). The size of the fish was also shown to influence the muscle amino acid content within a strain (Table 8).

The strong genetic influence of strain on the total lipid fatty acid composition reported (Table 4), has also been noted for the channel catfish (*Ictalurus punctatus* - Erickson, 1992). In a study on the fatty acid profiles of total lipids from three channel catfish strains, significant differences were found in the levels of PUFAs, $\omega 3$ and $\omega 6$ fatty acids. These differences were attributed to genetic factors, as age, diet and environment were similar between the strains (Erickson, 1992). Erickson (1992) also noted work done on rainbow trout (Lampi, 1986) where strain was shown to affect the degree of saturation of the lipid fatty acids.

In the investigation into influence of genetic strains on the muscle mineral composition (Table 5), no differences were found in the phosphorus and calcium contents between the strains (results consistent with that in Table 12). Size however, caused a significant difference in the phosphorus concentration within strain, the smaller sized fish yielding a higher concentration (Table 12). Similar to the present investigation (Table 5), significant strain influences were noted for magnesium, potassium, manganese and copper (Table 12). Size also influenced the concentration of these minerals within the same strain. In the present investigation (Table 5a and b), zinc and iron concentrations show significant differences between some of the strains, whilst in the latter investigation (Table 12), no differences were noted. In a comparison of the concentration of several metals in the muscle tissue of two strains of channel catfish, Erickson (1993) found no differences in the levels of copper and iron, but significant differences in the levels of manganese and zinc.

An external study of the golden African sharptooth catfish, *C. gariepinus*, has shown it to have characteristics typical of albinism, that is, complete absence of melanin in the chromatophores (Fig 4). Another characteristic

is the pink eye colouration observed, which is due to an absence of melanin in the iris or retina resulting in the visibility of the blood vessels in the eye. Albinism is not a rare phenomena in fish species and has been reported for, amongst others, tilapia, *Oreochromis mossambicus,* (Tave, Lovell, Smitheran & Rezk, 1990), the American catfish, *Ictalurus punctatus* (Heaton, Brunink & Richter, 1973) and the grass carp, *Ctenopharyngodon idella* (Rothard & Wohlfarth, 1993). The latter authors also note cases of albinism occurring in medaka *(Oryzias latipes)*, Siamese fighting fish (*Betta splendens*), paradise fish (*Macropodus opercularis*), swordtail (*Xiphpohorus helleri*), goldfish (*Carassius auratus*), catla (*Catla catla*) as well as rainbow trout (*Salmo gairdneri*).

**Figure 4:** A colour photo of a golden *Clarias gariepinus* specimen showing a complete absence of melanin.

The first albino catfish, a female, was seined in Turfloop Dam, Lebowa, during 1984 and bred artificially using standard techniques (Schoonbee *et al.,* 1980) to a normal coloured (wild-type) male from the same locality. The resulting progeny were all normal coloured. Siblings from this F1 generation were crossbred. The resulting F2 generation contained normal and albino offspring. The normal and albino strains have since been maintained and breed true at each successive spawning. The F1 offspring all being normal coloured, shows that the wild-type is dominant to albino. It also seems as if the albinism is determined by a single recessive allele as in

rainbow trout, grass carp (Rothard & Wohlfarth, 1993) and channel catfish (Tucker & Robinson, 1990), which is not sex-linked.

The lighter colouration of the muscle of the albino African catfish to the normal coloured (Fig 5), has also been reported for the American channel catfish (Heaton *et al.,* 1973) and for tilapia (Tave *et al.,* 1990). Similarly to the tilapia, the albino African catfish has a lighter coloured peritoneal lining compared to the normal coloured counter-part, this has significant advantages during the processing of fish flesh, especially mincing (Grantham, 1981 - Fig 6).

**Figure 5:** *Clarias gariepinus* fillets. The lighter coloured fillet being that of a golden coloured specimen and the darker that of a normal coloured specimen.

The muscle proximate composition of this investigation (Table 6) differs slightly from that of a previous study on the same species where a higher protein (18.2%) and lower lipid (2.4%) content was reported (Hoffman, Casey & Prinsloo, 1992). In the present investigation, the largest variation in the muscle proximate composition between the two size groups occurred between the moisture and total lipid content. The phenomena that older fish contain more muscle total lipid than young fish (Tables 6*a* & 6*b*), has also been reported for other fish species, such as channel catfish (*Ictalurus punctatus* - Tidwell & Robinette, 1990; Li & Lovell, 1992; Lovell

& Li, 1992), rainbow trout (*Salmo gairdneri* = *Oncorhynchus mykiss* - Reinitz 1983; Kiessling *et al.*, 1991) and silver salmon (*Oncorhynchus kisutch* - Karrick & Thurston, 1964). In these studies it was also noted that the fish showed a correspondingly decrease in muscle moisture content with size, an occurrence common to fish, where additional energy stored as fat simply replaces body water (Reinitz, 1983).

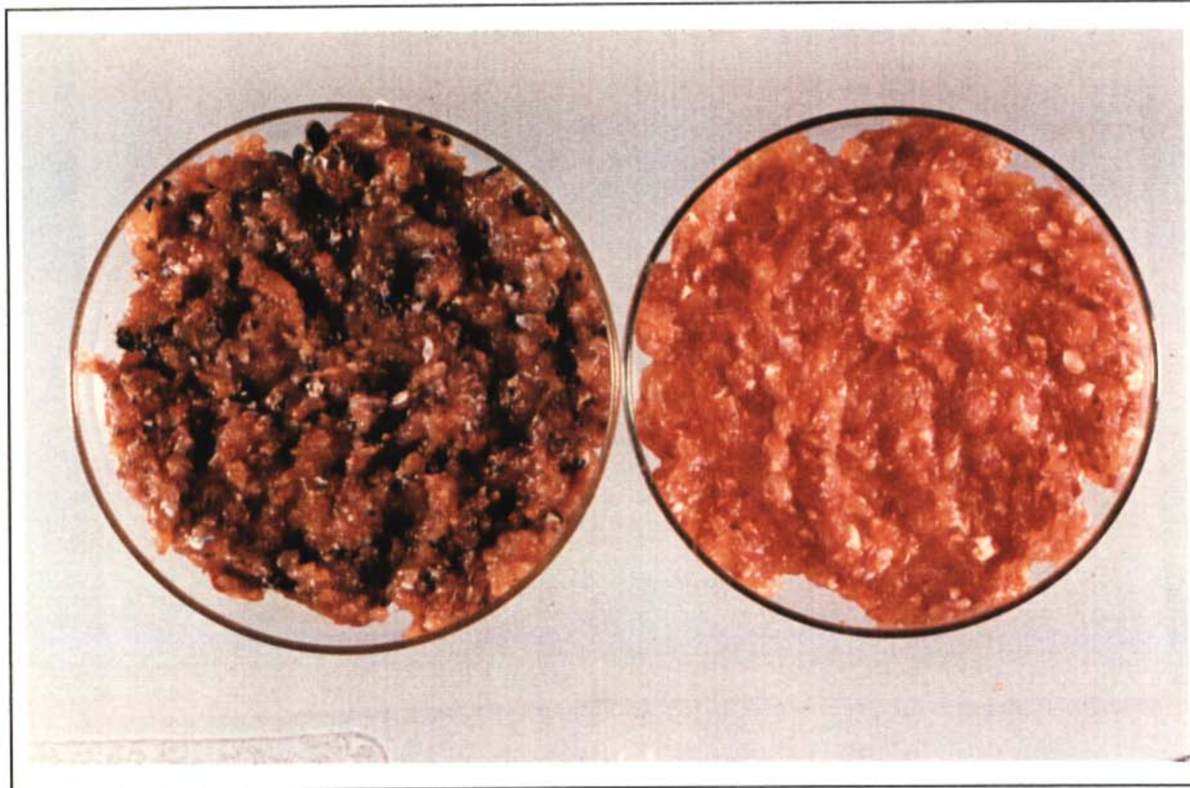

**Figure 6:** Minced *Clarias gariepinus* fillets (skin included). The darker samples are from normal coloured specimens and the lighter from golden specimens.

The higher lipid content of the golden catfish muscle (especially for the large size class, GML) compared to that of the normal coloured strain, could be attributed to a difference in the proportion of energy being channelled into various physiological processes. As fish become older, less energy is needed for growth (Smith, Kincaid, Regenstein & Rumsey, 1988), and more becomes available for sexual development (Shearer, 1994) or to be stored as an energy source in the form of muscle lipid (Love, 1970; 1980; 1988; Eliassen & Vahl, 1982; Pankhurst, 1982). The sexually mature golden catfish has a gonado-somatic index lower than that of the normal coloured strain (Prinsloo, Schoonbee & Hoffman, 1990) which implies that less energy is used for gonadal development and thus more energy is available to be stored as muscle lipid (Wolters, Libey & Chrisman, 1982). Henken *et al.*, (1987) have noted that triploid (sterile) *C. gariepinus* had a higher muscle total

lipid content than their normal (2N) contemporaries. In the study of Heaton *et al.*, (1973) no significant difference in the proximate composition of albino and normal channel catfish were noted.

The muscle amino acid composition of the investigation (Table 8) is similar to that noted previously for *C. gariepinus* (Hoffman *et al.*, 1992). The higher concentration of glycine, proline, hydroxyproline and alanine, in the muscle of the large size class (both strains) is a possible indication of aging. These four amino acids predominate in collagen and it has been noted that myotome collagen thickens as fish age (Love, 1988). The concentration of these four amino acids were also all statistically significantly higher in the skin than in the muscle. Hydroxyproline is normally used as an indication of collagen content (muscle, with a low collagen content, has <1%) and was present in the skin in concentrations above 6.5% and 7.4% in the small and large size classes, respectively. The skin from the larger sized class (both strains - Table 9) contained statistically significantly higher concentrations hydroxyproline, proline and glycine than the smaller sized classes. This phenomenon seems to indicate an increase in the skin collagen content with increasing size. Yoshinaka and co-workers (1990) found that collagen was contained abundantly in skin, scale, bone and fins of different fish species and ranged from 76.2% of whole body collagen for Japanese eel (*Anguilla japonica*) to 91.1% for red sea bream (*Pagrus major*). This increase in skin collagen content with size is an important factor to be considered if the golden catfish strain is to be utilised by the industry for minced (with the skin on) processing (Grantham, 1981).

The muscle total lipid fatty acid profile of the investigation (Table 10) is similar to that of the previous study on *C. gariepinus* (Hoffman *et al.*, 1992) where palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and docosahexaenoic acids predominated. A lack of significant differences between strains within size classes (with the exception of linoleic acid between NML and GML - Tables 10*a* & 10*b*) is in direct contrast to results noted for channel catfish (Erickson, 1992), where significant differences were noted between strains in their muscle tissue for fatty acid composition. In the three strains analyzed, there were significant differences in the level of triacylglycerol and in its fatty acid composition. Of interest was the significant differences noted in the sum of the fatty acid components, especially in the sum of the polyunsaturated fatty acids which varied from 22.07 to 24.45% between strains. There was also a strain influence in the ratio of $\omega3/\omega6$ fatty acids. Both these factors could have an important influence on the shelflife and health (human consumption) aspects of catfish meat (Erickson, 1992). The $\omega3$ polyunsaturated fatty acids are nutritionally desirable due to their association with a decreased risk of coronary and related diseases (Kinsella *et al.*, 1990).

The muscle mineral concentrations of the present investigation (Table 12) were similar to that reported previously for the same species (Hoffman *et al.*, 1992), with the exception of a higher zinc and lower iron and copper concentrations in the present investigation. Possible causes of these differences could be nutritional

and/or water source (Love, 1988). In the present investigation, the lower potassium content in the muscle of the larger sized fish (both strains) is supported by Love (1988), who in his review notes that some muscle mineral concentrations change (increase or decrease) with increasing size. The muscle mineral concentration changes that may occur with increasing size, are also determined by the fish species.

In the comparison of the farmed and wild catfish, the following compositions for the pooled data (male and female, farmed and wild - Table 14) for fillets were calculated: 77.9% moisture, 18.2% protein, 2.4% fat and 0.4% ash. The American channel catfish, *Ictalarus punctatus*, with a 76% moisture, 17.8% protein, 6% fat and a 1.2% ash content (Tucker, 1985), does not differ greatly from the African sharptooth catfish *C. gariepinus*.

The phenomenon of the wild catfish having uneven numbered carbon fatty acids (Table 15) is probably due to the diet of the fish (Van Senus, 1989), as it is known that plants contain some uneven numbered carbon fatty acids (Greene and Selivonchick, 1990). The percentage saturated fatty acids (SFA, 37.5%), mono-unsaturated fatty acids (MUFA, 39.8%) and polyunsaturated fatty acids (PUFA, 23.0%) for farmed *C. gariepinus* differ from that of beef (SFA 38.9%; MUFA 43.5% & PUFA 3.7%), lamb (SFA 37.6%; MUFA 42.1% & PUFA 7.4%) and pork (SFA 34.5%, MUFA 44.9% & PUFA 12.1%), especially in respect of the percentage PUFA (Breidenstein, 1987).

Numerous studies have attempted to formulate an "ideal protein" that contains all the essential amino acids in the correct concentrations for the human being. The relative amounts of the essential amino acids in such a protein are similar to those found in chicken whole eggs or cows' milk (Linder, 1985).

Apart from the proportion of essential amino acids present, the quality of a protein is also determined by its digestibility, being 85-100% for fish protein (Linder, 1985).

The mean essential amino acid concentrations of the farmed catfish found in the present investigation are compared with the essential amino acid profiles of other fish species, as well as that of whole chicken egg in Table 18. These species are *Cyprinus carpio* (European common carp), *Oncorhynchus mykiss* (= *Salmo gairdneri*, rainbow trout) and *Salmo salar* (Atlantic salmon) (Hepher, 1988). Of the essential amino acids, *C. gariepinus* has similar concentrations to these fish species, with the exception of histidine, isoleucine, phenylalanine and valine, which tend to be lower for *C. gariepinus*. Compared to chicken whole egg (6.8%), farmed catfish is a good source of lysine (10.0%), the amino acid that is essential in the crosslinking of proteins (as in collagen and elastin) and carnitine biosynthesis (Linder, 1985).

Mustafa and Medeiros (1985) analysed the mineral content of channel catfish fillets and found the following

mean values: Ca, 10; P, 221; Mg, 22; K, 259; Fe, 1,0; Na, 98; Cu, 0.23 and Zn, 1.1, all these values were expressed as mg element/100g wet weight fillet. From the investigation into the mineral content of farmed and wild catfish, expressed on the same basis, data for *C. gariepinus* showed the following; Ca, 3.7; P, 21.5; Mg, 4.8; K, 39.1; Fe, 1.9; Cu, 0.1 and Zn, 0.7. These values are all below those for the channel catfish (Mustafa & Medeiros, 1985), beef (P, 245; Mg, 27; K, 352 & Fe, 3.2), pork (P, 258; Mg, 22; K, 365 & Fe, 1.25) and lamb (P, 209; Mg, 26; K, 343 & Fe, 2.05) (Breidenstein, 1987).

**Table 18:** Comparision of essential amino acid composition of various fish muscle proteins versus whole egg proteins.

| Species | (% of total amino acids) | | | | | | | | | | | | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Arg | His | Iso | Leu | Lys | Met | Cys | Phe | Thy | Thr | Try | Val | |
| Chicken, whole egg | 6.4 | 2.4 | 6.3 | 8.8 | 6.8 | 3.2 | 2.4 | 5.7 | 4.2 | 4.9 | 1.6 | 7.2 | 1 |
| *Clarias gariepinus* | 6.1 | 1.9 | 3.7 | 6.7 | 10.2 | 2.7 | - | 3.6 | 3.1 | 4.1 | - | 4.0 | 2 |
| *Cyprinus carpio* | 7.1 | 3.1 | 6.2 | 7.7 | 6.2 | 3.2 | 1.7 | 4.5 | - | 4.9 | 1.3 | 5.6 | 3 |
| | 6.0 | 2.7 | - | - | 8.9 | 3.1 | 1.9 | 4.1 | - | 5.3 | 1.3 | 5.0 | 4 |
| | 6.0 | 2.2 | 5.1 | 9.2 | 11.6 | 3.3 | - | 5.1 | 3.8 | 5.9 | 1.1 | 6.8 | 5 |
| *Oncorhynchus mykiss* | 5.9 | 3.1 | 3.7 | 6.7 | 8.0 | 2.6 | 0.7 | 4.0 | 3.4 | 3.3 | - | 4.2 | 6 |
| *Salmo salar* | 5.9 | 3.3 | 5.1 | 7.8 | 8.6 | 3.3 | 0.6 | 4.5 | 3.1 | 3.9 | - | 5.6 | 7 |

Source: 1 MRC. (1983)*; 2 Present investigation; 3 Dupont, (1958)*; 4 Linder *et al.*, (1960)*; 5 Konossu *et al.*,(1956)*; 6 Rumsey & Ketola, (1975)*; 7 Ketola, (1982)*;
* quoted from Hepher (1988)

## CONCLUSION

This first investigation has shown that for the African sharptooth catfish *Clarias gariepinus*, strain may influence the growth rate. Strain also influences the body biochemical composition in terms of proximate (moisture, protein, total lipid and ash) composition, amino acid, fatty acid and mineral concentrations. The differences in the lipid composition of the four strains reported suggests that differences will be found in their nutritional value

(as pertaining to human consumption) as well as in their rate of oxidation during storage. The golden strain with its higher lipid PUFA content, may be the healthier (human nutrition) strain, but should also be the strain to show fastest lipid oxidation during storage.

In the second investigation, the results show that there are small chemical differences between the normal and golden strains and that fish size has a stronger influence on body composition than does strain. The larger sized class (both strains) had lower muscle moisture and higher total lipid content than the smaller. The muscle protein content was similar for both strains and sizes (±17%). The major fatty acids present in bothe the muscle and skin for both sizes and strains, were palmitic (>23%) and oleic (>28%). Of the muscle minerals, potassium was significantly higher in the larger sized fish. Iron and zinc concentrations showed high variations within the class groupings. The lack of melanin in the skin of the golden catfish strain proves that this strain is in fact an albino, that has been selected to breed true to skin colour. However, care should be taken in talking of the golden catfish as an albino, as albinism may lead to a consumer prejudice. From a marketing viewpoint, it is therefore correct to speak of this strain of catfish as a "golden catfish" as this strain does have a golden colour, especially when containing a reasonable subcutaneous fat layer. As mentioned, a comparable growth rate (Prinsloo & Schoonbee, 1989; Prinsloo *et al.*, 1989) and body composition, and higher dressout percentage (Hoffman & Prinsloo, 1990) coupled with its external appearance, makes the golden strain an ideal candidate for marketing as a fresh fish product. However, it has been noted that the skin of the golden catfish is prone to show handling marks more readily than the normal coloured strain. Care should therefore be taken in the handling of this strain during processing.

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# Chapter 6 THE INFLUENCE OF DIET ON THE BODY CHEMICAL COMPOSITION OF *CLARIAS GARIEPINUS*

CONTENTS

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**INTRODUCTION**

With the growth experienced in the aquaculture industry worldwide (Chamberlain, 1993) fishery waste disposal is an environmental and economic problem. In an American catfish processing plant, waste comprises at least 40 percent of the volume of product which enters the processing plant (Lovell & Ammerman, 1973; Lovell, 1980). This waste can be utilized in commercial animal or fish feeds when processed into a meal (Lovell, 1980; Dean, Nielsen, Helfrich & Garling, 1992). The African catfish, *Clarias gariepinus* has an even higher waste production, as preliminary results show this species to have a dressout percentage varying between 38-47%, giving a waste percentage of 53-61% (Hoffman, Casey & Prinsloo, 1993). The females had a gonad percentage (expressed as a percentage of body mass) of 17.7 compared to 0.6 for the males. A biochemical analysis of the gonads (roe) of female *C. gariepinus* (unpublished) has shown amino acid, fatty acid and mineral compositions favourable for its possible utilisation as a protein source in fish (larval) diets.

The use of fish roe as a major component in starter feed for larvae is not a new idea, for example, Garatun-Tjeldstø and co-workers (1989) used roe from different fish species successfully in a starter feed for salmon, cod and plaice larvae. In the feeding of cod, it was found that larvae fed roe diets gave better growth and survival than those fed commercial feed components. Similar results were also noted for salmon and plaice larvae.

With the increase in interest in the role that fatty acids, especially long chained polyunsaturated fatty acids, play in the lessening of cardiovascular related diseases (Hearn, Sgoutas, Hearn & Sgoutas, 1987; Singh & Chandra, 1988; Ackman, 1989; Kinsella, Lokesh & Stone, 1990; Tichelaar, 1993), the lipid fatty acid composition of fish muscle has been scrutinised world wide. Factors that have been found to influence fish fatty acid profiles include species (Love, 1988), genetic strain (Smith, Kincaid, Regenstein & Rumsey, 1988; Erickson, 1992), environment (Gallagher, McLeod & Rulifson, 1989; Morishita, Uno, Araki & Takahashi, 1989), water temperature (Stickney & Andrews, 1971; Stickney & Hardy, 1989), physiological (Sasaki, Ota & Takagi, 1989) and nutritional status (Tidwell, Webster & Clark, 1992). However, the most dominant factor that influences the lipid fatty acid composition is the diet of the fish (Gatlin & Stickney, 1982; Watanabe, 1982; Greene & Selivonchick, 1990).

In general, it has been found that marine fish species have a higher demand for $\omega 3$ fatty acids than freshwater fish, while coldwater fish have a higher demand for $\omega 3$ fatty acids than warmwater fish (Steffens, 1989). Higher $\omega 3$ concentrations are needed because of the important role they play in the membrane fluidity and composition (phospholipids) of fresh and marine fish species. The role that dietary fatty acid composition plays in determining fish muscle composition therefore varies from species to species and is determined by the essential fatty acid (EFA) requirement of that fish species (Watanabe, 1982). It is thus evident that manipulating the fish

muscle composition by applying the desirable fatty acids in the diet, is not always successful because of the role that the EFA play in the growth and general health state of the specific fish species (Greene, 1990).

As for other fish species (Bautista, del Valle & Rejana, 1991; Abrami *et al.*, 1992; Villarreal, Rosenblum & Fries, 1994) differences in the muscle fatty acid profile of wild and cultured African sharptooth catfish *Clarias gariepinus*, have been noted (Hoffman, Casey & Prinsloo, 1992). The major reason for these differences noted were attributed to diet.

In these investigations, the role of diet on the chemical composition of the African sharptooth catfish *Clarias gariepinus* muscle is examined. In the first investigation, the effect of replacing fish meal with *C. gariepinus* gonads in the traditional starter diet (Uys, 1988) of *C. gariepinus* larvae, on the chemical composition of the muscle is investigated. In the second investigation, various natural occuring lipids (sunflower, cod liver oil and tallow) are fed in an artificial diet to juvenile *C. gariepinus*, and the influence of the diets on the muscle fatty acid composition is examined.

## MATERIAL AND METHODS

*Larvae nutrition*

*Fish*

*Clarias gariepinus* broodstock were artificially spawned and the resulting larvae reared using standard hatchery techniques (Schoonbee, Hecht, Polling & Saayman, 1980, Prinsloo, Hoffman & Theron, 1993). Twenty days after hatching, 60 randomly selected larvae were transferred from the hatching tanks to each of ten 60 l aquaria. The initial mean mass of the larvae used was 0.0204 ± 0.0037g (± standard deviation).

*Sampling*

At the beginning of each week, 12 fish were randomly sampled from each aquarium and weighed. Mean body mass was determined for the calculation of feeding rate. The number of dead fish per aquarium was recorded daily. The experiment was terminated on day 50.

*Diet*

The formulated diets (Table 1) were based on the recommended dry feed of Uys (1988). The diets differed in the two fish meals used, arbitrary named FMA and FMB (purchased from two commercial suppliers) or in the replacement of fish meal with *C. gariepinus* female gonads: GOD being the left gonads, dried in an oven (50°C, 24h) and GVD, the right gonads, but dried in a freeze drier. The female donors were raised together in a recirculating system where they received the same commercial diet (38% protein). All female donors were

ready for spawning with their gonads at maturity stage 4 (Nikolsky, 1963). At this stage of development, the gonads have achieved their maximum mass, but eggs do not extrude when light pressure is applied to the abdomen. The four diets, FMA and FMB with three replicates and, GOD and GVD with two replicates per diet, were randomly allotted to the ten aquaria.

**Table 1**: The basic diet formulation for larval feeds (Uys 1988).

| Diet formulation | % |
|---|---|
| Torula yeast | 50 |
| Fish meal | 43 |
| Vitamin premix | 1 |
| Cod liver oil | 3 |
| Sunflower oil | 3 |

*Feeding regime*

The feeding regime of Schippers, Prajitno, Boon and Machiels (1992) was followed: satiation feeding of larvae during first 20 days with natural occurring plankton. From day 21 to 28, plankton was gradually replaced with the experimental diet. From day 29 until day 50, the fish were fed dry feed at a level of 25% of the total metabolic weight, which was calculated as: (mean weight of fish)$^{0.8}$*number of fish.

*Water Quality*

The 60 l aquaria were connected to a common recirculating system. A flow rate of between 1.0-1.2 l/min/aquarium was maintained. Weekly selected water quality parameters (ammonia, nitrite, nitrate, phosphate, pH, dissolved oxygen concentration) were tested according to APHA (1980) and were within the accepted limits for *C. gariepinus* larvae (Viveen *et al.*, 1985). The water temperature was maintained at 28 $\pm$ 0.1°C.

*Chemical Analysis*

The two different fish meals and two processed forms of gonads used in the compiling of the diets were analyzed (in triplicate) for proximate, mineral and fatty acids (Chapter 2). At the end of the experiment $\pm$60 g of fish from each tank, were pooled, as the individual fish were too small for separate analysis, and sampled

for proximate, mineral and fatty acids (in triplicate). Representative liver samples from fish in each aquaria (n=3/aquarium) were removed for the investigation of any pathological abnormalities that may have been caused by the diets.

*Liver Histopathological Evaluation*

The livers were fixed in 10% buffered formalin and divided into equal parts. One liver section was dehydrated with ethanol and embedded in paraffin in a vacuum oven. Thereafter, five micrometer sections were stained with Delafield haematoxylin and counter stained with eosin (Humason, 1979). The second liver part was sectioned on a cryostat microtome and these frozen sections stained with Sudan IV to demonstrate fat infiltration into hepatocytes.

*Statistical Analysis*

For the comparision of growth rates, the data were transformed to a logarithmic function, and the resulting linear regressions analysed for differences by means of a F test (Snedecor and Cochran, 1980). A linear model was fitted to the final body weights with diet as predictor. PROC ANOVA of the SAS package (SAS, 1985) was used for an analysis of variance. A mean and standard error were calculated for each diet and differences between diets tested by means of a Duncan grouping. Feed conversion was calculated as the amount of feed provided per unit fresh body weight gain.

*Influence of dietary lipid*

*Holding Facilitites*

Twelve 250 l aquaria connected to a 12 $m^3$ water recirculating unit were each stocked with 20 catfish. Water temperature was maintained at 29 ± 0.5°C at all times. Three times per week, 6 $m^3$ of the total recirculating water volume was replaced. A water flow rate of 4 l per min per aquarium was maintained. Weekly water ammonia, nitrite, nitrate and phosphate concentrations were tested according to Standard Methods (APHA, 1980) and were well below the accepted maximum limits for *C. gariepinus* (Viveen *et al.*, 1985).

*Fish*

The fish were bred and raised in the Mobil Aquarium at the University of the North and fed a commercial catfish diet (6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt) until the start of the experiment. Prior to stocking, the fish were sorted into three size classes, with live body masses of 50-85, 90-130 and 150-250 g, so as to minimise any aggression that could arise because of size differences within a tank.

*Diet Composition*

Four diets, which were identical except for lipid composition (the control diet, A, had no lipid), were tested. Diets (Table 2) were prepared from purified ingredients according to Stickney and Andrews (1971) and each contained 10% by weight of either sunflower oil (Diet B, a high level of oleic and linoleic fatty acids), cod liver oil (Diet C, a high level of 20 and 22 carbon ω3 fatty acids) or tallow (Diet D, predominantely saturated and monounsaturated fatty acids). The tallow was obtained from a commercial abatoir and contained cattle, pig and sheep fat. Following thorough mixing, the diets were frozen at -40°C to reduce the rate of oxidation of the lipids.

**Table 2:** Composition of experimental diets

| **Ingredient** | **Percentage of diet** |
|---|---|
| Casein | 32.0 |
| Cornstarch | 40.0 |
| Lipid supplement[1] | 10.0 |
| Agar (powdered | 1.0 |
| Methyl-cellulose | 6.9 |
| Oxytetracycline | 0.1 |
| Calcium phosphate dibasic | 2.5 |
| L-Arginine · HCl | 2.0 |
| L-Cystine | 0.5 |
| Vitamin premix[2] | 2.5 |
| Mineral mix[3] | 2.5 |

[1]Note that the control diet contained no lipid supplement

[2]Vitamin premix 1 - 50% (g/kg) vitamin A (4.4 MIU), vitamin D3 (2.2 MIU), vitamin E (55000 IU), folic acid (2.2), niacin (88), calpan (35), vitamin B1 (11), vitamin B2 (13), vitamin B6 (11), vitamin B12 (0.09), vitamin C (350) and antioxidant (200)

[2]Vitamin premix Vitastress® - 50% (g/kg) vitamin A (40 MIU), vitamin D3 (2 MIU), vitamin E (10 000 IU), vitamin K3 (8), folic acid (0.4), vitamin B1 (2), vitamin B2 (2), vitamin B12 (0.02), vitamin C (4), pantothenic acid (16), thiocticamide (1.2)

[3]mineral mix (g/kg) fe (10), Cu (1), Zn (20), Co (0.125), Mn (25), I (0.5), Se (0.125).

*Sampling*

At the beginning of each week, the individual body weight of the fish was measured. Mean weight per tank was determined for the calculation of feeding rate. The experiment continued for 60 days.

*Feeding Regime*

Prior to the experiment, the fish were fasted for a week, so as to minimise the influence of the commercial diet, thereafter, the fish were fed the experimental diets for a week at 0.1% body weight as a familiarisation period. Fish were thereafter fed at 3% (DM) of their live body weight daily, divided over three feedings (08:00, 12:00 and 16:00).

*Chemical Analysis*

Prior to feeding the fish with the experimental diet as a familiarisation period, four fish were randomly removed and the whole muscle of each fish chemically analysed (results designated by Z). At termination of the experiment, five fish from each tank were randomly removed and the muscle individually sub-sampled for chemical analysis, using the same procedures as that used prior to the commencement of the experiment. The chemical parameters analysed were proximate composition and fatty acids as described in Chapter 2.

*Statistical Analysis*

A linear model was fitted to the data with diet (lipid source) as predictor. PROC GLM of the SAS package was used and an analysis of variance was carried out. A mean and standard error was calculated for each diet and a matrix of excedence probabilities were calculated to test for pair-wise differences between diets. The FISHER LSD test was used for testing pairwise differences since it was recently shown that this test is optimal (Saville, 1990).

## RESULTS

In Table 3, the final mean mass and percentage survival of the larvae fed the different diets are shown. The diets containing fish meal FMA and FMB had higher percentages survival (93.9% and 86.7%, respectively) than the diets containing the gonads (GOD - 80.8% and GVD - 85.0%). The growth curves did not differ statistically within each treatment, and were therefore pooled for further statistical analysis. An exponential model fitted the different growth curves best (Fig 1). Table 4 shows the different exponential models for the four diets. The slopes did not differ statistically from each other at the 5% level, but, the slopes of diets FMA and GOD did differ statistically at the 10% level. At the end of the experiment, the larvae fed the GOD diet had the highest mean mass (9.4g) whilst those receiving diet FMA (6.9g - Table 2), had the lowest (significantly lower at $\alpha=0.05$). However, the food conversion ratio (FCR) showed the opposite trend. The use of the GOD

diet resulted in the highest FCR (2.64), whilst diet FMA recorded the lowest (1.90). The FCR's of diets GVD (2.39) and FMB (2.38) were very similar.

**Table 3:** The mean body mass (g) and percentage survival of *Clarias gariepinus* larvae fed different diets at termination.

| Diet | Mean body mass[*] | Standard Deviation | Percentage survival |
|---|---|---|---|
| FMA | 6.8872[a] | 3.0118 | 93.9 |
| FMB | 8.7981[b] | 3.2214 | 86.7 |
| GOD | 9.4098[b] | 4.0205 | 80.8 |
| GVD | 8.7331[b] | 4.1480 | 85.0 |

Where FMA = fish meal A in basic diet
FMB = fish meal B in basic diet
GOD = oven dried gonads in basic diet
GVD = vacuum dried gonads in basic diet
[*] values in coloumn with same letter are not significant at $\alpha=0.05$

The proximate and mineral composition of the different fish meals and gonads used in the diet and pooled fish meat samples are shown in Table 5. The amount of total nitrogen present (9% DM) in the fish meals and gonads was similar. At the termination of the experiment, the total body nitrogen for the fish receiving the fish meal diets were equal (12% DM) whilst that receiving the gonads differed significantly from that receiving the fish meal diets and each other (Table 5). There was a marked difference in the percentage lipid present in the diet components, FMA had the lowest (7.10% DM), whilst FMB had the highest (12.84% DM). The dietary lipid content of GVD and GOD did not differ greatly from each other (9.45 and 8.38%, respectively). At the end of the experiment, a somewhat different body lipid composition was noted, the fish receiving the gonads (GOD and GVD) had significantly ($\alpha=0.05$) higher percentages total body lipids (28.47 and 25.70% DM, respectively - also differed significantly from each other) than those receiving either of the fish meals (FMA - 13.97% DM and FMB - 16.30% DM; did not differ significantly from each other).

**Table 4**: The exponential growth models [$Y = \exp(a+bX)$] depicting the growth (g) of *Clarias gariepinus* larvae fed different protein sources.

| Diet | Intercept (a) | Slope (b) | Correlation Coefficient | SE of Estimate | $R^2$ for slope |
|---|---|---|---|---|---|
| **FMA** | -6.6105 | 1.0704 | 0.9873 | 0.3601 | 97.48 |
| **FMB** | -6.5801 | 1.1261 | 0.9780 | 0.5020 | 95.65 |
| **GOD** | -6.9325 | 1.1899 | 0.9865 | 0.4133 | 97.32 |
| **GVD** | -6.3527 | 1.0844 | 0.9692 | 0.5767 | 93.93 |

Where FMA = fish meal A in basic diet

FMB = fish meal B in basic diet

GOD = oven dried gonads in basic diet

GVD = vacuum dried gonads in basic diet

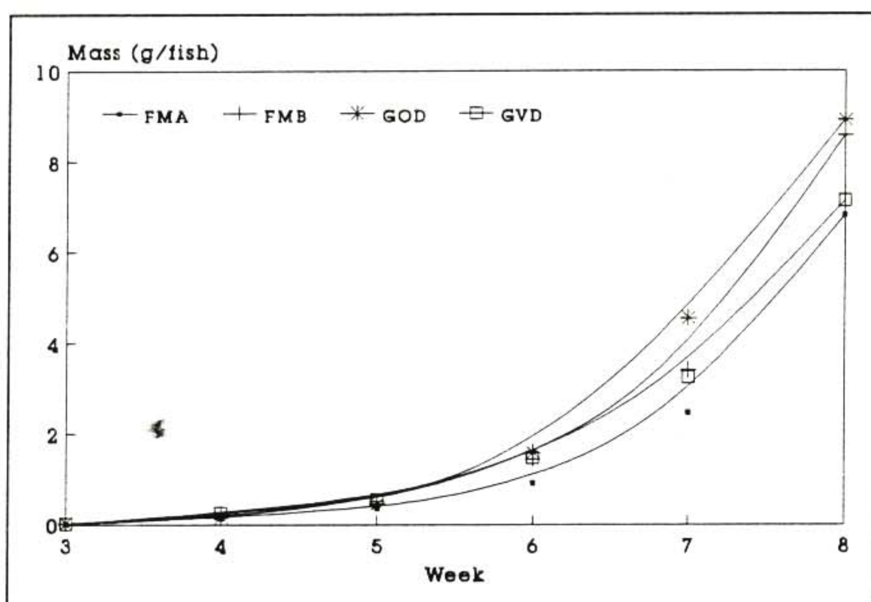


**Figure 1:** Growth curves of *Clarias gariepinus* larvae fed various protein sources.

The fatty acid composition of the lipids of the diets and pooled fish samples are shown in Table 6. The methods used for the drying of the gonads caused no major changes in the total lipid fatty acid profiles. As the diets contained the same amounts of sunflower and cod liver oils (Table 1), any difference in the fatty acid profiles of the body lipids, therefore, could only be as a result of the fatty acids being derived, or internally metabolized, from either the fish meals or the gonads in the diet. Generally, the same trend shown in the diet component is realised in the body lipid; both dried forms of gonad had higher concentrations stearic acid (C18:0, 7% compared to 5% for the fish meals), which resulted in the body lipids having 9 and 6% stearic acid, respectively. Similar results were experienced with myristic (C14:0), palmitic (C16:0), elaidic (C18:1), gondoic (C21:1ω9), eicosapentaenoic (C20:5ω3) and erucic (C22:1ω9) acids. Cetoloic acid (C18:4ω3), however shows the opposite trend, there was a lower concentration in both dried forms of gonads than the fish meals, but a higher concentration in the lipids of the fish receiving the gonads in their diets.

There were no distinct histopathological differences between the hepatocytes of the fish receiving either fish meal (FMA or FMB) or fish gonads (GOD or GVD). However, clear histopathological differences were noted between the hepatocytes of the larvae receiving the fish meals or gonads. The hepatocyte parenchymal cells of fish receiving diets FMA and FMB reveal normal hepatocytes. No inflammatory infiltrates were found in any of these liver samples. The Sudan IV staining of the liver sections (FMA and FMB) revealed an absence of fat droplets in the cytoplasm of the hepatocytes (Fig 2). Fat accumulation of metabolic type could therefore not be confirmed in these liver specimens. The livers of the fish receiving both diets GOD and GVD reveal moderate diffuse vacuolization of hepatocytes with severe hepatocellular cytoplasmic swelling due to the accumulation of fatty material in the cytoplasm of these cells (Fig 3). There was no morphological differentiation from the type of vacuolization recorded between the liver samples from specimens receiving diet GOD or GVD. The Sudan IV stained liver sections from the fish receiving the latter two diets show fat accumulation (neutral fats) in 100% of hepatocytes. There was marked variation in the amount of Sudan IV positive fat material accumulated in the cytoplasm between the different hepatocytes. Some of the cells show a large amount of fatty material present in the cytoplasm while small droplets could be demonstrated in other hepatocytes. Livers from both diets (GOD and GVD) showed this large variation in fat accumulation and size of fat droplets among the different hepatocytes. Sudan IV stains the nuclei blue, and fat orange to red (neutral fats - deep red; cholesterols - orange; phospolipids - slightly yellow). Table 7 gives a summary of the liver histopathological findings for the different diets.

**Table 5:** The total nitrogen, lipid and mineral profiles of the larval diets and fish body compositions at termination of the experiment (values expressed on a dry mass basis).

| Source | Nitrogen % | Lipid % | P % | Ca % | K % | Mg % | Fe ppm | Cu ppm | Zn ppm | Mn ppm |
|---|---|---|---|---|---|---|---|---|---|---|
| **Diet** | | | | | | | | | | |
| FMA | 9.45 | 7.10 | 1.68 | 0.20 | 1.54 | 0.35 | 198.34 | 4.70 | 89.88 | 136.13 |
| FMB | 9.24 | 12.84 | 1.99 | 0.19 | 1.59 | 0.36 | 232.55 | 10.46 | 144.46 | 144.84 |
| GOD | 9.30 | 8.38 | 1.52 | 0.68 | 1.42 | 0.39 | 254.74 | 9.64 | 131.46 | 136.00 |
| GVD | 9.62 | 9.45 | 1.35 | 0.54 | 1.41 | 0.30 | 244.57 | 12.74 | 124.94 | 139.58 |
| **Fish Body Composition*** | | | | | | | | | | |
| FMA | 11.80[a] | 13.97[a] | 2.30[a] | 2.03[a] | 1.28[a] | 0.22[a] | 64.54[a] | 7.24[a] | 77.08[a] | 13.71[a] |
| ±SD | 0.146 | 1.052 | 0.015 | 0.095 | 0.026 | 0.006 | 8.454 | 1.599 | 3.031 | 0.836 |
| FMB | 11.99[a] | 16.30[a] | 2.27[a] | 2.62[b] | 1.29[a] | 0.20[b] | 66.68[a] | 4.62[a] | 74.91[a] | 10.65[b] |
| ±SD | 0.152 | 0.371 | 0.139 | 0.091 | 0.009 | 0.004 | 3.371 | 1.101 | 0.846 | 0.858 |
| GOD | 10.23[b] | 28.47[b] | 1.58[b] | 1.36[c] | 1.08[b] | 0.16[c] | 41.08[b] | 5.63[a] | 56.12[b] | 9.01[b] |
| ±SD | 0.308 | 0.507 | 0.304 | 0.098 | 0.030 | 0.008 | 1.416 | 0.388 | 2.369 | 0.362 |
| GVD | 11.01[c] | 25.70[c] | 1.43[b] | 1.60[c] | 1.16[c] | 0.16[c] | 43.16[b] | 4.74[a] | 53.86[b] | 10.12[b] |
| ±SD | 0.400 | 1.146 | 0.073 | 0.039 | 0.035 | 0.001 | 1.939 | 0.394 | 1.153 | 0.402 |

Where FMA = fish meal A in basic diet
FMB = fish meal B in basic diet
GOD = oven dried gonads in basic diet
GVD = vacuum dried gonads in basic diet
* values in coloumn with same letter are not significant at $\alpha = 0.05$

**Table 6**: The fatty acid profiles of the two oils (sunflower and cod liver), the two fish meals (FMA and FMB) and the two gonadal protein sources (GOD and GVD) used in the compiling of the larval diet, and the body lipids of the larvae at the termination of the experiment (fatty acids identified expressed as % of total fatty acids).

| Fatty acid | Oil | | Diet Protein Source | | | | Body Total Lipid | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sunflower | Cod liver | FMA | FMB | GOD | GVD | FMA | FMB | GOD | GVD |
| **C14:0** | 0.14 | 6.85 | 3.31 | 4.41 | 1.71 | 2.08 | 2.77 | 3.26 | 1.28 | 1.37 |
| **C16:0** | 6.38 | 14.23 | 13.07 | 14.34 | 17.32 | 17.93 | 16.60 | 16.83 | 22.93 | 22.03 |
| **C16:1ω7** | 0.17 | 8.55 | 6.64 | 6.52 | 5.47 | 5.54 | 6.50 | 6.35 | 6.09 | 5.86 |
| **C16:2ω4** | nd | 0.17 | 0.14 | 0.13 | tr | tr | 0.23 | 0.23 | 0.14 | 0.15 |
| **C18:0** | 5.14 | 3.16 | 4.47 | 4.13 | 7.67 | 7.61 | 5.64 | 5.41 | 9.25 | 9.08 |
| **C18:1ω7** | tr | 0.30 | 0.72 | 0.59 | 0.58 | 0.60 | 0.56 | tr | 0.40 | 0.45 |
| **C18:1ω9** | 24.56 | 14.77 | 21.19 | 17.03 | 22.58 | 22.64 | 21.24 | 20.05 | 25.90 | 25.23 |
| **C18:2ω4** | nd | 1.89 | nd | nd | tr | tr | nd | nd | tr | tr |
| **C18:2ω6** | 1.47 | 1.38 | 25.84 | 20.45 | 20.45 | 20.06 | 20.29 | 19.08 | 12.80 | 14.07 |
| **C18:3ω3** | 60.27 | 7.68 | 2.99 | 4.02 | 2.12 | 2.18 | 2.85 | 3.31 | 1.59 | 1.65 |
| **C18:4ω3** | 0.11 | 0.94 | 1.20 | 1.05 | 0.85 | 0.89 | 0.93 | 0.90 | 0.53 | 0.56 |
| **C20:0** | nd | tr | tr | tr | 0.24 | 0.22 | 0.36 | 0.30 | 0.75 | 0.73 |
| **C20:1ω9** | 0.15 | 0.15 | 0.11 | 0.11 | 0.16 | 0.18 | 0.96 | 0.97 | 0.20 | 0.17 |

**Table 6**: The fatty acid profiles of the two oils (sunflower and cod liver), the two fish meals (FMA and FMB) and the two gonadal protein sources (GOD and GVD) used in the compiling of the larval diet, and the body lipids of the larvae at the termination of the experiment (fatty acids identified expressed as % of total fatty acids).

| Fatty acid | Oil | | Diet Protein Source | | | | Body Total Lipid | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sunflower | Cod liver | FMA | FMB | GOD | GVD | FMA | FMB | GOD | GVD |
| **C20:3ω3** | nd | 0.68 | tr | 0.14 | tr | tr | 0.70 | 0.69 | 0.43 | 0.50 |
| **C20:3ω6** | tr | 3.25 | 1.35 | 1.50 | 1.10 | 1.08 | 0.15 | 0.17 | 0.90 | 0.78 |
| **C20:4ω3** | nd | 0.85 | 0.47 | 0.65 | 2.57 | 2.32 | 0.35 | 0.32 | 2.52 | 2.55 |
| **C20:4ω6** | tr | 5.75 | 2.08 | 4.37 | 1.17 | 1.26 | 1.75 | 2.32 | 2.05 | 2.11 |
| **C20:5ω3** | 0.19 | 13.78 | 5.96 | 7.98 | 3.43 | 3.38 | 3.75 | 4.33 | 1.38 | 1.56 |
| **C22:0** | 0.73 | 0.18 | 0.27 | 0.27 | 1.54 | 1.44 | 0.76 | 0.57 | 0.41 | 0.33 |
| **C22:1ω11** | nd | 0.56 | 0.23 | 0.30 | 0.16 | 0.15 | nd | 0.18 | tr | nd |
| **C22:1ω9** | nd | 0.22 | 0.15 | 0.23 | 0.43 | 0.42 | nd | nd | 0.41 | 0.35 |
| **C22:5ω3** | nd | 2.17 | 1.18 | 1.09 | 1.11 | 1.06 | 1.39 | 1.32 | 0.89 | 0.95 |
| **C22:6ω3** | nd | 9.20 | 5.88 | 8.02 | 7.05 | 6.43 | 9.90 | 10.72 | 7.15 | 7.64 |
| **C24:0** | tr | 0.66 | 0.35 | 0.40 | 0.17 | 0.19 | 0.18 | 0.20 | 0.23 | 0.22 |

where nd = not detected, tr <0.1%, FMA = fish meal A in basic diet, FMB = fish meal B in basic diet, GOD = oven dried gonads in basic diet and GVD = vacuum dried gonads in basic diet

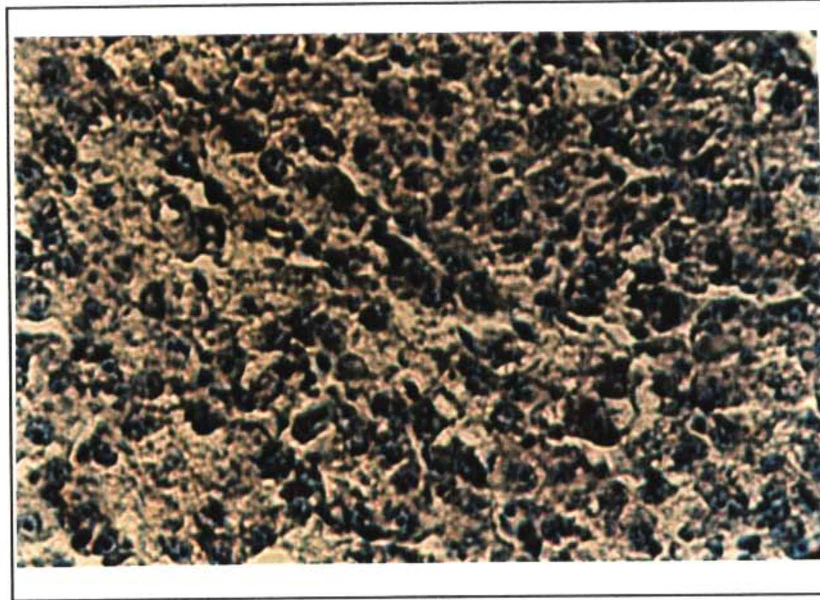

**Figure 2:** A light microscope section of the liver of catfish receiving diet FMA (x25). Note the abscence of any fat droplets.

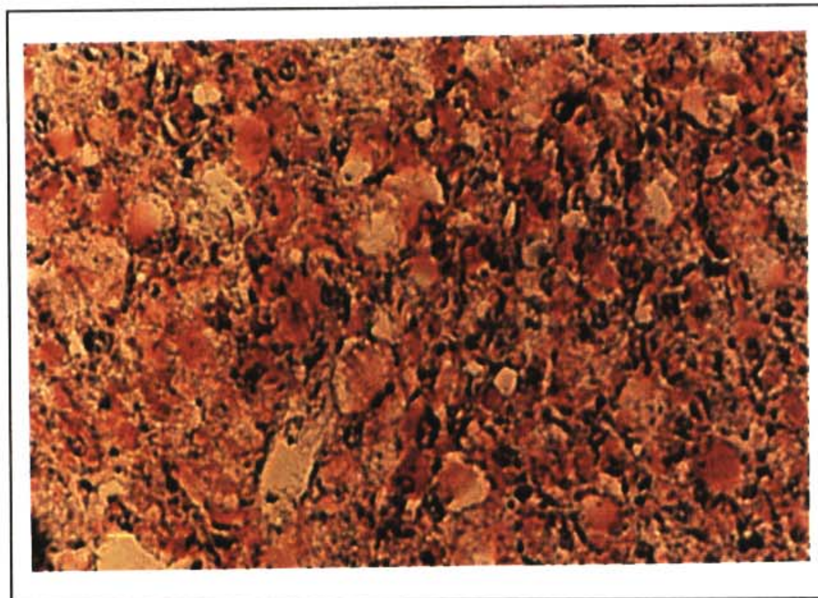

**Figure 3:** A light microscope section of the liver of catfish receiving diet GOD (x25). Note the presence of fat droplets (stained various shades of red).

**Table 7:** Histopathological evaluation of liver samples of *C. gariepinus* larvae receiving diets containing either fish meal or gonads as protein source.

| Diet | Degenerative changes recorded within hepatocytes | Percentage of hepatocytes with fat present in the cytoplasm |
|---|---|---|
| FMA1 | - | 0 |
| FMA2 | 1+ | 0 |
| FMA3 | - | 0 |
| FMB1 | - | 0 |
| FMB2 | - | 0 |
| FMB3 | - | 0 |
| GOD1 | 4+ | 100 |
| GOD2 | 3+ | 100 |
| GVD1 | 4+ | 100 |
| GVD2 | 3+ | 100 |

Legend - absent
1+ mild
2+ moderate
3+ severe
4+ very severe

*Influence of dietary lipid*

The chemical composition of the fish muscle was strongly influenced by that of the diet. All the fish showed a change in the muscle proximate composition (compared to that at the initiation of the experiment) which mainly consisted of a decrease in percentage moisture with a corresponding increase in percentage total lipid. The resulting fatty acid composition of the muscle total lipid tended to be similar to that of the dietary lipid.

The fatty acid profiles of the three oils (Table 8a, values incorporated in parenthesis) added to the experimental

diet (Table 1) differed greatly. The sunflower oil (Diet B) contained 12.8% saturated fatty acids (SFA) which was largely made up of C16:0 (6.8%) and C18:0 (5.8%), 23.1% mono-unsaturated fatty acids (MUFA) which was predominantely C18:1ω9 (22.9%). The high percentage polyunsaturated fatty acid (PUFA, 63.9%) was primarily constituted by C18:2ω6 (62.6%). The sunflower oil only showed trace concentrations, or no prescence, of the longer chained fatty acids. The cod liver oil (Diet C) had a SFA concentration intermediate to that of the sunflower oil and the tallow (Diet D). The main component of the SFA (30.8%) was C16:0 (16.1%), C14:0 (6.5%) and C18:0 (4.2%) with C22:0 and C24:0 also being present in lower concentrations. C18:1ω9 (17.1%) was the major mono-unsaturated fatty acid making up 30.4% of the MUFA. C16:1ω7 (9.9%) also formed a major component of the MUFA with lower concentrations of C20:1ω9, C22:1ω9 and C22:1ω11 also present. The cod liver oil had 33.2% PUFA, with the longer chained C20:5ω6 (13.8%) and C22:6ω3 (7.1%) being major constituents. C18:3ω3 (5.2%), C18:2ω6 (2.9%) and C22:5ω3 (1.3%) were also present in noticeable concentrations. The tallow (Diet D) had a high concentration of SFA (51.1%) which mainly consisted of C16:0 (23.7%) and C18:0 (23.9%). A lower concentration of C14:0 was also present. The principal MUFA (41.2%) was C18:1ω9 (37.2%) with a lower concentration of C16:1ω7 (3.8%). The low PUFA concentration (4.9%) came from C18:2ω6 (4.0%) and low concentrations of the shorter chained (C18) polyunsaturated fatty acids. Only minute or trace concentrations of the longer chained PUFA were detected.

None of the chemical parameters tested significantly different between the mass classes within a diet. The mass classes were therefore pooled per diet for further statistical analysis between diets.

The statistical analysis of the means of the muscle proximate composition of the fish receiving the different diets at termination of the experiment, and the initial composition (Z), are summarised in Tables 8a and 8b. The initial muscle moisture content of the fish was statistically significantly higher (78.34%) than that at the end (all four diets). Similarly, the fish recieving the control diet A, had a significantly higher muscle moisture content (76.91%) than those on diets B (74.32%), C (74.71%) and D (75.25%). The moisture content of the last three did not differ statistically from each other. There was no statisitical difference between the initial muscle protein content and that at termination (all four diets). The muscle protein content of fish on diets A and D (17.91% for both) was significantly higher than in the case of those on diets B (17.28%) and C (17.27%). All the muscle gained lipid during the feeding trial, including the control, which showed the smallest gain (all final lipid contents were significantly higher than Z). The fish receiving the sunflower oil showed the largest gain (Z=1.66% to B=7.21%). Livers of fish on diet C had the highest percentage of total lipids (12.89%), which differed significantly from all the livers of fish on the different diets except for those receiving diet D (9.47% - $p=0.1112$), the tallow. The ash content of the muscle of the fish receiving diet C also differed from that of the other diets, and was also the highest recorded (3.95%).

**Table 8a:** Mean proximate composition of the muscle and liver lipid content of catfish receiving diets containing different lipid sources (wet mass basis).

| **Parameter %** | **Z (n=4)** | **A (n=15)** | **B (n=15)** | **C (n=15)** | **D (n=15)** | **SE of Mean** |
|---|---|---|---|---|---|---|
| Moisture | 78.35 | 76.91 | 74.32 | 74.71 | 75.23 | 0.4033 |
| Protein | 17.75 | 17.91 | 17.28 | 17.27 | 17.91 | 0.2549 |
| Lipid | 1.66 | 3.52 | 7.21 | 6.39 | 5.60 | 0.5177 |
| Ash | 2.30 | 2.91 | 2.35 | 3.95 | 2.23 | 0.2158 |
| Liver Lipid | 4.37 | 7.14 | 6.03 | 12.89 | 9.47 | 1.5561 |

where Z = beginning of experiment, A = control diet, no lipid, B = sunflower oil, C = cod liver oil, D = tallow.

The means of the fatty acid profiles of the total lipids of muscle of the fish on the different diets are presented in Table 9a; the analysis of the differences between the means are summarised in Table 9b. The fatty acid profiles of the different oils incoorporated into the diets are also included in this table in parenthesis. The sum of the saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, as well as the ratio of ω3 to ω6 fatty acids are also included. The following fatty acids were present in the muscle lipids at concentrations below 1.0%, and were therefore not included in Table 9a: C16:2ω4, C18:1ω7, C18:4ω1, C20:0, C20:1ω9, C20:2ω6, C20:3ω3, C20:4ω3, C22:1ω9, C22:1ω11 and C24:0.

The fatty acid profiles of all fish muscle were strongly influenced by lipid in the diet. Generally, the most common fatty acid in the diet was most abundant in the muscle. The converse was true of the least abundant fatty acids.

The concentration of palmitic acid (C16:0) was high in the catfish muscle lipid for the initial analysis and all diets. This fatty acid was present in high concentrations in the oils of diets D and C (23.7 & 16.1% respectively), which resulted in correspondingly high concentrations in the muscle (24.71 & 22.42% respectively). However, the highest concentration was noted in the fish fed the control diet (A - 26.63%), which differed significantly from the 22.15% ($p=0.0001$) of the fish at the beginning of the experiment (Z). Similarly, the concentration of palmitoleic (C16:1ω7) acid was significantly higher ($p=0.0001$) in the muscle of the fish

receiving the control diet (10.01%) than that at the beginning of the experiment (5.34%).

Diet D had the highest concentration of stearic acid (C18:0 - 23.9%) which resulted in the muscle of the fish on this diet also having its highest concentration (10.45%), this amount being significantly higher ($p=0.0001$) than that from the other treatments.

In all the muscle samples (Z, A, B, C, D), the principle fatty acid present was oleic (C18:1ω9), with concentrations varying from 24.63% (Z) to 38.13% (D). All the muscle samples corresponding to the different diets had significantly higher oleic acid concentrations than Z. Diet D had the highest oleic acid (37.2%) concentration, as did the muscle of the fish fed on it (38.13% - significantly higher than other diets, Table 9a & 9b).

In diet B, linoleic acid (C18:2ω6) was present in very high concentrations, which produced muscle with significantly higher ($p=0.0001$) amounts (25.66%) than that of the fish on the other diets. There was a significant decrease in the final muscle linoleic acid concentration of the fish fed the other diets (A, C & D) when compared with that present at the beginning of the investigation. The muscle of the fish on diet C had a linolenic acid (C18:3ω3) concentration significantly higher (5.03% - $p=0.0001$) than that of the other diets (linolenic acid was also present in high concentrations in diet C - 5.2%).

Although C18:3ω4 was present in concentrations below 1% in the diet, the muscle of the catfish on diet B had a concentration of 2.74%, whereas the muscle samples of fish on the other diets were all below 1%. Arichidonic acid (C20:4ω6) was present only in diets C and D (0.8 & 0.1% respectively), yet all muscle samples had concentrations above 1.0%, with catfish receiving diet C, showing 2.28% in the muscle.

Diet C had a high concentration eicosapentaenoic acid (EPA, C20:5ω3 - 13.8%) which resulted in a muscle concentration (3.68%) that was significantly higher ($p=0.0001$) than the ±0.3% in the fish muscle from diets A, B and D. The muscle of the fish receiving these diets (A, B & D), showed a significant decrease in EPA concentration when compared to that at the start of the experiment (Z, 1.32%).

**Table 8b:** Probability values calculated for pairwise differences of the muscle proximate chemical composition of catfish recieving different dietary lipids.

| | $p >$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Z=A** | **Z=B** | **Z=C** | **Z=D** | **A=B** | **A=C** | **A=D** | **B=C** | **B=D** | **C=D** |
| Moisture | 0.0880 | 0.0001 | 0.0001 | 0.0005 | 0.0001 | 0.0001 | 0.0057 | 0.4703 | 0.1229 | 0.3445 |
| Protein | 0.7686 | 0.3798 | 0.3521 | 0.7642 | 0.0908 | 0.0663 | 0.9900 | 0.9671 | 0.0950 | 0.0705 |
| Lipid | 0.0862 | 0.0001 | 0.0001 | 0.0006 | 0.0001 | 0.0001 | 0.0076 | 0.2371 | 0.0351 | 0.2647 |
| Ash | 0.1794 | 0.9222 | 0.0004 | 0.8637 | 0.0713 | 0.0006 | 0.0327 | 0.0001 | 0.6989 | 0.0001 |
| Liver fat | 0.3913 | 0.6142 | 0.0085 | 0.1216 | 0.6307 | 0.0075 | 0.3054 | 0.0026 | 0.1483 | 0.1112 |

**Table 9a:** The fatty acid composition of the muscle total lipid from catfish receiving different dietary lipids (Diet lipid fatty acid concentration in parenthesis, (fatty acids identified as % of total fatty acids measured.

| Fatty acid | Z (n=4) | A (n=9) | B (n=9) | C (n=9) | D (n=9) | SE of Mean |
|---|---|---|---|---|---|---|
| C14:0 | 1.16 | 1.72 (0.0) | 1.39 (0.1) | 3.44 (6.5) | 2.49 (3.1) | 0.1634 |
| C16:0 | 22.15 | 26.63 (0.0) | 19.18 (6.8) | 22.42 (16.1) | 24.71 (23.7) | 0.3538 |
| C16:1ω7 | 5.34 | 10.01 (0.0) | 3.75 (0.1) | 7.48 (9.9) | 7.15 (3.8) | 0.3098 |
| C18:0 | 7.29 | 6.53 (0.0) | 7.49 (5.8) | 6.74 (4.2) | 10.45 (23.9) | 0.2300 |
| C18:1ω9 | 24.63 | 35.29 (0.0) | 29.60 (22.9) | 29.67 (17.1) | 38.13 (37.2) | 0.8112 |
| C18:2ω6 | 15.25 | 6.61 (0.0) | 25.66 (62.6) | 5.58 (2.9) | 5.97 (4.0) | 0.4514 |
| C18:3ω3 | 1.43 | 2.24 (0.0) | 1.15 (0.2) | 5.03 (5.2) | 1.59 (0.4) | 0.0707 |
| C18:3ω4 | 0.94 | 0.41 (0.0) | 2.74 (tr) | 0.34 (0.3) | 0.60 (tr) | 0.0822 |
| C20:3ω6 | 0.37 | 1.85 (0.0) | 0.37 (0.8) | 0.25 (0.1) | 1.10 (tr) | 0.1092 |
| C20:4ω6 | 1.78 | 1.08 (0.0) | 1.55 (nd) | 2.28 (0.8) | 1.06 (0.1) | 0.2129 |
| C20:5ω3 | 1.32 | 0.35 (0.0) | 0.33 (0.2) | 3.68 (13.8) | 0.30 (tr) | 0.0805 |
| C22:0 | 2.51 | 1.12 (0.0) | 2.45 (nd) | 0.67 (3.3) | 1.01 (0.1) | 0.1242 |
| C22:5ω3 | 1.23 | 0.47 (0.0) | 0.35 (nd) | 1.49 (1.3) | 0.35 (0.1) | 0.0456 |
| C22:6ω3 | 7.28 | 1.87 (0.0) | 1.26 (nd) | 4.94 (7.1) | 1.0 (0.1) | 0.2807 |
| SFA | 33.47 | 36.33 (0.0) | 30.78 (12.8) | 33.51 (30.8) | 38.87 (51.1) | 0.4866 |
| MUFA | 31.02 | 45.13 (0.0) | 34.54 (23.1) | 38.75 (30.4) | 46.03 (41.2) | 1.0545 |
| PUFA | 32.21 | 15.70 (0.0) | 33.96 (63.9) | 24.59 (33.2) | 13.06 (4.9) | 1.1379 |
| ω3/ω6 | 0.65 | 0.52 (0.0) | 0.11 (0.01) | 1.87 (7.42) | 0.44 (4.25) | 0.0403 |

where Z = beginning of experiment, A = control diet, no lipid, B = sunflower oil, C = cod liver oil, D = tallow, tr <0.10, nd = not detected

**Table 9b:** Probability values calculate for pairwise differences of the muscle fatty acids of catfish receiving different dietary lipids.

| Fatty acid | $p > \mid T \mid$ HO: $LSMean_i = LSMean_j$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Z=A | Z=B | Z=C | Z=D | A=B | A=C | A=D | B=C | B=D | C=D |
| C14:0 | 0.1176 | 0.5204 | 0.0001 | 0.0005 | 0.2348 | 0.0001 | 0.0099 | 0.0001 | 0.0003 | 0.0007 |
| C16:0 | 0.0001 | 0.0003 | 0.7128 | 0.0018 | 0.0001 | 0.0001 | 0.0030 | 0.0001 | 0.0001 | 0.0002 |
| C16:1ω7 | 0.0001 | 0.0211 | 0.0001 | 0.0103 | 0.0001 | 0.0024 | 0.0001 | 0.0001 | 0.0001 | 0.0099 |
| C18:0 | 0.1368 | 0.7434 | 0.2550 | 0.0001 | 0.0226 | 0.5677 | 0.0001 | 0.0534 | 0.0001 | 0.0001 |
| C18:1ω9 | 0.0001 | 0.0064 | 0.0038 | 0.0001 | 0.0001 | 0.0001 | 0.0503 | 0.9505 | 0.0001 | 0.0001 |
| C18:2ω6 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.1487 | 0.4172 | 0.0001 | 0.0001 | 0.5992 |
| C18:3ω3 | 0.0001 | 0.0207 | 0.0001 | 0.2144 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| C18:3ω4 | 0.0044 | 0.0001 | 0.0008 | 0.0686 | 0.0001 | 0.5916 | 0.1898 | 0.0001 | 0.0001 | 0.0554 |
| C20:3ω6 | 0.0001 | 0.9851 | 0.5868 | 0.0035 | 0.0001 | 0.0001 | 0.0002 | 0.4901 | 0.0003 | 0.0001 |
| C20:4ω6 | 0.1372 | 0.6275 | 0.2510 | 0.1336 | 0.1990 | 0.0006 | 0.9574 | 0.0314 | 0.1940 | 0.0008 |
| C20:5ω3 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.8524 | 0.0001 | 0.7190 | 0.0001 | 0.8576 | 0.0001 |
| C22:0 | 0.0001 | 0.8387 | 0.0001 | 0.0001 | 0.0001 | 0.0228 | 0.6230 | 0.0001 | 0.0001 | 0.0904 |
| C22:5ω3 | 0.0001 | 0.0001 | 0.0068 | 0.0001 | 0.1258 | 0.0001 | 0.1575 | 0.0001 | 0.9405 | 0.0001 |
| C22:6ω3 | 0.0001 | 0.0001 | 0.0002 | 0.0001 | 0.2020 | 0.0001 | 0.3361 | 0.0001 | 0.7790 | 0.0001 |
| SFA | 0.0018 | 0.0034 | 0.9565 | 0.0001 | 0.0001 | 0.0001 | 0.0007 | 0.0001 | 0.0001 | 0.0001 |
| MUFA | 0.0001 | 0.0984 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.9490 | 0.0033 | 0.0001 | 0.0001 |
| PUFA | 0.0001 | 0.3983 | 0.0002 | 0.0001 | 0.0001 | 0.0001 | 0.1172 | 0.0001 | 0.0001 | 0.0001 |
| W3/ω6 | 0.0842 | 0.0001 | 0.0001 | 0.0073 | 0.0001 | 0.0001 | 0.1887 | 0.0001 | 0.0001 | 0.0001 |

Similarly, diet C had the highest concentration docosapentaenoic acid (DPA, C22:5ω3 - 1.3%), which also resulted in a higher corresponding concentration ($p=0.0001$) in the muscle (1.49%). The concentration of DPA in the muscle of fish fed diet Z was also significantly higher (1.23%) than the ±0.4% of the muscle of the fish on diets A, B and D.

The concentrations of docosahexaenoic acid (DHA, C22:6ω3) reflected those of EPA and DPA: relatively high in diet C (7.1%) and so correspondingly elevated in the associated muscle (4.94%). There was significantly lower DHA concentrations in the fish muscle receiving diets A, B and D (±1%) than that at the atart of the experiment (Z - 7.28%).

In general, the SFA concentration of the diets were reflected in those of the lipids from the muscle. Diet D had the highest SFA concentration (51.1%) as did the corresponding fish muscle (38.87%). Of the three oils, B had the lowest SFA concentration (12.8%) which resulted in a SFA concentration of 30.78% in the muscle. There was a significant ($p=0.0018$) increase in the percentage SFA between the control (36.33%) and that of the muscle at the beginning of the experiment (33.47%). The same trend was noted for the MUFA, diet D having a concentration of 41.2% and 46.03% in the muscle, while diet C had the second highest concentration of it (30.4 & 38.75%, respectively). The higher muscle MUFA concentration in A (45.13) was significantly higher ($p=0.0001$) than that in Z (31.02%). Diet B had the highest concentration of PUFA (63.9% & 33.96% in the diet and muscle, respectively) whilst diet D had the lowest (4.9 and 13.06% respectively). There was a significant ($p=0.0001$) decrease in percentage PUFA between Z (32.21%) and A (15.70%).

The ratio of ω3 to ω6 fatty acids differed significantly between the diets and the total lipids of the muscle of the fish receiving the different diets. Diet B had a low ratio (0.01%), resulting in a 0.11 ratio in the muscle lipid. Diet C had the highest ratio (7.42) resulting in a 1.87 ratio in the lipid muscle. Diet D had a ratio of 4.25, which produced the second lowest muscle lipid ratio (0.44), which differd significantly from that of Z, B and C.

Although testing the influence of various lipid sources on the growth of *C. gariepinus* was not an objective of this investigation, the following trends were manifested. In all three mass classes; the fish receiving the sunflower oil (B) had the highest final mean mass (Table 10). In both the medium and large mass ranges, the fish receiving the control diet (A), with no lipid, showed the lowest mean mass followed by those receiving cod liver oil (C).

**Table 10:** The mean body mass (g) of the catfish at day 60 receiving diets containing different lipids.

| **Mass Class** | **Diet** | | | | **Std Err LSMean** | $p >$ \| T \| HO: $LSMean_i = LSMean_j$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **A (n=20)** | **B (n=20)** | **C (n=20)** | **D (n=20)** | | **A = B** | **A = C** | **A = D** | **B = C** | **B = D** | **C = D** |
| Small | 224.2 | 257.7 | 221.2 | 210.7 | 15.162 | 0.1277 | 0.8927 | 0.5357 | 0.1070 | 0.0363 | 0.6386 |
| Medium | 249.8 | 344.2 | 261.4 | 326.0 | 17.971 | 0.0004 | 0.6450 | 0.0037 | 0.0015 | 0.4749 | 0.0121 |
| Large | 411.5 | 543.9 | 461.3 | 467.3 | 25.030 | 0.0004 | 0.1631 | 0.1291 | 0.0223 | 0.0384 | 0.8706 |

Where Z = beginning of experiment, A = control diet, no lipid, B = sunflower oil, C = cod liver oil, D = tallow.

## DISCUSSION

The growth and survival noted (Fig 1, Table 3) for *Clarias gariepinus* larvae compare well with previous results obtained for this species (Uys & Hecht, 1985; Boon *et al.*, 1987; Polling *et al.*, 1988; Schippers *et al.*, 1992). Most of the deaths that occurred were from mechanical injuries (usually caused during netting for weighing purposes and manual cleaning of tanks) or of unknown causes. Only a small percentage of deaths were identifiable as the Ruptured Intestine Syndrome (RIS - Boon and Oorschot, 1986; Boon *et al.*, 1987; Schippers *et al.*, 1992). Schippers *et al.* (1992) noted in their studies that the highest mortalities caused by RIS occurred during weeks five to nine, with a peak in week seven. In the present investigation, most of the RIS deaths occurred during week six.

The feed conversion ratio (FCR) recorded in the present investigation (for all four diets) was relatively high for this species, and is probably as a result of the high feeding rate employed in the present investigation. This high FCR is analogous to that of Schippers *et al.* (1992), who also noted an increase in FCR, especially during weeks five to seven (FCR increased from 1.2 to above 5.8) at a similar feeding rate. In their studies, Boon *et al.* (1987) noted FCRs below one, however, their feeding rates differed from that of the present investigation (25% of total calculated metabolic weight), initially they started at feeding rates of 9, 18 or 27% of fresh body weight, with all the levels decreasing as the experimental period progressed.

The high body lipid content of the fish fed the gonads was also evident in the histopathalogy of the liver. The liver has been noted as a major lipid storage organ in a number of marine and freshwater fish species (reviewed by Sheridan, 1988). Fatty infiltration of liver cells is common in farmed fishes, especially during the period of maximum feeding. Roberts (1989) notes that fat infiltration normally causes the liver to enlarge, take on a yellow or light brown colour, and loose its sharp edges, descriptions that fit the livers of the fish receiving the GOD and GVD diets. Fatty infiltration of livers can also be caused by fish feeding on rancid feeds or by fish consuming diets containing toxic substances or vitamin deficiencies (Ferguson, 1989; Roberts, 1989). The present diets were not rancid as the different diets were stored in a freezer at -40°C before use and their respective lipid fatty acid profiles (Table 6) did not show a decrease in the amount of PUFA (Ferguson, 1989). Extra vitamins (Table 1) were also added to the diet before the daily feeding. A possible explanation for the variation in fatty acid profile may lie in the fact that in the present investigation, the fatty acids of the total lipids were analysed, and not that of the different lipid classes namely, phospholipids, cholesterol, free fatty acids, diglycerides, triglycerides and cholesterol esters (Love, 1988). The fatty acid profiles of these lipid classes could differ as could their utilisation by the larvae and warrants further investigation.

In a commercial fish production unit, slaughtering of sexually mature female *C. gariepinus* during summer will yield a large amount of gonadal material (Prinsloo *et al.*, 1990) that is at present classified as waste (Hoffman &

Prinsloo, 1990) and could be utilised in a starter diet for the same species' larvae. The accumulation of lipids in the hepatocytes of the larvae receiving the gonads, seems to indicate that fish meal should only be partially replaced with fish gonads in the traditional starter diet of Uys (1988). Alternatively, the time span that larvae are fed on a diet in which the fish meal is totally replaced with gonadal material, should be shortened. The proportional composition of these two protein sources or the time span fed on gonadal material only, so as to minimise liver lipid accumulation, warrants further study.

The second nutritional study has shown that the fatty acid profile of *C. gariepinus* can be manipulated to make the muscle more nutritionally desirable (Hoffman, Prinsloo & casey, 1993). Feeding the fish sunflower oil rather than tallow increased the PUFA concentration of the muscle lipid from 13.06 to 33.96%. From a human nutritional viewpoint, it is not only the concentration of PUFA present, but also the relationship between ω3 and ω6 fatty acids that are important (Tichelaar, 1993). In the present investigation, this ratio was manipulated from 0.11 for the sunflower oil diet to 1.87 for the cod liver oil diet.

The better growth experienced by *C. gariepinus* on a sunflower lipid diet (rich in C18:2ω6), compared to the other lipid sources, differs from other investigations reported on other warm-water fish species. Channel catfish (*Ictalurus punctatus*) fed on safflower oil (also rich in C18:2ω6) grew less than when on tallow or menhaden oil as a lipid base (Stickney & Andrews, 1971). The fatty acid profiles of the safflower oil, tallow and menhaden oil used by Stickney and Andrews (1971) was similar to that of this investigation, in that the same fatty acids predominated. In a latter study, Stickney and Andrews (1972) noted that high concentrations of either linoleic (C18:2ω6) or linolenic (C18:3ω3) led to inferior growth in channel catfish. The slower growth experienced by the catfish (*Clarias*) fed the cod liver oil as lipid source, may be caused by the high linolenic acid concentration (5.2%), as this component, when present at concentrations approaching 1%, retards growth in channel catfish (Stickney & Hardy, 1989).

The influence of dietary lipid and the relationships between ω3 and ω6 fatty acids on growth of *C. gariepinus* warrants further attention. In the present investigation, the similar growth rate displayed by the small mass class fed the control diet compared to the other lipid diets, may be attributed to the mobilisation of lipid depots so as to ensure sufficient EFA (Sheridan, 1988). It is therefore imperative that, as noted by Stickney and Hardy (1989), the duration of an investigation into the influence of lipid sources on growth rates be such that all lipid reserves are utilised so as not to influence the results. In the present investigation, a 60 day period would seem to be insufficient to test *C. gariepinus* with an initial total muscle lipid above 1.5%. A lipid concentration of 10% in the diet may also be too high for *C. gariepinus*, as the total lipid concentration in muscle (Table 8a) were higher than that previously noted for wild and cultivated specimens (Hoffman, Casey & Prinsloo, 1992) and led to large mesenteric fat depots forming (Hoffman, Casey & Prinsloo, 1993).

## CONCLUSION

The first investigation has shown that *Clarias gariepinus* gonads are suitable as a partial replacement for fish meal in the larval starter diet of the same species. The ratio of gonadal material to fish meal warrants further study. In the second investigation, it was indicated that the fatty acid composition of *C. gariepinus* muscle can be manipulated and changed through the dietary lipid. However, the influence that various fatty acids may have on the growth of *C. gariepinus* needs further investigation.

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# Chapter 7 HEALTH ASPECTS OF FISH RAISED IN FINAL EFFLUENT OXIDATION PONDS

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**INTRODUCTION**

In 1984, *C. gariepinus* had been identified as an important aquaculture species in South Africa (Bruton & Safriel, 1984) and in 1990, 1200 tonnes of catfish was produced locally (Hecht & Britz, 1990). Over the past number of years, research into the chemical variation of the muscle of the African sharptooth catfish *Clarias gariepinus* has been carried out in our laboratories (Hoffman, Casey & Prinsloo, 1992, 1993*a,b,c*).

During research investigations and farming of this species, isolated bacterial, fungal, viral and parasitic infections have been noted, both on wild and cultured fish. Although not all of these infections may be hazardous to human health, they decrease the value of the product in terms of consumer acceptability, especially because of their influence on the aesthetic appearance (Huss, 1988). Another factor that could play an important role in human health, is the presence of various pollutants, such as heavy metals (Turner, Sibbald & Hemens, 1986; Grobler, Kempster and van der Merwe, 1994) and pesticides in the fish muscle (Nettleton *et al.*, 1990; Grobler, 1994).

With the limited water resources available for aquaculture practices in South Africa, alternative water bodies need to be utilised. The use of final effluent oxidation ponds for the growth of various fish species is such a water body (Wood, 1986), and benefits both resource conservation and waste water disposal. The use of domestic sewage for aquaculture production is not common globally, but has gained in importance in recent years (Oláh, Sharangi & Datta, 1986) and in South Africa has been used successfully for this purpose (Sandbank & Nupen, 1984; Gaigher, 1985; Prinsloo & Schoonbee, 1987; Kilani & Tollow, 1993). The idea of introducing organic wastes and excreta from agricultural and human sources into pond systems seems to have originated in China (Wohlfarth & Schroeder, 1979). The use of domestic waste from animals and man has two important considerations: first, it is an inexpensive source of nutrients for promoting zoo- and phytoplankton growth; second, it leads to the introduction and concentration of various bacterial, viral, protozoal and helminth pathogens and parasites which may be transmitted via aquatic organisms to man (Naegel, 1990; Haard, 1992). With the incorporation of industrial and agricultural waste into the sewage purification systems, the additional contamination of the surface waters with metals, radioactive pollutants and pesticides becomes another factor to be considered (Bouwman, Coetzee & Schutte, 1990). Bryan (1977) has noted that waste-water contaminated shellfish have resulted in 28 international outbreaks of illness, watercress in 10, fish in three, and shrimp in one.

Where excreta are used in aquaculture, three groups of people may be at risk of infection (Naegel, 1990):

a) Persons who consume raw or insufficiently cooked aquatic organisms.

b) Persons who consume raw or inadequately cooked meat of animals that have been fed with raw, infected fish or contaminated plants.

c) Persons with occupational exposure to ponds laden with excreta, and people handling and preparing contaminated aquatic products.

There are a number of pre-requisites for a pathogen to cause an outbreak of an illness. These circumstances for the consumption of fish can be summarised as follows (Bryan, 1977; Naegel, 1990):

a) The infectious agent must be present in citizens of a community or in animals on farms, or toxic agents must be used for industrial or agricultural purposes; and wastes from these sources must reach sewerage or drainage systems.

b) The agents must survive and pass through all wastewater treatment processes to which they are exposed.

c) The waste-treatment effluent or water course receiving the effluent must be used as a growing environment for fish or for washing or freshening harvested fish.

d) The agents must be able to survive, and where applicable, multiply in the water environment or on/in the fish.

e) Many pathogens require one or even two intermediate hosts before becoming a threat to man. These hosts must be present in sufficient quantity.

f) Thereafter (i). The agents must be present on/in the contaminated fish in sufficient numbers to survive the remainder of the growing period, storage, and preparation and still cause illness. (ii). Bacteria on/in fish in insufficient numbers to cause illness must multiply and reach numbers that are necessary to cause illness. (iii). Bacteria, and perhaps other organisms, enter preparation areas on raw fish, where they may be transferred to workers' hands or to equipment surfaces, which if inadequately washed will contaminate other foods that they subsequently touch.

g) Sufficient quantities of the contaminated food that contain enough of the agent to exceed a person's susceptibility threshold must be ingested.

When insufficient numbers of pathogens are ingested, the infected individuals may become carriers and subsequently contaminate other foods/environments that they touch (Bryan, 1977). If the use of waste water for aquaculture practices is to succeed, then the chain of events that need to develop for an outbreak of illnesses to occur as described above, require to be broken at as many links as possible, especially towards the end, where harvesting of fish occurs.

As noted, many of the pathogenic organisms that infect man reach him by being conveyed by more than one vehicle. For example, eggs of some parasitic organisms can appear in the faeces of infected persons, reach water where they hatch into a form that infects a vector (such as a snail), and, after a period of development,

metamorphize into a free-swimming form which penetrates tissues of fish or water vegetables (Bryan, 1977). In Table 1, various diseases that are transmitted to man via such pathways are listed.

**Table 1:** Parasitic diseases maintained by the cycle: Human faeces to water or soil; water or soil to food; food for human consumption (from Bryan, 1977)

| Agent | Intermediate vehicle | Intermediate host | Food |
|---|---|---|---|
| *Clonorchis sinensis* | fresh water | snail | fish |
| *Diphyllobothrium latum* | fresh water | copepods | fish |
| *Echinostoma ilocanum* | fresh water | snail | snails, clams, limpets, fish, tadpoles |
| *Fasciola hepatica* *F. gigantica* | fresh water | snail | water cress, water vegetables |
| *Fasciolosis buski* | fresh water | snail | water vegetables |
| *Opisthorchis felineus* | fresh water | snail | fish |
| *Paragoniums westermani* | fresh water | snail | crabs, crayfish |
| *Taenia saginata* | soil (grass) | | beef |
| *Taenia solium* | soil (grass) | | pork |

Most of the incidences of diseases communicated to man via fish have been noted in the Far East (Naegel, 1990). This is understandable as animal and human waste utilisation for aquaculture is an ancient practice there, dating back several centuries where traditional systems involved cartage, the removal of nightsoil by bucket or cart, and more recently by vacuum truck, for use as fertilizer for fish ponds. In a number of countries in Asia, the overhung latrine, a superstructure over a fish pond, can still be seen (Anon, 1990).

Natural waters also contain many different bacterial species, including some known to be pathogenic to fish and/or human beings. Studies have been conducted on the bacterial populations of aquatic ecosystems, ranging from freshwater, i.e. lakes, ponds, rivers and groundwater, to brackish and estuarine conditions, and the pelagic

waters and benthic zones of the oceans (reviewed by Austin & Allen-Austin, 1985; Jones & Watkins, 1985; Lewis, 1985).

This paper is firstly, a review of pertinent literature on possible organisms and materials that have, and can cause, outbreaks of illnesses, and secondly, an investigation into the body chemical composition of the African sharptooth catfish *Clarias gariepinus* found in such a water body. Although many of these organisms/materials have not been noted in South Africa, knowledge of their existence is of vital importance for two major reasons:

i). With the optimum utilisation of water resources, the use of wastewater for fish production and the integration of agriculture with aquaculture will become a more common practice (Wood, 1986).

ii). With modern means of transport, especially aircraft, the conveyance of parasites and pathogens by carriers into South Africa, and subsequently into our environment, is a reality (Masterton & Green, 1991), as manifested by the latest international outbreak of cholera.

## ENTERIC BACTERIA AND VIRUSES

It is generally accepted that human and warm-blooded animal bacterial and viral pathogens do not cause acute diseases in aquatic organisms. Aquatic organism can be considered passive carriers and mechanical transmitters of pathogens to man (Ward, 1989; Naegel, 1990). Fish grown in excreta-laden ponds carry these pathogens passively in their intestines, gills and in the mucus of their skin. It is only when the pathogens reach a certain concentration in the water that the peritoneal fluid and muscle of the fish become infected. Buras and co-workers (1987) named this level the "threshold level" and it expresses the limit of the capacity of the reticuloendothelial (RE) system of the fish. When this limit is surpassed, the RE is no longer effective, no barrier is present and bacteria and viruses from the water can then penetrate the muscle of the fish.

The threshold concentration can vary with fish species. In a study on the bacteria infection of silver carp (*Hypophthalmichthys molitrix*), tilapia aurea (*Sarotherodon aureus* = *Oreochromis aureus*) and common carp (*Cyprinus carpio*), Buras and co-workers (1987) noted the following important points: Before being introduced into the experimental ponds (containing aerated excreta) the fish already contained a number of bacteria - in the digestive tract of the carp, bacteria was present in concentrations of $10^6$ $g^{-1}$, for tilapia the concentrations were $5,0 \times 10^4$ $g^{-1}$. However, none of the muscle was contaminated. (Good reviews of fish bacterial pathogens are available in the literature - Austin & Allen-Austin, 1985; Roberts, 1989). At the end of the investigation, when the concentration of the bacteria in the water was high ($10^6$-$10^7$/ml), the number of bacteria recovered from the organs and muscles of the fish was high and varied between species. When the concentration of the bacteria in the water was low ($10^4$/ml), small numbers of bacteria was recovered from the kidneys, liver, pronephros and

spleen. None was recovered from the fish muscle. Bacteria was usually only recovered from the muscle when they were present in high concentrations in the other organs. In the above mentioned experiment, the threshold concentration for the mentioned fish species was between 1.0 and 2.0 x $10^4$/ml total bacteria (not total coliforms or faecal coliforms but SPC/ml). The bacteria concentration in the pond water that caused the appearance of bacteria in the muscle of fish is known as the critical concentration.

In South Africa, a combination of mirror carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambicus*) and silver carp (*Hypophthalmichthys molitrix*) reared in human tank effluent showed no faecal coliforms or salmonella in the prepared sterile fish fillets. The fish ponds had a bacterial total plate count of 11 x $10^4$/ml, a total coliforms of 4 x $10^2$/100ml and a faecal coliforms count of 4 x $10^1$/100ml (Sandbank & Nupen, 1984). Similarly, tilapia (*Oreochromis mossambicus*) showed no muscle bacteria when raised in sewage stabilization ponds that contained 2.0 x $10^3$/ml faecal coliforms (Slabbert, Morgan & Wood, 1989). Van den Heever and Frey (1994) also found no muscle contamination in African catfish (*Clarias gariepinus*) raised in water containing 6.3 x $10^2$/100ml faecal coliforms and 7.2 x $10^2$/100ml coliphages. Cloete, Toerien and Pieterse (1984) examined the muscles of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) raised in ponds receiving a cattle feedlot's effluent and noted no bacterial presence therein. In their investigation, the total aerobic bacteria of the fish pond water ranged from 3 x $10^5$/ml at the inflow and 0.25 x $10^2$/ml at the outflow, whilst the total anaerobic bacterial numbers ranged from 1.5 x $10^5$ to 10 x $10^5$/ml. The coliform numbers ranged from 2.5 x $10^2$ to 17 x $10^2$/ml and the presumptive salmonellae numbers from 80 to 520/ml.

In America, Hejkal and co-workers (1983) found no correlation between the bacterial level of the water and the bacterial contamination of the fish species raised therein. The two filter feeder fish species of Chinese carp studied, *Hypophthalmichthys molitrix* (silver carp) and *Aristichthys nobilis* (bighead carp) accumulated faecal coliforms and faecal streptococci in their digestive tracts and skin at concentrations as great or greater than that in the waste water. Only low levels of faecal coliforms and streptococci were found in the fish muscle tissue (maximum 25 faecal streptoccoci per 100g) even when concentrations in the gut exceeded $10^5$ per 100g. The presence of these pathogens were attributed to contamination from the skin and gut during the killing and dressing processes. Similarly, in a study of the bacterial contamination of farm raised channel catfish *Ictalurus punctatus* at various retail outlets in America, it was found that the catfish had low incidences of bacterial enteric pathogens which most probably resulted during the processing (Andrews, Wilson, Poelma & Romero, 1977).

In their study on the bacterial contamination of two large fish farming enterprises in the United Kingdom, Austin and Allen-Austin (1985) found that the fish farming practice did not produce a major imbalance in the

aquatic bacterial communities.

Jones and Watkins (1985) list pathogenic agents (Table 2) that may be present in sewage and notes that the whole range may not be present at any one time and levels may not be high enough to cause infection.

In their respective reviews, Bryan (1977), Velasquez (1980), Austin and Allen-Austin (1985), Tyler (1985) and Naegel (1990) list the various bacterial and viral pathogens that have been noted in waste water and which could cause possible epidemics (summarised in Table 3).

Tuberculosis has also been observed in a large group of animals including fish, alligators, frogs, snakes and turtles (Velasquez, 1980). The following enteric bacteria could also conceivably be transmitted by foods, although proof is inconclusive (Bryan, 1977): *Streptoccocus faecalis, S. faecium, Proteus* spp., *Providencia* spp., *Klebsiella* spp., *Citrobacter freundii, Enterobacter* spp., *Pseudomonas aeruginosa*. Other pathogenic bacteria that have also been found to be carried by fish include: *C. tetani*, *Staphylococcus aureus* (Velasquez, 1980), *Listeria monocytogenes* and *Campylobacter fetus* (Haard, 1992), and *Clostridium botulinum* type E (Austin & Allen-Austin, 1985; Hackney & Garrett, 1985). The latter organism is widely distributed in marine and lake sediments and is a frequent contaminant of fish. Fortunately it does not appear to grow and produce toxin in living animals and has been a health hazard only in processed fish, and not in fresh and frozen products (Brown, 1986). This is generally a result of inadequate heat treatment that kills competing organisms but allows *C. botulinum* spores to live. The toxin produced is easily destroyed by heat, therefore, if cooked properly, even fish infected with this organism may be safely eaten (Wheaton & Lawson, 1985).

*Edwardsiella tarda* is the causative agent of the disease "emphysematous putrefactive disease of catfish" and is frequently found in organically polluted water (Roberts, 1989). This organism has been implicated in gastroenteritis in humans, wound infections and meningitis (Ward, 1989). In our various investigations we have identified isolated cases of *Edwardsiella tarda*, on the African sharptooth catfish *Clarias gariepinus*.

*Aeromonas hydrophila* is the causative agent of the disease "motile aeromonad septicemia" which is common in cultured species world wide (Tucker, 1985). There is speculation on the possible role of species of the *Aeromonas hydrophila* group (*A. hydrophila, A. sobria,* and *A. caviae*) as a cause of human gastroenteritis. *A. hydrophila* can produce fatal septicemia in individuals debilitated by some other disease or condition (Ward, 1989).

**Table 2:** Pathogenic agents that may be present in sewage (from Jones & Watkins, 1985)

| Bacteria | Viruses | Intestinal parasites |
|---|---|---|
| *Salmonella typhi*<br>*Paratyphi*<br>Other spp. | Enteroviruses | *Schistosoma* spp. |
| *Shigella* spp. | Poliovirus | *Ascaris lumbricoides* |
| *Vibrio cholerae* | Echovirus | *Trichuris trichuria* |
| *Mycobacterium tuberculosis* | Coxsackieviruses | *Taenia* spp. |
| *Leptospira icterhaemorrhagiae* | New enteroviruses | *Diphyllobothrium latum* |
| *Campylobacter* spp. | Hepatitis type A | *Ankylostoma duodenale* |
| *Listeria monocytogens* | Norwalk virus | *Necator americanus* |
| *Candida albicans* | Rotavirus | *Entamoeba histolytica* |
| *Yersinia enterocolticia* | Reovirus | *Giardia lamblia* |
| Enteropathogenic *Escherichia coli* | Adenovirus | *Naegleria* spp. |
| *Pseudomonas aeruginosa* | Parvovirus | *Acanthamoeba* spp. |
| *Klebsiella* spp. | | *Cryptosporidia* |
| *Staphyloccocus aureus* | | |
| *Aeromonas hydrophila* | | |
| *Mycobacterium paratuberculosis* | | |
| *Erysipellothrix shusopathiae* | | |
| *Bacillus anthracis* | | |
| *Clostridium* spp. | | |
| *Yersinia pestis* | | |
| *Brucella* spp. | | |

**Table 3:** Important pathogens with potential for spread by the use of excreta in aquaculture

| Pathogens | Diseases |
|---|---|
| | ***Bacteria*** |
| *Arizona hinshawii* | *Arizona* infection |
| *Bacillus cereus* | *Bacillus cereus* gastroenteritus |
| *Campylobacter jejuni* | diarrhoea, vomiting |
| *Clostridium perfringens* | *Clostridium perfringens* gastroenteritus |
| *Escherichia coli* | Enteropathogenic *Escherichia coli* infection |
| *Salmonella paratyphi* A, B, C<br>*S. sendai* | Paratyphoid fever |
| *Salmonella* (+1500 serotypes) | Salmonellosis |
| *Salmonella typhi* | Typhoid fever |
| *Shigella sonnei*<br>*S. flexneri*<br>*S. dysenteriae*<br>*S. boydii* | Shigellosis, diarrhoea, dysentary |
| *Vibrio cholerae* | Cholera, diarrhoea |
| *Yersinia enterocoliticia*<br>*Y. pseudotuberculosis* | *Yersinia* gastroenteritis |
| | **Viruses** |
| Adenoviruses | Adenovirus infection |
| Coxsackie viruses | Coxsackie infection |
| ECHO viruses | ECHO virus infection |
| Enteroviruses | Poliomyelitis, meningitis, fever, diarrhoea |
| Hepatitis virus A | Viral hepatitis |
| Norwalk agent | Winter vomitting disease |
| Polioviruses | Poliomyelitis |
| Reoviruses | Reovirus infection |
| Rotaviruses | Diarrhoea |

*Plesiomonas shigelloides* is a member of the Vibrionaceae and has been implicated as the agent of human gastroenteritis (Brock, 1986). *P. shigelloides* has also been known under several names: *Aeromonas shigelloides*, *Pseudomonas shigelloides*, *Fergusonia shigelloides*, *Pseudomonas michigani* and C27 (Ward,

1989). Bacterial dysentery (shigellosis) and its cause were first recognised in Japan at the end of the nineteenth century during an epidemic involving 90 000 cases (Abel, 1989). Today the disease remains common throughout the world. Shigellosis is caused by the consumption, in faecally-contaminated water or food, of live bacteria of the genus *Shigella*; known pathogenic species include *Sh. dysenteriae, Sh. flexneri* and *Sh. sonnei.*

The following summaries are of diseases of special interest as they are applicable to people working with excreta-laden water bodies (Velasquez, 1980):

**Erysipelas** - a communicable disease of swine and poultry caused by corneabacteria, *Erysipelothrix insidiosa* Langford and Hensen, *E. mursepticum* Rosenbach, *E. rhusiopathiae, Bacillus erysipelatus suis* and *B. rhusiopathiae.* Infected fish show no symptoms. Man contracts the infection through skin abrasions in handling materials of animal, fish or shellfish origin. It is often called "fish handlers disease" in the United States and produces a condition called "Fish Rose". Sever inflammation may result when wounds are infected from mucus of dead fish with a burning and itching skin sensation which may last for up to 3 weeks.

**Leptospirosis** (Weil's disease, Leptospirosus, canicola fever, haemorrhagic jaudice, Swine's head disease) - This disease is an occupational hazard of agricultural, fish and abattoir workers, sewer workers, etc., from exposure to water contaminated by the urine of wild or domestic animals. Velasquez (1980) lists 16 serotypes of Leptospira that has been isolated from human cases and animals in the Philippines (Table 4). Cases of people swimming in water contaminated by *L. pomona*-infected animal excreta have been reported (Velasquez, 1980). The possibility of fish being implicated in the dissemination of the disease is not remote if they are reared in contaminated water.

**Salmonellosis** - About 45 species of salmonellae have been reported in the Philippines (Velasquez, 1980). A number of fish species have been found to take up salmonellae and fish could therefore be important in the epidemiology of salmonellae (Wyatt, Nickelson & Vanderzant, 1979). Lewis (1975) as quoted by Ward (1989) has reported that certain species of freshwater and marine fish could harbour *Salmonella* for 30 days after exposure to high levels ($10^7$ per ml) and some species developed apparent salmonellosis 10-14 days after exposure. Typhoid infection, caused by *Salmonella typhi*, is usually caused by ingestion of bacteria from faecally-contaminated water or food. If the bacteria survive passage through the acid of the stomach, they colonise the intestine and enter the epithelial cells of the gut lining. Very few bacteria (between 100 and 1000 cells) are required to establish a *Salmonella typhi* infection; for other *Salmonella* infections, the minimum infective dose is rather higher, approximately $10^5$ to $10^6$ cells (Abel, 1989). An interesting feature of this disease is that in some cases the symptoms of infection can be mild and undiagnosed, although the patient becomes chronically infected without showing symptoms of the disease. These "carriers" are a persistent source of

infection in the community, and if diagnosed the disease in such patients does not readily respond to the normal treatment with antibiotics (Abel, 1989).

**Table 4:** *Leptospira* reported in the Philippines from farm animals and man (Velasquez, 1980)

| Serotypes | Host(s) |
|---|---|
| *L. australis* | pig, man |
| *L. autumnalis* | wild rat, dog, pig, sheep, cat |
| *L. bataviae* | wild rat, dog, pig, man |
| *L. canicola* | dog, cattle, man |
| *L. grippotyphosa* | wild rat, cat, dog, sheep, shrew, pig, man |
| *L. hyos* | wild rat, pig, man |
| *L. icterohemorrhagiae* | wild rat, dog, carabao, pig, man |
| *L. javanica* | wild rat |
| *L. javiana* | wild rat, man |
| *L. manilae* | wild rat, man |
| *L. pomona* | cattle, carabao |
| *L. poi* | dog |
| *L. pyrogenes* | wild rat, pig, man |
| *L. sejroe* | cattle |
| *L. tarassovi* | wild rat, pig, carabao |
| *L. wolfii* | cattle, man |

**Influenza pandemics** - Every 10 to 20 years pandemic influenza A viruses with new surface antigens suddenly appear in nature against which no neutralising antibodies are present in the human population (Scholtissek & Naylor, 1988). This antigenetic shift is caused by genetic readjustment between human and avian viruses in pigs. Pigs can become infected by and may transmit both human and avian influenza viruses not only amongst other pigs but also back to the original hosts. Pigs are therefore the "mixing vessels" where the two separate reservoirs meet and where reassortment between avian and human influenza A viruses occurs, giving rise to

the antigenetic shift creating new human pandemic influenza strains with new surface antigens (Scholtissek & Naylor, 1988).

The concept of integrated agriculture-aquaculture practices is based on the optimum utilisation of all resources and normally consists of an animal-bird-fish-crop combination or part combination (Schroeder, 1980). A frequent combination found is that of pigs, ducks or chickens and various fish species. These combinations have been implemented successfully in India (Jhingran & Sharma, 1980), Hungary (Woynarovich, 1980*a, b*; Sharma & Oláh, 1986), China, Israel and Europe (Wohlfarth & Schroeder, 1979), Taiwan (Chen & Li, 1980), Singapore (Delmendo, 1980), Philippines, (Cruz & Shehadeh, 1980), Hong Kong (Wai-Ching Sin, 1980), Indonesia - West Java (Djajadiredja, Jangkaru & Junus, 1980), Malaysia (Tan & Huat, 1980), Nepal (Rajbanshi & Shrestha, 1980), Thailand (Wetcharagarun, 1980), and South Africa. Such a combination produces the potential for the genetic reassortment between human and avian pandemic influenza A viruses, which may well result in the creation of a considerable human health hazard which could have global implications for the recurrence of influenza pandemics (Scholtissek & Naylor, 1988).

## PROTOZOAL INFECTIONS

The two major diseases, Amoebiasis (Amoebic dysentery - *Entamoeba histolytica*) and Balantidiasis (Balantidial dysentery -*Balantidium coli*) occur in areas of poor environmental sanitation. Association with pigs and the use of animal manure as fertiliser may result in higher incidence of the diseases. Coccidiosis (Isospora infection) caused by *Isospora belli* and *I. hominis*, Dientamoeba infection (*Dientamoeba fragilis*) and Giardiasis (*Giardia lamblia*) have also been responsible for disease outbreaks associated with foods contaminated by sewage or wastewater (Bryan, 1977; Velasquez, 1980). Other protozoa that have been found in effluent water also include *Entamoeba coli*, whilst *Endolimax nana, Iodamoeba butchel* and *Chilomastix mesorili* are protozoa that normally inhabit the human intestine, but have also been found in the pond water (Bartone & Khouri, 1990).

## PARASITES

The presence of parasites in fish, particularly wild fish, is very common. The great majority are of no public health significance, but they represent a quality problem since their appearance is unaesthetic and causes rejection and complaints from buyer and/or consumer (Huss, 1988).

### Flatworms (*platyhelminths*)

Most of the numerous infectious helminths which can be transmitted to man, require at least one intermediate host for completion of their life cycle. This fact reduces the potential for transmission. If however, the appropriate host or hosts are present in the water the potential for spread increases. Transmission to man can

only occur if appropriate host/hosts are present and if man consumes either raw or partially cooked flesh from the intermediate host that contains the helminth larva (Naegel, 1990). Most flukes and all tapeworms have complex life histories involving stages in different animals in addition to fish. The flatworms found in fish are almost all harmless to man (Connell, 1980, 1990).

Velasquez (1980) lists 17 fish species that were found to be carriers of human helminthic infections, none of the helminth parasites were host specific (Table 5):

**Table 5:** List of helminth parasites found in 17 fish species in the Philippines (adapted from Velasquez, 1980)

| **Helminth parasites** | |
|---|---|
| **Trematodes** | **Nematodes** |
| *Procerovum calderoni** | *Gnathostoma spinigerum** |
| *Stellantchasmus amplicaecalis** | |
| *Centrocestus caninus* | |
| *Haplorchis yokogawai** | |
| *Haplorchis pumilio* | |
| *Haplorchis taichui** | |
| *Stictodora guerreroi* | |
| *Stictodora manilensis* | |
| *Heterophyopsis expectans* | |

Parasites indicated with * were also found in humans in the Philippines. Velasquez (1980) also notes cases of humans being infected by: *Echinostoma ilocanum, Fasciola hepatica, F. gigantica, Carneophallus brevicacea, Paragonimus westermanii, Philophthalmus* sp. *Schistosoma japonicum, Ascaris lumbricoides* and *Gnathostoma spinigerum*. In the majority of these mentioned parasites, the first intermediate host was a snail.

Of special note are the Schistosoma trematodes (*Schistosoma japonica* or blood fluke, *S. mansoni* or hepato-intestinal fluke, *S. haematobium* Bilharziose or urinary fluke, *S. mekongi* and *S. intercalatum*) as their mode of infection is via cercaria swimming and penetrating the skin of humans or animals (Maar, Mortimer & Van der Lingen, 1966; Naegel, 1990). These parasites are also prevalent in South African water bodies (Pretorius, Joubert & de Kock, 1989; de Kock *et al.*, 1993). McCullough (1990) gives a thorough review of the interaction

between schistosomiasis and aquaculture and the possible steps that need to be taken to control this parasite (from an aquacultural perspective). These steps can be summarised as:

1) A strong management programme that is closely linked to a well informed workforce and that has a strong community involvement (includes informative health programmes, strong primary health care, etc.). The community involvement is essential in all stages of the schistosomiasis control programme, as the infection is "man-made".
2) Physical control measures that include a more ecological approach to disease/pest/vector control, such as snail habitat modifications, measures to reduce or eliminate human-water contact and/or pollution at transmission sites and improved sanitation and water supplies. (This will also play a major role in the prevention of other communicable diseases).
3) The elimination or suppression of snail host populations by environmental, chemical and biological means.
4) Chemotherapy using one or other of the safe and effective antischistosomal drugs which have become available during the last 20 years.

**Roundworms (Nematodes)**

Roundworms or nematodes cannot survive long periods of freezing and cold storage (Wheaton & Lawson, 1985). For this reason, Japanese, Dutch, and Norwegian regulations require that certain fish products which are to be eaten raw are to be frozen and stored at -20°C for at least 24 hrs before being sold (Connell, 1980). Connell (1990) noted that nematodes are not readily killed by modern methods of curing involving only moderate concentrations of salt.

In the South African context, nematode infections are very common in the sharptooth catfish (especially wild) and have been reported on by Mashego and Saayman (1981) and van As and Basson (1984). Van As and Basson (1988) note that in their surveys in the Northern Transvaal, every catfish (wild) examined had an infection of the larval nematode, *Contracaecum*, found attached to the viscera. Our own studies have noted this nematode in catfish from both man-made dams and final effluent oxidation ponds. This nematode has also been noted in farmed catfish where the management practices have not been of a high standard. The final hosts are piscivorous birds (and most probably large vertebrates such as crocodiles) that feed on catfish. Eggs are deposited into water from these birds, where the fish then become infected with the resulting larvae. When catfish feed on other fish that have been infected, *Contracaecum* larvae are accumulated. The ingested worms migrate from the stomach to the viscera where high numbers can occur (Fig 1), Mashego and Saayman (1981) noted up to 2860 larvae per fish. In the latter study, the stomach musculature of heavily infected fish also contained *Contracaecum* larvae. Van As and Basson (1988) note that internal infections are evident as black

spots in the muscle. What the influence of these *Contracaecum* larvae could be when consumed (raw or partially cooked meat) by humans is not clear, although Rodrick and Cheng (1989) report cases of marine fish being infested with nematodes that resulted in various human diseases and deaths. The problem arises with *Contracaecum* larvae infested fish when the whole fish are sold as this would lead to consumer resistance.

**Figure 1:** The viscera of an African catfish *Clarias gariepinus* showing a heavy *Contracaecum* infection.

The Mozambique tilapia *Oreochromis mossambicus* was found to be infested with clinostomatid trematode metacercariae. Two distinct metacercarial types were found, one was encysted in the muscles and was identified as *Euclinostomum heterostomum* (Britz, Saayman & Van As, 1984). The second type, prevalent on the gills of the fish, was identified as *Clinostomum tilapiae* (Britz, van As & Saayman, 1984). *E. heterostomum* has also been identified in the musculature of *Clarias gariepinus* (Mashego & Saayman, 1989) where the parasites were found encysted in the muscles ventro-lateral to the dorsal fin immediately below the skin (Fig 2 & 3). The latter authors note that although human infestations by members of the genus *Euclinostomum* (Clinostomatidae) have

not yet been reported, records of human infestations by members of a closely related genus, *Clinostomum* (Clinostomatidae) exist and probably resulted from the eating of uncooked fish. The latter genus have been reported in the following fish: tilapia (*Oreochromis* spp.), orangefin barb (*Barbus eutaenia*), papermouth (*B. mattozi*), straightfin barb (*B. paludinosus*), Beira barb (*B. (Beirababus radiatus*), threespot barb (*B. trimaculatus*), longbeard barb (*B. unitaeniatus*), bulldog (*Marcusenius macrolepidotus*), silver catfish (*Eutropius depressirostris*) and the sharptooth catfish (*Clarias gariepinus*) (van As & Basson, 1984). A large number of these fish species are either fished by local fisherman, farmed or have been identified as aquaculture candidate species in South Africa (Bruton & Safriel, 1984).

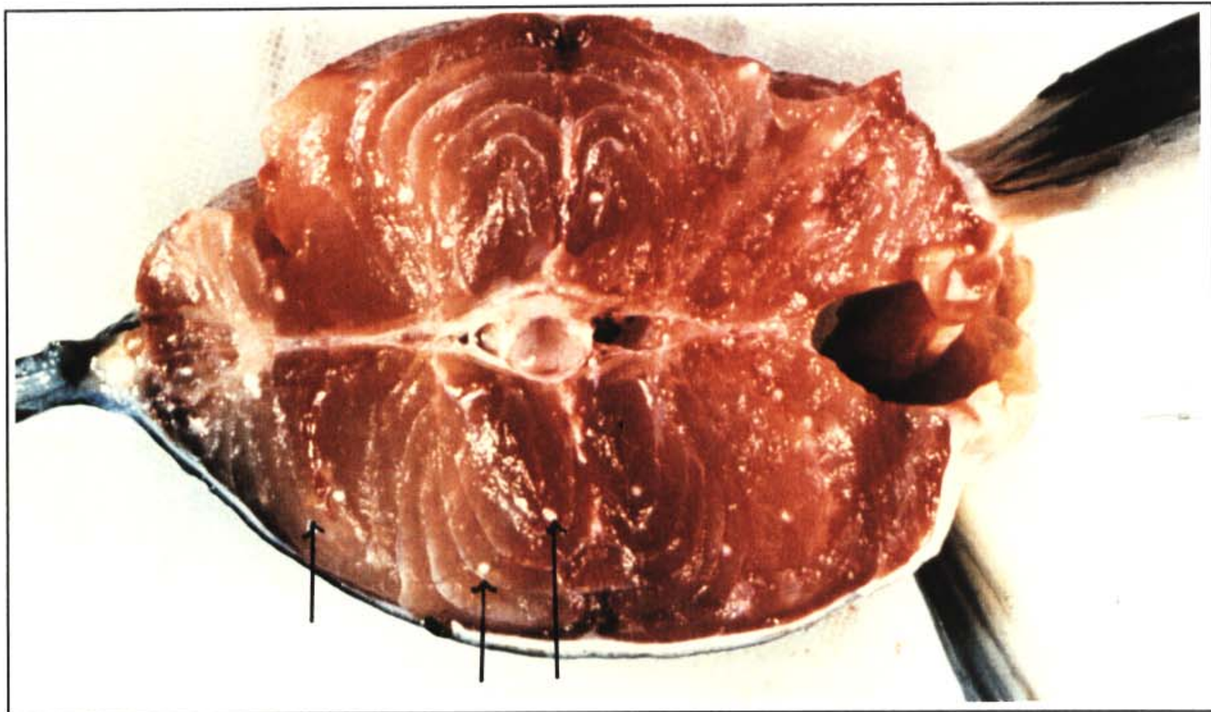

**Figure 2:** Cross section of *C. gariepinus* showing an *Euclinostomum* infection.

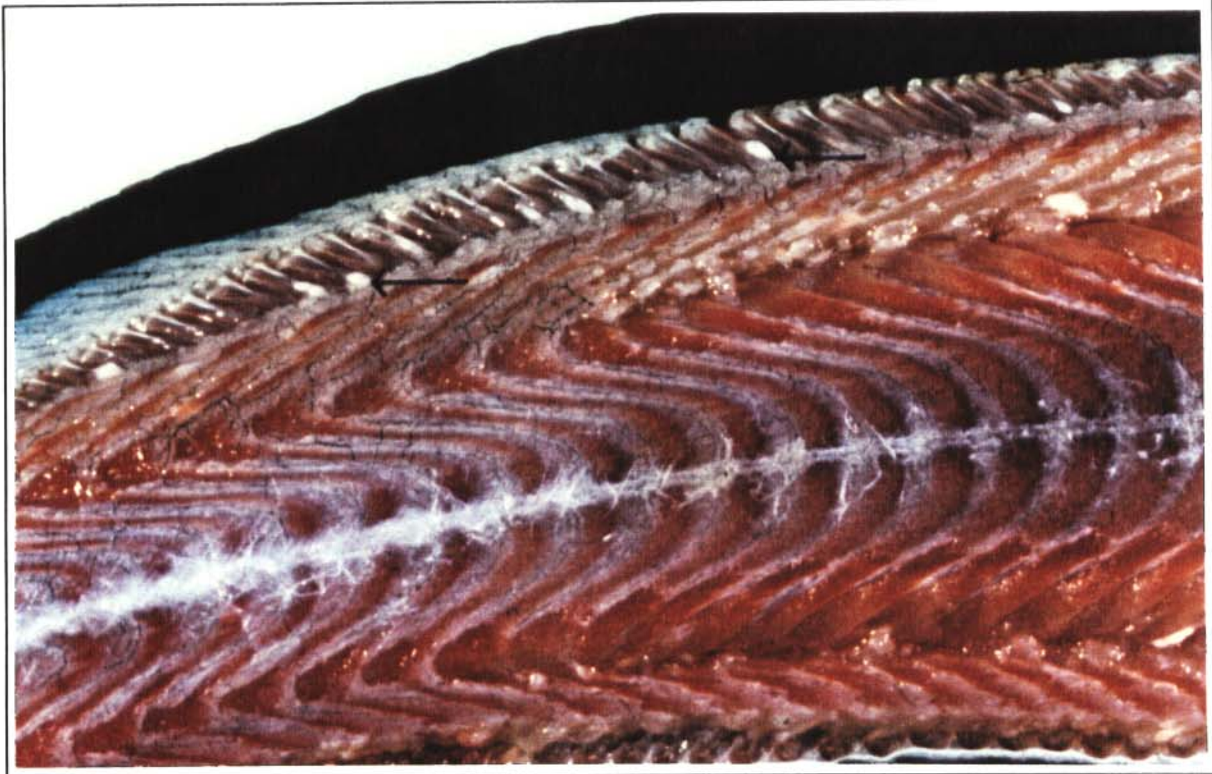

**Figure 3:** Skinned *C. gariepinus* showing a subcutaneous *Euclinostomum* infection.

## POLLUTANTS

### Detergents

One of the most troublesome substances in municipal wastewater is detergent which originates from multiple dispersed sources in domestic wastewater. Detergents may be toxic to fish, affecting the mucus coat of the fish and rendering them more susceptible to attack from diseases and parasites (Hepher & Pruginin, 1981).

### Trace Minerals

The possibility of bio-accumulation of some metals in toxic amounts in fish is a matter of concern to scientists. The information on this aspect in relation to aquaculture, however is diverse in nature. The major concern on heavy metal concentrations and possible toxic effects thereof originated from Japan, where in 1953 and 1961 a mysterious disease affecting the central nervous system occurred in persons living in the region of Minamata Bay, Japan. This was ultimately shown to be caused by the consumption of marine fish contaminated with high

concentrations of mercury. A similar outbreak occurred in Niigata, Japan in 1964-65 (Connell, 1990). These epidemics were caused by contaminated industrial effluent being discharged into the sea. In a study on numerous fish species from both freshwater and marine water in South Carolina, the freshwater fish species had trace metal (Fe, Zn, Mn, Cd & Cu) concentrations well below that of marine species (Koli *et al.*, 1978).

In a study on the occurrence of heavy metals in fishponds in Calcutta supplied with sewage effluent, it was observed that except for Fe, Zn and to some extent Pb, concentrations of other elements like Cd, Cu, Co and Ni were not high in these fish ponds (Ghosh, Chattopadhyay & Chakraborti, 1990). In South Africa, the levels of Cu and Zn in *Clarias gariepinus* muscle from a Transvaal lake (Germiston Lake) affected by mine and industrial effluents were below concentrations that could pose a threat for human health (Bezuidenhout, Schoonbee & de Wet, 1990; RDA, 1989). Similarly, van den Heever and Frey (1994) found that the muscle of *C. gariepinus* raised in cages in treated effluent contained $0.246 \pm 0.217$ mg/g wet mass Zn and $0.006 \pm 0.006$ mg/g wet mass Cu. The same authors however, noted that the concentrations of these two metals in the livers and kidneys of *C. gariepinus* were high enough to pose possible human health risks if consumed.

In terms of other fish species in South Africa: tigerfish *Hydrocynus vittatus* muscle showed concentrations of Fe, Zn, Pb, Ni, Cu, Cd and Mn well below that which could be hazardous for human consumption (du Preez & Steyn, 1992). The southern mouthbrooder *Pseudocrenilabrus philander*, also had low muscle concentrations of Fe, Mn, Zn, Cu, Ni and Pb and was found to be suitable for human consumption (de Wet, Schoonbee, de Wet & Wiid, 1994).

Since the research by Slabbert and co-workers (1989), a number of municipalities and fish farmers have considered using municipal stabilization pond systems for aquaculture and the production of fish for human consumption (Prinsloo & Schoonbee, 1992). Fish already exist in a wild state in a number of these ponds, though little data on their chemical composition is yet available. In the present investigation, a number of naturally occurring African sharptooth catfish were netted from a municipal sewage stabilization pond in Pietersburg, Northern Transvaal, South Africa. These were then chemically analyzed in terms of fillet proximate composition, lipid fatty acid, and mineral profiles in order to evaluate their suitability for human consumption. The influence of sex (if any) on these concentrations were also evaluated.

## MATERIALS AND METHODS

*Fish used:*

Thirty specimens of a wild population of the African sharptooth catfish, *C. gariepinus*, were captured by gill net from the Pietersburg Municipal final effluent oxidation ponds during two periods - the first at the end of

winter and the second a month later. The fish were of varying sizes, had bred naturally in the ponds, and had a natural diet of mainly zooplankton, debris, and smaller specimens of *C. gariepinus* and *O. mossambicus*.

*Chemical analysis*

The myotomal muscles of the 30 specimens were individually analyzed for proximate composition and mineral profiles. The muscle samples of 16 were also analyzed for fatty acid composition. Of the 30 fish, 19 were male and 11 female. The methodologies employed in the chemical analysis are as described in Chapter 2.

*Statistical analysis*

The influence of sex on the different chemical parameters was tested by means of a students *t*-test.

## RESULTS

Data on the proportional composition of the catfish in terms of fillet, head, viscera (gut), gut fat, bone, gonads and skin of males and females is presented in Table 6. Although the males were on average heavier than the females, this difference was not significant ($n=30$, $p=0.0919$). A large variation in live body mass was noted for both sexes, with the females varying from 999 to 4390g, and the males, 1644 to 6650 g. The fillet yield provided no sexual influence ($p=0.7568$) and varied between 41 and 51%. Similarly, no sexual influences were noted for the percentages of head, gut, skin, and gut fat. There were, however, significant differences in the percentages of bone, GSI (Gonado Somatic Index) and liver, with the males having the heavier percentage of bone and the females a heavier GSI and percentage of liver. The gonads of the females were in various stages of development, an observation further manifested by the large standard deviation in the GSI (2.3324 - Table 6).

The mean fillet proximate composition of the male and female catfish, as well as the total mean for the pooled data, is presented in Table 7. Although the percentages of protein and ash for the pooled data show relatively low standard deviations, values for both the moisture and total lipid percentages yielded large standard deviations - the fillet total lipid varied from as low as 0.89% to as high as 21.73%. None of the proximate composition parameters varied significantly between the sexes and were $73.76 \pm 4.8087$, $17.31 \pm 1.4374$, $7.84 \pm 5.0311$ and $2.82 \pm 0.5278$% for the pooled percentages of moisture, protein, total lipid and ash, respectively.

**Table 6:** The mean percentage body components (% of body weight) of female (n=19) and male (n=11) African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds.

| | Mean | SD | Minimum | Maximum | SE | T | Prob> \| T \| |
|---|---|---|---|---|---|---|---|
| **Body Mass (g)** | | | | | | | |
| Female | 2629 | 1062.66 | 999 | 4390 | 243.79 | 1.7450 | 0.0919 |
| Male | 3430 | 1442.33 | 1644 | 6650 | 434.88 | | |
| **% Fillet** | | | | | | | |
| Female | 46.93 | 2.4373 | 41.27 | 50.77 | 0.5592 | 0.3128 | 0.7568 |
| Male | 47.21 | 2.2245 | 43.43 | 49.88 | 0.6707 | | |
| **% Head** | | | | | | | |
| Female | 26.34 | 2.6343 | 21.46 | 32.30 | 0.6044 | 0.5500 | 0.5867 |
| Male | 26.94 | 3.2146 | 21.53 | 31.83 | 0.9692 | | |
| **% Gut** | | | | | | | |
| Female | 2.33 | 0.4354 | 1.45 | 3.39 | 0.0999 | 0.2220 | 0.8259 |
| Male | 2.37 | 0.3729 | 1.77 | 3.00 | 0.1124 | | |
| **% Bone** | | | | | | | |
| Female | 12.48 | 0.9754 | 10.84 | 14.54 | 0.2238 | 2.2828 | 0.0316 |
| Male | 13.35 | 1.0632 | 11.81 | 15.13 | 0.3206 | | |
| **% Skin** | | | | | | | |
| Female | 5.45 | 0.5042 | 4.38 | 6.37 | 0.1157 | 1.3157 | 0.1989 |
| Male | 5.69 | 0.4709 | 4.85 | 6.24 | 0.1420 | | |
| **% Gut Fat** | | | | | | | |
| Female | 1.28 | 1.3385 | 0.00 | 4.16 | 0.3071 | 0.6872 | 0.4976 |
| Male | 1.76 | 2.47 | 0.00 | 8.32 | 2.4690 | | |
| **GSI (% Gonads)** | | | | | | | |
| Female | 2.63 | 2.3324 | 0.24 | 7.26 | 0.5351 | 3.1085 | 0.0043 |
| Male | 0.42 | 0.1669 | 0.14 | 0.74 | 0.0503 | | |
| **% Liver** | | | | | | | |
| Female | 1.03 | 0.1758 | 0.72 | 1.46 | 0.0403 | 2.6022 | 0.0140 |
| Male | 0.87 | 0.1412 | 0.66 | 1.19 | 0.0426 | | |

**Table 7:** The fillet mean proximate composition of female (n=19) and male (n=11) African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds (% wet mass).

| | Mean | SD | Minimum | Maximum | SE | T | Prob > \|T\| |
|---|---|---|---|---|---|---|---|
| **Moisture** | | | | | | | |
| Female | 74.00 | 4.5629 | 62.92 | 80.14 | 1.0468 | 0.3474 | 0.7309 |
| Male | 73.35 | 5.4124 | 64.24 | 92.08 | 1.6319 | | |
| Pooled | 73.76 | 4.8087 | 62.92 | 82.08 | | | |
| **Protein** | | | | | | | |
| Female | 17.36 | 1.5281 | 13.12 | 19.90 | 0.3506 | 0.2569 | 0.7992 |
| Male | 17.22 | 1.3323 | 14.88 | 19.08 | 0.4017 | | |
| Pooled | 17.31 | 1.4374 | 13.12 | 19.90 | | | |
| **Lipid** | | | | | | | |
| Female | 7.35 | 4.2614 | 1.35 | 17.21 | 0.9776 | 0.6934 | 0.4938 |
| Male | 8.68 | 6.2833 | 0.89 | 21.73 | 1.8945 | | |
| Pooled | 7.84 | 5.0311 | 0.89 | 21.73 | | | |
| **Ash** | | | | | | | |
| Female | 2.92 | 0.5703 | 1.90 | 3.91 | 0.1308 | 1.4929 | 0.1466 |
| Male | 2.63 | 0.4034 | 2.07 | 3.37 | 0.1216 | | |
| Pooled | 2.82 | 0.5278 | 1.90 | 3.91 | | | |

The mean fatty acid composition (n=16) of the total lipids derived from the catfish fillets is presented in Table 8. As an imbalance occurred between the numbers analyzed from either sex (13 male, 3 female), the influence thereof on the total lipid fatty acid composition was not evaluated. The catfish had high concentrations of palmitic (C16:0 - 20.00%), palmitoleic (C16:1ω7 - 12.6%), stearic (C18:0 - 7.45%), oleic (C18:1ω9 - 19.74%) and linolenic (C18:3ω3 - 7.02%) acids. The longer chained polyunsaturated fatty acids - arachidonic (C20:4ω6 - 2.97%), eicosapentaenoic (C20:5ω3 - 4.32%), docosapentaenoic (C22:5ω3 - 2.50%) and docosahexaenoic (C22:6ω3 - 4.87%) - were also present in high concentrations. The total lipid of the catfish contained 32.85% saturated, 35.83% mono-unsaturated, and 28.62% poly-unsaturated fatty acids. The ω3/ω6 fatty acid ratio was 2.57.

**Table 8:** The fillet mean total lipid fatty acid composition of African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds (% fatty acid/% total fatty acid, N=9).

| Fatty Acid | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| C14:0 | 4.21 | 1.6385 | 1.90 | 7.25 |
| C16:0 | 20.00 | 1.1091 | 18.47 | 21.17 |
| C16:1ω7 | 12.62 | 0.9736 | 11.19 | 13.70 |
| C18:0 | 7.45 | 0.3818 | 6.67 | 7.81 |
| C18:1ω7 | 0.70 | 0.2000 | 0.39 | 1.02 |
| C18:1ω9 | 19.74 | 2.1976 | 15.81 | 22.96 |
| C18:2ω4 | 0.22 | 0.0430 | 0.17 | 0.29 |
| C18:2ω6 | 4.57 | 1.5688 | 3.39 | 8.02 |
| C18:3ω3 | 7.02 | 1.0156 | 5.38 | 8.07 |
| C18:3ω4 | 0.54 | 0.2384 | 0.33 | 1.12 |
| C18:4ω3 | 0.09 | 0.0492 | nd | 0.17 |
| C20:1ω9 | 1.95 | 0.6908 | 1.18 | 3.26 |
| C20:3ω3 | 0.10 | 0.0173 | 0.07 | 0.13 |
| C20:3ω6 | 0.26 | 0.3338 | nd | 0.97 |
| C20:4ω3 | 1.16 | 0.1981 | 0.77 | 1.42 |
| C20:4ω6 | 2.97 | 0.5915 | 2.18 | 4.00 |
| C20:5ω3 | 4.32 | 0.8129 | 2.94 | 5.23 |
| C22:0 | 0.67 | 0.1152 | 0.52 | 0.84 |
| C22:1ω9 | 0.61 | 0.1496 | 0.43 | 0.87 |
| C22:1ω11 | 0.21 | 0.0213 | 0.18 | 0.24 |
| C22:5ω3 | 2.50 | 0.3894 | 2.10 | 3.31 |
| C22:6ω3 | 4.87 | 1.0315 | 3.62 | 7.11 |
| C24:0 | 0.52 | 0.1527 | 0.34 | 0.82 |
| Unidentified | 2.7 | | | |
| SFA | 32.85 | | | |
| MUFA | 35.83 | | | |
| PUFA | 28.62 | | | |
| ω3/ω6 | 2.57 | | | |

The mineral profile of the muscle of the catfish is shown in Table 9. Of the minerals measured, only zinc differed significantly between the two sexes (n=30, p=0.0162), with the males (32.70 vs 22.61ppm) having a higher concentration of the metal than the females.

**Table 9**: The fillet mean mineral composition of female (n=19) and male (n=11) African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds (dry mass basis).

| Mineral | Mean | SD | Minimum | Maximum | SE | T | Prob> \| T \| |
|---|---|---|---|---|---|---|---|
| **Ca %** | | | | | | | |
| Female | 3.33 | 0.9545 | 2.24 | 5.88 | 0.2190 | 0.0678 | 0.9483 |
| Male | 3.31 | 1.0686 | 2,02 | 5.52 | 0.3222 | | |
| Pooled | 3.32 | 0.9795 | 2.02 | 5.88 | | | |
| **K %** | | | | | | | |
| Female | 1.05 | 0.4314 | 0.25 | 1.84 | 0.1078 | 0.4655 | 0.6458 |
| Male | 1.13 | 0.5169 | 0.37 | 1.96 | 0.1635 | | |
| Pooled | 1.08 | 0.4580 | 0.25 | 1.96 | | | |
| **Mg %** | | | | | | | |
| Female | 0.12 | 0.0312 | 0.07 | 0.16 | 0.0078 | 0.0558 | 0.9560 |
| Male | 0.12 | 0.0324 | 0.07 | 0.16 | 0.0102 | | |
| Pooled | 0.12 | 0.0310 | 0.07 | 0.16 | | | |
| **P %** | | | | | | | |
| Female | 0.65 | 0.2122 | 0.48 | 1.36 | 0.0515 | 0.4792 | 0.6358 |
| Male | 0.69 | 0.2708 | 0.48 | 1.44 | 0.0816 | | |
| Pooled | 0.66 | 0.2331 | 0.48 | 1.44 | | | |

**Table 9**: The fillet mean mineral composition of female (n=19) and male (n=11) African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds (dry mass basis).

| Mineral | Mean | SD | Minimum | Maximum | SE | T | Prob > \| T \| |
|---|---|---|---|---|---|---|---|
| **Cd ppm** | | | | | | | |
| Female | 0.78 | 0.2348 | 0.45 | 1.12 | 0.0783 | 0.2534 | 0.8039 |
| Male | 0.74 | 0.3005 | 0.48 | 1.33 | 0.1227 | | |
| Pooled | 0.76 | 0.2531 | 0.45 | 1.33 | | | |
| **Co ppm** | | | | | | | |
| Female | 1.48 | 0.3804 | 1.04 | 2.03 | 0.1268 | 0.6389 | 0.5340 |
| Male | 1.34 | 0.4115 | 1.00 | 2.16 | 0.1680 | | |
| Pooled | 1.42 | 0.3843 | 1.00 | 2.16 | | | |
| **Cr ppm** | | | | | | | |
| Female | 3.39 | 0.5528 | 2.80 | 4.28 | 0.1843 | 0.6657 | 0.5172 |
| Male | 3.09 | 1.1704 | 2.34 | 5.40 | 0.4778 | | |
| Pooled | 3.27 | 0.8285 | 2.34 | 5.40 | | | |
| **Cu ppm** | | | | | | | |
| Female | 1.32 | 0.6016 | 0.74 | 2.73 | 0.1380 | 0.9190 | 0.3660 |
| Male | 1.52 | 0.5402 | 0.82 | 2.73 | 0.1629 | | |
| Pooled | 1.40 | 0.5789 | 0.75 | 2.73 | | | |
| **Fe ppm** | | | | | | | |
| Female | 44.32 | 13.2011 | 23.82 | 79.80 | 3.0285 | 0.8883 | 0.3819 |
| Male | 48.69 | 12.5455 | 29.56 | 75.13 | 3.7826 | | |
| Pooled | 45.92 | 12.9235 | 23.82 | 79.80 | | | |

**Table 9**: The fillet mean mineral composition of female (n=19) and male (n=11) African sharptooth catfish, *Clarias gariepinus*, from final effluent oxidation ponds (dry mass basis).

| Mineral | Mean | SD | Minimum | Maximum | SE | T | Prob> \| T \| |
|---|---|---|---|---|---|---|---|
| **Mn ppm** | | | | | | | |
| Female | 0.95 | 0.5849 | 0.00 | 1.90 | 0.1342 | 0.0422 | 0.9666 |
| Male | 0.96 | 0.6104 | 0.00 | 1.9* | 0.1841 | | |
| Pooled | 0.96 | 0.5838 | 0.00 | 1.90 | | | |
| **Ni ppm** | | | | | | | |
| Female | 4.29 | 1.1967 | 2.54 | 6.15 | 0.3989 | 0.4959 | 0.6283 |
| Male | 3.95 | 1.4620 | 3.12 | 6.89 | 0.5969 | | |
| Pooled | 4.16 | 1.2695 | 2.54 | 6.89 | | | |
| **Pb ppm** | | | | | | | |
| Female | 7.94 | 2.6667 | 4.16 | 11.71 | 0.8889 | 0.3681 | 0.7187 |
| Male | 7.37 | 3.2736 | 5.01 | 13.93 | 1.3365 | | |
| Pooled | 7.71 | 2.8237 | 4.16 | 13.93 | | | |
| **Zn ppm** | | | | | | | |
| Female | 22.61 | 4.0248 | 16.57 | 29.77 | 0.9233 | 2.5577 | 0.0162 |
| Male | 32.70 | 16.5574 | 16.21 | 72.53 | 4.9922 | | |
| Pooled | 26.31 | 11.3588 | 16.21 | 72.53 | | | |

## DISCUSSION

The lack of a sexual influence on the fillet yield is similar to an earlier study's findings, where wild populations of *C. gariepinus* males and females yielded 44.2 and 44.0% fillet respectively (Hoffman *et al.*, 1993*c*). Although the wild catfish sampled in the earlier study had a lower body mass (1395.8 g for males and 1006.1 g for females), and came from a different location, the same filleting procedures were employed. In the earlier

study, the sexual dimorphism was more pronounced on farmed catfish, where smaller-sized fish (672.2 g for males and 632.5 g for females) yielded 46.7 and 38.9% fillets respectively. The significant difference in the fillet yield of farmed males and females was attributed to the difference in gonad mass (Hoffman & Prinsloo, 1990; Prinsloo, Schoonbee & Hoffman, 1990). In the case of the American channel catfish (Dunham, Joyce, Bondari & Malvestuto, 1985), *Ictalurus punctatus,* it was found that the sex of the fish had no influence on the proportional representation of the head. In *I. punctatus* it was noted that the percentage head mass (males 21.6%, females 22.3%) remained approximately the same between the sexes, while the dressing percentages of the males were slightly higher (68.5%) than those of the females (67.2%). Thus it would seem that *C. gariepinus* has a lower yield (±47%) than *I. punctatus* (±67%), possibly due to *C. gariepinus* having a much heavier head. The skull of adult *C. gariepinus* as a whole is solid bone and a number of abdominal vertebrae forming a 'complex vertebra' are fused to its base, thus providing additional weight (Mills, 1966).

The mean fillet chemical composition for the present investigation differs from that of an earlier study on *C. gariepinus* (77.9% moisture, 18.2% protein, 2.4% fat and 0.4% ash - Hoffman *et al.*, 1992) and is similar to that reported for *I. punctatus* (76% moisture, 17.8% protein, 6.0% fat and a 1.2% ash content - Tucker, 1985). The present investigation yielded the highest muscle total lipid yet reported for *C. gariepinus* (21.73%). This is surprising, especially since the fish were netted at the end of winter when water temperatures were below those noted for the active growth of *Clarias gariepinus* (Viveen *et al.*, 1985), and when it would have been presumed that the fish were largely slow swimming and not actively feeding (Hecht, Uys & Britz, 1988). That *C. gariepinus* muscle can attain such high total lipid contents is an important consideration for the processing industry in terms of product development and quality maintenance where taste and shelf life are concerned.

In the present investigation, the predominant fillet total lipid fatty acid was palmitic acid (C16:0 - 20.00 ± 1.1091%) with oleic acid (C18:19 - 19.74 ± 2.1976%) being very similar in concentration. An earlier investigation (Hoffman *et al.*, 1992) found that farmed *C. gariepinus* females (1702.0 ± 162.0 g mean body weight) had oleic acid (29.6 ± 0.648%) as the predominant total lipid fatty acid while palmitic acid (26.3 ± 2.131%) was present at a lower concentration. The catfish from the present investigation had lower concentrations of SFA and MUFA and higher concentrations of PUFA than those reported for the farmed females (36.6, 37.6 and 19.6%, respectively - Hoffman *et al.*, 1992). Diet would most probably be the major cause of this difference (Gatlin & Stickney, 1982; Watanabe, 1982; Stickney & Hardy, 1989; Greene & Selivonchick, 1990), though it has been noted that factors found to influence fish fatty acid profiles include species (Love, 1988), genetic strain (Smith, Kincaid, Regenstein & Rumsey, 1988), environment (Gallagher, McLeod & Rulifson, 1989; Morishita, Uno, Araki & Takahashi, 1989), water temperature (Stickney & Andrews, 1977; Stickney & Hardy, 1989), and physiological (Sasaki, Ota & Takagi, 1989) and nutritional status (Tidwell, Webster & Clark, 1992). This high concentration of especially the longer chained PUFAs

(docosapentaenoic and docosahexaenoic acids) and the high $\omega 3/\omega 6$ ratio (2.57) could be an important consideration in the utilisation of these catfish from effluent oxidation ponds as a source of PUFAs. The latter play an important role in the reduction of human cardiovascular diseases (Hearn, Sgoutas, Hearn & Sgoutas, 1987; Singh & Chandra, 1988; Ackman, 1989; Kinsella, Lokesh & Stone, 1990; Tichelaar, 1993). The high total lipid ($7.84 \pm 5.0311\%$) content of the catfish fillets would ensure that high quantities of PUFAs were consumed (Booysens & van der Merwe, 1991).

A major objection to the consumption of fish raised in oxidation ponds is the presence of macro and micro elements in concentrations perhaps hazardous for human consumption. In a study on the accumulation of copper and zinc in various organs of *C. gariepinus*, the muscle (Cu: $9 \pm 2.6$; Zn: $59 \pm 31.7$ $\mu$g/g DM) and body fat (Cu: $13 \pm 7.0$; Zn: $50 \pm 41.5$ $\mu$g/g DM) contained the lowest concentrations of these heavy metals (Bezuidenhout, Schoonbee & de Wet, 1990). The fact that the catfish muscle of the present investigation contained lower concentrations of both copper and zinc than those reported by Bezuidenhout and co-workers (1990) is not surprising, however, since Pietersburg has no local mining or heavy industry which could contaminate the effluent water.

In the present investigation, the lower zinc concentration in the muscle of the females may have been due to mobilisation of zinc in the developing ovaries (Fletcher & King, 1978). In the earlier investigation, for both the farmed and wild catfish, no sexual differences were noted for the various minerals analyzed, though the farmed males had a higher phosphorus concentration than the farmed females (Hoffman *et al.*, 1992).

Concentrations of the macro-elements in the muscle of *C. gariepinus* were similar to those noted in the earlier investigation (Hoffman *et al.*, 1992). Calcium is an important cation balance regulator between cells and milieu. It also fulfils a number of other functions in humans and was present in concentrations well below toxicity levels. The RDA (1989) for calcium for both sex groups from ages 11 to 24 years is 1200mg. Similarly, the muscle concentrations of magnesium and phosphorus were well below the RDA of 350 and 800-1200mg respectively (RDA, 1989). The concentration of trace elements in particular may play an important role in toxicity, especially when these include the heavy metals cadmium, mercury, and lead (Schweinsberg & von Karsa, 1990). In the present investigation, however, these metals were all well within accepted limits (RDA, 1989). Similarly, concentrations of the other elements measured (Table 9) in the muscle of the catfish from the present investigation were also well within the limits set for human consumption (RDA, 1989).

## OTHER SUBSTANCES

Herbicides and pesticides used in agricultural practices are readily taken up by living organisms where they are stored in fatty tissue. The maximum permissible levels of chlorinated hydrocarbon pesticides in foods as set out in the "Foodstuffs, Cosmetics and Disinfectants Act, No. 54 of 1972, Regulation No. R2064 as amended 2 January 1981" are: DDT, TDE and DDE singly and in combination, 3 ppm in meat fat; dieldrin 0.2 ppm in meat fat and lindane 2.0 ppm in meat fat.

In South Africa, DDT was used for indoor malaria control in the northern part of KwaZulu. DDT levels in fish muscle were monitored before and after spraying in fish that occurred in a number of pans in the area. Prior to the application, low levels of DDT were measured in the muscle of tiger fish (*Hydrocynus vittatus*), bream (*Oreochromis mossambicus*) and butter catfish (*Eutropius depressirostis*) and there was no increase after application (Bouwman, Coetzee & Schutte, 1990). In another study in the Olifants River, no polychlorinated biphenyls (PCBs) or chlorinated pesticides were detected in the muscle of tiger fish (*Hydrocynus vittatus*), bream (*Oreochromis mossambicus*) and African catfish (*Clarias gariepinus*). Low levels of DDT and its metabolites were found to be present in the muscles of these fish species, but it was deemed that these concentrations did not constitute a health hazard in terms of fish consumption (Grobler, 1994).

## RECOMMENDATIONS

The present review may have given the impression that the aquaculture industry is beset with too many problems to have much of a future. What must be remembered, is that the above-mentioned are lists of potential parasites and factors that may be hazardous to human health. In the intensive review of Bryan (1977), freshwater fish were only implicated in two outbreaks of illness (the third outbreak was caused by mercury poisoning in marine fish species in Japan). Many of the issues described may not in fact be issues at all, and even if they are proven to be, they should be manageable, for example thorough cooking, or freezing of fish if the fish is to be consumed raw, will kill all helminths. In almost every instance, for microorganisms to cause food-borne illness, abusive conditions and/or cross-contamination would have to occur (Ward, 1989). These are the same factors that other muscle protein food industries must contend with. Although aquaculture has components that make it unique, proper management practices will ensure that this growing industry in South Africa does not suffer any major setbacks. Some of these management practices are discussed below, they all include attempts at breaking the chain of events (described at the beginning of this manuscript) needed to cause an epidemic:

The first step towards the control of these parasites is to stop the reinfection of water bodies. The chain of transmission may be broken by safe disposal of faeces as well as by protection of the water supplies. Another means of breaking the transmission, is to treat infected individuals within the community. This implies that

national primary health care principles will have to be implemented effectively.

Secondly, where possible, the animals utilised in an integrated agriculture-aquaculture system should be regularly treated for parasites. For example, pigs that were utilised in such an investigation were found to have light to moderate infestations of *Balantidium*, a protozoan parasite. Treatment with a broad spectrum antibiotic eradicated the problem (Hopkins & Cruz, 1982). The problem however arises when there is uncontrolled movement of animals (wild and domestic), or when the people utilising the animals in such integrated systems cannot afford various antibiotics, or through lack of proper training, are not aware of possible parasitic infections in their animals.

Proper design and management of effluent waters to eliminate various bacteria, virus and parasitic pathogens will also play an important role in minimising the potential for epidemics (Osborn, 1988). There are many different designs and management activities that can ensure an effective system, for example, the layout of the oxidation ponds will also play an important role in the removal of parasites. A total of twenty days retention time with a minimum of two ponds is considered to ensure an effluent free of protozoan cysts and helminth eggs (Pearson, 1990). Where modern treatment methods and improved sewage disposal have been introduced, infections from parasite protozoa and helminths have disappeared (Lewis, 1985).

More research is needed for the determination of bacteria threshold levels for the various fish species that could be cultivated in final effluent ponds in South Africa.

The technique of holding fish in a densely stocked freshwater pond for a depuration period of several days prior to marketing has been the pragmatic method used as a safeguard against transmission of pathogens by fish. The actual effectiveness of and duration for this process needs to be clarified (Hepher & Pruginin, 1981; Little & Muir, 1987). The rate of depuration depends to a large extent on the degree of contamination. If the pathogens have invaded the flesh of the fish, a very long period (sometimes months) will be required to depurate them.

The single most important factor to control the potential of an epidemic occurring, is the eradication of bacteria from both the fish and fish processing environment. Numerous studies have repeatedly stressed the importance of proper sanitary procedures during the processing of the fish to ensure minimum contamination of the end product (Andrews *et al.*, 1977; Wyatt *et al.*, 1979; Connell, 1990). A properly designed abattoir or processing plant that is well managed will minimize potential hazards. Similarly, the workers should also be trained in personal hygiene and also made aware of the role that they could play in being passive carriers of pathogens (Howie, 1979).

Regular chemical analysis of fish for bacteria, trace metals, pesticides and other hazardous pollutants will ensure a healthy product being produced for the consumer. It is suggested that the end product, where it enters the consumers market, be regularly analyzed for bacterial infection, and not the fish prior to processing.

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# Chapter 8 THE INFLUENCE OF COOKING METHODOLOGY ON THE CHEMICAL COMPOSITION OF *CLARIAS GARIEPINUS* MUSCLE

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## INTRODUCTION

Recently, interest has been growing in fish and fish products as sources of polyunsaturated fatty acids (PUFA), mainly of the ω-3 family. This interest stems from studies suggesting that ω-3 PUFA may have an important role in the prevention and control of cardiovascular disease (Kinsella, Lokesh & Stone, 1990). Extensive existing literature suggests favourable effects of dietary n-3 fatty acids on a diversity of factors that include blood rheology, hyperlipidaemia, atopic dermatitis, early delivery in pregnancy, rheumatoid arthritis, dysmenorrhoea, and human breast cancer (Sing & Chandra, 1989; Ackman, 1989; Simopoulos, Kifer, Martin & Barlow, 1991; Nielsen, 1993; Tichelaar, 1993).

With the stabilisation in volume of marine fish landed over the last few years (Hempel, 1993), the potential of freshwater aquaculture as a possible replacement for marine fish has been thoroughly investigated in South Africa (Hecht, Uys & Brits, 1988) and elsewhere (Haylor, 1992). In 1984 the African sharptooth catfish, *Clarias gariepinus*, was indicated as a potential aquacultural candidate in South Africa (Bruton & Safriel, 1984). Although a number of catfish farms were then established (Hecht & Britz, 1990), total production has decreased during the past year, mainly as a result of inadequate marketing strategies (Uys, 1993). To help the producers in the development of a marketing strategy, the chemical composition of the muscle and fillets of this species has been analyzed for the compilation of nutrient tables (Hoffman & Prinsloo, 1990; Hoffman, Casey & Prinsloo, 1992; 1993*a,b,c*).

The aim of the present investigation was to determine the influence, if any, of different cooking methods such as shallow fat frying, deep fat frying, baking and microwaving on the proximate, lipid fatty acid and mineral profiles of *C. gariepinus*.

## MATERIAL AND METHODS

### *Fish*

The fish used in this investigation were bred and maintained in the Aquaculture Research Unit's water-recirculating facilities. The fish had been maintained under identical conditions and were fed the same commercial diet (6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt) for 18 months prior to sampling. Sixteen fish (678.2 - 1209.0 g body weight) were slaughtered and filleted according to standard methods (Hoffman *et al.*, 1993*c*). This range in body weight was chosen as it represents the size that is commercially utilised. The fillets were cut into small thin blocks (maximum 2.5 cm thickness) and mixed thoroughly. Thereafter the sub-samples (150 g) were randomly allocated to the different cooking treatments. Each cooking treatment, unless otherwise stated, was replicated four times.

**Cooking methods**

Standard cooking methods as described in the Food and Cookery manual were used (Anon, 1984).

*Control* ***(A)***

Uncooked fillet sub-samples were used as a control.

**Fried fish**

*Shallow fat fry* ***(B)***

A thin batter was prepared by mixing 25 ml of cake flour (®Snowflake containing 14% moisture, 11% protein DM, 0.55% ash DM - Premier Milling company) with 50 ml of tap water. The fish sub-samples were lightly covered in the cake flour prior to being rolled in the batter. The fish were then fried in a preheated (2 mins - 180°C) shallow teflon-lined pan containing 17.5 ml of sunflower cooking oil (®Sunvalley - Nola Oils) for four minutes. Every minute the fillet pieces were turned. At the end of the frying period, the fillets had an internal temperature of 85°C and the oil a temperature of 180°C.

*Deep fat fry* ***(C)***

The fish were prepared as for shallow fat frying and then deep fat fried for 5 minutes in 1350 ml of preheated (7 mins - 180°C) sunflower cooking oil (®Sunvalley) in an aluminium 2 l ®Hart pot.

**Baked fish**

*Aluminium foil covered* ***(D)***

The fish sub-samples in 250 ml of tap water were wrapped in aluminuim foil (20 $\mu$ thickness) and placed on an aluminium baking sheet. The fish were then baked in a preheated (200°C) convection oven (®Aloe convectomat model number KFA 16 - Aloe catering equipement Pty Ltd) at 180°C for 10 minutes.

*No covering* ***(E)***

The same method as above was used, except that the fish were not wrapped in aluminium foil but placed on the aluminium baking sheet in 250 ml of tap water.

**Microwave (F)**

The fish were placed on a clear pyrex microwave glass plate in 12.5 ml of tap water and cooked at 80% power for 2 minutes. A Litton commercial microwave (model E3100i, 1400 watt) was used.

**Shallow fat fry (G)**

A single sub-sample of fish was fried as per method B, with the difference that, prior to frying, the gut fat from the fish sampled (Hoffman *et al.*, 1993*a*) was melted and used as the oil source.

[Although it is noted that using a batter will significantly increase the oil absorption of the sample and influence the fatty acid profile analyzed (Mai, Shimp, Weihrauch & Kinsella, 1978), this cooking method was used as it is the method suggested to the consumers in a nutritional study being jointly conducted by the Aquaculture research Unit, Nutritional Department, University of the North, and the Medical Research Council. This cooking method was recommended as Steyn and co-workers (1992) have noted that there is an energy shortage in the diet composition of the children in the northern Transvaal, and that using this method should increase their energy intake - Prof N Steyn, pers comm].

After cooking, all samples were placed on absorbent paper for two minutes so as to remove all excess moisture/oil. Thereafter the fish sub-samples were thoroughly homogenised and the various chemical analyses performed.

*Chemical analyses*

The following chemical analyses were performed on each of the four replicates of the various cooking methods tested: proximate analysis, lipid fatty acid and mineral profiles (according to the methods described in Chapter 2).

*Statistical Analyses*

A linear model was fitted to the data with cooking methods as predictor. PROC GLM of the SAS package (SAS, 1985) was used and an analysis of variance was carried out. A mean and standard error was calculated for each cooking method and a matrix of excedence probabilities were calculated to test for pair-wise differences between cooking methods. The FISHER LSD test was used for testing pairwise differences since it was recently shown (Saville, 1990) that this test is optimal.

As the shallow frying in fish gut fat cooking method (G) only involved a single sample, a statistical comparison was not run between this cooking method and the mean values of the other methods for the various chemical parameters. The values of these parameters, however, are included in the various tables to show possible trends.

## RESULTS

*Proximate composition*

The influence of the different cooking methods on the mean proximate composition (moisture, protein, fat and ash) of the fillet portions is shown in Table 1a, while Table 1b includes the excedence probability values of the hypothesis that the difference between two means does not differ significantly from zero. There was a significant ($p \leq 0.05$) total moisture loss in all the cooking methods when compared to the uncooked control (A; 77.31% moisture), with the shallow fried (B) having the greatest loss (58.82%), and cooking methods C (deep fried; 61.06% moisture) and G (shallow fried in fish fat; 61.10% moisture) showing the second largest loss. The mean moisture of the two frying methods (B and C) did not differ significantly ($p=0.4591$). Similarly, the percentages of moisture in the fish fillets from cooking methods D, E and F did not differ significantly from each other, but did differ from the other cooking methods.

The mean protein percentage of the samples from the different cooking methods all differed significantly from that of A (control; 17.16%) with the exception of cooking method C (deepfried; 18.07%), whilst G (shallow fried in gut fat; 18.13%) also showed a similar protein content. The percentage of protein from the shallow fried fish (B; 14.76%) differs significantly ($p=0.0150$; Table 1b) from that of the deep fried (C; 18.07%). The two baking methods (D and E) did not differ significantly ($p=0.4171$) in their percentages of protein (21.74 and 22.76%), which were very similar to the percentage of protein obtained after the microwave (F; 22.11%) cooking method (these differences in protein percentage were not significant - Table 1b).

The two cooking methods that involved frying (B and C) had significantly higher percentages of total lipid than the control (A), baking (D and E), and microwave (F) cooking methods. The latter four (A, D, E and F) did not have significantly different percentages of total lipid (all $\pm 3\%$). The two shallow frying methods (B and G) showed the highest percentages of total lipid (11.74 and 10.94%, respectively) while that of the deep frying cooking method (C; 8.77% total lipid) was slightly lower. The latter (C) differed significantly from that of the fish shallow fried in the same type of oil (B; $p=0.0018$).

The percentage of ash showed a variation between the different cooking methods employed in the investigation. The fish baked in tin foil (D; 0.59%), and the control (A; 0.63%) had the lowest mean percentages of ash, while that shallow fried in fish gut fat (G) had the highest (1.42%). All three frying methods (B, C and G) that involved covering the fish with a batter prior to frying had a higher ash content than the control, baking, and micro-waving methods.

**Table 1a:** The mean proximate composition (%) of uncooked and cooked *Clarias gariepinus* fillets.

| Parameter (%) | Means of various cooking methods | | | | | | | Standard Error of Mean |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | |
| Moisture | 77.31 | 58.82 | 61.06 | 72.38 | 70.61 | 73.05 | 61.10 | 1.349 |
| Protein | 17.16 | 14.76 | 18.07 | 21.74 | 22.76 | 22.11 | 18.13 | 0.869 |
| Lipid | 3.21 | 11.74 | 8.77 | 3.58 | 3.30 | 3.04 | 10.94 | 0.576 |
| Ash | 0.63 | 1.02 | 0.83 | 0.59 | 0.77 | 0.64 | 1.42 | 0.107 |

Where A=raw control, B=shallow fried, C=deep fried, D=baked in tin foil, E=baked open, F=microwaved, G=shallow fried in fish gut fat.

**Table 1b:** Probability values calculated for pairwise difference between cooking methods for the proximate chemical parameters.

| Parameter | Pr > \| T \| HO: $LSMean_i=LSMean_j$ | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A=B | A=C | A=D | A=E | A=F | B=C | B=D | B=E | B=F | C=D | C=E | C=F | D=E | D=F | E=F |
| Moisture | 0.0001 | 0.0001 | 0.0186 | 0.0025 | 0.0382 | 0.2547 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.3659 | 0.7305 | 0.0009 |
| Protein | 0.0667 | 0.4707 | 0.0015 | 0.0002 | 0.0008 | 0.0150 | 0.0001 | 0.0001 | 0.0001 | 0.0079 | 0.0013 | 0.0040 | 0.4171 | 0.7636 | 0.6059 |
| Lipid | 0.0001 | 0.0001 | 0.6506 | 0.9108 | 0.8417 | 0.0018 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.7326 | 0.5156 | 0.7554 |
| Ash | 0.0213 | 0.2147 | 0.7577 | 0.3937 | 0.9481 | 0.2321 | 0.0110 | 0.1165 | 0.0244 | 0.1272 | 0.6851 | 0.2382 | 0.2506 | 0.7090 | 0.4297 |

*Fatty acids*

The influence that the heating process of the different frying methods may have had on the fatty acid composition of the oils used was investigated and the resulting fatty acid profiles (B = shallow fry; C = deep fry; G = shallow fry using gut fat as oil base) are summarised in Table 2. The same sunflower oil source was used for cooking methods B and C (the fatty acid of this sunflower oil before heating is also shown in Table 2). There are noticeable differences between the sunflower oil used (methods B and C) and the oil derived from the gut fat of the fish. A major difference is the higher percentage of palmitic acid (C16:0) present in the gut fat (22.18%) compared to that of the sunflower oil (6.80%). The sunflower oil had double the concentration of linoleic acid (C18:2ω6) than found in the gut fat oil, although the latter had a higher concentration of oleic acid (C18:1ω9) than the sunflower oil, this difference being in the 5-6% range. The sunflower oil had only trace concentrations (if present) of the longer-chained polyunsaturated fatty acids, whereas the gut fat oil had measurable concentrations thereof, the highest being 1.76% docosahexaenoic (C22:6ω3) acid. The influence of the different heating methods employed (shallow fry with a small volume to surface area compared to deep fry with a large volume to surface area ratio with similar temperatures at 180°C) was negligible. The major difference was that the oil used in the shallow pan fry cooking method tended to have slightly lower concentrations of the unsaturated oleic (C18:1ω9) and linoleic (C18:2ω6) fatty acids than that analyzed from the deep fry cooking method and from the oil prior to frying.

The fatty acid profiles of the fried fish (B, C and G - Table 3a) were strongly influenced by the oil used. No significant difference in myristic acid (C14:0) concentration between the uncooked control (A) and the different baking (D and E) and microwave (F) cooking methods was measured. However, the frying methods using sunflower oil (B and C) had significantly lower (Table 3b) concentrations thereof, as did the sunflower oil used in these two frying methods (Table 2). The same trend is noted with palmitic (C16:0), palmitoleic (16:1ω7), linoleic (C18:2ω6), gondoic (C20:1ω9), behenic (C22:0), eicosapentaenoic (C20:5ω3), docosapentaenoic (C22:5ω3) and docosahexaenoic (C22:6ω3) acids. The most noticeable differences (statistically significant - Table 3b) were the 11% palmitic acid concentration for the two frying methods B and C (difference between B and C being non-significant - Table 3b) compared to a 21-22% concentration for the control (A) and all the other cooking methods (D-G; Table 3a). The concentration of palmitoleic acid (C16:1ω7; ±1%) from frying methods B and C was a quarter that of the other cooking methods. The linoleic acid (C18:2ω6) concentration (50%) in the fried fish (methods B and C) was more than double that in the control (A; 19.72%) or other cooking methods (19-20%). Shallow frying in fish gut oil (G) also resulted in a higher concentration (24.01%) of linoleic acid than that measured in the control. The concentration of linolenic acid (C18:3ω3) was below 1% in the control and in all the cooking methods employed, the exception being the fish fried in gut fat (G) that had a concentration of 1.26%. As can be seen in Tables 3a and 3b, the concentration of gondoic acid (C20:1ω9) was significantly lower in the fried fish samples (B and C; <0.75%) than that found in the other

cooking methods (all ± 1.36%). Similar results were noted for eicosapentaenoic acid (C20:5ω3), behenic acid (C20:0), docosapentaenoic acid (C22:5ω3), and docosahexaenoic acid (C22:6ω3); that is, there were lower fatty acid concentrations in the shallow (B) and deep fried (C) cooking methods when compared to the control (A) and other cooking methods (D-F) employed. However, frying in fish gut fat (G) also resulted in the fish fillets containing lower concentrations of these fatty acids than the control (A), baked fish (D and E), and microwaved fish (F), but higher concentrations in the two sunflower oil fried fish (B and C).

*Minerals*

The mean mineral content of the fillets subjected to the various cooking methods is given in Table 4a while the probability values of the differences between the means are given in Table 4b. The concentrations of the minerals are expressed on a dry mass (DM) basis. The percentage of phosphorus in the control (A; 1.03% DM) is significantly higher than in the two frying methods (B; 0.90 and C; 0.93% DM) and significantly lower than that of the open baking method (D; 1.10% DM)) and the microwave cooking method (F; 1.18% DM). The two frying methods (B and C) differ statistically from all the other cooking methods in the percentage of phosphorus present, but not between themselves (Table 4b). The percentage of calcium does not differ statistically between the different cooking methods, with the exception of the shallow fry (B) and baking (E) methods (both 0.23% DM) which have significantly higher concentrations than the microwave cooking method (F; 0.10% DM). The percentage of potassium differs significantly in all the different cooking methods employed, with the exception of the comparison of the two baking methods (D and E) with the microwave cooking method (F). All the cooking methods have a significantly lower potassium concentration than that of the uncooked control (A - Table 4a and 4b). The percentage of magnesium was also higher in the uncooked control than in the different cooking methods, although these differences were not always significant (Table 4a and 4b). Similarly, with the exception of the microwave cooking method (F; 26.08 ppm DM), the concentration of iron was higher in the uncooked control (A; 24 ppm DM) than in the different cooking methods investigated. Although the two frying methods (B and C) had higher copper concentrations than the uncooked control, neither of these two cooking methods, nor any of the other methods, tested significantly different from the control or between each other. The only two mean copper concentrations that differed significantly ($p=0.0278$ - Table 4b), were between the shallow fried (B; 2.65 ppm DM) and the micro-waved (F; 1.40 ppm DM) cooking methods employed. The concentration of zinc in the uncooked control (A; 21.225 ppm DM) was similar to that of the microwave cooking method (F; 21.325 ppm DM, $p=0.9604$) and the covered baking method (D; 20.950 ppm DM, $p=0.8913$). The three frying methods tested had the lowest zinc concentrations. All the samples had low concentrations of manganese (≤0.750 ppm), with cooking methods E, F and G having a mean value of zero. There was no significant difference in manganese concentration between the cooking methods that registered a manganese concentration and those that measured zero manganese.

**Table 2:** The fatty acid profiles of oils obtained from the three frying methods (fatty acids expressed as percentage of total fatty acids).

| Fatty acid | Sunflower oil | Method of Frying | | |
|---|---|---|---|---|
| | | B | C | G |
| **14:0** | 0,08 | 0,13 | 0,08 | 1,80 |
| **16:0** | 6,80 | 6,90 | 6,82 | 22,18 |
| **16:1ω7** | 0,15 | 0,34 | 0,17 | 4,95 |
| **18:0** | 5,80 | 5,70 | 4,41 | 6,52 |
| **18:1ω9** | 22,95 | 21,88 | 23,13 | 28,19 |
| **18:2ω6** | 62,58 | 57,03 | 61,91 | 24,55 |
| **18:3ω3** | 0,16 | 0,42 | 0,14 | 1,35 |
| **18:4** | 0,70 | 0,62 | 0,79 | 0,66 |
| **20:1ω9** | 0,10 | 0,60 | 0,30 | 1,30 |
| **20:5ω3** | 0,23 | 0,07 | 0,02 | 0,85 |
| **22:0** | 0,80 | 0,74 | 0,72 | 0,16 |
| **22:1ω9** | nd | 0,03 | nd | 0,93 |
| **22:5ω3** | nd | 0,04 | nd | 0,77 |
| **22:6ω3** | nd | 0,08 | nd | 1,76 |

Where nd = not detected, B=shallow fried, C=deep fried, G=shallow fried in fish gut fat.

**Table 3a:** The fatty acid profiles of uncooked and cooked *C. gariepinus* fillets (fatty acids expressed as percentage of total fatty acids).

| Parameter | Means | | | | | | | Standard Error of Mean |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | |
| C14:0 | 1.69 | 0.35 | 0.59 | 1.68 | 1.66 | 1.76 | 1.56 | 0.050 |
| C16:0 | 22.05 | 11.05 | 10.79 | 21.50 | 21.49 | 22.03 | 22.39 | 0.273 |
| C16:1ω7 | 4.80 | 1.14 | 1.40 | 4.68 | 4.83 | 4.95 | 4.90 | 1.754 |
| C18:0 | 7.39 | 6.04 | 5.19 | 7.28 | 7.28 | 7.33 | 6.66 | 0.438 |
| C18:1ω9 | 25.95 | 22.72 | 23.87 | 25.25 | 25.77 | 25.60 | 27.94 | 0.546 |
| C18:2ω6 | 19.72 | 50.22 | 50.33 | 20.91 | 20.24 | 19.25 | 24.01 | 0.869 |
| C18:3ω3 | 0.88 | 0.51 | 0.36 | 0.87 | 0.85 | 0.83 | 1.26 | 3.212 |
| C18:4 | 0.69 | 1.47 | 0.77 | 0.67 | 0.70 | 0.69 | 0.65 | 0.086 |
| C20:1ω9 | 1.36 | 0.75 | 0.61 | 1.35 | 1.37 | 1.36 | 1.30 | 0.109 |
| C20:5ω3 | 1.50 | 0.31 | 0.40 | 1.41 | 1.41 | 1.47 | 0.97 | 0.073 |
| C22:0 | 1.48 | 0.31 | 0.41 | 1.52 | 1.50 | 1.53 | 1.08 | 0.097 |
| C22:5ω3 | 1.17 | 0.23 | 0.32 | 1.09 | 1.16 | 1.11 | 0.82 | 0.097 |
| C22:6ω3 | 5.25 | 0.95 | 1.34 | 4.89 | 4.92 | 4.95 | 2.27 | 0.419 |

Where A=raw control, B=shallow fried, C=deep fried, D=baked in tin foil, E=baked open, F=microwaved, G=shallow fried in fish gut fat.

**Table 3b:** Probability values calculated for pairwise difference between cooking methods for the various fatty acids identified.

| Parameter | Pr > \| T \| HO: $LSMean_i = LSMean_j$ | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A=B | A=C | A=D | A=E | A=F | B=C | B=D | B=E | B=F | C=D | C=E | C=F | D=E | D=F | E=F |
| C14:0 | 0.0001 | 0.0001 | 0.8605 | 0.6236 | 0.3656 | 0.0023 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.7520 | 0.2828 | 0.1698 |
| C16:0 | 0.0001 | 0.0001 | 0.1683 | 0.1611 | 0.9440 | 0.5088 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.9796 | 0.0001 | 0.1815 |
| C16:1ω7 | 0.1553 | 0.1856 | 0.9611 | 0.9921 | 0.9524 | 0.9168 | 0.1689 | 0.1527 | 0.1399 | 0.2013 | 0.1826 | 0.1678 | 0.9532 | 0.9136 | 0.9603 |
| C18:0 | 0.0420 | 0.0200 | 0.8639 | 0.8639 | 0.9301 | 0.1813 | 0.0594 | 0.0594 | 0.0502 | 0.0029 | 0.0029 | 0.0024 | 1.0000 | 0.9333 | 0.9333 |
| C18:1ω9 | 0.0005 | 0.0142 | 0.3739 | 0.8231 | 0.6599 | 0.1505 | 0.0038 | 0.0008 | 0.0013 | 0.0908 | 0.0231 | 0.0366 | 0.5025 | 0.6485 | 0.8280 |
| C18:2ω6 | 0.0001 | 0.0001 | 0.3416 | 0.6768 | 0.7107 | 0.9248 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.5876 | 0.1919 | 0.4335 |
| C18:3ω3 | 0.9350 | 0.9091 | 0.9983 | 0.9948 | 0.9918 | 0.9740 | 0.9367 | 0.9402 | 0.9432 | 0.9108 | 0.9143 | 0.9173 | 0.9965 | 0.9935 | 0.9970 |
| C18:4 | 0.0001 | 0.5041 | 0.8706 | 0.9189 | 1.0000 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.4079 | 0.5702 | 0.5041 | 0.7914 | 0.8706 | 0.9189 |
| C20:1ω9 | 0.0008 | 0.0001 | 0.9618 | 0.9490 | 0.9618 | 0.3842 | 0.0009 | 0.0007 | 0.0007 | 0.0001 | 0.0001 | 0.0001 | 0.9110 | 0.9236 | 0.9873 |
| C20:5ω3 | 0.0001 | 0.0001 | 0.4044 | 0.4044 | 0.7917 | 0.4044 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 1.0000 | 0.5657 | 0.5657 |
| C22:0 | 0.0001 | 0.0004 | 0.7737 | 0.8998 | 0.7329 | 0.4747 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.8714 | 0.9570 | 0.8292 |
| C22:5ω3 | 0.0001 | 0.0001 | 0.5673 | 0.9714 | 0.6935 | 0.5553 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.5915 | 0.8576 | 0.7200 |
| C22:6ω3 | 0.0001 | 0.0001 | 0.5538 | 0.5873 | 0.6247 | 0.5161 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.9602 | 0.9172 | 0.9596 |

**Table 4a:** The mineral content of uncooked and cooked *C. gariepinus* fillets (Dry Mass).

| Parameter | Means | | | | | | | Standard Error of Mean |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | |
| P % | 1.03 | 0.90 | 0.93 | 1.10 | 1.18 | 1.18 | 0.90 | 0.035 |
| Ca % | 0.20 | 0.23 | 0.18 | 0.13 | 0.23 | 0.10 | 0.10 | 0.035 |
| K % | 1.78 | 0.73 | 0.98 | 1.53 | 1.60 | 1.58 | 0.80 | 0.033 |
| Mg % | 0.25 | 0.02 | 0.18 | 0.18 | 0.23 | 0.02 | 0.10 | 0.210 |
| Fe ppm | 24.00 | 15.93 | 18.95 | 22.35 | 22.03 | 26.08 | 16.20 | 1.126 |
| Cu ppm | 2.20 | 2.65 | 2.33 | 1.63 | 1.73 | 1.40 | 0.80 | 0.369 |
| Zn ppm | 21.23 | 12.53 | 15.73 | 20.95 | 19.60 | 21.33 | 13.30 | 1.403 |
| Mn ppm | 0.50 | 0.75 | 0.70 | 0.23 | 0.00 | 0.00 | 0.00 | 0.264 |

Where A=raw control, B=shallow fried, C=deep fried, D=baked in tin foil, E=baked open, F=microwaved, G=shallow fried in fish gut fat.

As a means of comparison with other data, and for the compilation of nutritional tables, Table 5 is included. The mineral values in Table 5 are expressed as either mg or $\mu$g per 100 g wet fillet and are the means of the four replicates, with the exception of G, which was from a single replicate. The standard deviations of the means are also given.

**Table 4b:** Probability values calculated for pairwise difference between cooking methods for the various minerals analysed.

| Parameter | Pr > | T | HO: $LSMean_i = LSMean_j$ | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A=B | A=C | A=D | A=E | A=F | B=C | B=D | B=E | B=F | C=D | C=E | C=F | D=E | D=F | E=F |
| P | 0.0233 | 0.0608 | 0.1510 | 0.0077 | 0.0077 | 0.6231 | 0.0008 | 0.0001 | 0.0001 | 0.0026 | 0.0001 | 0.0001 | 0.1510 | 0.1510 | 1.0000 |
| Ca | 0.6231 | 0.6231 | 0.1510 | 0.6231 | 0.0608 | 0.3306 | 0.0608 | 1.0000 | 0.0223 | 0.3306 | 0.3306 | 0.1510 | 0.0608 | 0.6231 | 0.0223 |
| K | 0.0001 | 0.0001 | 0.0001 | 0.0014 | 0.0004 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.1234 | 0.2954 | 0.5966 |
| Mg | 0.1134 | 0.0225 | 0.0225 | 0.4163 | 0.1134 | 0.4163 | 0.4163 | 0.4163 | 1.0000 | 1.0000 | 0.1134 | 0.4163 | 0.1134 | 0.4163 | 0.4163 |
| Fe | 0.0001 | 0.0053 | 0.3139 | 0.2308 | 0.2090 | 0.0736 | 0.0008 | 0.0012 | 0.0001 | 0.0468 | 0.0694 | 0.0003 | 0.8406 | 0.0311 | 0.0204 |
| Cu | 0.4001 | 0.8135 | 0.2853 | 0.3749 | 0.1428 | 0.5414 | 0.0653 | 0.0934 | 0.0278 | 0.1967 | 0.2655 | 0.0934 | 0.8502 | 0.6712 | 0.5414 |
| Zn | 0.0004 | 0.0126 | 0.8913 | 0.4234 | 0.9604 | 0.1241 | 0.0005 | 0.0022 | 0.0003 | 0.0169 | 0.0665 | 0.0113 | 0.5048 | 0.8522 | 0.3960 |
| Mn | 0.5116 | 0.5987 | 0.4708 | 0.1971 | 0.1971 | 0.8949 | 0.1767 | 0.0598 | 0.0598 | 0.2194 | 0.0771 | 0.0771 | 0.5542 | 0.5542 | 1.0000 |

**Table 5:** Mean mineral composition, on a wet mass basis, for uncooked and cooked *C. gariepinus* fillets (±SD)

| Parameter | Means | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| P (mg/100g) | 239 ± 17.9 | 356 ± 29.0 | 346 ± 29.6 | 307 ± 21.3 | 346 ± 30.6 | 320 ± 19.7 | 363 |
| Ca (mg/100g) | 50 ± 1.1 | 94 ± 12.3 | 67 ± 28.4 | 30 ± 9.4 | 69 ± 25.6 | 21 ± 2.3 | 29 |
| K (mg/100g) | 401 ± 11.9 | 309 ± 35.0 | 372 ± 22.9 | 414 ± 9.0 | 472 ± 18.7 | 424 ± 22.3 | 325 |
| Mg (mg/100g) | 61 ± 9.1 | 68 ± 5.5 | 66 ± 13.2 | 44 ± 3.5 | 60 ± 9.3 | 46 ± 1.7 | 40 |
| Fe (μg/100g) | 545 ± 18.3 | 655 ± 62.2 | 739 ± 99.9 | 615 ± 58.6 | 647 ± 68.5 | 702 ± 84.3 | 629 |
| Cu (μg/100g) | 49 ± 14.4 | 108 ± 17.7 | 90 ± 34.0 | 46 ± 17.2 | 50 ± 17.3 | 38 ± 9.4 | 32 |
| Zn (μg/100g) | 781 ± 35.9 | 516 ± 57.5 | 614 ± 100.6 | 578 ± 65.7 | 572 ± 29.7 | 577 ± 123.0 | 516 |
| Mn (μg/100g) | 11 ± 19.2 | 30 ± 17.9 | 29 ± 16.8 | 6.3 ± 10.8 | 0 | 0 | 0 |

Where A=raw control, B=shallow fried, C=deep fried, D=baked in tin foil, E=baked open, F=microwaved, G=shallow fried in fish gut fat.

## DISCUSSION

Generally speaking, the chemical composition of the fish fillets used (A) was within the normal range reported for this species (Hoffman *et al.*, 1993*a,b,c*).

*Proximate composition*

The phenomenon of fish losing different amounts of moisture during different cooking methods has been reported for a number of different fish species(mai *et al.*, 1978; Gall, Otwell, Koburger & Appledorf, 1983; Mustafa & Medeiros, 1985; Hearn, Sgoutas & Hearn, 1987; Shiau & Shue, 1989). In a study of the influence of different cooking methods on the proximate composition of marine fish species (Gall *et al.*, 1983) deep frying was found to cause the highest moisture loss and highest fat gain when compared to an uncooked control. Mai *et al.* (1978) found that although deep frying resulted in a significant moisture loss, this was not as great as the loss that occurred during pan frying. This result is consistent with the findings of the present investigation (Table 1).

It has been noted (Mustafa & Medeiros, 1985; Mai *et al.*, 1978) that the gain or loss of lipids from fish fillets to the cooking medium is related to the lipid content of the raw fillet. The catfish used in the present investigation had a reasonably low lipid content (3.21% on a wet mass basis) and should thus have lost only a small, amount of lipid, if any at all, during the different cooking processes (but not in the frying methods). When the total lipid content of the fillets from the different cooking methods (Table 1 - excluding the frying methods) are expressed on a dry mass basis, there is no decrease in total lipid content when compared to the uncooked control, a result consistent with the findings of Anthony, *et al.* (1983) Gall *et al.* (1983) and Hearn *et al.* (1987).

Shiau and Shue (1989) noted a significant increase in the amount of lipid absorbed by tilapia fillets with increasing frying times. In their study, a frying time of ten minutes resulted in a twofold increase in the absorbed total lipid on a dry mass basis. Gall *et al.* (1983) also noted that, as the amount of lipid in the fish fillet increased, the amount of absorbed lipid from the cooking medium decreased. In their study, Mai *et al.* (1978) noted that trout, with a high lipid content (36.12% DM at an anterior position), lost lipids during frying (33.27% DM same position), while sucker and bluegill fillets, with a low initial lipid content (8.54 and 3.45% DM, respectively), gained lipids (21.56 and 18.15% DM, respectively). In the present investigation, the shallow frying cooking method resulted in a fourfold increase in total lipid on a dry mass basis (4.15 to 19.96% DM). The use of cake flour as a batter source may also have contributed to this large increase in total lipid, as batter is known to absorb lipid (Mai *et al.*, 1978).

Similarly, the higher ash content of the fried samples could be attributed to the presence of the batter. The increased protein percentages (cooking methods D,E and F) could be directly attributed to the general moisture loss experienced, whilst the decrease in protein content, in especially in the pan fried samples (B), is caused by the diluting effect of the absorbed lipid.

*Fatty acids*

The reflection of the fatty acid profile of the frying oil (Table 2) in the lipid extraction of the fried fillets (Table 3a) is consistent with the results of a number of investigations (mai *et al.*, 1978; Gall *et al.*, 1983; Mustafa & Medeiros, 1985). Mai *et al.* (1978) noted that the fatty acid profiles of sucker (*Catastomus commersoni*) and bluegill (*Lepomis macrochirus*), which absorbed frying oil, tended to reflect the fatty acid composition of the oil. The decrease in the percentage of polyunsaturated fatty acids (PUFA) noted in the fried samples was mainly attributed to their quantitative dilution by the absorbed fatty acids.

In their study on the influence of baking on the fatty acid content of the fish fillets, Mai *et al.* (1978) found that baking resulted in an apparent increase in all the component fatty acids, a direct result of the moisture loss experienced. The results of the present investigation support this data, as none of the fatty acids identified in the different baking methods differed significantly ($p \leq 0.05$) in their proportions (percentages) from those of the uncooked control (Tables 3a and b). However, there was an increase in the total lipid content (and a decrease in the moisture content) when the two baked methods employed were compared to the control (Table 1a). This increase in total lipid (and protein) content, and decrease in moisture content, can be attributed directly to moisture (evaporation and drip loss) being lost during the cooking process (Anthony *et al.*, 1983).

Hearn *et al.* (1987) also reported that the fatty acids, including the sought-after PUFAs, are not destroyed in the process of microwave cooking results which are consistent with the present investigation. Gall *et al.* (1983) also reported no difference in the fatty acid profile between raw, baked, broiled and microwaved red snapper (*Lutjanus campechanus*), Florida pampano (*Trachinotus carolinus*), and Spanish mackerel (*Scomberomorus maculatus*) fillets. However, in their study (Gall *et al.*, 1983), grouper (*Epinephelus morio*) fillets cooked in a microwave oven had significantly higher ($p \leq 0.05$) amounts of C17:0, C22:0 and C20:2 than the raw fillet and a significantly lower ($p \leq 0.05$) level of the polyunsaturated C22:6.

*Minerals*

In the study conducted on the American channel catfish (*Ictalurus punctatus*), an increase in phosphorus content with the different cooking treatments when compared to the raw fillet was noted (Mustafa &

Medeiros, 1985). Frying gave a higher phosphorus content than baking. Similarly, Gall *et al.* (1983) noted an increase in phosphorus content with the different cooking methods in all four species studied (grouper, red snapper, pompano, and Spanish mackerel) as did Anthony *et al.* (1983) for the six species studied (bluefish *Pomatomus saltatrix*, croaker *Micropogon undulatus*, flounder *Pseudopleuronectes americanus*, sea bass *Centropristes striatus*, grey sea trout *Cynoscion nobilis*, and spot *Leiostomus xanthurus*). In the present investigation, frying (B, C and G) resulted in a decrease in phosphorus content, while baking and microwave cooking resulted in an increase (when expressed on a dry mass basis, Table 4). This decrease was most probably a result of the dilution effect of oil absorption and the increase because of the concentrating effect caused by the moisture loss. When the phosphorus concentration is expressed on a wet mass basis (Table 5), an increase is noted for all the different cooking methods, when compared to the raw fillet, a result consistent with the investigations mentioned above.

No statistical difference in the calcium concentration (DM) between the raw fillet and the different cooking methods tested was found for *C. gariepinus* fillets. The results of Gall *et al.* (1983) and Mustafa & Medeiros (1985) support this observation. Anthony *et al.* (1983), however, noted an increase in the calcium content of the cooked fillets for all the species studied when expressed on a wet weight basis. The latter authors attribute this increase to moisture loss. The results of this study (Table 5) support their finding in that the cooking methods that resulted in large moisture losses (cooking methods B, C, E and G - Table 1) also showed an increase in calcium content when expressed on a similar basis.

All the cooking methods resulted in a significant decrease in potassium content when compared to the raw control, a result differing from that of Gall *et al.* (1983), Mustafa & Medeiros (1985) and Anthony *et al.* (1983), although Gall and co-workers (1983) noted that when expressed on a dry weight or moisture free and fat free basis the potassium concentration decreased in the baked, broiled and microwave cooked grouper and red snapper fillets, a result comparable with that from the present investigation.

For magnesium content, the results of Gall *et al.* (1983) are comparable with those of this study, where the different cooking methods resulted in a decrease in magnesium content, but differ from those of Anthony *et al.* (1983) and Mustafa & Medeiros (1985) who noted an increase with cooking.

In the present investigation, the iron content of the fish fillets when expressed on a wet weight basis (Table 5) resulted in an increase for the various cooking methods. This result is similar to that of Gall *et al.* (1983), Mustafa & Medeiros (1985), and Anthony *et al.* (1983). These authors also found no fixed trend in the concentrations of copper for the different cooking methods studied.

Although no fixed trend manifested itself in the zinc concentration when expressed on a dry mass basis (Table 4a), an increase in concentration was noted when the concentration was expressed on a wet mass basis (Table 5), a result similar to the findings of the three mentioned authors (Anthony *et al.,* 1983; Gall *et al.,* 1983 and Mustafa & Medeiros, 1985).

## CONCLUSION

Overall, this investigation indicates that the major change in the proximate composition of the African sharptooth catfish, *Clarias gariepinus* fillets, during cooking, is a decrease in moisture percentage, while frying also leads to an increase in total lipid content. This increase is caused by the absorption of the frying oil, and the resulting lipid tends to have a fatty acid composition similar to that of the cooking medium. The loss of moisture during the cooking methods (and the absorption of oil during frying) also influences the mineral composition of the fillets by either having a concentratiing or diluting effect. The trends manifested are not always the same when the results are expressed on a dry mass versus a wet mass basis. Phosphorus concentration, for example, when compared to the control, decreases for the different frying methods tested (B and C), when expressed on a dry mass basis (Table 4a), but shows a higher increase for both frying methods when expressed on a wet mass basis (Table 5).

The findings reported here are of value to nutritionists and dieticians for calculating the chemical content and composition of this fish species in the formulation of diets when different cooking methods are employed.

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# Chapter 9 CONSUMER PERCEPTIONS OF *CLARIAS GARIEPINUS*

**CONTENTS**

**Introduction** 9.2

**Study area and survey methods** 9.2

**Results** 9.5

**Discussion** 9.9

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## INTRODUCTION

The phenomenal growth experienced in commercial production of the African sharptooth catfish (*Clarias gariepinus*) during the late 1980s and early 1990s (Hecht & Britz, 1990) has now gone into reverse (Uys, 1993). Although high feed costs have played a role in this, a major contributory factor could be a lack of proper market research and development (Hecht, 1993; Uys, 1993). Most major producers of catfish concentrated on marketing their product in a highly processed form, targeting the higher income bracket as potential consumers (Hoffman, Prinsloo & Casey, 1993). The targeting of this market was further encouraged by a report from a syndicate of MBA students at WITS Business School (Manicom *et al,* 1992) which argued that there was a potentially high demand for processed catfish, especially in a smoked form, in restaurants and upper income supermarkets. However, catfish had to compete there not only with marine fish but also with trout, a fish species cultivated in South Africa since 1875 (Hecht & Britz, 1990). None of the commercial producers was strong enough to succeed alone against such well-established competition.

In another report published by an MBA student (Visser, 1992), it was suggested that the future of the catfish industry might lie in marketing this species among the lower income groups, though to compete successfully the fish would have to be competitively priced and acceptable to these consumers.

This paper investigates consumer behaviour of lower income groups in rural and urban Northern Province, as this relates to meat products, and especially, the African sharptooth catfish, *Clarias gariepinus*.

## STUDY AREA AND SURVEY METHODS

To establish whether fish meat preference in this region is linked to socio-economic milieu, personal interviews were conducted in both a rural and an urban setting. Two study areas, Giyani and Ga-Mamphaka, were arbitrarily selected.

Giyani, capital of the former self-governing national state of Gazankulu, may be classified as a commercial and administrative town, though, according to Maphophe (1992), it is nothing more than an accommodation centre for bureaucrats and would, without them, be a ghost town (Fig 1). There is a possibility that Giyani may forfeit its administrative function in a post-apartheid South Africa. Data from 1987 indicate that Gazankulu has an annual per capita GGP[1] of R494 compared to R4 258 for the rest of the Northern Province. Although Giyani

[1]Geographical Gross Product - The value of all final goods and services produced within a region or province of a country.

is located in the poorest area of the region, the overall quality of life of its residents is much higher than in the rest of Gazankulu. Proof of this can be seen in a well-established infrastructure supported by commercial, administrative and educational facilities. According to the 1991 Population Census, there are slightly more than 15 000 people residing in the town (RSA, 1991).

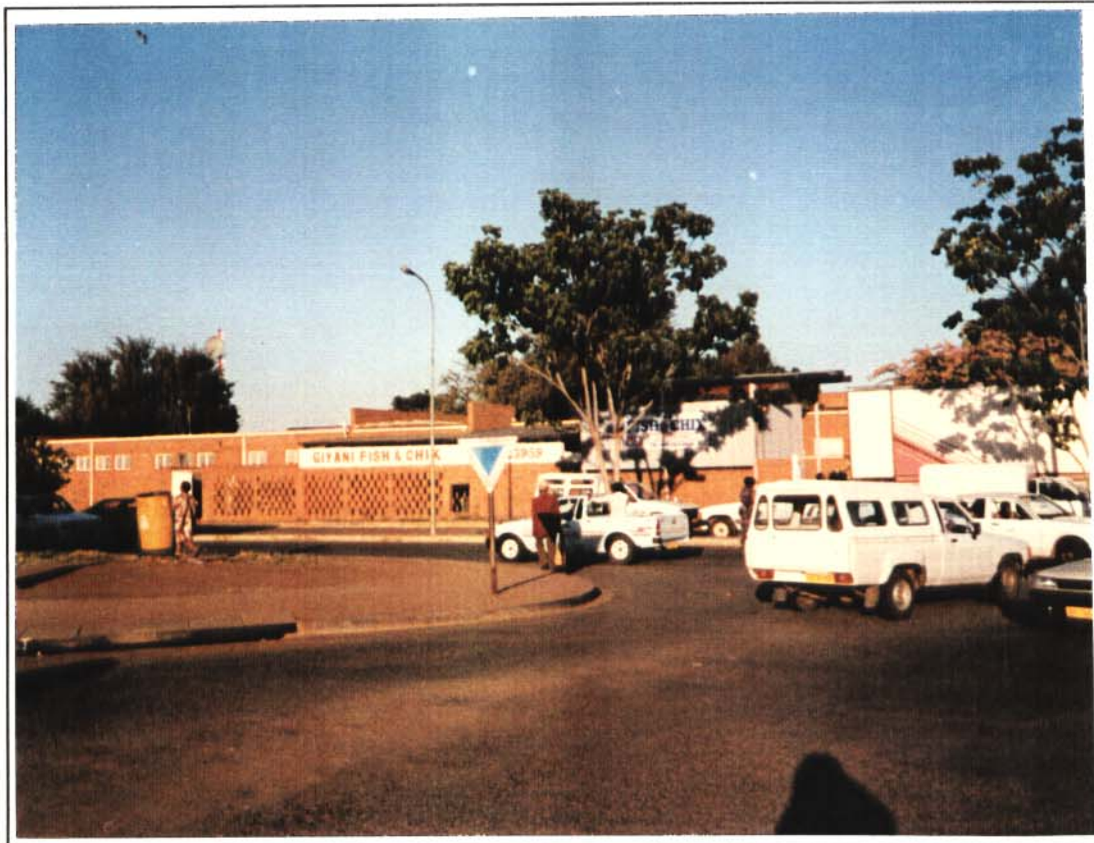


**Figure 1:** A typical Gyani street scene showing the local fresh fish store.

The rural settlement of Ga-Mamphaka, on the other hand, situated in the Thabamoopo district of the former homeland of Lebowa, typifies the low standard of living in peripheral areas of South Africa (Fig 2). A socio-economic survey conducted in the settlement illustrates its poverty and generally low living standards (Donaldson, 1992). The majority of its economically active people are migrant workers (73%) while 84% of households have an income below R1 000 per month. Inadequate provision of such services as electricity, water,

roads and shopping facilities contributes to the lack of development of such rural communities. There are approximately 112 households in Ga-Mamphaka.

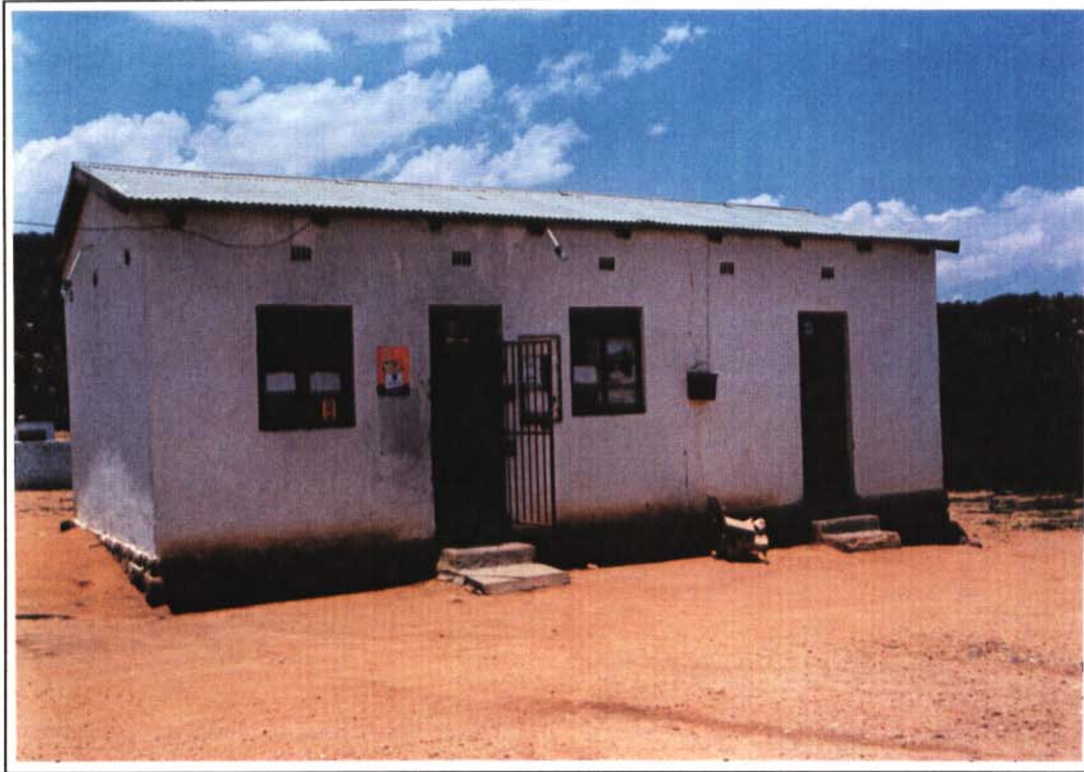

**Figure 2:** Ga-Mamphaka showing the single trade store.

The above background information reveals major differences in living standards between urban and rural people. The two study areas also show marked differences in the provision of fish. Besides numerous cafe and supermarkets that sell fried fish, Giyani also has a distributor who sells fresh fish and a viable freshwater fish-farming enterprise adjacent to the town. Ga-Mamphaka, has but two cash stores that sell fish products and only in canned form. Water is scarce and the local stream has been dry for a number of years. Close to Giyani, on the other hand, there are two dams, the Nsami ($\pm 8$ km) and Middle Letaba ($\pm 50$ km).

A systematic sample of 100 households in a Giyani residential area and 50 households in Ga-Mamphaka was selected for an in-house questionnaire survey, which was conducted by trained interviewers from the University of the North proficient in the respondents' home language.

The primary aim of the questionnaire was to determine the respondents' consumer behavioural patterns relating to meat products, with special reference to the African sharptooth catfish, *Clarias gariepinus*, also commonly known as the barber in this region. The questionnaire was constructed to determine the following information from respondents: socio-economic background; preference with regard to fish products; perception of other meat products (the competition market); and place of purchase.

## RESULTS

*Household and Income*

Comparisons of rural and urban household sizes and monthly incomes are summarised in Figures 3 and 4 respectively. Although, according to Figure 3, there was no significant difference in household size between the two community types, it can clearly be seen in Figure 4 that the urban community's income is higher than its rural counterpart's. In both communities, the majority of the households (33% and 53%, respectively) had more than seven residents (Fig 3). However, in the rural community, 43% of the households had a monthly income below R500, whereas only 5% of the urban households had such a low income (Figure 4). In the urban community, 39% of the households earned a monthly income above R1 500, compared to 4% in the rural community.

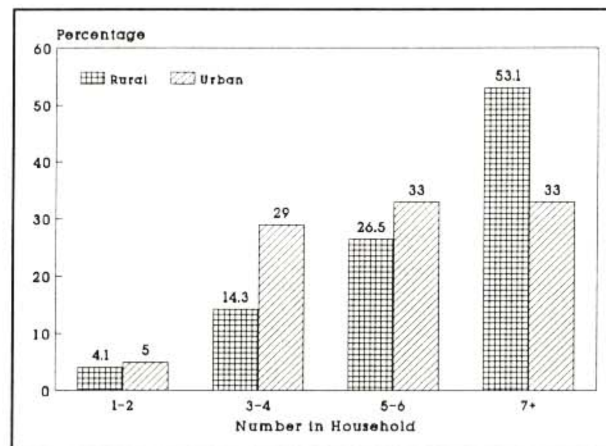


**Figure 3:** Number of people per household in a rural and urban region in Northern Province, South Africa.

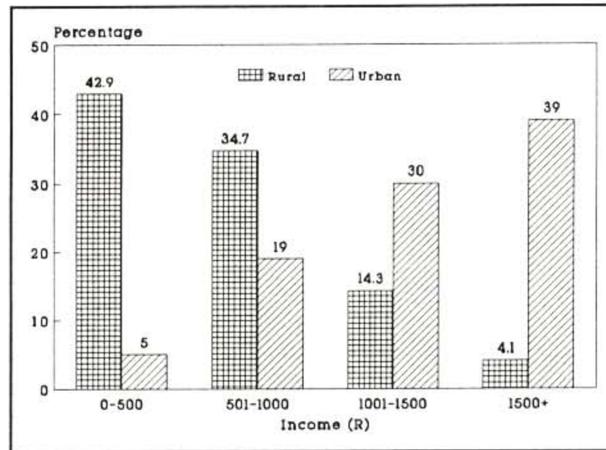


**Figure 4:** Monthly income per household in a rural and urban region in Northern Province, South Africa.

A comparison of amount spent monthly on food between the two communities is summarised in Figure 5. In the rural community, 41% of the households spend between R101-200 per month on food. In the urban community, however, 32% of the households spend between R201-300 and 31% spend more than R301. The Pearsons correlation between amount spent on food and household income is 0,2843 and 0,3835 for rural and urban communities respectively, suggesting that in the urban areas either food is more expensive or living standards are higher. Price sampling of local goods suggests the latter, a point also supported by the type of meat purchased by the urban community.

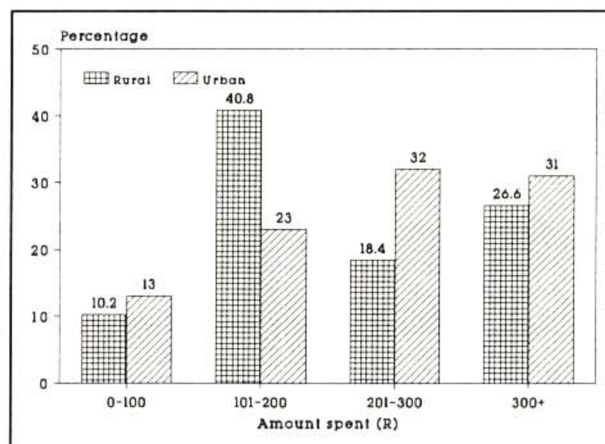


**Figure 5:** Monthly expenditure per household on food in a rural and urban region in Northern Province, South Africa.

*Meat Types Purchased*

The consumption of different meat types by the residents in the two communities is summarised in Figure 6. The most popular meat type purchased by both communities is chicken, with the rural community being its bigger purchaser (71.4 and 46.0% respectively). Beef was the next most popular meat (a higher consumption was found in the urban community), with lamb, fish and pork figures statistically insignificant. To test consumers' perception of costs involved in purchasing meat, respondents were asked to classify meat types in order of cheapness. In the rural community, 79.6% of respondents identified chicken and 14.3% fish as the cheapest meat source, with beef (49.0%) and lamb (47.0%) as the most expensive. In the urban community, 45.0% identified fish as the cheapest meat form while 31.0% chose chicken. Beef (35.0%) and lamb (45.0%) were also identified as the most expensive options.

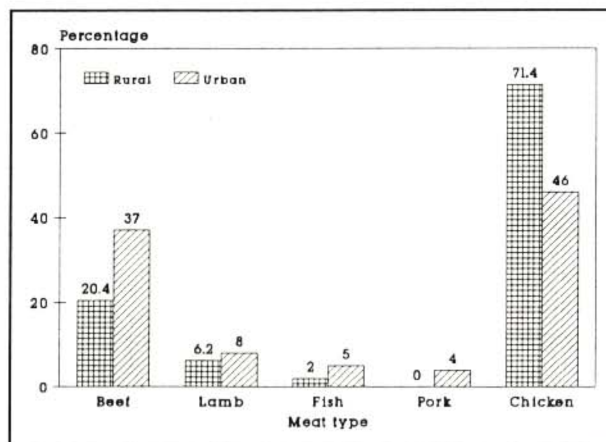


**Figure 6:** Meat type purchased in a rural and urban region in Northern Province, South Africa.

*Processed Form*

In the rural community 36.7% of households prefer to purchase meat in a frozen form, 34.7% prefer purchasing live animals, while 28.6% like to buy their meat fresh. Meat is bought either at a cafe or butchery (28.6% and 28.6%, respectively). Sixteen percent of the rural community buy their meat from a supermarket and the same percentage from a hawker. However, in the urban community, 73.0% of households prefer buying their meat fresh while 20.0% prefer buying it frozen. The rest of the households prefer to purchase their meat alive, canned or cooked. The majority of urban households (73.0%) purchase their meat from a butchery or supermarket (18.0%). Only 4% of the urban households buy their meat from a cafe.

Fridges are rare in rural households (18.4%) compared to the urban community (80.0%). Thus of the 81.6% of rural households without fridges, 38.8% eat their meat as soon as possible after purchase, while 22.5% dry their meat as a method of storage. Alternative forms of processing practised (22.4%) include smoking and salting.

*Consumption of Fish*

Although the respondents in neither the rural nor urban communities regularly purchase fish, the majority (88%) in both communities consumed it. Those few who do not, either dislike the taste or have never tasted it before. In the rural community, fish is normally prepared as a meal once (32.7%), twice (16.3%), three times (8.2%) and more than three times (28.6%) per month, while in the urban community the corresponding figures are 15.0% (once), 18.0% (twice), 16.0% (three times) and 41.0% (more than three times), respectively.

Fish consumed in the rural community is normally in canned form (77.6%) or fresh (6.1%). Fried (4.1%) or frozen (2.0%) fish does not seem to be very popular. In the urban community, canned fish (37.0%) is also the major processed form purchased, followed by frozen (28.0%), while 21% of respondents purchase fresh fish and only 3% purchase it fried and 1% smoked. In the urban community, 22.0% of respondents also catch their own fish from nearby rivers and the Middle Letaba and Nsami dams. None in the rural community catch fish, probably because of the settlement's distance from rivers and dams.

*Consumption and Purchase Preference of the African Sharptooth Catfish, Clarias gariepinus*

Only 40.8% of respondents in the rural community currently eat the African sharptooth catfish, *Clarias gariepinus* (also known here as the barber) compared to 64.0% from the urban community. Of the respondents who do not eat it, 52% do not do so because it is unavailable, while it is unknown to 35%, and only 8% of respondents dislike the taste. No respondents gave religion as a reason for not eating catfish. In the urban community, 48% of respondents listed "unknown" as a reason for not eating it, 32% did not like its taste, and 20% did not eat it because it was unavailable.

Of the 57.1% rural respondents who would buy catfish if it was readily available, 46.9% would prefer it in a canned form, while 14.3% would like it fresh. A small percentage would like to purchase it either dried (8.2%), fried (6.1%), frozen (6.1%) or filleted (6.1%), while no respondents would purchase it whole or gutted. In the urban community, 69.0% would purchase the fish if it became readily available. The preferred processed form in the urban community is fresh (37.0%), followed by frozen (27.0%) and canned (16.0%). Only 4.0% of respondents would like the fish filleted and 3.0% fried.

The rural community would like to purchase its catfish at the local cafe (32.7%) or at a supermarket (24.5%).

Only 14.3% would like to buy it at a butchery and 2.0% from a hawker. In the urban community, the supermarket is the preferred place (37.0%) of purchase, followed by the butcher's (28.0%).

## DISCUSSION

The present investigation indicates that as a result of differences in living standards between urban and rural areas (Fig 3 & 4), more money is spent on food in the urban area (Fig 5). Rural households studied in the present investigation were similar to those noted by Ladzani and co-workers (1992) in the same region, where it was found that the majority of households comprised five to seven occupants with a monthly income below R250 per household.

There was a reliable perception of meat prices in both communities, with beef and lamb being correctly indicated as the most expensive. The identification of fish as the cheapest meat form in the urban area (45.0%) is most probably a result of the fish-farming enterprise adjacent to the town marketing its produce at a competitive price, especially *vis à vis* chicken. This would also seem to indicate that the potential market for the African catfish would be at a price competitive with that of chicken.

The high number of urban residents who prefer to purchase their meat in a fresh form (73%) could be attributed to the proximity of the butcheries and supermarkets to their respective homes (as well as their possession of fridges) so that shelf-life would not be an important consideration when purchasing meat. However, shelf-life is an important consideration in the rural area where meat is either purchased frozen (36.7%) or alive (34.7%), and consumed as soon as possible, or dried as a means of storage. In the urban community, marketing catfish in a fresh form, which is currently the norm at production farms (known as gate sales), would seem a viable and cost effective option for the producer. Marketing in the rural area, however, could be more expensive since fish would need to be processed (frozen or canned) or a means devised to deliver it fresh closer to rural residential areas.

It is interesting to note that no respondents gave religion as a reason for not eating catfish, though this has been postulated by a number of unsuccessful farmers (pers. comm.). It seems rather that lack of a competitive price and ignorance of the fish species are the deciding factors.

The fact that the African sharptooth catfish is acceptable to the majority of both urban (69.0%) and rural (57.1%) respondents should be heartening for the South African catfish production industry. Yet, although most respondents also prefer to purchase catfish processed in a canned form, this preference has not yet been addressed by the industry. A major reason perhaps is that canning industries need a large and constant supply

of fish to set up an economically viable production line. At present South African catfish producers do not supply enough tonnage to the canning industry. Hecht (1993), however, suggests that when the catfish marketing problems have been overcome, total production of catfish could increase to 5000-6000 tons *per annum* within two to three years.

The only way out of the current marketing bottleneck, according to Hecht (1993), is a considerable injection of funds into a dynamic marketing campaign. The present investigation also shows that if catfish could be marketed at a competitive price, there is very little consumer prejudice against purchasing it among lower income groups in northern South Africa.

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# Chapter 10 CONCLUSIONS AND RECOMMENDATIONS.

The recommendations made in this chapter are mainly directed to academics, who through their research and findings will hopefully help the industry to surpass its present production and overcome the marketing problems experienced.

*Chapter 3*

In Chapter 3 of this study, the muscle chemical composition within the catfish was analyzed in three different investigations, *viz*, the muscle heterogeneity within a fillet, comparison of light and dark muscle and comparison of the fatty acid composition of various lipid depots. The following trends were found within the fillet: a decline in moisture and protein percentages and an increase in total lipid percentage caudally in the musculature (Fig 2). Similarly, a decrease in the percentages SFA and MUFA and an increase in the percentage PUFA, caudally (Table 3). The concentration of the amino acids glycine, alanine, proline and hydroxyproline increased caudally along the musculature (Table 2). These increases were attributed to an increase in the collagen content of the muscles. The concentrations of the minerals calcium, copper, zinc and manganese showed no fixed trends caudally, whilst phosphorus, potassium and magnesium decreased and iron increased (Table 4).

Most of these trends were attributed to a difference in the proportion of dark and light muscle, the proportion of the former increasing caudally (from 7% to 9%). The dark and light muscle were found to have similar moisture (78%) and ash (3%) contents, but significantly different protein (14 & 19% respectively) and lipid (5 & 1% respectively) contents (Table 5). The dark and light muscle also had similar SFA (34%) but different MUFA (32 & 26% respectively) and PUFA (30 & 35% respectively) contents (Table 6).

The only major incongruity between these two investigations, is the trends manifested by the fatty acids, in particular, the PUFAs, that increased in concentration caudally (Table 3). This increase was attributed (in the initial investigation) to dark muscle having a higher concentration PUFA, which, from the latter investigation (Table 6), does not seem to be true. A possible explanation for this phenomenon could lie in the relative proportions of the different lipid classes namely, phospholipids, cholesterol, free fatty acids, diglycerides, triglycerides and cholesterol esters present (Love, 1988). The fatty acid profiles of these lipid classes differ and warrants further investigation in terms of their concentration and composition in *C. gariepinus* muscle.

The results of the chemical analysis of the right fillet and different parts of the left fillet, as well as that of the two muscle types, support the recommendation of Love (1988) who suggests that ideally, white and dark muscle should be investigated separately, failing which the entire fillet, or a carefully specified part thereof, should be analyzed. In all our investigations into the muscle chemical composition, samples of minced and homogenised whole fillets were analyzed as the investigations were launched to study *Clarias gariepinus* as a "protein" source

from a nutritional viewpoint. In this context, the composition of the total fillet is of more value than that of the part as often the whole fillet is consumed.

The third investigation into the fatty acid composition of the different lipid depots, showed that there are differences, although no fixed trends could be found. These differences need to be quantified, and the whole metabolism of lipids in the catfish warrants further investigation.

*Chapter 4*

In Chapter 4, a number of investigations looked at the yield attained by various strains of catfish. The definition of yield also varies, in the first investigation, the yield was described in terms of drawn and dressed masses, whilst in the other investigations, yield was defined as the fillet produced. By definition, the drawn mass (Fig 3) consisted of the weight of the fish with its viscera removed and the dressed mass also had the head removed. This dressed mass is frequently referred to as yield in the literature, especially as pertaining to channel catfish *Ictalurus punctatus*. This differs from our definition where yield relates to the fillet of the fish.

In the first investigation, the golden strain gave higher dressed masses (as a percentage) than the Turfloop normal coloured strain (Table 3). For both strains, the males gave higher dressed yields than the females. As these fish were sexually mature, these differences were due to gonadal development. The high drawn masses of the males (92%) indicate that the viscera of males make up 8% of the total weight. This value is low because the fish were fasted for three days thereby ensuring that the gut were empty. Unfortunately, the data did not give good fits to the regression equations for percentage drawn and percentage dressed mass (Table 5) thereby making a comparison of "yield" between the sexes and strains of little value to the industry. For these regression equations to be of value more data would have to be collected.

In the second investigation, the data on the various proportions found in the catfish for all the fish investigated in this study, was pooled and the influence of size and sex thereon, monitored. Although the data covered a wide body weight range (Table 1), it would have been of more value if the total sample numbers could have been larger, especially when applicable to comparison of strains. However, a strong linear relationship ($R^2=0.9934$) between fillet weight with increasing body weight (BWt) for the population as a whole was found (Table 6):

$$\text{Fillet weight (g)} = -13.2828 + 0.46699 \times \text{BWt (g)}$$

Similarly, regression equations for the prediction of the other carcass components were also calculated (Table 8). These regression equations will enable the processing trade to predict the fillet yield (and waste yield) of various sized fish. Further research is needed to see whether these equations will hold true for the catfish population as a whole, or whether strain will have an influence thereon.

The influence of sex on the chemical composition of the fillet showed no fixed trends with the exception of the females gaining moisture at a significantly faster rate that the males with increasing live weight (Table 9). However, what is interesting, is the low correlations noted between the condition factor (total length/body weight x 100) and the other chemical parameters (Table 10). A possible explanation could be that these correlations were calculated for the pooled data and not for the individual strains and sexes. Similarly, physiological maturity would also influence the condition factor. The influence of these factors (strain, sex, physiological condition) on the condition factor, and its correlation with the chemical proximate composition warrants further investigation. If high correlations could be found, it would enable the producers to monitor the chemical composition of the catfish, without having to slaughter and analyze the fish.

Similar to other fish species, *C. gariepinus* also has a strong body water - lipid line (Fig 23).

The last part of Chapter 4 reported the chemical composition of two waste products: female gonads and mesenteric lipid depots. The chemical results show that these so called waste products, with the proper marketing strategy, could become viable income generating products. The female gonads could not only be used as a fish meal replacer in larvae starter diets (Chapter 5), but also has a potential for processing as caviar. Initial results in our laboratories has shown that catfish roe is acceptable as caviar in the market. Similarly the mesenteric lipid is also being used locally as a cooking oil source. Another facet that is being researched at present, is the tanning of the catfish skins for the exotic leather market. Preliminary results show that the skin tans well and results in a thin but strong product. The only potential obstacle that can be foreseen, is that at present a 600g catfish delivers a skin that is too small. Slaughtering catfish of a larger size raises other research opportunities such as nutrition requirements, influence of age on body composition, etc.

*Chapter 5*

In this chapter the influence of genetic strain on chemical composition was investigated. In the first experiment, four different genetic strains were raised together under identical conditions to investigate the influence of strain on the whole body chemical composition. The faster growing strains (Fig 1) were shown to have higher moisture (and lower lipid) levels (Table 2). Although the comparison of the amino acids, fatty acids and mineral profiles showed statistically significant differences, no fixed trends were found. It would be interesting to see whether these differences become more (or less) pronounced as the fish increase in live weight.

In the second investigation between two strains (gold and Turfloop normal), size was found to influence the chemical composition of the muscle. An investigation into the chemical changes with increasing body weight (growth) for different strains would be of value.

In the third investigation in this chapter, a commercially farmed strain was compared to a wild strain. Unfortunately, there were large differences in the sizes of the two groups, which made comparisons difficult. The large deviation in the body lipid content (Table 14) is worth noting. This is a factor that needs to be monitored closely as it could be influenced by consumer preference.

*Chapter 6*

This chapter consisted of two investigations into the role that diets (especially dietary lipid) has on the body chemical composition. In the first investigation, *C. gariepinus* gonads were fed as a replacement for fish meal in the starter diet of catfish larvae. Although the gonads were suitable as a replacement, they caused heavy liver lipid deposition. It is recommended that catfish roe only partially replace fishmeal in these starter diets.

The second investigation consisted of feeding naturally occurring lipids in an artificial diet to catfish so as to see how easy the body lipid composition can be manipulated. Both this investigation and the earlier investigation showed that the lipid fatty acid profiles of *C. gariepinus* can be manipulated. This experiment needs to be repeated with naturally occurring protein sources and the influence of diet composition on the growth also needs investigation. For the latter, it is recommended that the duration of the feeding trial be longer than 60 days, thereby ensuring that the influence of the lipids already present in the muscle at the start of the experiment will not influence the results.

*Chapter 7*

A water source that has great aquaculture potential is the final effluent oxidation ponds of municipal sewage works. In this chapter an extensive review is given on potential health hazards that may arise from the utilisation of such ponds for the production of fish for human consumption. In the latter part of this chapter, catfish that occur naturally in the final oxidation ponds of Pietersburg's municipality were caught and chemically analyzed. The muscle was found to be fit for human consumption in terms of the chemical composition. As was noted in the chapter, if care is taken during the harvesting and processing of the fish, no bacterial contamination should occur. However, it must clearly be stated that the suitability of different municipal ponds for aquaculture needs to be monitored individually, especially in terms of the presence of potentially harmful substances.

*Chapter 8*

The influence of different cooking methods on the chemical composition of the catfish fillets was investigated in this chapter. The major changes noted were a decrease in the moisture content with cooking, while frying gave an increase in the oil content. In the latter, the fatty acid profile resembled that of the oil absorbed. These concentrating and diluting effects also influenced the mineral content of the fillets. The chemical composition tables in this chapter will be of value to human dieticians in the compilation of balanced diets.

*Chapter 9*

This chapter is important in that it is the first survey conducted in an alternative market (lower income) for the catfish in South Africa. The future of the catfish industry lies in a sound marketing strategy and the application of good basic scientific practices. Some of the results from this chapter on the consumer behavioural patterns as pertaining to meat products, with special reference to *C. gariepinus*, in a rural (Ga-Mamphaka) and urban (Giyani) setting in Northern Province, South Africa, was read at the last Aquaculture '94 Congress. (This congress was organised by the producers and not scientists). At this conference, there was an overwhelming response from the catfish producers to this paper, as this was the first marketing survey done amongst the lower income group in South Africa. Traditionally catfish producers have been concentrating on selling catfish as value added products for the higher income market. The consensus reached at the conference was that a wider, more in-depth survey needs to be conducted amongst the lower income groups to investigate their possible consumption of fish. One of the problems identified in the survey conducted, was that the fish species was unknown to many of the respondents.

In the survey conducted, it seems as if the potential market for catfish would be at a price competitive with that of chicken. For this to be achieved, the production costs would have to be lowered. This implies that the catfish production would have to be more efficient, which can only be achieved by applying sound scientific knowledge. Another means of lowering the production costs would be to lower the feed costs. This can be achieved by feeding alternative cheaper protein sources, such as waste products (abattoir-, tomato-, paw paw-, orange wastes, etc).

In the marketing survey, most respondents also indicated that they would like to purchase catfish processed in a canned form. The present study has provided good background information for the development and processing of various catfish products, an area that needs to be researched.

*General*

The South African catfish producers have not yet entered the international market on a large scale. A number of producers have used results from the present study in their initial market surveys. The problem with the international market seems to be, that although there is a competitive market, the tonnage involved is to large. This implies that the South African producers will have to increase their production, however before this can be achieved, more research needs to be carried out, not only on the production, but also on the processing of this fish species.

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