THE CHEMICAL COMPOSITION

OF <u>Haplochromis</u> spp.

by

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ABSTRACT

The Haplochromis genus constitutes about 80% by weight of the
fish in Lake Victoria yet only small amounts are utilised directly
for food.   There are over 150 species of Haplochromis in the lake
forming a species flock and any typical catch is made up of a
mixture of many species.   Morphological resemblance of the species
makes identification of individual species very difficult.   Little
information has been published about the chemical composition of
Haplochromis.

The present study provides chemical composition data for lioid,
protein and other nutritionally important constituents of Haplochromis
as an aid to the processing of the resource and its nutritional
evaluation.

Four batches of Haplochromis from Lake Victoria were received.
The first batch was analysed only for total lipid.   Fish from the
other three batches, caught over a five month period, were   each
split into four weight groups.   Data were obtained for the weight
distribution in the batches, the relationship between weight and
length of the fish, weight and maximum depth of the fish and for the
proportions of head, gut and headless gutless portions in the
different weight groups.

Total lipid determinations were carried out on the head, gut
and headless, gutless portions for all four groups for each batch.
On average 54% of the total lipid in the whole fish was present in
the head, 19% in the gut and 27% in the headless, gutless portion.
Small variations were found in the total lipid content and distribution
between size groups and batches, although on average the whole fish
contained about 6% total lipid.

Analyses for the lipid fatty acids profile, crude protein, true
protein, amino acid profile, ash, calcium, vitamin A and moisture were
carried out on the head, gut, fillet and residue for the major group
and on the whole fish for other groups.

Processing and nutritional implications of the results are discussed.
The results generally indicate that for practical processing purposes
the species flock may be treated as a single species.

DEDICATION

To FATHER and MOTHER;
the former for being an excellent
teacher and philosopher, and the
latter for her unusual tolerance
and unfailing loyalty.

ACKNOWLEDGEMENTS

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1. <u>INTRODUCTION</u>

The rapid growth in population and income since the Second
World War resulted in a rapid expansion of fishery production
during the 1950's and 1960's. As shown in Figure 1, total world
catches (marine and inland) rose from about 20 million tonnes in
1948 to about 70 million tonnes in 1970 (FAO, 1949-1979; FAO,
1980), contributing 6% of the total world supply of protein and
18% of animal protein (FAO, 1978). Since 1970, however, the
total world catch has remained at about 70 million tonnes. In
1980 the total catch was estimated at just over 71 million tonnes,
and FAO does not expect significant change in the 1981 level of
world catches, compared with those of 1980 (Anon., 1981a). One
of the reasons for stagnation in the world total fish catches is
the over-fishing of some fish stocks. During the 1960's, for
example, large quantities of Peruvian anchoveta (<u>Engraulis ringeus</u>)
were landed for reduction to meal and oil. Catches rose from
approximately 4 million tonnes in the early 1960's to just over
13 million tonnes by 1970. After 1970 catches started to decline
such that by 1979 only 1.4 million tonnes of Peruvian anchoveta
were landed (FAO, 1949-1979; FAO, 1980). This decline has been
attributed to over-fishing and environmental changes (FAO, 1978).
Over-fishing has also occurred with some common fishes caught for
human food, such as hake and herring, whose stocks have been
considerably reduced. Cod, haddock and saithe, and many other
stocks, are reported as "fully exploited" and some are threatened
with depletion. Consequently quotas have had to be agreed upon for
many stocks (Bailey, 1976).

Depletion of the conventional fish stocks has resulted in fish
species being investigated that had hitherto been under-utilised,

Figure 1 <u>Total world catch of fish over period 1948-1979</u>
(FAO 1949-79 ; FAO 1980)
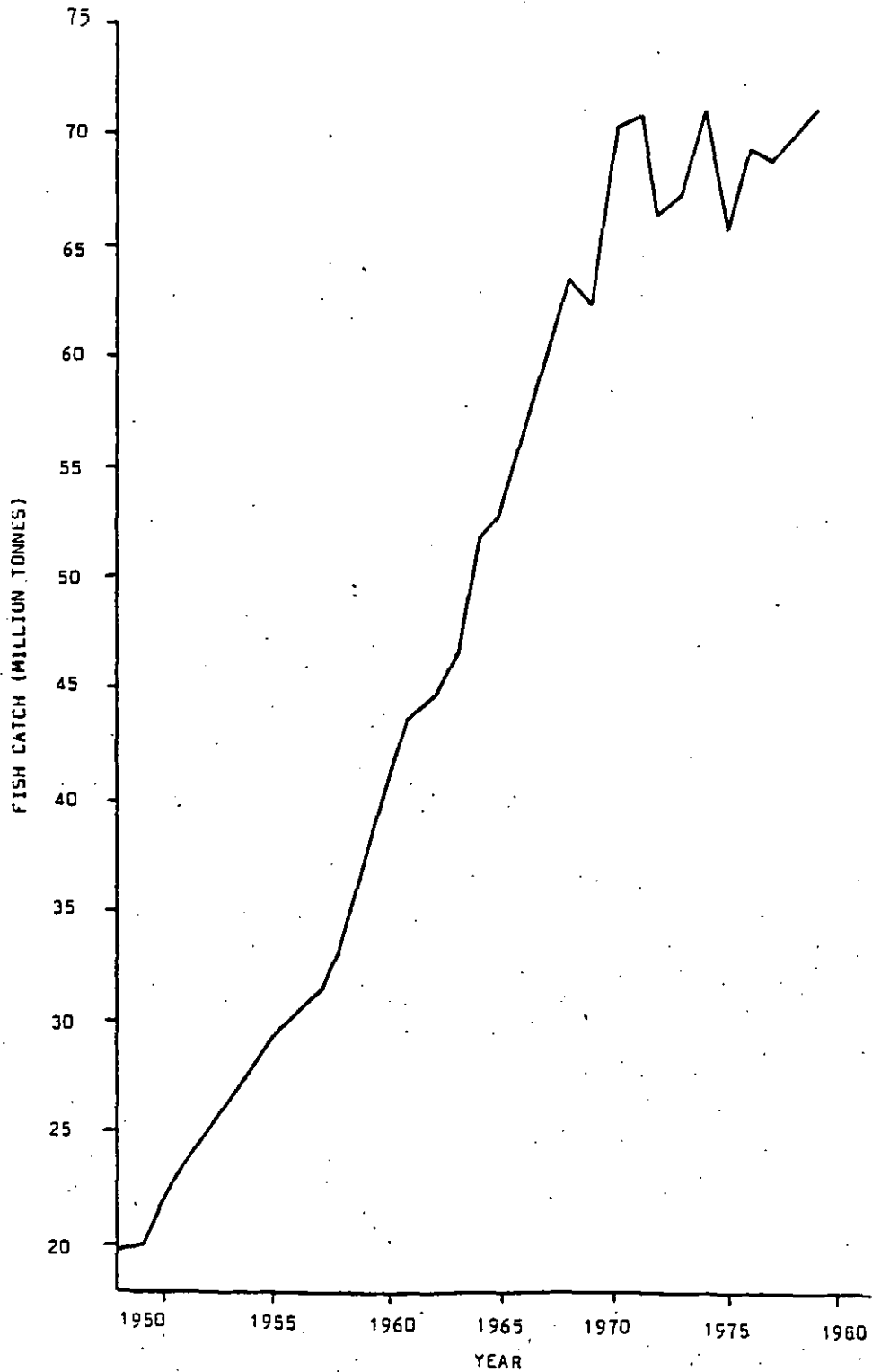
for example, in the North Atlantic the blue whiting (_Micromesistius poutassou_), which has a spawning stock of about 10-15 million tonnes, the greater and lesser silver smelts (_Argentina silus_ and _A. sphyraena_) and horse mackerel (_Trachurus trachurus_) (Bailey, 1976).

It is difficult to assess the situation on fish stocks in tropical waters "because of the many countries involved and their differing socio-economic structures; and their insufficient catch data" (Chikuni, 1977). However, it has been estimated that the total catch from tropical waters (excluding Peruvian anchoveta) rose from 10 million tonnes in 1965 to 18 million tonnes in 1973. The rapid increase in catch was attributed mainly to marine rather than inland waters. Certain species of fish, such as the yellowfin tuna (_Thunnus albacares_), have been over-exploited in tropical waters while other species, such as the skipjack (_Katsowonus pelamis_) and bonitos (_Sarda_ spp.) have not been fully exploited (Chikuni, 1977; Anon., 1981b).

Although information on the potential of inland waters in the tropics is scanty, there are indications that substantial amounts of species of small fish remain largely under-exploited. Species of the genus _Haplochromis_ are thought to be the most abundant freshwater fish species in Africa, but relatively small amounts are utilised for food.

Before embarking on full exploitation of such under-utilised species, however, stock assessment surveys, fishing gear trials and chemical composition and processing studies on the fish must be undertaken. Information about the chemical composition is important to the processor and the nutritionist (Stansby, 1961). The processor needs to know the chemical composition of the raw material in order to decide on the most appropriate processing procedures and storage conditions. The nutritionist needs to

know the amounts of the nutrients present in the fish and their distribution, when producing food tables or formulating diets.

The work described in this thesis is concerned with the chemical composition of <u>Haplochromis</u> species caught in the Ugandan waters of Lake Victoria and aims to produce compositional data that will be of value in the effective utilisation of this resource.

2. LITERATURE SURVEY

2.1 Fish resources in Uganda

2.1.1 General survey

Uganda is a land-locked country but endowed with many lakes, rivers and swamps providing a great potential for fish (see Figure 2). The total catch in Uganda in 1971 was estimated at 162,310 tonnes valued at about 200 million shillings (8 shillings = 1 U.S. $ in 1980) at the lake shore. This gave a per capita consumption of 14.8 kg (Dhatemwa, 1975). In 1979 the catch was estimated at 223,830 tonnes (FAO, 1980).

Table 1 gives a breakdown of the fish production in Uganda according to the body of water for 1971, the latest date for which figures are available (Uganda Ministry of Animal Resources, 1974). Over 50% of the total catch was taken from L. Kyoga, the main species being nile perch (Lates spp.) and Tilapia spp. Catches from L. Victoria were dominated by Bagrus docmac and Tilapia spp. (see Section 2.1.2). The main species caught in the other bodies of water were Tilapia spp., Lates spp. and Protopterus spp. In addition Hydrocynus spp. and Alestes spp. were caught mainly in L. Albert and the Albert Nile. Although these figures relate to 1971, the present distribution of catch between bodies of water and the species caught are not expected to differ greatly.

The production of L. Victoria in 1971 was less than half that of L. Kyoga, although the area of the former is about 12 times that of the latter (see Table 1). Several factors account for the

Figure 2   <u>Uganda - Major bodies of water and rivers</u>

Scale 1:4,000,000   1 cm to 40 km approx.
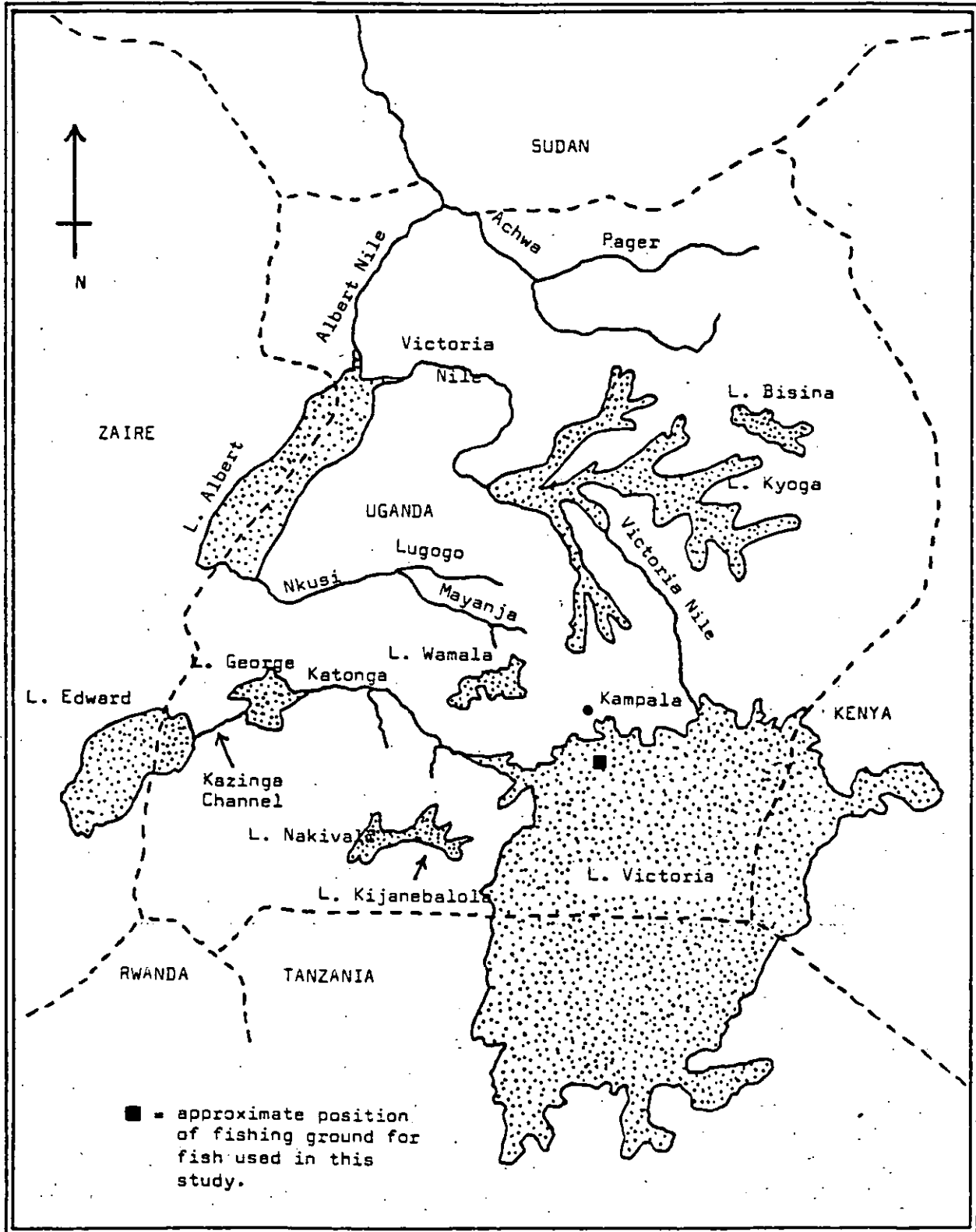
Table 1   Uganda fish production in 1971 [1]

Body of water	Area (km^2) [2]	Fish production (tonnes)	Percentage of the total fish production
L. Kyoga	2,279	89,724	55.3
L. Victoria [3]	28,389	38,070	23.5
L. Albert and Albert Nile [3]	3,587	13,670	8.4
L. Edward [3]/George/ Kazinga Channel	878	11.726	7.2
L. Wamala	30	5,197	3.2
Minor lakes	–	2,435	1.5
Rivers, dams and ponds	–	1,495	0.9
Total		162,317	100

Notes: (1) Uganda Ministry of Animal Resources (1974).

(2) Semakula (1967).

(3) Uganda sector.

lower production of Lake Victoria including the fact that Lake Victoria is much deeper than Lake Kyoga, which leads to lower primary production. Another important factor is that only negligible amounts of the major species in Lake Victoria, _Haplochromis_ spp., were landed. This is discussed further below.

There is some evidence now that Lakes Kyoga, George and Edward are being almost fully exploited (Uganda Ministry of Animal Industry and Fisheries, Fisheries Department, 1981). The best opportunity for increasing fish production in Uganda probably rests on the full exploitation of _Haplochromis_ from Lake Victoria.

2.1.2 Lake Victoria fishery

The fish fauna of Lake Victoria fall under 12 familes with 27 genera and 50 species excluding _Haplochromis_ spp. (Kanyike, 1972). Of the 50 species, 20 are fished for marketing as food fish. The species of commercial importance are _Bagrus docmac_, _Tilapia_ spp., _Clarias_ spp., _Protopterus aethiopicus_ and _Synodontis_ spp. (Bergstrand and Cordone, 1971; Uganda Ministry of Animal Industry and Fisheries, Fisheries Department, 1981), although no detailed figures have been published for the catches of the individual species. There are several species which used to be of commercial importance in the past but their numbers have declined due to over-fishing. These include _Labeo victorianus_, _Schilbe mytus_, _Alestes jacksonii_ and _Alestes sadleri_ (Kanyike, 1972).

Certain species of fish, though existing in large quantities, have not been fully exploited, either because the demand for them is low, or because efficient methods of catching them have not been employed. The most notable examples are species of the _Haplochromis_

genus and, to a lesser extent, _Engraucypris argenteus_.

Fish stocks assessment surveys carried out in Lake Victoria, using trawling, showed that _Haplochromis_ formed about 80% by weight of the total fish stock of the lake (Gee and Gilbert, 1968; Bergstrand and Cordone, 1971; Okedi, 1972). It was estimated that the standing stock of _Haplochromis_ was about 600,000 tonnes (Okedi, 1972) and about 160,000 to 200,000 tonnes could be harvested annually with no damaging effect on the stocks (Jiwani and Dhatemwa, 1972; Kudhongania, 1973).

It is difficult to obtain data for the present total catch of _Haplochromis_ from L. Victoria. However, the total _Haplochromis_ catch, in all bodies of water, in Kenya and Tanzania (two of the three countries sharing L. Victoria) was reported as 65,599 tonnes in 1979 (FAO, 1980), although possibly half of this total is taken from waters other than L. Victoria. No figures were given by FAO for _Haplochromis_ caught in the Ugandan waters, although the figures quoted for species of fish "not included elsewhere" was 11,500 tonnes. This gives an absolute maximum figure for the _Haplochromis_ catch in Uganda. It is apparent that the total catch of _Haplochromis_ from L. Victoria is far below the estimated maximum possible catch of 160,000 to 200,000 tonnes and that much of the possible increased catch could be taken in the Uganda waters which cover about 42% of the total lake area.

2.2 Haplochromis

2.2.1 General information and biology

Haplochromis is the generic name for a bony and scaled fish
of the family Cichlidae. The majority of adult individuals of
most species are small fishes ranging between 7 and 11 cm in total
length, but some piscivorous predators grow to lengths of 18-22 cm
(Greenwood, 1974). The genus is extremely large and varied and
the exact number of species is not known. In Lake Victoria alone
Greenwood (1974) estimates a total of 150-170 Haplochromis species.

The success of Haplochromis in Lake Victoria has been
attributed to rapid and successful adaptive radiation, a process
by which they have managed, with only small morphological changes
to colonise every available niche and utilise almost every available
source of food (Greenwood, 1951; Fryer and Iles, 1972). The
morphological changes are most apparent with respect to the
mouth features as can be seen in the fish shown in Figure 3.

The taxonomy of the group is very complicated as exemplified
by the numerous works of Greenwood (1956; 1957; 1959; 1960;
1962; 1965; 1967; 1974; Greenwood and Gee, 1969) on the subject.
The complication in taxonomy arises from the fact that one is
dealing with a "species flock"where there are many species involved,
and some species are so morphologically similar, that-they are
almost impossible to distinguish by a non-specialist (Gee and
Gilbert, 1968; Basasibwaki, 1974). Yet identification of fish
species is normally considered essential for biologists when
carrying out ecological and stock assessment studies. Identification
of species is normally also important to processing technologists

Figure 3 Haplochromis spp.

Note the different mouth features adapted for different diets
(from Greenwood, 1974).
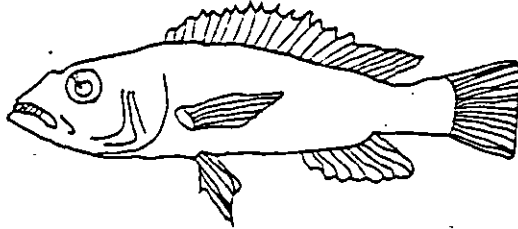
H. estor, piscivore (x ¼)
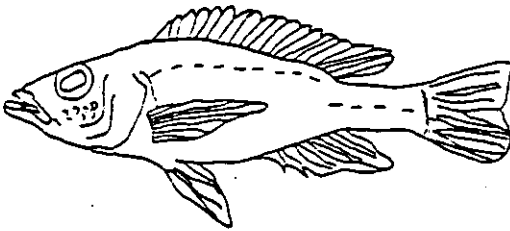
H. dolichorhyncus, benthic
crustacean eater (x ½)
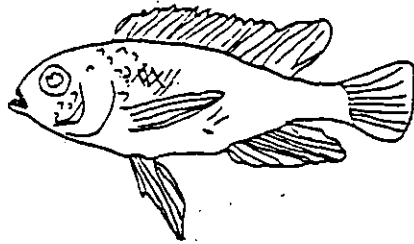
H. sauvagei, mollusc eater
(x ½)
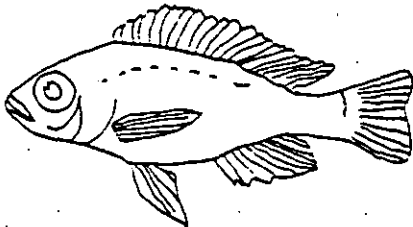
H. macrops, insectivore
(x ½)
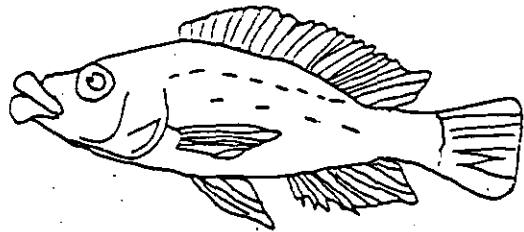
H. chilotes, specialised
insectivore (x ½)

since processing techniques depend on the chemical composition
of the fish which, in turn, depends on the species (Meinke, 1974;
Young and Romero, 1979). Simple approaches to get round the
problem of dealing with the species flock of Haplochromis have
been suggested. Basasibwaki (1974) suggests "lumping" rather than
"splitting" the genus into convenient groups. Such grouping, for
example, could be based on trophic groups. Parameters such as
body length, head length, length of the lower jaw, number of teeth,
etc., have been shown to be species specific and even trophic
group specific. Hence piscivores are elongate, standard length
in the range 9.2-23.2 cm, with large mouth (see Figure 3), while
paedophagus and phytophagus species tend to be smaller, ranges
6.3-17 cm and 5.8-13 cm respectively (Basasibwaki, 1974). Body
depth tends to be lowest in the piscivores. By using sizes to
group the fish, therefore, a practicable system of "classification"
of Haplochromis can be established.

There is very little information about the breeding habits
of Haplochromis. Greenwood (1974) suggests that "at least part
of a population is breeding at any one time." Probably the
majority of the species flock breed during the major rainy season
of the lake which occurs from May to July (Beadle, 1974).

2.2.2   Processing of Haplochromis

The most important method of processing Haplochromis in Uganda
is sundrying. This is applied to the smaller species (7-11 cm)
which form the bulk (approx. 70%) of the catches. The larger
species are sometimes consumed fresh, salted or smoked. Sundried
Haplochromis, however, has limited marketability. It is eaten

predominantly along the shores of Lake Victoria, and even in that
narrow belt of acceptance, various taboos and customary habits
limit its consumption. A marketing survey showed that the percent-
age demand for the fish along the lake shores was 5% compared with
55%, 25% and 15% for _Tilapia_ spp., _Bagrus docmac_ and _Clarias_ spp.
respectively (Jiwani and Dhatemwa, 1972).

The limited acceptance of sundried _Haplochromis_ in Uganda has
necessitated the development of other methods of processing the
fish (on an experimental basis) to render it more acceptable to a
larger part of the population.

Experimental canning of _Haplochromis_ was carried out by
Beatty (1964). The fish were canned in tomato puree and quality
evaluation of the resulting product showed that it was of inferior
quality compared with, for example, South African canned pilchards
(Cole, 1967). It was found, for example, that the _Haplochromis_
bones "although softened and crumby were slightly spiky in the
mouth". This was not the case with the pilchards. It was also
noticed that _Haplochromis_ flesh darkened markedly two hours after
exposure to air, probably due to oxidation, a phenomenon not observed
in canned S. African pilchards opened at the same time.

Production of fish powder for human consumption from _Haplochromis_
was investigated on a laboratory scale (Uganda Ministry of Animal
Industry and Fisheries, Fisheries Department, 1981). The fish were
beheaded, gutted, washed and smoked until brittle, after which they
were crushed in a hammer mill.. It was found necessary to remove
the heads and guts prior to smoking in order to prevent clogging
of the hammer mill sieve. The clogging was thought to be due to a
high lipid content in the heads and guts. However the high cost
involved in the manual removal of heads and guts made the price of
the product prohibitive.

Reduction of _Haplochromis_ to fish meal was considered by the

Uganda Government. However, the capital expense of such a venture
has until now prevented it going ahead (Uganda Ministry of Animal
Industry and Fisheries, Fisheries Department, 1981). Some of the
Haplochromis caught in the Tanzanian section of Lake Victoria is
reduced to fish meal at Manzini.

2.3 Chemical composition of fish

Analysis of the lipids, protein, minerals, vitamins and
other chemical constituents in fish provides information which is
valuable to processors and nutritionists when determining the
most effective ways of utilising a fish resource. In this section,
the analytical methods and chemical composition data are discussed
first, followed by a discussion of the relationship between
chemical composition and nutritive value and between chemical
composition and handling, processing and storage.

2.3.1 Analytical methods and chemical composition data

In general chemical analytical methods used for other foods
apply to fish except in a few instances, particularly with respect
to lipid analysis, where methods have been developed specifically
for fish.

2.3.1.1 Lipids

Estimation of the total lipid in fish tissue involves

three steps:-

1) preliminary treatment of the sample to
   render the lipid extractable,
2) extraction of the lipid with organic
   solvent systems,
3) estimation of the lipid dissolved in the
   solvent.

The accuracy of the results is mainly affected by the effectiveness
of the extraction of the lipid from the tissue.

Several methods have been used to determine total lipid in
fish (Ackman, 1980). The classical routine method involves
drying a weighed amount of fish sample overnight at $105^{o}C$, grinding
and extracting for 3 to 4 hours in a soxhlet apparatus with a low
boiling point petroleum ether or ether/petroleum ether mixture.
The lipid content is determined gravimetrically after evaporation
of the solvent. However, phospholipids, which total about 0.7% in
fish muscle, are only partially extracted by this method. This can
give considerable error when lean fish are analysed (Cutting, 1969).

Bligh and Dyer (1959) described a method whereby lipids
(including phospholipids) of biological materials can be extracted
and purified in a single operation. A modified method was
developed by Hanson and Olley (1963). The basis of the method is
the formation of a monophasic solution when the sample is
homogenised in a mixture of chloroform and methanol. The resulting
homogenate is diluted with water and/or chloroform to produce a
biphasic system where the chloroform contains the lipids and the
methanol-water layer the non-lipids. The lipid content is

determined gravimetrically after evaporation of an aliquot of the chloroform solution under reduced pressure.

Usher et al. (1973) described a rapid method for estimating total lipids in various foods, using the Foss-Let density apparatus. The technique is based on measurement of the density of a perchloroethylene extract of lipid using a magnetic float cell and a magnetic float.

Comparison of the Bligh and Dyer method with other lipid extraction/estimation methods showed that more lipid was extracted in the former than in the latter (Bligh and Dyer, 1959). Hla (1981) also found that more lipid was extracted from fish samples using a modified Bligh and Dyer method than both soxhlet and Foss-Let methods. An additional advantage of the Bligh and Dyer method is that the fish sample and lipid extract can be kept at room temperature or below. The method is therefore particularly suitable for extraction and estimation of fish lipids, which are characteristically highly unsaturated and therefore susceptible to autoxidation, unlike the soxhlet method, where the sample is normally subjected to high temperatures.

The Bligh and Dyer method is now generally the preferred method for fish total lipid determination particularly when the lipid is to be further analysed, for example for peroxide value or fatty acid profile (Torry Research Station, 1980).

The total lipid content of fish varies considerably, not only from one species to another, but also, particularly with fatty fish, from one individual of the same species to another. Typically for lean fish, such as cod, plaice, Tilapia, the lipid content of the flesh is around 1%, whereas for fatty fish, such as mackerel, herring, the lipid content can vary between about 2 and 20%.

This variation is caused by factors such as the season of the year when the fish are caught, the fishing ground, the age, sex, size and nutritional status of the fish (Stansby and Olcott, 1963; Jangaard et al. 1967; Love, 1970; Hardy and Keay, 1972; De Silva and Rangoda, 1979). It has been observed that in all species of fish fat content can vary much more widely than the water, protein or mineral content (Stansby and Olcott, 1963).

Few studies have been carried out in total lipid distribution in whole fish. However available data, such as that given for mackerel and capelin in Table 2, show that lipids are not evenly distributed within the fish (Ackman, 1980).

Fish lipids are complex mixtures of triglycerides, phospholipids, diacyl glyceryl ethers, hydrocarbons, sterols, carotenoids, vitamins and wax esters (Malins, 1967). If the fish lipids are to be refined to give edible oil then it is useful to know the different classes of lipid present. Various techniques have been adopted to fractionate fish lipids, including thin layer chromatography (TLC) (Amenta, 1964; Freeman and West, 1966) and column chromatography (McCarthy and Duthie, 1962; Hardy et al. 1971).

The total lipid or the lipid fractions (triglycerides, phospholipids, etc.) can be analysed for the fatty acid chains present by trans-esterification to give methyl esters, which are then separated by gas-liquid chromatography (GLC) (Stoffel et al. 1959; Luddy, et al. 1960). Ackman, (1974, 1980) gives fatty acid profile data for several species of fish. Palmitic acid (16:0) is the principal saturated fatty acid in most species of fish, accounting for 10-30% of the total fatty acids, with some degree of interchangeability with myristic acid (14:0), which may account

Table 2 Total lipid distribution in various parts of spring
         mackerel and summer capelin[1]

	Composition in relation to anatomical location	
	portion analysed	% of total lipid
mackerel	head	18
(Scomber	backbone	6.5
scombrus)	intestine	2.5
	liver	2.4
	gonads	0.5
	light muscle	19
	dark muscle	5
	belly flap	6
	skin	44
capelin	head	5.2
(Mallotus	backbone	6.2
villosus)	intestine	8.2
	liver	1.8
	gonads	0.5
	light muscle	5
	dark muscle	5
	belly flap	43
	skin	26

(1) Ackman (1980).

for 5-10% of the total. Stearic acid (18:0) usually accounts
for no more than 1-3% of the total fatty acids, while the 20:0
22:0 and 24:0 acids are barely detectable at levels of 0.01-0.1%.
The four common monoethylenic fatty acids are 16:1, 18:1, 20:1
and 22:1. They may exist in different isomeric forms although
the 9, 10 cis double bond isomers, which account for 10-30% of
the total are the most common. The polyethylenic fatty acids
give marine lipids their distinctive character, in particular
C20:4, C20:5, C22:5 and C22:6.

Most published work on lipid content and fatty acid composition
in fish has been done on marine species. Information regarding
freshwater species is scarce and there is a need for further work
to be carried out in this area (Stansby, 1967; Kinsella et al.
1977).

2.3.1.2   Protein

The protein content of fish is normally determined by a
standard Kjeldahl procedure (Cutting, 1969; Association of Official
Analytical Chemists, 1970; Commission of European Communities,
EEC, 1979). For most routine analyses protein content is quoted
as "crude protein" which is derived by multiplying the total
nitrogen by an empirical factor of 6.25. Cutting (1969) points
out that the conventional factor of 6.25 may be too high for fish
since "total nitrogen" includes a considerable amount of non-
protein nitrogen. A factor of 6.1 is considered to be more realistic,
but is hardly ever used.

"True protein" can be isolated by precipitation with, for
example, trichloroacetic acid; the trichloroacetic acid-insoluble
matter representing "true protein" (Cutting, 1969). The difference

between the crude protein nitrogen and the true protein nitrogen
is the non-protein nitrogen.

Protein content may vary from one part of a fillet to another
within the same fish. Hence Cutting (1969) recommends that for
accurate determination of protein content in fish flesh sampling
should be done from a particular part of the fish. In practice
this is only necessary if very accurate protein figures are
required, for example, when determining seasonal variation in
protein content (Love, 1970).

Crude protein in fish flesh is reported to range from 13.1 to
18.5% of the flesh muscle weight, with clupeids and elasmobranchs
having the lower values (Dyer and Dingle, 1961). Stansby and Olcott
(1963), on the other hand, quote far wider ranging figures of
crude protein content in fish flesh, from 6 to 28%, with the
normal range being 18 to 20%. The percentage crude protein in
whole fish is in general slightly lower (1-2%) than that of the
fish flesh (Murray and Burt, 1969; Windsor and Barlow, 1981).

Non-protein nitrogen accounts for from 9.2 to 18.3% of the
total nitrogen of the flesh of teleost fishes and from 33 to 38.6%
in elasmobranch fishes (Simidu, 1961). The true protein content
is lower than the crude protein content by a corresponding
percentage. The main components of the non-protein nitrogen
fraction in fish are: trimethylamine oxide (in marine fish), urea
(in elasmobranch fish), creatine, carnosine, anserine, free
amino acids and nucleotides and derivatives (Shewan, 1951; Ikeda,
1980). They are important in the spoilage of fish (Shewan, 1951)
and breakdown products (trimethylamine, ammonia, hypoxanthine)
are often used in assessing the freshness of fish (Connell, 1980).

Evaluation of the nutritional quality of a given protein requires a knowledge of the amino acid profile in order to assess the balance of the essential amino acids.

Amino acid determination involves three steps:-

1) hydrolysis of the protein, normally using hydrochloric acid,

2) separation of the amino acids in the hydrolysate,

3) estimation of the individual amino acids.

The latter two steps are usually done by an automatic amino acid analyser. The acid hydrolysis step causes destruction of some of the amino acids such as cystine and tryptophan, therefore different procedures have to be used for estimation of these amino acids. Cystine may be determined by a method described by Schram et al. (1954) and Moore (1963), while tryptophan may be determined after alkaline hydrolysis or by the method of Miller (1967). Table 3 gives the essential amino acid content in the flesh proteins of some freshwater and marine fish; nutritional aspects are discussed below (see Section 2.3.2.2).

2.3.1.3 Minerals

Ash determinations are used to give a rough estimate of total mineral content of fish and standard analytical methods are used for determining the various minerals present. Table 4 gives the ash and mineral content of some fish and fish products. Nutritional

Table 3 Essential amino acid content of the flesh of some species of fish and FAO/WHO suggested pattern of amino acid requirements in man

Essential amino acid (g/100g protein)	Freshwater species [1]					Marine Species [1]			FAO/WHO suggested pattern [6] of requirements (g/100g protein)		
	Tilapia[2]	White sucker[3]	Burbot[3]	Black crappie[3]	Rainbow trout[3]	Cod[4]	Herring[5]	Mackerel[5]	Infant (0-2yrs)	Child (10-12yrs)	Adult
Histidine	1.8	2.2	2.5	1.8	1.2	3.5	2.4	3.8	1.4	0	0
Isoleucine	} 12.4 {	3.7	4.3	3.4	3.3	4.9	4.9	5.2	3.5	3.7	1.8
Leucine		8.0	8.5	6.9	6.6	9.3	7.1	7.2	8.0	5.6	2.5
Lysine	10.6	8.5	8.3	6.9	6.6	10.3	7.8	8.1	5.2	7.5	2.2
Methionine[7]	1.1	2.7	3.2	2.3	2.4	2.1	2.7	2.7	2.9	3.4	2.4
Phenylalanine[8]	3.3	4.0	4.2	3.7	3.3	4.7	3.4	3.5	6.3	3.4	2.5
Threonine	3.9	3.9	4.1	8.3	3.2	5.3	4.4	4.9	4.4	4.4	1.3
Tryptophan	-	1.0	1.0	1.0	1.0	1.3	0.8	1.0	0.9	0.5	0.7
Valine	5.4	4.9	5.1	3.9	4.1	5.8	5.0	5.4	4.7	4.1	1.8
Protein g/100g flesh	18.7	15.8	17.4	18.0	18.3	14.6	18.1	19.0	-	-	-

(1) Tilapia = Tilapia nilotica,

White sucker = Catostomus commersoni,

Burbot = Lota lota,

Black crappie = Pomoxis nigromaculatus,

Rainbow trout = Salmo gairdneri,

Cod = Gadus morhua,

Herring = Clupea harengus,

Mackerel = Scomber scombrus.

(2) Khalil et al. (1980).

(3) Mai et al. (1980).

(4) Geiger and Borgstrom (1962).

(5) Jacquot (1961).

(6) FAO/WHO (1973).

(7) The FAO/WHO figures are for total sulphur amino acids (methionine and cystine). The cystine content of fish flesh is normally about 1g/100g protein.

(8) The FAO/WHO figures are for total phenylalanine and tyrosine. The tyrosine content of fish flesh is normally about 3g/100g of protein.

Table 4 <u>Ash and mineral content of some fish and fish products and mineral requirements of man</u>

Species	Portion analysed	% ash	Na	K	Ca	Mg	mg per 100g P	Fe	Cu	Zn	S	Cl	I
Cod[1]	fillet	1.2	77	320	16	23	170	0.3	0.06	0.4	200	110	0.15
Herring[1]	fillet	1	67	340	33	29	210	0.8	0.12	0.5	190	76	0.05
Salmon[1]	fillet	1	98	310	27	26	280	0.7	0.2	0.8	170	59	-
Portuguese sardines[2]	whole fish (canned)	-	-	-	580	-	624	-	-	-	-	-	-
Various West African species[2]	whole fish	-	-	-	888	-	350	-	-	-	-	-	-
White fish meal[3]	-	20.0	-	900	8000	-	4800	30	0.7	1.0	-	-	-
Peruvian anchovy meal[3]	-	15.4	870	650	3950	250	2600	24.6	1.1	11	-	-	-
Mineral requirements (mg/day)[4]													
Male adults recommended daily intake			2,300 -6,900	800	1,950 -5,850	300	800	18	-	-	-	-	0.15-0.3

(1) Paul and Southgate (1978). (2) Causeret (1962). (3) Windsor and Barlow (1981). (4) Nutrition Search Inc. (1979).

aspects of the data are discussed below (see Section 2.3.2.3).

2.3.1.4 Vitamins

The water-soluble vitamins in fish are normally determined by standard methods (Association of Vitamin Chemists, 1966).

The fat-soluble vitamins particularly A and D, tend to receive more attention than the water-soluble ones due to the fact that they occur in relatively higher amounts in fish than in most other foodstuffs. A number of methods for determining the two fat-soluble vitamins have been described including spectro-photometry, colorimetry and chromatography (Association of Vitamin Chemists, 1966; Bell, 1971; Kamangar and Fawzi, 1978). The Carr-Price method, in which a blue colour is formed with antimony trichloride and subsequently measured using the U.V. absorbance, is widely used to determine vitamin A.

A complicating factor in the determination of vitamin A in fish, however, is the presence of different forms of the vitamin (Higashi, 1961; Jacquot, 1961), namely vitamin A_1 and vitamin A_2 (see Figure 4). While it was first thought that vitamin A_1 was specific to marine fish and A_2 typical of freshwater fish, subsequent studies have revealed exceptions. For example, in the liver oil of Indian freshwater fish it was found that vitamin A_2 was predominant in carnivorous fish and vitamin A_1 in herbivorous fish. It has also been suggested that in general fish living in the sea throughout their life contain only A_1, those living entirely in freshwater have only A_2, while those which live part of their life in the sea and part in freshwater contain both A_1 and A_2 (Higashi, 1961).

Figure 4   <u>The two different forms of Vitamin A</u>
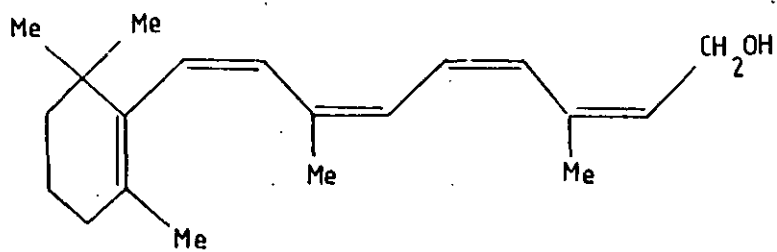
Vitamin A$_1$
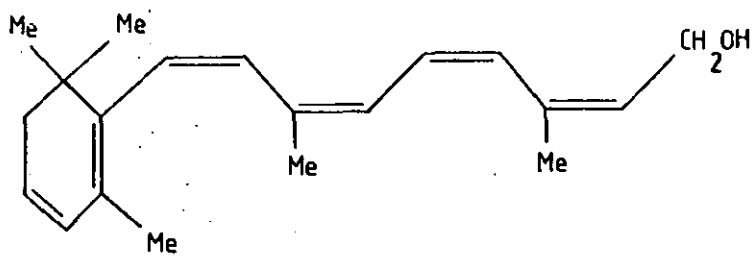
Vitamin A$_2$

The concentrations of both water-soluble and fat-soluble
vitamins in fish varies with species, age, season and fishing
ground (Higashi, 1961; Cutting, 1969). The concentrations of
vitamins im the internal organs, particularly the liver, are
normally higher than in the muscle (Ikeda, 1980) (see Table 5).
In certain species of fish, for example, up to 99.95% of the
total vitamin A is found in the liver (Cutting, 1969). Nutritional
aspects of the data in Table 5 are discussed below (see Section
2.3.2.4).

2.3.1.5 Other components

Proximate analysis data includes, in addition to total lipid,
crude protein and ash, also carbohydrate and moisture. Carbohydrate
in foodstuffs is determined either by difference or by specific
methods for glycogen, glucose, etc. Data for fish show that
carbohydrate is a minor component present in amounts normally less
than 0.5%. Since it occurs in such small amounts in fish, it is
not considered to be nutritionally significant, and is generally
not included in composition tables.

Moisture is the main constituent of fish flesh and a number
of methods are available for its determination. The preferred
methods, such as that recommended by the Commission of European
Communities, EEC (1979), involve mixing a fish sample (5-10g) with
a known weight of dry sand and ethanol to give a homogeneous
paste, which is dried in an air oven at $103 \pm 2^{o}C$ to constant weight.
The usual range of moisture contents in fish flesh and whole fish
is 60-80% (Murray and Burt, 1969; Windsor and Barlow, 1981).
Moisture determinations are often performed to check other proximate
analysis data for fish samples since in general the sum of total

Table 5 <u>Vitamin content in some fish and fish products and recommended daily intake for man</u>

Species	Portion analysed	Vitamin content per 100g											
		Vit. A µg (retinol)	Vit. D µg	Vit. E mg	Thiamine mg	Ribof-lavin mg	Nicotinic acid mg	B_6 mg	B_{12} µg	Pantothe-nic acid mg	Biotin µg	Folic acid µg	Vit. C
Cod[1]	fillet	Tr	Tr	0.44	0.08	0.07	1.7	0.33	2.0	0.2	0.3	12	Tr
Herring[1]	fillet	45	22.5	0.3	Tr	0.18	4.1	0.45	6	1.0	10	5.0	Tr
Salmon[1]	fillet	Tr	Tr	-	0.2	0.15	7.0	0.75	5.0	2.0	5.0	2.6	Tr
White fish meal[2]	-	-	-	-	-	0.65	5.0	-	7	1.5	30	50	-
Peruvian anchovy meal[2]	-	-	-	-	-	0.66	9.5	-	18	0.9	8	16	-
Cod liver[3]	-	3,600-180,000	30	-	-	0.34	-	-	11	0.43	-	-	-
Cod roe[3]	-	-	-	-	0.25	0.55	-	-	15	1.97	17	-	-
Recommended daily intake for man													
Male moderately active[4]		750	2.5	-	1.2	1.7	18	-	-	-	-	-	30

(1) Paul and Southgate (1978) (2) Windsor and Barlow (1981)   (3) Murray and Burt (1969)

(4) Ministry of Agriculture, Fisheries and Food (1976).

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lipid, crude protein, ash and moisture is within 1 or 2% of 100%.

2.3.2   Chemical composition in relation to nutritive value

2.3.2.1 Lipids

Nutritionally lipids serve a number of functions.   They
provide energy and they are a source of the essential fatty acids.
In addition they have a cholesterol-lowering effect in man and
laboratory animals (hypocholesterolenic agents), are a source of
vitamins A, D and E (see Section 2.3.2.4) and provide a medium
for their absorption.

Lipids are a good source of energy providing approximately
9.0 kcal/g compared with 3.75 kcal/g and 4.0 kcal/g from
carbohydrate and protein respectively (Ministry of Agriculture,
Fisheries and Food, 1976). Although the major source of energy
in the flesh of a typical lean fish (1% lipids, 17% protein) is
protein (9 kcal/100g from lipids, 68 kcal/100g from protein) in
a typical fatty fish (10% lipids, 17% protein) the lipid fraction
is the major source of energy (90 kcal/100g from lipids, 68 kcal/100g
from protein).

Linoleic (18:2ω6) and arachidonic (20:4ω6) are considered
"essential fatty acids" (EFA) because they can prevent or cure
the symptoms giving rise to the EFA syndrome. They restore growth,
prevent damage to kidney and reproductive tissues and "cure the
characteristic dermal symptoms in EFA deficient animals"   (Aaes-
Jørgensen, 1967). It has been estimated that 1-2 per cent of the
energy intake should be in the form of these essential fatty acids
(Ministry of Agriculture, Fisheries and Food, 1976). Assessment,

therefore, of the nutritional value of a sample of fish lipids requires fatty acid profile data, particularly with respect to linoleic and arachidonic acids.

Oil from many species of fish has been shown to have a cholesterol-lowering effect in laboratory animals and man, a factor that could reduce coronary heart disease. The hypocholester olenic effect is attributed to the presence of a large proportion of polyunsaturated fatty acids in fish lipids (Peifer, 1967; Carpenter, 1980).

There are a few instances where ingestion of fish lipids has resulted in undesirable effects in animals. The "castor oil" fish, Ruvettus pretiosus, for example, contains wax esters which are responsible for a purgative effect (Ackman, 1980).

Fish lipids are very susceptible to autoxidation, due to their high degree of unsaturation, resulting in formation of peroxides which may be toxic (Toyama and Kaneda, 1962). The presence of high quantities of polyunsaturates in the diet has been reported to cause growth retardation under certain conditions (Stansby, 1967, and references therein).

2.3.2.2 Protein

Evaluation of the nutritive value of fish protein is complicated by the presence in fish flesh of a significant proportion of non-protein nitrogen including free amino acids (see Section 2.3.1.2). Hence results of nutritive evaluation experiments must be treated with some reservation if related to crude protein content. However, fish flesh clearly rates as a good source of high quality protein (Guha, 1961; Ministry of Agriculture, Fisheries and Food, 1976).

Growth experiments carried out on rats to assess the nutritive value of fish showed that fish protein had greater nutritive value than casein. In one of the experiments, for example, the body weight of 22 rats fed on purified cod protein increased 121% during a 59-day period, while that of a group fed on casein increased 103% (Geiger and Borgstrom, 1962). In feeding trials with chicks, also, high quality fish meal was found to be capable of supporting normal chick growth entirely by itself (Geiger and Borgstrom, 1962).

Table 3 shows the essential amino acid profiles of protein from a number of fish and the FAO/WHO suggested pattern of amino acid requirements for man. It can be seen that for most species of fish given in Table 3 the fish protein provides all the essential amino acids in sufficient quantities. It is significant that there are high amounts of lysine and appreciable amounts of methionine, two of the amino acids deficient in cereals (Combs, 1961; Ministry of Agriculture, Fisheries and Food, 1976). Fish meal is said to be the "richest natural source of lysine and methionine practically available to feed mixers" and purchasers of fish meal need to know the content of lysine and methionine in the protein in order to estimate its quality (Windsor and Barlow, 1981).

Assessment of the nutritive value of a fish species should include determination of true protein, in addition to crude protein, and determination of the amino acid content, particularly lysine and methionine. In the preparation of fish flour, and other protein-rich fishery products, knowledge of proximate composition is required to determine whether the yield of protein will be sufficient to make the operation economically viable (Stansby, 1961). In this regard the true protein content and amino acid profile of different parts of the fish may prove valuable in determining the economic viability of, for example, beheading the fish before processing.

2.3.2.3 Minerals

Fish flesh contains all of the nutritionally essential
elements, though most of them in trace amounts (see Table 4).
Fish is most notable as a source of iodine, calcium and
phosphorus (Ministry of Agriculture, Fisheries and Food, 1976).
The calcium and phosphorus are found mainly in the bones and
scales of the fish and hence whole fish and fish meal are
particularly good sources of these minerals.

In assessing the contribution that a fish species can make
to the mineral requirements of a diet, the ash content can
provide a rough guide, with further information being obtained
from specific analyses, for calcium, iodine, etc.

2.3.2.4 Vitamins

As a vitamin source fish is most notable for the fat-soluble
vitamins A, D and E which predominantly occur in fish liver oil,
and the flesh of fatty fish, such as mackerel. For decades fish
liver oil has been known to be a valuable source of the vitamins
A, D and E (Ackman, 1974). Vitamins A and D are most important
in this respect and many contractors for fish liver oils specify
a minimum quantity of these two vitamins (Windsor and Barlow, 1981).

As can be seen from Table 5, with the exception of vitamin C,
fish can make a significant contribution to the total vitamin
intake of man, although this depends very much on species and on
the part of the fish that is eaten.

2.3.3   Chemical composition in relation to handling, processing and storage

A knowledge of the chemical composition of the raw material plays an important role in determining the most effective methods of processing and storage of fish (Stansby, 1961). Of particular importance is the lipid content and its distribution in the fish.

Fish meal manufacturers need to know the total lipid content of the raw material and its seasonal variation, since the oil produced as a by-product is a source of income and also the fish meal is standardised to close tolerances in lipid content (Stansby, 1961; Windsor and Barlow, 1981). Knowledge of total lipid content may also be of value in deciding the type of machinery to be installed. A fish meal manufacturer, for instance, using fish with a high lipid content (greater than 15%) as the raw material needs centrifuges to separate the oil (to meet the lipid content limits in the meal and also to recover the oil to maximise profits) whereas one using raw material with low lipid content (less than 5%) may not need centrifuges (Windsor and Barlow, 1981).

The effectiveness of dehydrating preservative processes such as sundrying, salting and smoking depends to some extent, on the chemical composition of the fish. Sundrying of fish is practised very widely yet it has been noted that fatty fish dry more slowly than lean fish and often may not give satisfactory products (Wood, 1981). Fatty fish should be brined or pickled rather than dry salted in order to retard rancidity development (Burgess et al., 1965), and in general it is observed that cured fish products with a low lipid content have a longer shelf life than those with a high lipid content due to a slower rate of oxidative rancidity development.

Lipid oxidation also takes place in fish kept frozen for long periods of time. In general, fatty fish flesh develops rancid flavours rapidly during frozen storage (Labuza, 1971; Hardy, 1980). The fillets of lean fish such as cod, on the other hand, can be stored for much longer periods, before they develop off-flavours and off-odours. These "cold-storage flavours" and "cold-storage odours" are caused by products, such as cis-4-heptenal, from the oxidation of structural phospholipids predominant in lean fish lipids (McGill et al., 1974; Hardy, 1980).

The amounts and distribution of the nutritionally important components of fish (protein, minerals and vitamins) can affect the processing of the fish in that they may determine the commercial viability of a process, e.g. protein with respect to fish flour production (see Section 2.3.2.2) or vitamin A content with respect to the processing of viscera.

2.3.4 Chemical composition of Haplochromis species

Information on the chemical composition of Haplochromis is very scanty. To the author's knowledge there is no published systematic data on the subject. Table 6, however, summarises the small amount of information available, which refers to deboned, pressed dried and ground Haplochromis or to mixtures of Haplochromis and Lethrinops. No information is given as to the species of Haplochromis concerned, or their sizes, or the time of the year they were caught. The amino acid profile shows a good balance of essential amino acids when compared with the FAO/WHO figures given in Table 3.

Table 6   Chemical composition data for Haplochromis

	%[1]	%[2]	%[3]
Protein (Nx6.25)	65.8	-	16.0
Moisture	11.6	-	69.9
Total lipid	9.6	8.7 wet basis	8.0
		28.5 dry basis	
Ash	12.0	-	-

Amino acid	g/100g protein[1]
Isoleucine	5.0
Leucine	7.5
Lysine	7.9
Available lysine	7.8
Methionine	3.2
Cystine	1.1
Phenylalanine	5.1
Tyrosine	3.7
Threonine	4.6
Tryptophan	1.1
Valine	5.6
Arginine	6.2
Histidine	2.3
Alanine	7.2
Aspartic acid	9.9
Glutamic acid	14.3
Glycine	8.8
Proline	5.5
Serine	4.8

(1)   Freshwater Fisheries Institute, Nyegezi, Tanzania (1975),
      for deboned, pressed, dried and ground Haplochromis from
      Lake Victoria.

(2)   Disney (1974) for mixture of Haplochromis and Lethrinops
      from Lake Malawi.

(3)   Meynell (1979) for mixture of Haplochromis and Lethrinops
      from Lake Malawi.

2.4 Aims of the present work

There is need to improve the present processing methods and to develop new processing methods to render Haplochromis species from Lake Victoria more acceptable as food to a larger part of the population of Uganda (and Kenya and Tanzania).

Information about the chemical composition of Haplochromis, including distribution of components, and the variation of chemical composition with fish size and with season is important for determining the most effective ways of processing the fish. The information would also be of value to nutritionists.

This study was concerned with the following aspects of composition:

A. Establishing whether there is a significant variation in the chemical composition of Haplochromis within the species flock, or whether the genus can be treated as a single species as far as processing and nutritional value are concerned.

B. Providing information about the seasonal variation in the composition of the fish, particularly with reference to total lipid content.

C. Providing quantitative and qualitative information about some of the chemical components that are of importance with respect to processing, storage and nutritive-value, i.e. lipids, protein, ash, vitamin A and moisture.

3.   EXPERIMENTAL

3.1 Materials

Four batches of <u>Haplochromis</u> were caught by bottom-trawling
(depth 25-45 metres) from the same area of Kitubulu-Nsazi, an
inshore fishing ground of Lake Victoria (see Figure 2), during
May, 1980; October, 1980; November, 1980 and February 1981
(hereafter referred to as MAY 80, OCT 80, NOV 80 and FEB 81 batches).
The fish were frozen, packed in insulated boxes and flown to the
U.K. They were kept in cold store ($-25^{\circ}C$) until analysed. Fish
in batch MAY 80 were analysed only for the total lipid content
in the whole fish, whereas fish in batches OCT 80, NOV 80 and
FEB 81 were more fully analysed after splitting into weight groups.

3.2 Methods

3.2.1 Species identification by gel electrophoresis

Fourteen <u>Haplochromis</u> from batch MAY 80 were selected on the
basis of outer appearance and size. They were grouped as: "light",
"dark", "lined" or "spotted"; "small", "medium" or "large". Sarco-
plasmic proteins from each fish were extracted and separated by
gel electrophoresis according to Torry Method 17 (Torry Research
Station, 1980) using a Bio-Rad Laboratories Gel Electrophoresis
Apparatus (Model 500).

3.2.2   Grouping of Haplochromis

Thawed fish were individually weighed, to the nearest 0.1g, and measurements of total length and maximum depth taken to the nearest mm. The fish were sorted into four weight groups viz. I: 5.9g and less, II: 6.0-8.9g, III: 9.0-13.9g and IV: 14g and over.

3.2.3   Portioning of fish

Fish were portioned into either, a) head, gut (used throughout this report to mean viscera) and headless, gutless portions (see Figure 5), or b) head, gut, skinless fillets and residue. Any visceral material cut off with head portion was removed from the head portion and included with the gut portion.

3.2.4   Lipids

3.2.4.1   Total lipid

Total lipid was determined according to a modified Bligh and Dyer method. Approximately 10g of the sample was accurately weighed into a 200 cm^3 homogenising flask. The flask was held in ice water. 8 cm^3 of water, 40 cm^3 methanol and 20 cm^3 of chloroform were added and the mixture homogenised for 1 minute at constant speed using an Ultra-Turrax homogeniser. A further 20 cm^3 of chloroform was added by pipette and the mixture homogenised for 30 seconds. Finally 20 cm^3 of water was added by pipette and the mixture homogenised for a further 30 seconds. The homogenate was

Figure 5 <u>Haplochromis specimen showing the portions analysed and</u>
<u>defining measurements taken</u>
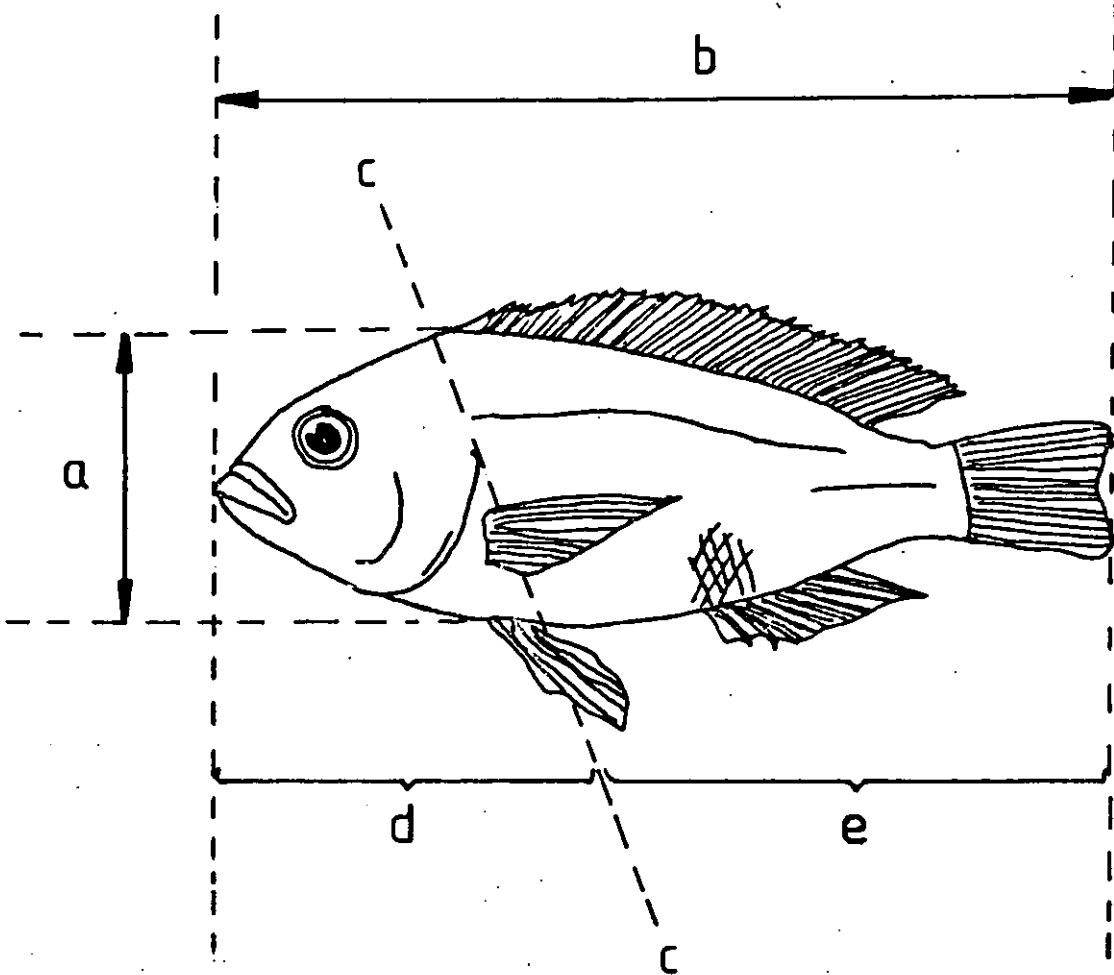
a   =   maximum depth

b   =   total length

C--C   =   line of cut

d   =   head portion (after removal of
        visceral material)

e   =   headless, gutless portion (after
        removal of visceralmaterial)

transferred to capped centrifuge tubes and centrifuged at 2000 r.p.m. for 20 minutes after which 20 cm^3 of the chloroform layer was pipetted into a tared beaker and the solvent evaporated off over a steam bath and finally in an oven at 105°C for 15 minutes. After cooling in a desiccator the beaker was weighed and the lipid content determined by difference.

3.2.4.2 Fatty acid profiles

The lipid was extracted according to the method outlined in 3.2.4.1, however 0.01% of butylated hydroxytoluene (BHT) was added prior to homogenising and the solvent was evaporated off using a rotary evaporator and vacuum pump at room temperature.

Methylation: 100-150 mg of lipid was saponified by adding 2 cm^3 toluene and 4 cm^3 of sodium hydroxide in methanol (1.5:228 w/v) in a 50 cm^3 round bottomed flask and refluxing for 30 minutes. After cooling, 5 cm^3 boron trifluoride-methanol was added and the mixture was refluxed for a further 30 minutes. The methyl esters were extracted three times with 35 cm^3 portions of n-hexane and dried with anhydrous sodium sulphate prior to injecting on to the gas chromatography column.

Gas chromatography: The content and composition of fatty acid methyl esters were analysed by a Perkin Elmer F11 (flame ionisation detector) gas chromatography apparatus fitted with a 2 metre column packed with 10% SP 2330 on Chromosorb W.AW 80-100 mesh. The temperatures of the injection part and detector were 150 and 210°C, respectively. Hydrogen, air and nitrogen flow rates were 20, 25 and 42 (at 3.5 bar) cm^3/min respectively. The fatty acid methyl ester peaks were identified by comparison with two standards: "PUFA I" supplied by Chromatography Services, Carr Lane

Industrial Estate, Hoylake, Wirral and GLC Reference Mixture No. 3 (AOCS), Ref. No. 32246, supplied by Chrompack U.K. Ltd., Quantification was by cutting out peaks and weighing them.

3.2.5   Protein

3.2.5.1   Crude protein

Total nitrogen was determined by the Commission of European Communities, EEC (1979) recommended method ISOR 937-1969, a standard macro-Kjeldahl procedure, and crude protein was calculated using a factor of 6.25.

3.2.5.2   True protein and non-protein nitrogen

True protein were determined by the method outlined by Cutting (1969). Approximately 2g of the sample was accurately weighed and ground with a pestle and mortar in 10 cm^3 of 15% trichloroacetic acid (TCA). The TCA soluble material was recovered by filtration and the solid residue was washed with four 10 cm^3 aliquots of 15% TCA. The total nitrogen content (true protein nitrogen) of the solid residue was determined by the standard method given above and non-protein nitrogen was calculated by difference between crude protein nitrogen and true protein nitrogen. True protein was calculated using a factor of 6.25.

3.2.5.3 Amino acid profiles

The fish samples (whole fish or different portions) were minced using a hand mincer and representative samples were hydrolysed by the standard 6M hydrochloric acid method. The amino acids were separated and the amounts determined using an LKB amino acid analyser with norleucine as an internal standard.

3.2.6 Ash and calcium

Ash was determined according to the Association of Official Analytical Chemists (1970) method 3.1.013.

The ash was dissolved in conc. HCl : H_2O : conc HNO_3 (3:1:3) and calcium was determined using an EEL Atomic Absorption Spectrophotometer with a calcium hollow cathode lamp, wavelength 422.7 nm, slit width 0.06 mm and operating current 3mA, acetylene pressure 8 lb/in^2 and air pressure 10 lb/in^2.

3.2.7 Vitamin A

Vitamin A was determined by the Carr-Price blue colour colorimetric method (Association of Vitamin Chemists, 1966).

3.2.8 Moisture

Moisture was determined according to the Commission of European Communities, EEC (1979) recommended method ISO R.1442-1973.

4.   RESULTS AND DISCUSSION

4.1 Species identification by gel electrophoresis

     In order for the chemical analysis results to have general
applicability for the Haplochromis species flock from Lake Victoria
it was necessary to confirm that the batches of fish received
from the particular fishing ground (Kitubulu-Nsazi) did indeed
contain a large number of species and did not atypically
contain only one or a few species. There are over 150 species of
Haplochromis in Lake Victoria and their morphological resemblance
makes identification using the classical taxonomic techniques
very difficult (see Section 2.2.1). Gel electrophoresis has been
found to be an effective technique for identifying fish species
(Mackie, 1980, and references therein). Gel electrophoresis was
therefore used to check that the batches of Haplochromis received
did in fact contain a mixture of many different species.

     The sarcoplasmic proteins from 14 Haplochromis selected
according to appearance and size from batch MAY 80 were subjected
to electrophoresis and 8 distinctly different patterns of protein
bands were obtained indicating at least 8 different species.
N'dene (1981) working in the same laboratory and using the same
procedure carried out gel electrophoresis on the sarcoplasmic
proteins from 17 Haplochromis from batch OCT 80 and 14 distinctly
different patterns of protein bands were obtained indicating at
least 14 different species. There was no clear correlation
between appearance and size of fish and the pattern of protein
bands indicating that these factors are of little value in
identifying the Haplochromis fish species.

     Although gel electrophoresis was carried out on a relatively
small number of Haplochromis in only two batches, these results

indicate that there were a very large number of species in the batches, although it was not possible to make any accurate estimate of how many.

4.2 Grouping of Haplochromis

Most chemical analyses of fish have been carried out on single species, which represent clearly defined biological material. Processing is also normally carried out on batches of single species. Haplochromis catches, however, comprise a mixture of species, as shown in this investigation by gel electrophoresis, and it is not practical, for analysis or processing purposes, to work with individual species. However, grouping according to a size parameter may be very useful in processing, particularly if there is significant variation in composition with size.

Length was used as a basis for grouping mixed species of Haplochromis for canning by Beatty (1964). During the experimental canning of Haplochromis it was found that in a haul of Haplochromis species 84% of the trawl catch was suitable for canning (10 to 20 cm in total length) whereas 2% and 12% were too small and too large respectively to be used for canning.

In the study reported here, three parameters of size, viz. weight, length and maximum depth, were measured to establish a basis of grouping Haplochromis for analytical and processing purposes.

4.2.1   Relationship between weight, length and maximum depth

Figure 6 shows the relationship between weight and length (see Figure 5 for definition of length) of 90 Haplochromis, 30 taken randomly from each of the batches OCT 80, NOV 80 and FEB 81.   Although the points plotted represent many different species of Haplochromis, the curve obtained is very similar to that observed for single species such as cod (Waterman, 1968), herring (Stroud, 1972) and haddock (Waterman, 1975).   There was no marked difference in the relationship between weight and length of fish caught during the different months.

Figure 7 shows the relationship between weight and maximum depth (see Figure 5 for definition of maximum depth) for 90 Haplochromis, 30 randomly selected from each of the batches OCT 80, NOV 80 and FEB 81.   It can be seen that a number of fish with the same maximum depth may vary greatly in weight.   Basasibwaki (1974), when discussing simple practical methods of grouping Haplochromis, noted that predators were larger, standard length ranging from 9.2-23.2 cm, but they had the lowest depth.   Therefore, for practical purposes, maximum depth may not be a satisfactory parameter for grouping the fish since relatively large-sized piscivorous predators would be grouped together with small non-predatory fish.

In the present study, weight was chosen as the grouping parameter since it could be measured more quickly than length and since it gave more satisfactory grouping than maximum depth.

Most mechanical size graders select fish on the basis of their thickness (Hewitt, 1980).   Measurement of thickness of Haplochromis was not considered in this study because the samples used had been frozen and cold-stored, resulting in loss of firmness of the fish.   It would therefore not have been possible to get

Figure 6 Relationship between weight and length for Haplochromis
batches OCT 80, NOV 80 and FEB 81
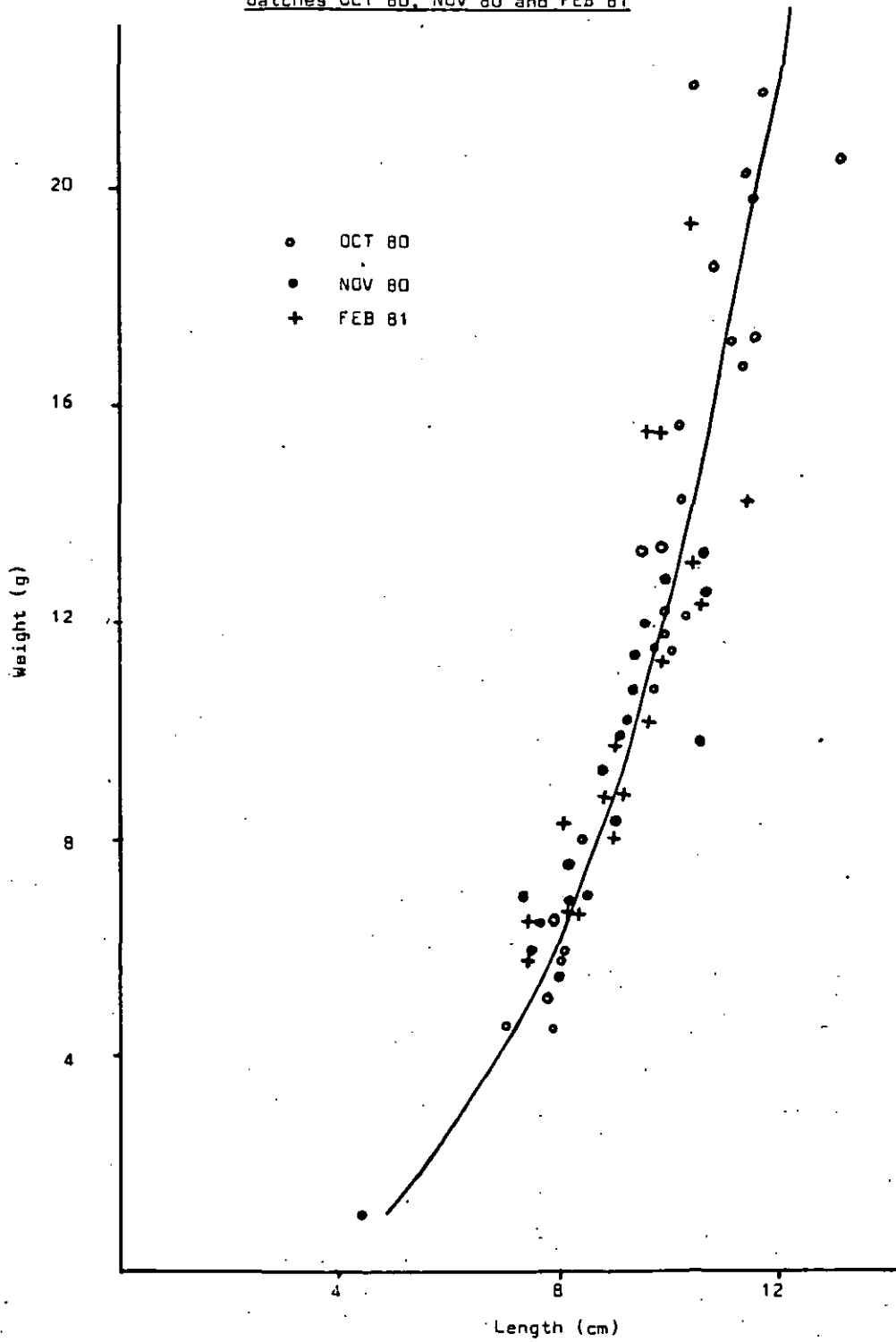
Figure 7 Relationship between weight and maximum depth of
Haplochromis batches OCT 80, NOV 80 and FEB 81
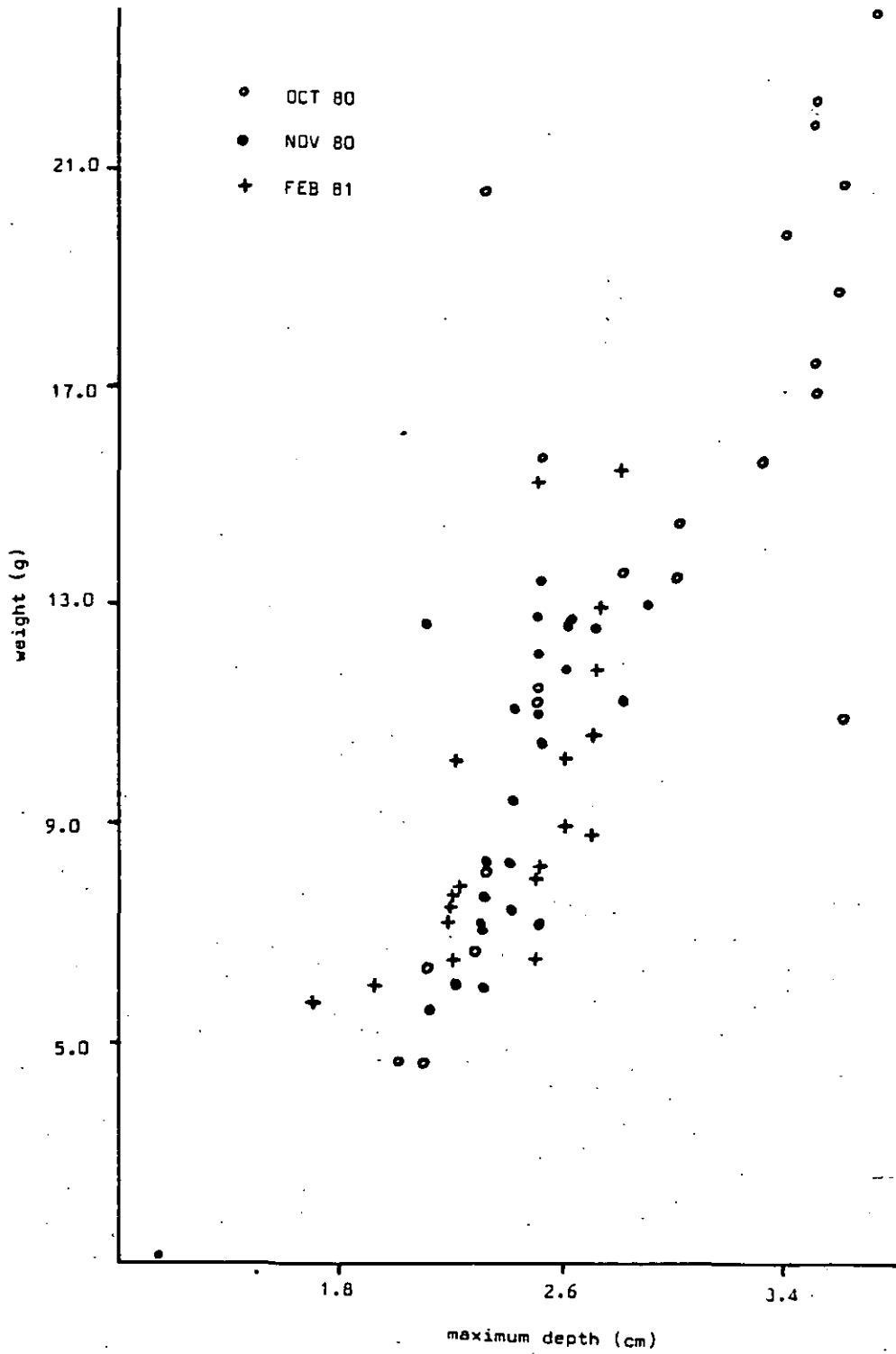
accurate results, but such studies could be carried out in Uganda with freshly caught fish.

4.2.2   Weight distribution in Haplochromis batches

Figure 8 illustrates the weight distribution pattern from a sample , `(3137 fish) taken from three batches OCT 80, NOV 80 and FEB 81.   It was on the basis of a similar distribution pattern for batches MAY 80 and OCT 80 only that the four weight groups used to group the species flock for the purposes of this study were determined.   The rationale was to put the smallest fish (less than 6.0g) in one group, the bulk of the species in a single group and to split the remainder, which varied widely in weight, into two groups.   The results of the grouping (see Table 7) showed that for the 3 batches OCT 80, NOV 80 and FEB 81, group I. (5.9g and less) constituted 15.0% of the total number of fish in the whole sample, group II (6.0 to 8.9g) 61.7%, group III (9.0-13.9g) 15.0% and group IV (14.0g and over) contributed 8.3% of the total sample.   When the total weight of fish is considered rather than numbers of fish, then group I constitutes 8.6%, group II 51.0%, group III 17.6% and group IV 22.8%.   Greenwood (1974) reported that the majority of adult Haplochromis species are small fishes, between 7 and 11 cm long but some piscivorous predators grow to lengths of 18 to 22 cm.   When the weights are related to total lengths of the fish (see Figure 6) it can be seen that fish measuring between 7 and 11 cm long correspond to a weight range of 4 to 14g approximately.   This weight range contributes about 75% of the total sample weight (Table 7 and Figure 8), in agreement with Greenwood (1974).   The fish are in general smaller than those caught by Beatty (1964) for canning.

Figure 8 Weight distribution in batches OCT 80, NOV 80, and FEB 81
(the points plotted are the percentage of total number of
fish represented by the one gram weight ranges given)

Note: 1.3% of the catch weighed
       between 21 and 24.9g
       2% weighed between 25-29.9g
and   2.5% weighed over 30g
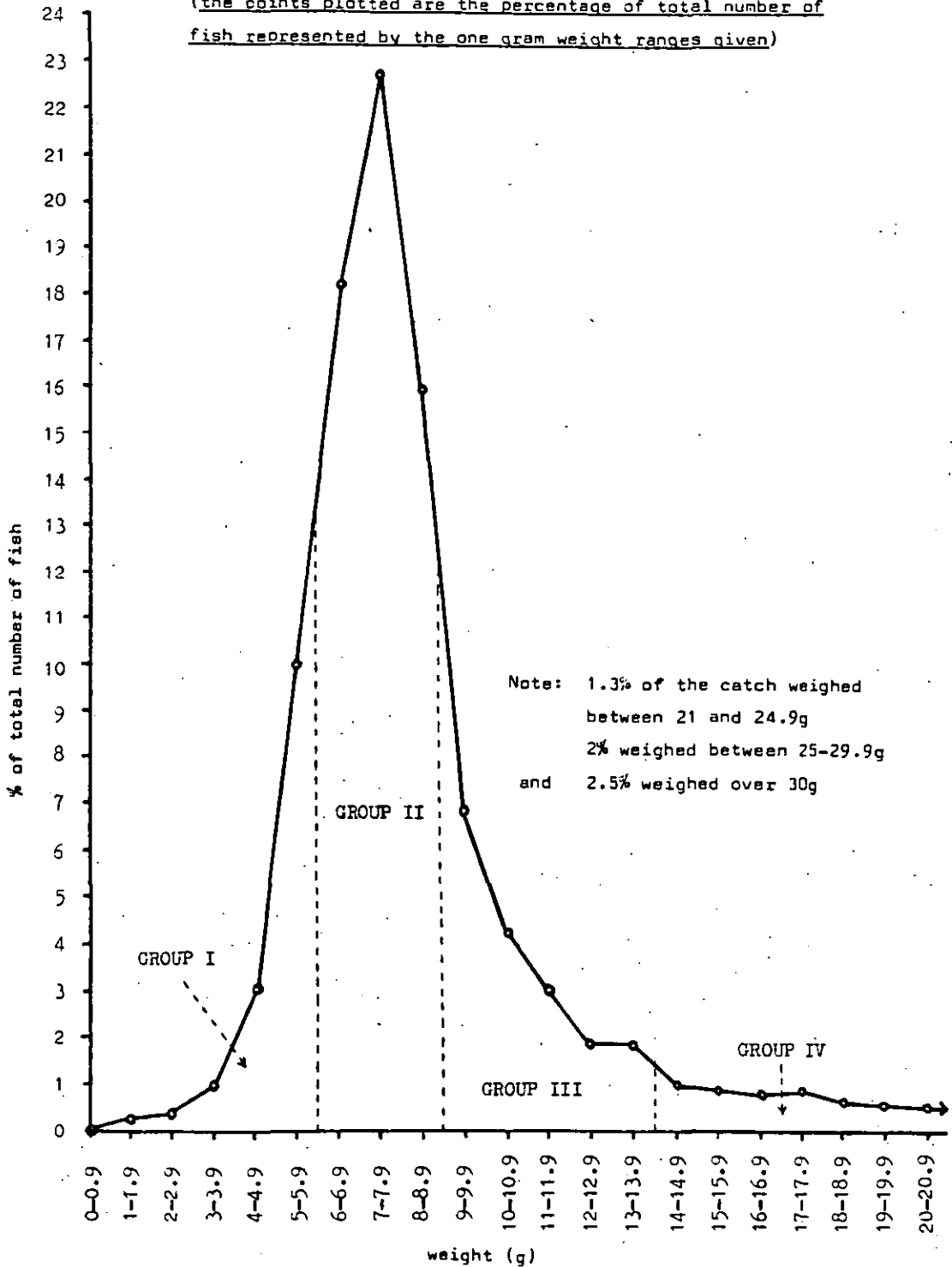
Table 7 Composition of Haplochromis, by percentage number of fish and by weight, from batches OCT 80, NOV 80, FEB 81

Group[1]	OCT 80		NOV 80		FEB 81		AVERAGE[2]	
	% no. of fish	% of total wt. of fish	% no. of fish	% of total wt. of fish	% no. of fish	% of total wt. of fish	% no. of fish	% of total wt. of fish
I (5.9g and less)	23.3	13.4	7.2	4.4	8.6	4.5	15.0	8.6
II (6.0-8.9g)	54.8	44.3	74.2	65.2	61.9	50.5	61.7	51.0
III (9.0-13.9g)	12.8	15.1	13.4	17.1	19.7	21.7	15.0	17.6
IV (14g and over)	9.1	27.2	5.2	13.3	9.8	23.3	8.3	22.8

(1) Fish weights were measured to the nearest 0.1g.

(2) Based on all fish weighed (unequal amounts from each batch).

Although there are some marked differences in distribution
of fish between groups for the different batches, particularly
with respect to group I, no general conclusions can be made
regarding, for example, seasonal variation in size profile,
since this would require that the fish catching operations were
carefully controlled and monitored. It is clear, however, for
all three batches, that group II is the major group and therefore
several analyses, reported below, were carried out on this
group only.

4.3 Relative proportions of parts of fish

Except for a few species of very small fish it is generally
the case that only parts of the fish e.g. fillets, steaks, etc.,
are consumed directly as food. The processor therefore needs to
know the relative proportions of the fillet, head, gut, etc., in
order to estimate the yield in such processes as filleting. In
the present study the relative proportions of the head, gut and
headless, gutless portions for Haplochromis (see Figure 5) were
determined, in order to provide information which will be of
value in estimating the yield after, for example, beheading and
gutting the fish and also to establish the distribution in the
fish of various chemical components such as lipid. The headless,
gutless portion was further split into skinless fillet and
residue for some of the groups and batches. The splitting of
Haplochromis into fillets has little practical application for
such small fish but chemical composition data obtained on the
fillet can be of value in assessing the efficiency of processes

such as flesh-bone separation. The relative proportions of head,
gut and headless, gutless portions of _Haplochromis_ from batches
OCT 80, NOV 80 and FEB 81 are shown in Table 8. The table also
shows the relative proportions of fillets and residue for all
groups batch NOV 80 and groups II and III only batch FEB 81.

It can be seen that there is some variation in the percentage
contribution of each portion to the whole fish both between the
batches and groups. However, statistical analysis (Snedecor's F
test) showed that the variation in the percentage of the head and
headless, gutless portions both between the batches and groups was
not significant. The variation in percentage of the gut between
the batches, however, was statistically significant at the 1%
level. Variation between the groups for percentage of gut was not
significant.

The proportion of head, about 37% on average, is relatively
high, for example for cod (_Gadus morhua_) the figure is 21%
(Waterman, 1968), for kampango (_Bagrus_ spp.) 28.5% and for catfish
(_Clarias_ spp.) 32% (Disney, 1974). However, a figure of 34.7%
was obtained for chisawasawa which is a mixture of _Lethrinops_ spp.
and _Haplochromis_ spp. (Disney 1974). The proportion of head
obviously depends on how it is defined; in this study the
pectoral and pelvic fins were included with the head (see Figure 5).

Variation in weight and chemical composition according to season,
size, etc., is a characteristic feature of fish, as has been shown
by various studies such as those of Love (1970, and references
therein) and Whittle _et al._ (1980). The variation is particularly
marked in the gut. Whittle _et al._ (1980), for example, found
that in blue whiting (_Micromesistius poutassou_) variation in
gross percentage composition over a five month period (February to
June) occurred more in the true guts, liver and gonads than in the

Table 8 The relative proportions of head, gut and skinless fillet and residue or headless, gutless portions of Haplochromis batches OCT 80, NOV 80, FEB 81

| Group | Batch | Relative proportions as % of whole fish | | | | |
		head	gut	skinless fillet	residue	gutless headless
I	OCT 80	36.4	7.2	–	–	56.4
	NOV 80	38.9	7.5	25.8	27.8	53.6
	FEB 81	36.2	10.5	–	–	53.3
II	OCT 80	37.0	7.1	–	–	55.9
	NOV 80	37.6	8.1	26.3	28.0	54.3
	FEB 81	36.7	9.0	28.1	26.2	54.3
III	OCT 80	43.0	7.4	–	–	49.6
	NOV 80	37.9	8.5	24.9	28.7	53.6
	FEB 81	36.0	10.4	25.4	28.2	53.6
IV	OCT 80	36.0	8.9	–	–	55.1
	NOV 80	36.5	8.6	28.8	26.1	54.9
	FEB 81	36.9	9.6	–	–	53.5
Weighted[1] averages	OCT 80	37.6	7.6	–	–	54.8
	NOV 80	37.6	8.2	26.4	27.9	54.2
	FEB 81	36.6	9.5	–	–	53.9

(1) Calculated using the data in Table 7.

fillets. During the period when fish are either feeding, starving
or spawning these organs are the ones expected to show most
variation in size and weight. The significant variation in the
percentage of the Haplochromis gut between the months (batches)
is, therefore, consistent with observations on other fish species,
although an explanation of the factors causing an increase
between October and February requires further studies.

4.4 Lipids

4.4.1 Total lipid

Of all the chemical components of fish, the lipid fraction
is the most important in determining the most appropriate
processing and storage procedures (see Section 2.3.3). Detailed
studies on the total lipid content, distribution and variation in
Haplochromis size groups and batches were, therefore, carried out
on three batches, OCT 80, NOV 80 and FEB 81. Total lipid content
only for whole fish of mixed groups was carried out on batch
MAY 80.

4.4.1.1 Variation between parts of the fish

Table 9 shows the total lipid content of Haplochromis and its
distribution in the different parts of the fish. The distribution
of total lipid in the fish is very uneven and in particular it
is noticeable that although the head contributes about 37% of the
total weight of the fish (see Table 8) it contributes about 54%
of the total lipid of the fish. Although data on lipid distribution

Table 9 Total lipid content and distribution in batches MAY 80, OCT 80, NOV 80 and FEB 81

Group	Weight range of groups (g)	Batch	Experimental[1],[2] % lipid content					Whole fish[3]	Distribution of total lipid as %		
			head	gut	skinless fillet	residue	headless, gutless fish		head	gut	headless gutless fish
I	5.9 and less	OCT 80	7.8 (0.42)	12.7 (3.6)	-	-	2.3 (0.29)	5.1	56.2	18.1	25.7
		NOV 80	6.0 (0.47)	6.9 (1.6)	-	-	1.8 (0.08)	3.8	61.2	13.6	25.3
		FEB 81	7.8 (1.1)	9.8 (4.2)	-	-	2.2 (0.40)	5.0	56.2	20.4	23.3
II	6.0-8.9	OCT 80	9.6 (1.1)	15.6 (0.57)	-	-	3.4 (0.17)	6.6	54.1	16.9	29.0
		NOV 80	8.5 (0.73)	13.5 (0.70)	-	-	3.0 (0.16)	5.9	54.0	18.5	27.5
		FEB 81	8.9 (0.59)	12.7 (0.7)	1.6 (0.78)	4.2 (0.33)	2.9 (0.16)	6.0	54.6	19.1	26.3
III	9.0-13.9	OCT 80	10.6 (0.32)	18.3 (1.8)	-	-	3.4 (0.25)	7.6	60.0	17.8	22.2
		NOV 80	7.9 (0.81)	14.2 (2.5)	-	-	2.8 (0.37)	5.7	52.5	21.2	26.3
		FEB 81	8.1 (1.5)	12.7 (1.6)	1.3 (0.05)	4.1 (0.65)	2.7 (0.25)	5.7	51.3	23.2	25.5
IV	14.0 and over	OCT 80	11.3 (0.5)	16.9 (4.2)	-	-	3.4 (0.65)	7.4	54.6	20.2	25.2
		NOV 80	9.7 (1.0)	13.4 (3.6)	-	-	2.8 (0.81)	6.2	56.3	18.5	24.7
		FEB 81	6.0 (0.76)	9.0 (7.4)	-	-	2.6 (1.5)	4.5	49.5	19.3	31.1
Weighted averages[4]		MAY 80	-	-	-	-	-	4.7[5]	-	-	-
		OCT 80	10.0	16.0	-	-	3.3	6.6	55.4	17.9	26.7
		NOV 80	8.4	13.3	-	-	2.9	5.8	54.3	18.7	27.0
		FEB 81	8.0	11.7	-	-	2.8	5.5	52.8	20.0	27.2

(1) Average of 3 determinations.

(2) Standard deviation in parentheses.

(3) Calculated using data in Table 8.

(4) Calculated using data in Table 7.

(5) Average of 6 determinations (Std. deviation = 0.57).

in whole fish is very limited (see Section 2.3.1.1) the
figure of 54% of total lipid in the head appears to be
exceptionally high.

The gut is about 8% by weight of whole fish but it
contributes about 19% of the total lipid in the fish. Correspond-
ingly the headless, gutless portion which is about 54% by weight
of the fish contributes about 27% of the total lipid in the fish.
For each of the parts analysed the variation in the lipid
distribution between the groups and batches is not statistically
significant (Snedecor's F test).

The distribution pattern of total lipid in Haplochromis has
some important processing implications. By removing the head
and gut, nearly three quarters of the total lipid in the fish is
removed, leaving a headless, gutless portion with a low lipid
content (about 3%) compared with the whole fish (about 6%).
Products produced from the headless, gutless portion (e.g. minced
fish) would therefore have a lower lipid content and would be
less susceptible to oxidation. The head and gut, on the other
hand, could be used as raw material for the production of fish
oil, animal feeds, etc. Dhatemwa (1981) in parallel work on
Haplochromis, found that mince from whole fish contained about
5-6% total lipid. It is probable that if a simple means of
removing the head and gut could be developed then the lipid content
of the mince could be reduced to about half this value.

4.4.1.2   Variation between size groups

Table 9 and Figure 9 show the lipid content of the head, gut,
headless and gutless fish, and whole fish for the four groups

Figure 9. The total lipid content of Haplochromis batches OCT 80 NOV 80 and FEB 81 (values in percentages)
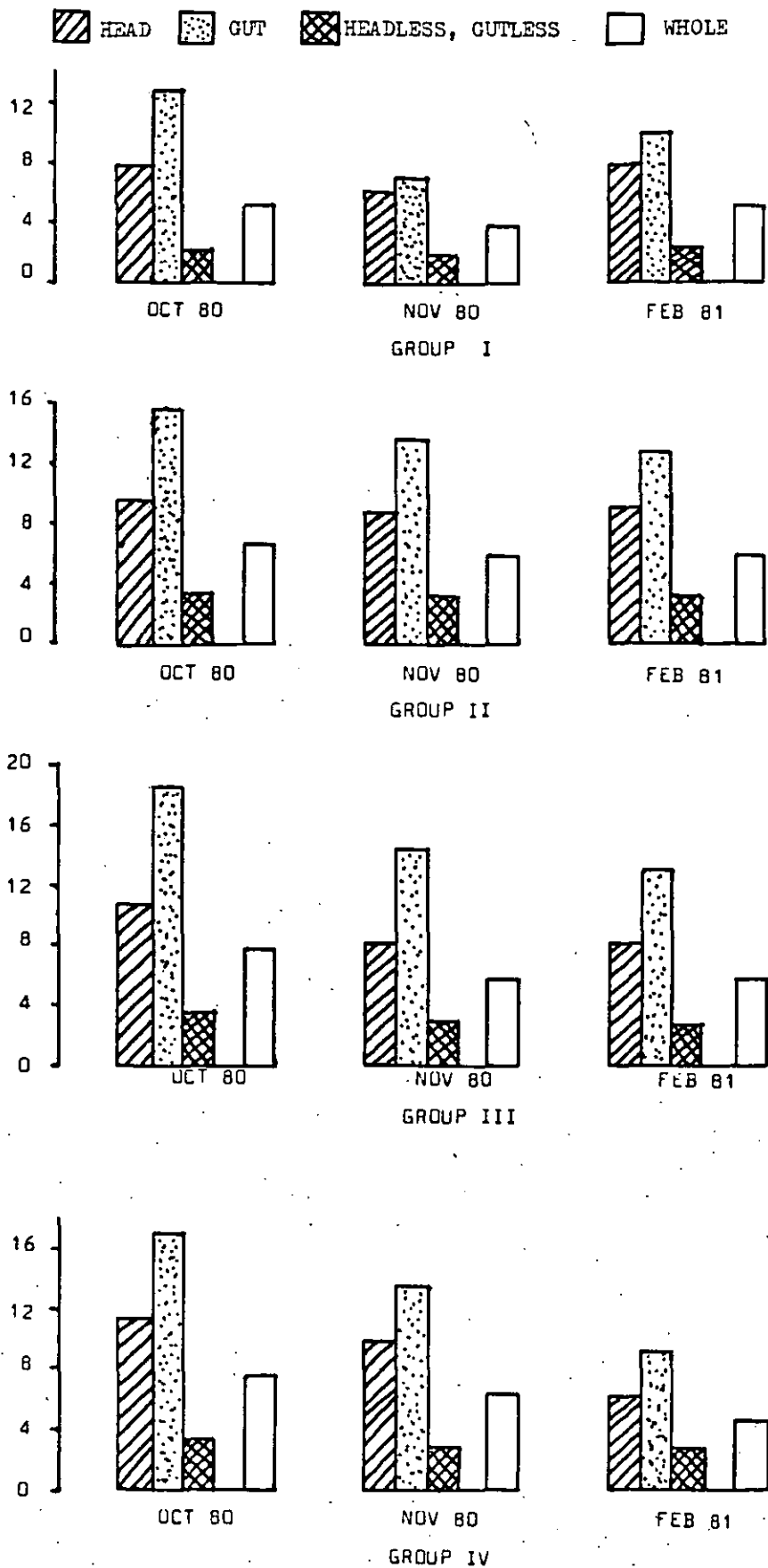
from batches OCT 80, NOV 80 and FEB 81. In Figure 10 is plotted
the average values for each group (averaged over three batches)
for the head, gut, headless and gutless portions, and whole fish.
The variation in the lipid content of the whole fish and head was
not statistically significant (Snedecor's F test), while that of
the gut was significant at the 5% level and that of the headless,
gutless portions was significant at the 1% level. Although
significance in variation of total lipid for whole fish between
groups could not be shown, the data in Figure 10 indicate that
the group I fish have a lower total lipid content (4.6%) than the
other three groups, which have very similar total lipid contents
(6.2%, 6.3% and 6.0%). This is consistent with observations
made by the author previously in Uganda that smaller sized
Haplochromis were preferred for fish powder production because
of their lower lipid content. The relatively large variation in
the gut lipid content was to be expected for reasons discussed
above (see Section 4.3).

The variation in total lipid content with size group for
the Haplochromis samples is relatively small and less than has
been demonstrated for some single species of fish (Love, 1970;
Zain, 1980).

4.4.1.3 Variation between batches

The lipid content of Haplochromis from batches MAY 80,
OCT 80, NOV 80 and FEB 81 for the different parts of the fish
(whole fish only for batch MAY 80) and whole fish is shown in
Table 9 and Figures 9 and 11. The variation in lipid content of

Figure 10 Variation in total lipid content of Haplochromis between
size groups for the head, gut, headless gutless fish and
whole fish. The values plotted are averages for batches
OCT 80, NOV 80 and FEB 81
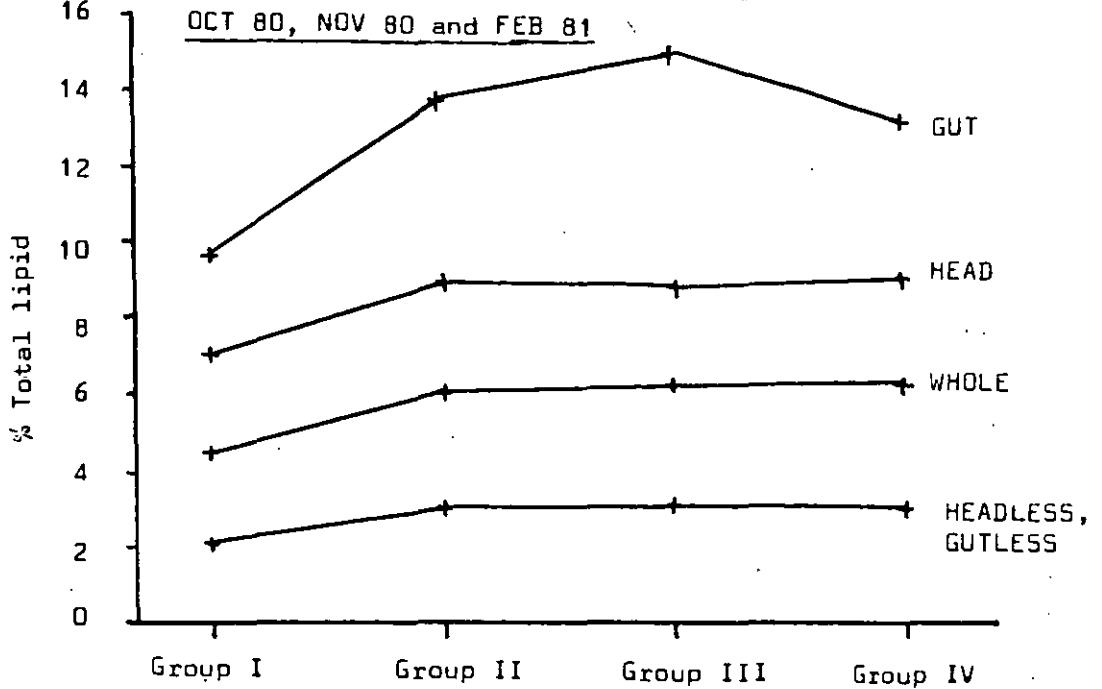
Figure 11 Total lipid content of whole Haplochromis (mixed groups)
from batches MAY 80, OCT 80, NOV 80, FEB 81
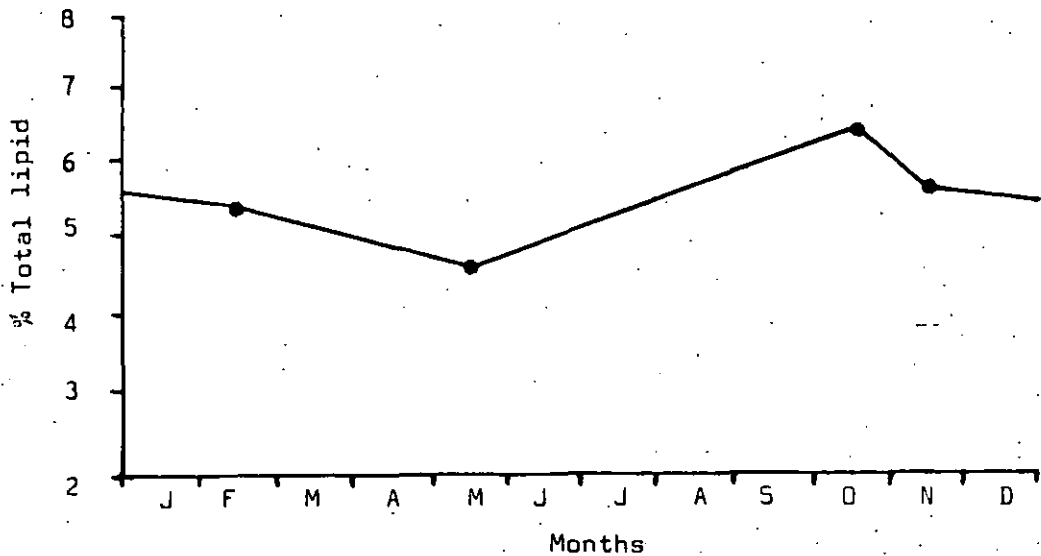
whole fish and the head between the batches was not significant, while variation in the lipid content of the gut was significant at the 2.5% level, and variation of the lipid content of the gutless, headless portions was significant at the 1% level (Snedecor's F test).

Seasonal variation in lipid content of fish flesh has been observed in many species, particularly fatty fish. Hardy and Keay (1972), for example, found that the total lipid content of Cornish mackerel (Scomber scombrus) varied from 6.3% to 23.4% over a ten-month period. Andrade and Lima (1980) observed seasonal variation in the total lipid content of mandi (Pimelodus clarias), a Brazilian freshwater fatty fish. In lean fish the variation in the lipid content of the flesh is very small but large variations occur in the lipid content of the liver as found for cod (Jangaard et al., 1967). The depletion of fat in many fish species is presumed to be due to mobilisation of fat which accompanies spawning (Hardy and Keay, 1972, and references therein).

The trend shown in Figure 11 for the whole Haplochromis, if confirmed by further studies, indicates a relatively small variation in total lipid content with season, with an increase occurring some time between May and October, followed by a gradual decrease throughout the rest of the year. This may be explained by the differences in the breeding status of the various Haplochromis species, since Beadle (1974) suggested that most Haplochromis breed during the rainy season, May to July. However Greenwood (1974) suggested that part of the Haplochromis species flock is breeding at any one time. Another factor that may be important in seasonal variation in lipid content is the rise in

primary production in the open water of Lake Victoria which occurs
in June and July as a result of the heavy south-east trade winds
stirring up the nutrients from below (Beadle, 1974). Monthly
analyses need to be carried out, preferably in Uganda, in order
to confirm these trends in total lipid content and no definite
conclusions should be drawn at this stage.

4.4.2   Fatty acid profiles

Fatty acid profiling was carried out on the head, gut, fillet
and residue of group II the major group, and whole fish only for
groups I, III and IV, from batch FEB 81.

The individual peaks on the gas chromatograms of the fatty
acid methyl esters (FAME) of _Haplochromis_ total lipids were
identified by comparing them with corresponding peaks of two
standards (PUFA I and Chrompack No. 32246). Some peaks on the gas
chromatograms of the _Haplochromis_ FAME could not be identified
because there were no corresponding peaks on the standard
chromatograms. However the unidentified peaks were marked
a, b, c, d, e and f and their percentages worked out as given in
Table 10.

Figure 12 shows the gas chromatogram of FAME of the fillets
(group II). The patterns of the chromatograms of FAME of the head,
gut, residue and whole fish were similar to that in Figure 12.

Table 10 shows the fatty acid profiles for the total lipid
of the head, gut, fillet and residue (group II) and whole fish of
groups I, III and IV. The data given are the means of three
samples in each case. The values for the individual three samples
were normally within $\pm$ 10% of the mean value. Table 11 shows the
fatty acids according to the number of carbon atoms and number of

Figure 12 Chromatogram of the FAME of the fillet lipids of group II
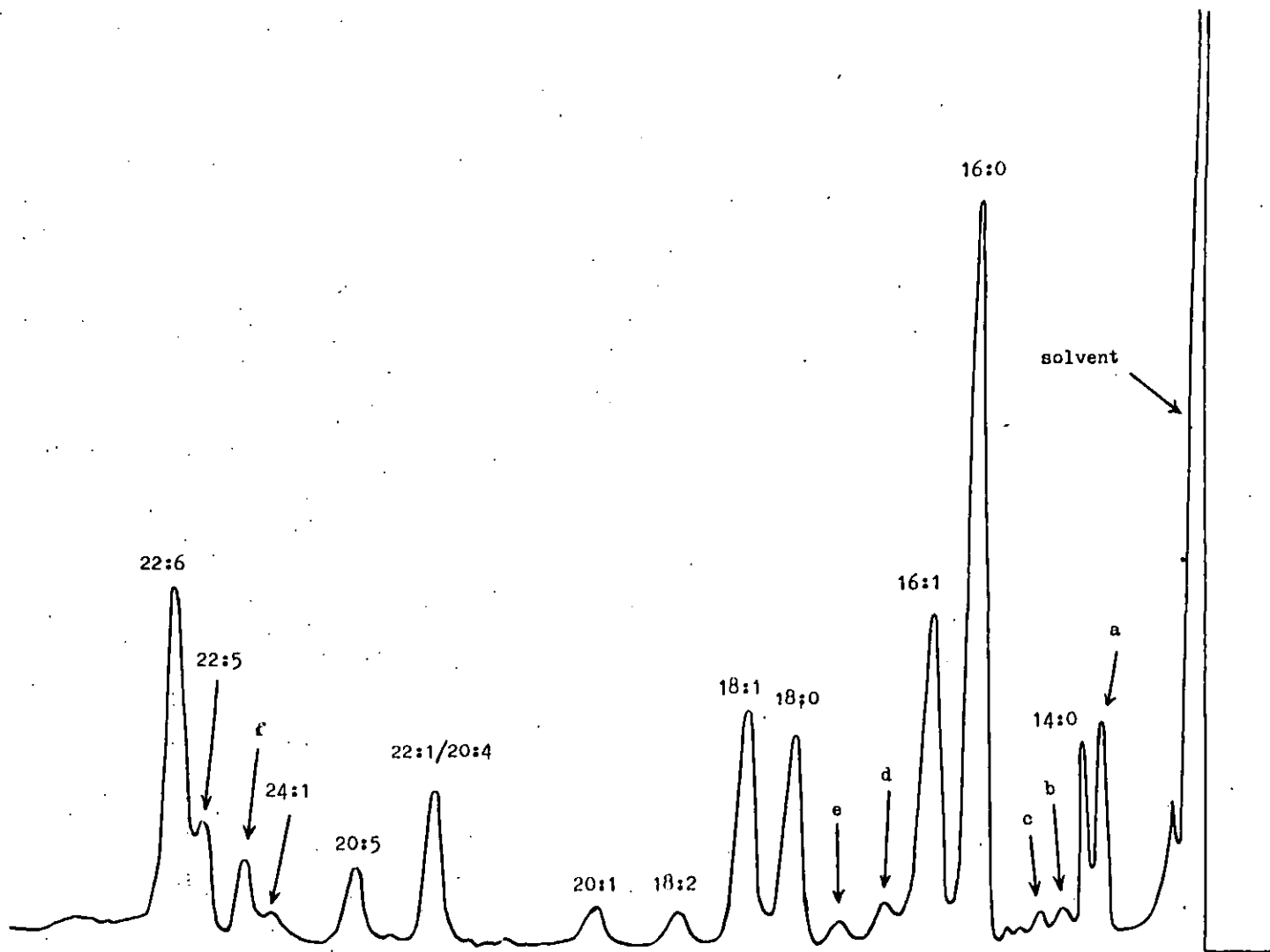
Table 10   Fatty acid profiles of Haplochromis lipids from batch FEB 81 fish[1]

Sample	identified												unidentified						Total (%)
	14:0	16:0	16:1	18:0	18:1	18:2	20:1	20:5	22:1[2]	22:5	22:6	24:1	a	b	c	d	e	f	
II head	5.9	21.6	19.9	5.1	10.3	2.4	3.7	3.1	2.8	3.7	5.2	1.3	0.8	1.5	1.1	–	1.7	1.2	91.8
II gut	3.1	25.8	17.9	5.8	22.4	2.0	1.4	1.8	2.2	2.1	3.6	0.5	0.8	0.7	0.7	1.5	1.4	0.8	94.0
II skinless fillet	3.0	21.9	12.8	7.9	9.8	1.5	1.7	3.2	5.2	3.5	11.4	0.8	3.0	0.9	0.7	1.6	1.1	2.8	92.8
II residue	6.0	21.5	19.6	5.8	11.0	2.3	3.0	3.1	3.2	3.4	6.3	0.7	0.4	1.4	1.2	2.0	2.2	1.7	95.3
I whole	5.0	23.3	16.6	5.1	10.0	2.1	3.9	3.9	3.6	4.0	9.1	0.8	0.9	1.3	0.9	2.0	1.1	–	95.5
III whole	4.6	20.8	18.8	5.3	13.1	2.4	2.9	3.4	3.4	4.0	6.8	0.6	0.7	1.0	0.9	1.7	1.8	–	93.3
IV whole	3.2	21.7	17.6	5.4	19.8	2.9	1.4	1.7	2.6	2.4	6.0	0.5	4.5	0.8	0.8	–	1.8	–	93.7
PUFA I standard	4.0	4.6	13.4	0.6	21.5	3.8	5.7	12.1	3.0	1.1	14.2	0.8	0.9	–	–	–	1.0	0.3	95.3
Chrompack No. 32246	2.0	4.9	–	3.2	42.1	8.9	–	–	19.1	–	–	–	–	–	–	–	–	–	90.0

(1) Figures given for Haplochromis lipids are the means of 3 determinations.   (2) Probably includes some 20:4.

Table 11 <u>General fatty acid composition of Haplochromis[1] mackerel, anchovy, capelin and white perch lipids</u>

	Haplochromis Group II				Haplochromis Whole Fish				Mackerel[3]	Anchovy[3]	Capelin[3]	White perch[4]
	head	gut	skinless fillet	residue	I	II[2]	III	IV				
$\sum$ C-14	6	3	3	6	5	5	5	3	9	7	8	3
$\sum$ C-16	42	44	35	41	40	40	40	39	38	30	19	33
$\sum$ C-18	18	30	19	19	17	19	21	28	21	24	25	37
$\sum$ C-20	7	3	5	6	8	8	6	4	15	23	24	17
$\sum$ C-22	9	6	15	10	13	10	11	8	11	15	19	5
$\sum$ Δ 0	33	35	33	33	33	33	31	30	24	35	23	25
$\sum$ Δ 1	35	42	25	34	34	33	38	41	48	30	60	40
$\sum$ Δ 5	7	4	7	7	8	6	7	4	7	18	4	12
$\sum$ Δ 6	5	4	11	6	9	7	7	6	9	12	3	4

$\sum$ C-14 = % total fatty acids having carbon chain lengths of 14

$\sum$ Δ 6 = % total fatty acids having six unsaturated linkages

(1)   C22:1 is not included, since the peak probably includes some 20:4.

(2)   Calculated using data in Table 8.

(3)   Windsor and Barlow (1981).

(4)   Kinsella et al. (1977).

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double bonds. The same data for the total lipids of mackerel, anchovy, capelin and one freshwater species (white perch) are given for comparison.

Palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1) decosahexaenoic (C22:6), stearic (C18:0) and myristic (C14:0), comprising approximately 70% of the total fatty acids, were the major component acids in the fish. Kinsella et al. (1977) made a similar observation on 18 species of freshwater fish fillets although there were marked variations in fatty acid composition between the species.

Most of the fatty acids (over 60%) were unsaturated, with about 15% containing 5 or 6 double bonds, a characteristic feature of fish lipids (see Section 2.3.1.1).

The fillet contained more poly-unsaturated fatty acids than the other portions indicating a high phospholipid content (Ackman, 1974). However analysis for the lipid classes in Haplochromis lipids would have to be carried out in order to confirm this.

Freshwater fish are reported to contain significant quantities of the essential fatty acids C18:2 and C20:4. Kinsella et al. (1977) found that the C18:2 content of 18 species of fish was about 3.3% (average) ranging from 1.1% to 5.5% and the C20:4 content was 6.4% (average) ranging from 4.3% to 15.8%. However, as Table 10 shows, the C18:2 content of the Haplochromis lipid averaged about 2.1% and was particularly low in the fillet while the C20:4 could not be clearly identified because of the overlap with the 22:1 peak, although it may be present in significant amounts.

Generally Haplochromis lipids had about the same amounts of C-16 as mackerel, anchovy and white perch but more than capelin (see Table 11). Haplochromis lipids had slightly lower amounts of

the long chain fatty acids (C-18 to C 22) than mackerel, anchovy, capelin and white perch. The total amount of saturated ($\sum \Delta_0$) fatty acids of Haplochromis lipids was similar to that of anchovy but was less than that of mackerel, capelin and white perch. The total amount of polyunsaturated fatty acids ($\sum \Delta_5$ & $\sum \Delta_6$) of Haplochromis was similar to those of mackerel and white perch.

Since the fatty acid profiles of the total lipid of Haplochromis does not differ appreciably from those of, for example, mackerel, anchovy and capelin (see Table 11), it is likely that Haplochromis oil would be acceptable on the commercial fish oil market, however further analytical information on the oil would be required, such as the phospholipid and the unsaponifiable matter content. This is an area which requires further investigation.

4.5 Protein

Determinations of crude protein and true protein were made on the head, gut, fillet, residue and whole Haplochromis. Non-protein nitrogen was calculated from the difference between crude protein nitrogen and true protein nitrogen values. Two batches were analysed for crude protein. The head, gut, fillet, residue and whole fish for all groups were analysed for batch NOV 80 and the head, gut, fillet and residue for group II only, and whole fish for the other groups, were analysed for batch FEB 81. The crude protein determination for the FEB 81 batch was part of an assessment of true protein and non-protein nitrogen.

The amino acid profiles of the head, gut, fillet and residue of group II and whole fish of group IV, for batch NOV 80, were also determined.

4.5.1 Crude protein

Table 12 and Figure 13 give the crude protein content for the
NOV 80 batch and Table 13 gives the crude protein for the FEB 81
batch.

The apparent variations in the crude protein content of
whole fish and the four different portions of the fish, between
the groups were not statistically significant for the NOV 80 batch
(Snedcor's F test). Similarly for the FEB 81 batch the variation
between the groups for crude protein content of the whole fish
was not significant. Although there are differences between the
two batches for individual crude protein values, these differences
are not significant and the weighted average of crude protein for
the two batches (16.8% for NOV 80 and 16.6% for FEB 81) are in good
agreement with each other and with the results obtained by
Dhatemwa (1981) for fish mince obtained from the same batches of
Haplochromis. He found that the mince (about 65% of the whole
fish using a 3 mm drum) contained about 15% of crude protein
whereas the residue (about 30%) of the whole fish contained about
18.5% of crude protein.

There was a significant difference in the weighted average
crude protein content between the different parts of the fish for
the NOV 80 batch (see Table 12). The gut contained the least
amount of protein (weighted average 9.3%) and the fillet and the
residue contained the highest amounts (18.9% in each case).

The distribution of crude protein between the different parts
of the fish in the total NOV 80 batch was 34% in the head, 5% in
the gut, 30% in the fillet and 31% in the residue i.e. the head,
fillet and residue each contained about a third of the total crude
protein.

Table 12 <u>Crude protein content of Haplochromis batch NOV 80</u>

Weight group	% Crude protein [1]				
	Experimental				Calculated for whole fish [2]
	head	gut	skinless fillet	residue	
I	17.1 (0.66)	11.1 (0.40)	20.2 (1.2)	19.4 (0.51)	18.1
II	15.6 (0.11)	8.8 (0.75)	18.3 (0.85)	19.1 (1.2)	16.7
III	14.6 (1.2)	9.9 (1.1)	19.3 (2.0)	18.1 (2.5)	18.7
IV	14.2 (1.0)	10.0 (3.3)	21.0 (0.43)	18.8 (0.6)	17.0
Weighted averages [3]	15.3	9.3	18.9	18.9	16.8

(1) Mean of 3 determinations. Standard deviations are given in parentheses.

(2) Calculated using the data in Table 8.

(3) Calculated using the data in Table 7.

Figure 13 Crude protein content of batch NOV 80 head, gut, fillet,
          residue and whole fish for all four groups
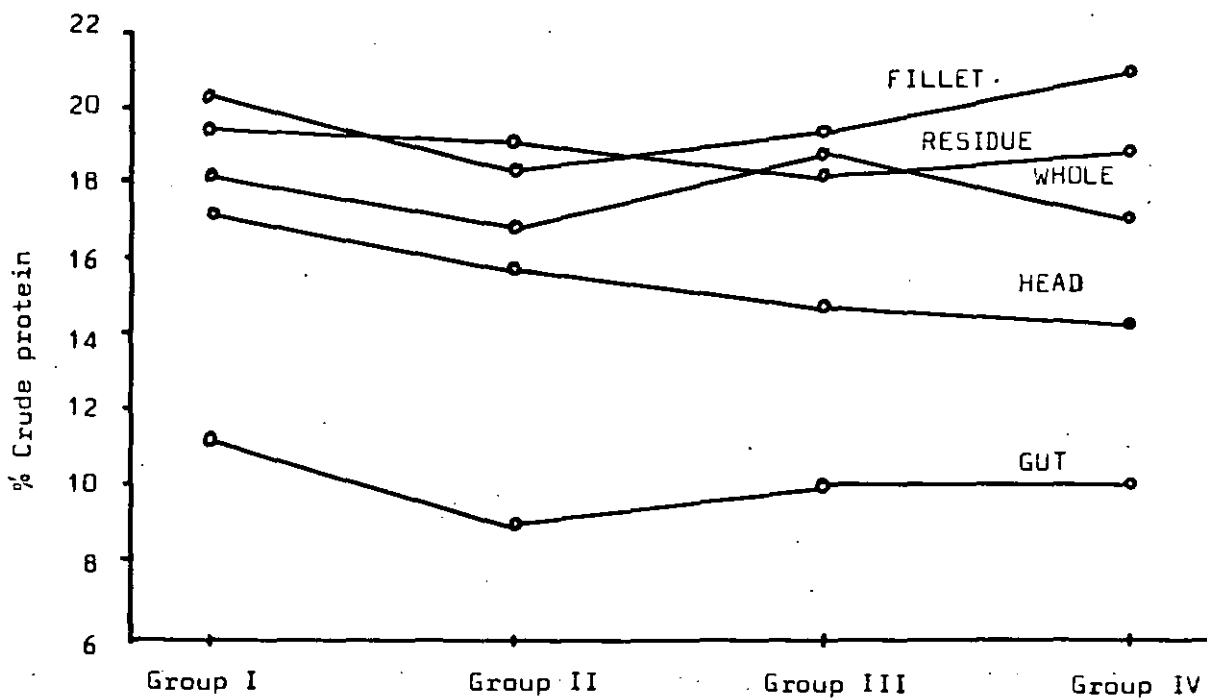
Table 13   <u>True-protein and non-protein nitrogen content of Haplochromis batch FEB 81</u> [1]

Group	Crude Protein % Total nitrogen (x 6.25)					True Protein % TCA-insoluble nitrogen (x 6.25)					Non-protein nitrogen (calculated) % TCA-soluble nitrogen					% non-protein nitrogen (whole fish)
	head	gut	skinless fillet	resi -due	whole fish	head	gut	skinless fillet	resi -due	whole fish	head	gut	skinless fillet	resi- due	whole fish	
I	-	-	-	-	16.0 (0.05)	-	-	-	-	13.5 (0.26)	-	-	-	-	0.49	15.6
II	15.0 (0.54)	10.8 (0.44)	19.2 (0.73)	17.2 (0.73)	16.4 (0.28)	12.6 (0.40)	6.9 (0.82)	16.8 (0.68)	14.8 (1.7)	13.9 (0.69)	0.39 (0.11)	0.62 (0.06)	0.38 (0.15)	0.38 (0.26)	0.40	15.2
III	-	-	-	-	17.1 (0.69)	-	-	-	-	13.5 (1.1)	-	-	-	-	0.57	21.1
IV	-	-	-	-	16.8 (0.60)	-	-	-	-	13.3 (1.56)	-	-	-	-	0.56	20.0
weighted averages [2]	-	-	-	-	16.6	-	-	-	-	13.7	-	-	-	-	0.46	17.0

(1) The figures quoted are the means of three values.   Standard deviations are given in parentheses.

(2) Calculated using data in Table 8.

The values for the crude protein content of the fillets are very similar to those quoted for other freshwater fish (see Section 2.3.1.2 and Table 3) such as _Tilapia nilotica_ 18.7% Khalil _et al._, 1980).

Proper assessment of _Haplochromis_ as a source of protein should take into account the part of the fish being considered for food. Sundried _Haplochromis_ (see Section 2.2.2) is consumed whole except for the head (personal observation), which gives a crude protein content of about 66% of the whole fish. It was mentioned in Section 2.2.2 that fish powder was produced from headless, gutless _Haplochromis_; this product would contain about 61% of the crude protein of the whole fish whereas the offals from fish powder production, which would contain 39% of the crude protein could be useful for animal feeds.

4.5.2   True protein and non-protein nitrogen

Table 13 shows the true protein and non-protein nitrogen content of the head, gut, skinless fillet and residue of group II and whole _Haplochromis_ for the four groups for batch FEB 81.

The average true-protein content of the whole _Haplochromis_ is 13.7% compared with 16.6% crude protein. Similarly for the fillet the protein content is 16.8% compared with 19.2%. Any calculations of protein yield in, for example fish flour production, should relate to true protein, since most of the non-protein nitrogen is of no nutritional value (Shewan, 1951) or will be lost during processing.

Non-protein nitrogen in groups I and II (whole fish) was found to be 15.2% and 15.6% of the total nitrogen respectively.

NPN in groups III and IV accounted for 21.1% and 20.8% of the total nitrogen with a weighted average of 17%. The differences between the groups were not significant but result from determining NPN by difference rather than directly.

For the group II fish the gut contained the highest amount of NPN (36.5%) and the fillets contained the least amount (12.2%).NPN accounted for 16.2% of the nitrogen in the head and 14.1% in the residue. The high content of NPN in the gut is in part probably due to the presence of various short peptides (which are TCA-soluble) in different stages of digestion since these would be included in the NPN fraction.

The value of 12.2% obtained for the NPN content of the Haplochromis fillet is lower than that of the cichlid Iilaoia nilotica (15.3%) but is within the range commonly quoted for teleost fish e.g. 9.2-18.3% (Simidu, 1961).

It should be noted that the NPN was analysed in fish which had been stored frozen for 2-3 months and there is a possibility that the NPN had increased at the expense of true protein due to autolysis, or had decreased due to leaching or spoilage reactions. It is clear, however, that the gut has a higher concentration of NPN than the other parts of the fish and hence this part would be expected to be the most susceptible to spoilage reactions (Shewan, 1951).

4.5.3   Amino acid profiles

Table 14 shows the amino acid profiles of the head, gut, fillet and residue of group II and whole fish of group IV Haplochromis for batch FEB 81. Amino acid profiling for the gut

Table 14   The amino acid profiles of the head, gut, fillet, residue and whole fish of group II and whole fish of group IV Haplochromis batch FEB 81

Amino acid (g/100g protein[1])	Group II					Group IV
	head	gut	skinless fillet	residue	whole[2]	whole
Isoleucine	4.4	5.0	5.8	4.6	5.0	4.9
Leucine	7.8	8.5	10.1	8.0	8.7	8.6
Lysine	8.1	9.3	11.2	7.5	9.0	9.4
Methionine	3.2	2.2	3.7	3.1	3.3	3.3
Phenylalanine	4.8	5.3	4.9	4.4	4.7	4.8
Tyrosine	3.8	4.6	4.3	3.3	3.9	3.6
Threonine	4.9	6.1	5.6	5.1	5.2	5.2
Valine	5.3	6.7	5.6	5.1	5.4	5.2
Arginine	8.6	7.0	7.7	8.5	8.2	7.5
Histidine	2.4	2.7	2.7	2.5	2.5	2.5
Alanine	9.2	8.2	8.0	9.6	8.8	9.7
Aspartic acid	11.2	11.7	11.8	11.0	11.4	10.9
Glutamic acid	16.2	15.0	20.0	17.7	17.8	18.4
Glycine	13.1	11.8	5.6	12.6	10.3	10.4
Proline	7.2	5.6	3.7	7.3	6.0	6.2
Serine	5.6	5.8	5.0	5.2	5.3	5.1

(1)   Calculations based on the assumptions that: a)   100g protein ≅ 118g amino acids

                                  b)   cystine + tryptophan = 2% of total amino acids.

(2)   Calculated from the figures for head, gut, fillet and residue using the values given in Table 7 for fish composition and in Table 13 for true protein.

was done on two samples (from the same batch) and the two sets
of figures agreed within about $\pm$ 5% for each amino acid.

It can be seen that the figures for the whole fish amino
acid profile are not very different to those in Table 6 for
Haplochromis, although the figures for Table 6 were obtained from
deboned, pressed, dried and ground Haplochromis.

When the results for the Haplochromis in Table 14 are
compared with the essential amino acid values for other fish
and the FAO/WHO suggested amino acid requirements, given in
Table 3, it can be seen that the whole Haplochromis and the
different parts provide all the essential amino acids (excluding
tryptophan which was not analysed) in good quantities.

Lysine and methionine, the two amino acids particularly
deficient in cereals, are relatively high in the Haplochromis
fillet, whereas in the head and residue the glycine and proline
are high (due to the collagen) and lysine content relatively low.

When the distribution of lysine in the whole fish is
considered then it is found that the fillet contains 42% of the
lysine compared with 33% of the crude protein (FEB 81 figures
from Table 13). The equivalent figures for the head are 30% lysine
and 34% crude protein, for the gut 4.5% and 6%, and for the
residue 23% and 27%.

On comparing the figures for group I with group IV it can be
seen that size does not appear to significantly affect the amino
acid profile.

4.6 Ash and calcium

Ash was determined on batch FEB 81 on the head, gut, fillet
and residue portions of group II only and whole fish for all the
groups. The results are shown in Table 14. Calcium was determined
in the ash and the results are shown in Table 15.

The head and residue, which included bones and scales,
contained the highest percentage of ash (8.7% and 7.6% respectively)
and the fillet and gut contained the least (1.1% and 1.6%
respectively). The variation between the groups was not significant
(Snedecor's F test).

The value obtained for the fillet was comparable to those
obtained for other species of fish (see Section 2.3.1.3 and Table
4).

The ash content of the whole fish was relatively high when
compared with the limited number of values quoted in the
literature for whole fish e.g. values in Murray and Burt (1969)
and values quoted by Meinke (1974) for 17 species of Gulf Coast
trash fish (ranging from 2.5 to 6.4% with an average of 4.1).
This is consistent with Haplochromis being considered a small "bony"
fish.

The head and residue also contained the highest amount of
calcium (747 and 690 mg/100g respectively) corresponding to the
high ash content, while the gut and fillet contained the least
amount (18 and 23 mg/100g respectively). There was some variation
in calcium content between the groups, with groups I and IV
apparently containing less calcium, however the differences were
not significant (Snedecor's F test).

The value for the calcium content of fillets (23 mg/100g) was

Table 14 Ash content of Haplochromis batch FEB 81

Group	Ash content (%)[1]				
	head	gut	fillet	residue	whole fish
I	-	-	-	-	5.1 n = 5 (0.4)
II	8.7 (0.07)	1.6 (0.09)	1.1 (0.09)	7.6 (0.62)	5.4/5.6[2] n = 3 (0.11)
III	-	-	-	-	5.8 n = 7 (0.56)
IV	-	-	-	-	5.1 n = 7 (1.05)

(1) Mean of 3 determinations except where n (number of determinations) given.
(standard deviation in parenthesis).

(2) Calculated using data in Table 8.

Table 15   Calcium content of Haplochromis batch FEB 81

Group	Calcium content (mg/100g fish)[1]				
	head	gut	fillet	residue	whole fish
I	-	-	-	-	357
					(32)
II	747	18	23	690	431/481[2]
	(192)	(10)	(4)	(286)	(61)
III	-	-	-	-	457
					(38)
IV	-	-	-	-	344
					(50)

(1)   Mean of 3 determinations (standard deviations in parentheses).

(2)   Calculated using data in Table 8.

within the normal range quoted in the literature for other fish
species (20-40 mg/100g, Murray and Burt, 1969); see also Table 14.
The calcium content of the whole fish was rather low compared to
the very few figures quoted in the literature for whole fish
e.g. for Portuguese sardines preserved in oil, 580 mg/100g,
various West African species average 888 mg/100g (Causeret, 1962).

It is clear that for <u>Haplochromis</u>, as with most other species
of fish investigated, consumption of the fillet alone would lead
to very little of the calcium in the fish being consumed and
presumably also phosphorus.

4.7 Vitamin A

The gut of group IV only for batch FEB 81 was analysed for
vitamin A using the Carr-Price reagent. Surprisingly the UV
spectrum of the blue complex formed showed that the vitamin A
present was vitamin A_1 ($\mathcal{E}$ max 615 nm) and not vitamin A_2
($\mathcal{E}$ max 693 nm). Previously it had been stated that in freshwater
fish vitamin A_2 is the major vitamin A compound present (see
Section 2.3.1.4).

Vitamin A_1 was found to be present at 500 IU/g oil (150 μg/g
oil) this is equivalent to 5,000 IU/100g gut (1500 μg/100g gut).
This figure may be low due to the fact that vitamin A is lost on
storage as a result of destruction by UV light and oxidation
(Cruickshank, 1962; Association of Vitamin Chemists, 1966). It
is possible also that any vitamin A_2 present in the fresh fish
may have been lost on storage. Further work on freshly caught

<u>Haplochromis</u> needs to be carried out to elucidate this point. It
is clear, however, that the oil from the gut of <u>Haplochromis</u>
could be used as a vitamin A supplement.

4.8   <u>Moisture</u>

Moisture was determined on the head, gut, fillet and residue
of group II only and whole fish for all the groups for batch
FEB 81, and the results are shown in Table 16 along with the
proximate analysis values for ash, total lipid and crude protein.

The gut and fillet contained the highest amount of moisture
(79.5% and 77.2% respectively) due to their low ash content, and
to the low protein content of the gut (10.8%) and the low lipid
content of the fillet (1.6%). The head and residue contained the
least amount of moisture (68.6% and 69.7% respectively) due to
their high ash content.

The figures for "Total" are all within ± 2.5 of 100% except
for the gut, and whole fish of groups I and IV. The anomalous
figure obtained for the gut (104.6%) could be explained by the
variation introduced by the fact that the gut showed the most
variations in its proportion of the whole fish and in its
composition (see Sections 4.3 and 4.4.1.2). The anomalous figures
in the totals for whole fish of groups I and IV (96 and 96.8%) are
probably due to the variation introduced by the fact that for each
group relatively few fish were available for analysis (see Table 7).

When the proximate analysis (for group II) is considered on
a dry weight basis it can be clearly seen that protein is the major
component of the fillet (88%) compared with the head, gut and

Table 16 <u>Moisture and other proximate analysis data for Haplochromis batch FEB 81</u> (values are percentages)

	Group II				Whole fish				
	head	gut	fillet	residue	Gp I	Gp II	Gp III	Gp IV	Weighted[2] averages
Moisture[1]	68.6	79.5	77.2	69.7	69.9	71.0	73.9	70.5	71.4
	(68.4,68.7)	(79.2,79.7)	(77.0,77.2)	(69.0,70.4)	(4.0)	(0.4)	(1.7)	(0.4)	
Ash (wwb)[3]	8.7	1.6	1.1	7.6	5.1	5.4	5.8	5.1	5.4
(dwb)	27	6	5	26	20	19	20	19	19
Total lipid (wwb)	8.9	12.7	1.6	4.2	5.0	5.9	5.7	4.4	5.7
(dwb)	27	51	7	14	19	21	20	17	21
Crude protein (wwb)	15.0	10.8	19.2	17.2	16.0	16.4	17.1	16.8	16.6
(dwb)	46	43	88	60	61	59	60	64	60
Total (wwb)	101.2	104.6	99.1	98.7	96.0	98.7	102.5	96.8	99.0

(1) For the head, gut, fillet and residue the values given in parentheses are the experimental values. For the whole fish the values given in parentheses are standard deviations of 3 replicates for groups II and IV and 5 replicates for groups I and III.

(2) Calculated using data in Table 7.

(3) wwb - wet weight basis; dwb - dry weight basis.

residue whose respective protein contents are 46%, 43% and 60%.
It is also significant to note that on a dry weight basis 51% of
the gut is lipid compared with 27%, 7% and 14% lipid content of
the head, fillet and residue respectively.

The proximate analysis data for the fillet of group II
indicate that Haplochromis fits with Stansby's (1961) "low oil-high
protein fish" grouping, which includes typical lean fish such as
gadoids, however the data for the whole batch, given under the
heading "Weighted averages" are more in line with fatty species
such as menhaden (protein 15.3%, lipid 6.4%, ash 4.7%) or
hard heads (Micropogon undulatus) (protein 16.3%, lipid 5.1%, ash
6.4%) (Meinke, 1974).

5.   CONCLUSIONS

It was confirmed, by gel electrophoresis, that in a single
trawl catch from Lake Victoria very many species of Haplochromis
can be present. No attempt was made to carry out analytical work
on single species, but weight was used to group the fish for
analysis. The relationship between weight and length of the fish
for the batches was determined and found to be very similar to
those obtained for single species of fish.

Data was obtained for the physical composition of Haplochromis
in terms of head, gut, skinless fillet and residue.

Although it was not possible to carry out analytical work on
as many batches of fish as had originally been planned, from the
data obtained for the four batches analysed, the following
conclusions can be drawn in relation to the aims of the present
work as set out in Section 2.4.

A.   Significant differences in chemical composition between
     groups, and therefore according to size, were only observed
     with respect to the total lipid content. Even these
     differences were less than those that have sometimes been
     observed for single species. In general, therefore, the
     Haplochromis species flock can be treated as if it were a
     single species as far as processing and nutritive value
     are concerned.

B.   The only significant difference observed for the total
     lipid content between the batches, an indication of
     seasonal variation, was for the gut and headless, gutless

portions. For the whole fish the between batch
variation was small and insignificant (5% level).
The percentage of gut in the fish was found to show
a significant seasonal variation. This increased
from October to February.

C.   The average total lipid content of the whole Haplochromis
was about 6% and approximately three quarters of this was
in the head and gut, (54% in the head and 19% in the gut).
The fatty acids profiles of the Haplochromis lipids were
predominantly unsaturated (over 60%) with about 15%
containing 5 or 6 double bonds and generally they did not
differ significantly from those of other species of fish.
These results indicate that if it can be produced economically,
Haplochromis oil has a commercial potential.
The crude protein content of the skinless fillet was about
19% and for whole Haplochromis was about 17%. The true
protein content of the whole Haplochromis was about 14%
and that of the skinless fillet about 17%. Non-protein
nitrogen accounted for about 17% of the total nitrogen in
the whole fish, the highest content being in the gut
(about 35%).
The Haplochromis skinless fillet was found to be a
particularly good source of lysine and methionine, the
amino acids deficient in cereals.
The highest percentage of ash was found in the head and
residue, due to their high bone and scale content (8.7%
and 7.6% ash respectively) and the fillet and gut contained

the least ash (1.1% and 1.6% respectively). The head and
residue contained the highest amount of calcium, and the
gut and fillet the least, corresponding to their respective
ash contents.

The lipid from the <u>Haplochromis</u> gut contained appreciable
amounts of Vitamin A_1 and not A_2 as would be expected of.
freshwater fish.

The highest amounts of moisture were found in the fillet
and gut and the least in the head and residue, due to the
respective low and high ash contents of the parts. On a
dry weight basis the crude protein was the major component
of the fillet and lipid the major component of the gut.
The data obtained in this report can be used as a basis for
determining the most effective ways of utilising <u>Haplochromis</u>.

6. <u>SUGGESTIONS FOR FURTHER WORK</u>

1) Monthly total lipid determinations should be carried out
    on fish caught from the same area of Lake Victoria in
    order to obtain detailed data for seasonal variation in
    lipid content. Particular attention should be paid to
    June-September period since it covers the Lake Victoria
    "turnover" period. Lipid analyses should also be carried
    out on fish caught from different fishing grounds to
    establish whether there is variation in the lipid content
    with fishing ground.

2) In order to assess the commercial value of <u>Haplochromis</u>
    oil, lipid class separation and further fatty acid
    profiling should be carried out.

3) Vitamin A should be determined on freshly caught fish to
    establish the actual amounts present in the various parts
    of the fish and also to resolve the question of whether any
    vitamin A_2 is present. Some other vitamins and nutritionally
    important elements (such as iodine) should also be
    determined.

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