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THE PRESERVATION OF SOME EAST AFRICAN FRESHWATER FISH

A. HOFFMAN, J. G. DISNEY, A. PINEGAR and J. D. CAMERON

Quality changes during storage were investigated for several commercially important East African freshwater fish. *Lates*, *Bagrus*, *Protopterus*, *Tilapia esculenta* and *T. nilotica* were examined during storage in ice and at ambient temperature (25°C). After 24 hours at ambient temperature *Lates* and *Bagrus* were completely spoilt but *Protopterus* was still edible. In iced storage most fish were acceptable for at least 20 days. Organoleptic examination showed that *T. nilotica* was acceptable after 22 days storage in ice and that gutting was only marginally beneficial.
Changes in physical appearance, which could form the basis of a fish inspection system, were recorded during storage. Possible chemical quality control indices were also investigated. It was found that total volatile bases and hypoxanthine are unlikely to be useful quality indices for the species studied with the possible exception of *Lates*. The bacterial counts of the flesh and skin of *T. esculenta* and *T. nilotica* were found to be low (a maximum of 10^7 organisms per sq cm of skin or per g of flesh) after 22 days storage in ice.

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INTRODUCTION

The use of ice and the application of refrigeration are gradually being introduced to the developing fisheries in Africa in response to an increasing demand for fresh fish of good quality. The realisation that fish inspection and quality control are essential for the enforcement of high quality and hygiene has followed this trend. In recent years much research has been carried out on temperate marine fish to improve quality and develop systems to monitor changes in quality. However, the relevance of this work to tropical freshwater species has not been established (JONES, 1969). Indeed, in many cases, the pattern of spoilage of the latter, under various conditions of handling and storage, has not been investigated.

The work described in this report was carried out in Uganda at the East African Freshwater Fisheries Research Organisation (EAFFRO) at Jinja and in the United Kingdom at the Tropical Products Institute (TPI) in London. The spoilage of several commercially important species was studied and the work falls into two parts.

1. The spoilage of *Lates* (Nile perch), *Bagrus* and *Protopterus* (lung fish) was investigated in Uganda.
2. Quality changes in iced *Tilapia esculenta* and *T. nilotica* were studied in fish which were sent by air to TPI in London.

MATERIALS

So far as possible fish were taken alive for these experiments in order to ensure that no prior spoilage had occurred. Although the most important method of fishing is gill-netting, fish caught in this way may spend a number of hours alive or dead in the net before they are removed. Consequently trawled or line-caught fish were used whenever possible.

The following experimental material was used:

a. Nine *Bagrus* (0.5 kg to 1.5 kg) were caught by trawl net in Lake Victoria. Three of these fish were gutted, washed and stored in ice; three further fish were iced ungutted. The remaining three fish were kept ungutted at ambient temperature (25°C.)

b. Nine *Protopterus* (4 kg to 6 kg) caught in gill-nets by local fishermen in Lake Victoria, but landed alive, were treated similarly.

c. Three small *Lates* (approximately 5 kg each) which were caught by line in Lake Kyoga, were stored at ambient temperature (25°C). One further fish caught by line was stored ungutted in ice.

d. Twenty four small *T. esculenta* (200 g) were taken alive from a trawl catch and divided into two groups. One group was gutted and washed prior to icing and the other group was stored in ice as whole fish.

e. Fourteen gill-netted *T. nilotica* (450 g) were obtained in a very fresh condition from local fishermen and similarly divided into gutted and ungutted groups. The two batches of iced *Tilapia* (groups d and e) were flown to London in insulated containers. They were sampled periodically during 22 days storage in ice.

METHODS

Visual and other organoleptic quality changes: *Bagrus*, *Protopterus* and *Lates* were all examined at intervals during storage for signs of quality deterioration. Particular attention was paid to the colour and odour of the gills (excluding *Protopterus*) and the odour and firmness of the flesh.

Visual signs of quality deterioration of both species of *Tilapia* were recorded and in addition *T. nilotica* was tasted on four occasions during the storage period. Fillets from two gutted and ungutted fish were steamed over boiling water for 30 minutes. After tasting, the sample were awarded scores according to a ten point hedonistic scale as judged by their flavour and texture, fish scoring less than four being regarded as unacceptable.

Analyses: During the experimental storage of *Lates*, *Bagrus* and *Protopterus* samples were removed for the preparation of perchloric acid extracts (DISNEY *et al*, 1969). These extracts were then frozen at –24°C for subsequent analyses in London. The neutralised perchloric acid extracts were used for the estimation of hypoxanthine (JONES *et al*, 1965).

Further extracts were prepared for the estimation of pH and total volatile bases (TVB), using the microdiffusion method of Conway (BEATTY and GIBBONS, 1937). Both species of *Tilapia* were sampled periodically during iced storage and neutralised perchloric acid extracts were prepared for the estimation of hypoxanthine.

Bacteriological examination: Changes in total viable count on the skin and in the flesh were followed during iced storage in both species of *Tilapia*. A swab wiped over 25cm of skin was washed in 10 ml of physiological saline. A 5g sample of muscle was then removed using sterile instruments. This sample was macerated in 45 ml of saline. After sampling, serial dilutions were carriep out in saline and counts were made using the pour plate method on plate count agar after incubation at 27°C for three days. A limited qualitative examination of the plates was carried out after counting based upon Gram staining and colony morphology.

RESULTS AND DISCUSSION

Preservation and fish inspection

The changes in physical appearance of *Lates*, *Bagrus* and *Protopterus* during storage at ambient temperature (25°C) are given in Table 1. *Lates* and *Bagrus* were completely spoiled after 24 hours, the point of incipient spoilage occurring after approximately twelve hours. The colour and odour of the gills and the firmness of the flesh, although they vary considerably, are the most useful criteria upon which to base a fish inspection system. Although *Protopterus* gave the impression of complete spoilage after 24 hours this was due to the odour of the surface slime. Once this was removed the flesh was firm and had a fresh odour *Protopterus* is apparently more resistant to spoilage than *Lates* and *Bagrus*.

Physical changes during iced storage of gutted and ungutted fish of the same species are shown in Table 2. Whole gutted *Lates* were still acceptable after 20 days storage when the experiment terminated. Gutted *Bagrus* was still acceptable after 20 days storage when the trial ended. There was not sufficient time to keep the ungutted *Bagrus* for more than 16 days. After 16 days the flesh on the underside was becoming soft, although the fish was not spoiled. Gutted and ungutted *Protopterus* approach the limits of acceptability after 25 days iced storage.

Previous work (DISNEY, *et al*, 1969 and unpublished work) has shown that at ambient tropical temperatures *Tilapia* become unacceptable within 24 hours. Under iced conditions *Tilapia* have been kept up to 23 days (under ideal conditions up to 4 weeks) and were still acceptable to the taste panel.

Table 1. Physical changes in *Lates*, *Bagrus* and *Protopterus* during ambient temperature storage (25°C) ungutted.

	Lates (3 fish)	*Bagrus* (3 fish)	*Protopterus* (3 fish)
Initial Condition	Gills red Flesh firm Fresh odour	Gills red Flesh firm Fresh odour	Flesh firm Fresh odour
5 hours	Gills red Flesh firm Fresh odour	Gills pink Flesh firm Fresh odour	Flesh firm Fresh odour
8 hours	Gills reddish Flesh firm Fresh odour	Gills faintly pink Flesh firm Fresh odour	Flesh firm Fresh odour
12 hours	Gills pink-grey Flesh firm Fresh odour	Gills pink-grey Flesh firm Slight off-odour	Flesh firm Slight off-odour
24 hours	Gills grey and slimy Flesh soft off-odour (completely spoilt)	Gills grey and slimy Flesh soft off-odour (completely spoilt)	Off-odour due to slime but flesh still has a fresh odour

Table 2. Physical changes in *Lates*, *Bagrus* and *Protopterus* during iced storage

	Lates	*Bagrus*		*Protopterus*	
	Ungutted (one fish)	Gutted (3 fish)	Ungutted (3 fish)	Gutted (3 fish)	Ungutted (3 fish)
1 day	Gills red Flesh firm Fresh odour	Gills red Flesh firm Fresh odour	as for gutted	Flesh firm Fresh odour Slightly slimy	as for gutted
2 days	Gills red Flesh firm Fresh odour	Gills reddish-pink, Fresh firm Fresh odour	as for gutted	Slimy body Slimy mouth Eyes glassy Fresh odour	as for gutted
3 days	Gills pink Flesh firm Fresh odour	Gills pink-grey Eyes glassy Flesh firm Fresh odour	as for gutted	Body slimy Tailfin turing blue-mauve Fresh odour	as for gutted
5 days	Gills pink Flesh firm Fresh odour	Gills pink-grey Flesh firm Fresh odour	as for gutted	Slimy Flesh firm Fresh odour	as for gutted
8 days	Gills pink Flesh firm Fresh odour	Gills pink-grey Flesh firm Fresh odour	as for gutted except flesh slightly firmer	Slimy Flesh firm Fresh odour	as for gutted
16 days	Gills pink Flesh firm Fresh odour	Gills pink-grey Flesh firm Fresh odour	as for gutted but flesh softer on underside	Slimy Flesh firm Fresh odour	as for butted
20 days	Gills pink Flesh firm No off-odour	Gills pink-grey Flesh firm Fresh odour	—	Slimy Flesh firm Fresh odour	as for gutted
25 days	—	—	—	Slimy, flesh firm, slight off-odour on the underside	underside turning yellow and soft. No off-odour

The spoilage at ambient temperature of *Lates* and *Bagrus* is similar to the *Tilapia* previously examined. *Protopterus* appears to behave differently and further work on this species would be of interest.

In *Tilapia nilotica* no organoleptic difference was apparent between gutted and ungutted fish after 14 days storage, but there was a difference after 22 days, the ungutted fish having an inferior flavour (Table 3). There was no difference in appearance between gutted and ungutted *Tilapia* stored in ice, and observations from the two treatments have been combined (Table 4).

These experiments using four important food fish, illustrate the value of ice in fish

Table 3. Organoleptic Assessement of Iced *T. nilotica* (average of 2 fish tasted by a panel of five people)

Days of Storage	Overall Score		Hypoxanthine Concentration (*Micro-moles/g*)
	Gutted	*Ungutted*	*Average of 4 fish (2 gutted and 2 ungutted) except on day 22 when only 2 fish (1 gutted and 1 ungutted) were used*
2	6.9	6.9	2.7
8	4.7	5.0	5.1
14	4.8	5.1	4.8
22	4.8	3.9	3.4

Table 4. Physical Changes in *T nilotica* and *T. esculenta* during iced storage

	T. nilotica (*gutted and non-gutted*)	*T. esculenta* (*gutted and non-gutted*)
2 days	Eyes slightly opaque. Gills pink, little mucus. Fresh fish odour.	Eyes slightly opaque. Gills crimson-pink, little mucus. Fresh fish odour.
8 days	Eyes sunken and cloudy. Gills dirty pink, some mucus. Fresh fish odour.	Eyes cloudy, beginning to become sunken. Gills dirty pink, some mucus. Slight rancid smell.
14 days	Eyes very cloudy, sunken. Gills brownish, pale at edges, copious mucus, unpleasant odour with faint marine tang.	As for *T. nilotica*.
21 days	Eyes very cloudy and discoloured. Gills brownish-pink, discoloured, very copious mucus. Unpleasant "off"-odour.	As for *T. nilotica*, although oduor not so pronounced.

preservation in the tropics; one hour at ambient temperature being roughly equivalent to one day in ice. These long periods of acceptable iced storage indicate that all the species examined could be distributed throughout East Africa in a fresh condition provided the ice is properly used. Gutting appears to be only marginally beneficial with *Tilapia* and does not significantly extend the storage life of the other species investigated.

Quality control index

The introduction of any fish inspection system will probably be based initially upon a visual examination of the fish by trained personnel, using the type of data presented in Tables 1, 2 and 4. Such a system could be supplemented by regular olfactory and tasting assessment. The deficiencies of such a system are the reliance upon a subjective judgement and the range of individual preference. In the longer term an objective quality control test may be desirable. One chemical test which has been proposed as a quality index for certain marine species is the estimation of hypoxanthine, a nucleotide degradation product (JONES *et al*, 1964). Several quality tests, including hypoxanthine, have been studied at the Tropical Products Institute for *Tilapia* spp. The accumulation of hypoxanthine has been found to correlate with organoleptic flavour scores under some conditions (DISNEY *et al*, 1969).

The changes in hypoxanthine concentration in *Tilapia* species during iced storage are shown in Figure 1. As there was no consistent difference between the gutted and ungutted fish the results have been combined in this figure. The hypoxanthine increased during the first ten days of iced storage as the quality deteriorated (organoleptic score 5). Subsequently, however, there was a gradual fall in the hypoxanthine concentration which considerably limits the usefulness of this test as a quality indicator for these species. A similar pattern has been observed previously with *T. nilotica* (DISNEY *et al*, 1969).

The usefulness of hypoxanthine levels as a quality index was also studied in *Lates*, *Bagrus* and *Protopterus*. The changes in hypoxanthine during ambient and iced storage are shown in Figures 2a and 2b. The ultimate concentration of hypoxanthine was much higher under ambient storage conditions.

The levels of hypoxanthine in *Bagrus* and *Protopterus* were very low which, therefore, reduces the value of this compound as a quality control index for those species. There was, however, a steady rise in the concentration of hypoxanthine in *Lates*. Hypoxanthine levels in *Lates* may prove to be a useful quality index for this species.

The results obtained from estimations of TVB for *Lates*, *Bagrus* and *Protopterus* showed that this test does not lend itself to be used as a quality index for these species. The values for TVB showed an increase from 0 to 0.7 mgm N/1g of muscle sample during ambient temperature storage, and from 0 to 0.26 mgm during iced storage of *Bagrus*, and from 0 to 0.26 mgm during ambient storage of *Protopterus*. There was no increase in the value of TVB during iced and ambient storage of *Lates*.

Decreases in pH values during iced and ambient storage treatments were small. That of *Bagrus* decreased from 7.2 to 6.9 during ambient and from 6.9 to 6.7 during iced storage. It decreased in *Protopterus* from 6.5 to 6.3 for ambient temperature and from 6.6 to 6.4 for iced storage. There was no change in pH value during the ambient and iced storage of *Lates*.

These results represent no more than a preliminary study on the possiblilities for the development of an objective quality test. A much more comprehensive study correlating the rate of spoilage with organoleptic testing and a number of possible physical and chemical analyses such as the thiobarbituric acid test and determinations of total volatile bases (TVB), hypoxanthine and peroxide values would be required before any firm conclusions can be drawn.

Bacteriological spoilage in Tilapia

Total viable bacterial counts in gutted and ungutted *T. nilotica* and *T. esculenta* are shown in Figure 3. These results confirm earlier findings on *Tilapia* species (TPI unpublished information) that an initial lag phase is followed by an increase in population up to a maximum of 10^6 or 10^7 organisms per sq cm of skin or per g of flesh, after 22 days storage in ice. A similar pattern of bacterial increase has also been reported for iced *T. mossambica*. (WATANABE, 1965–66). These levels contrast markedly with figures published for temperate marine fish. Haddock, for example, has approximately 10^8 organisms per g of flesh after 9 to 10 days storage in ice (SHEWAN, 1961).

Tilapia stored in ice for 22 days was still acceptable to the taste panel, scoring 4 and above on the hedonistic scale, whereas it is well known that many species of fish from temperate and cold waters reach the limit of acceptability within 16 days of storage in ice. The microflora on temperate

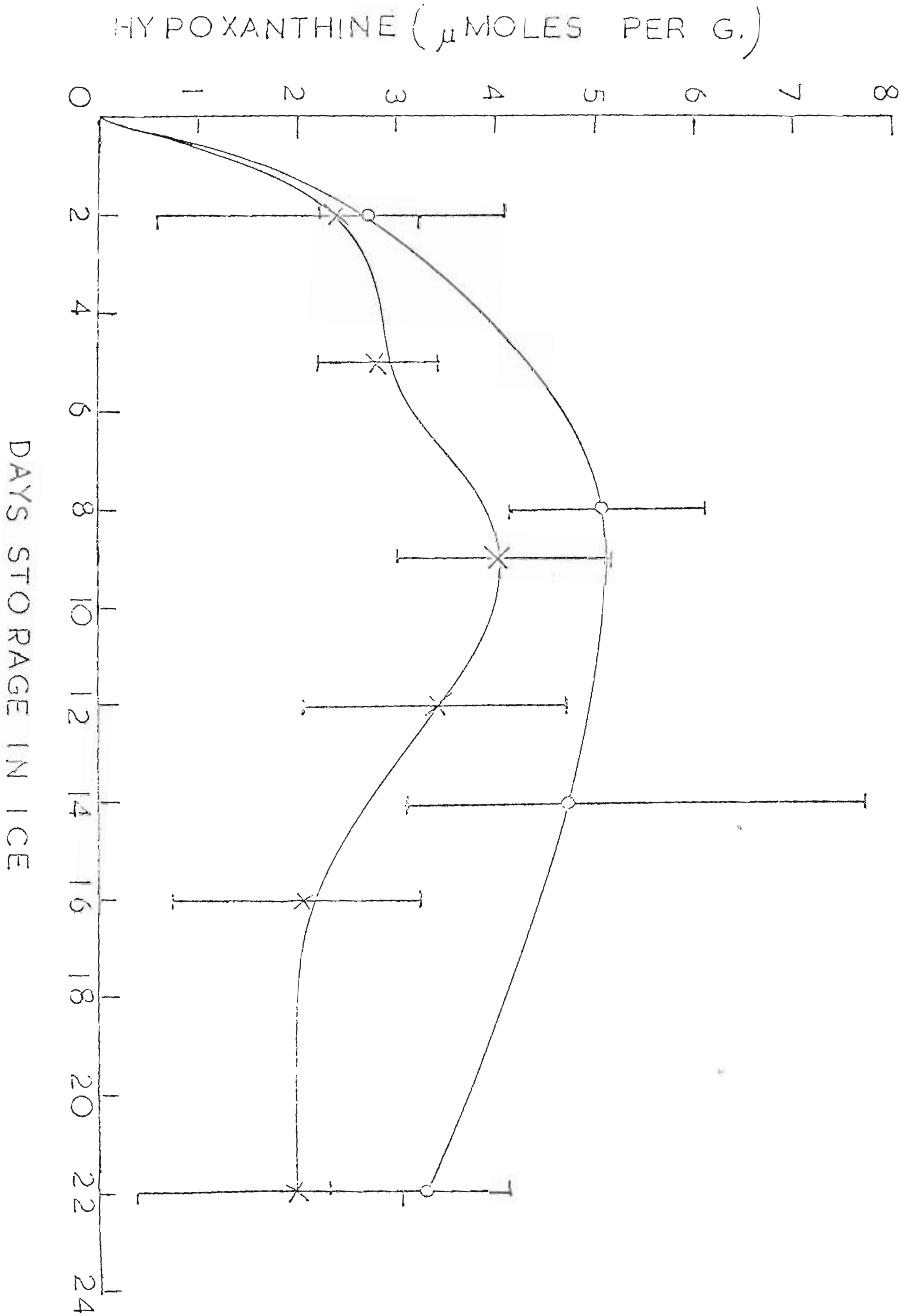
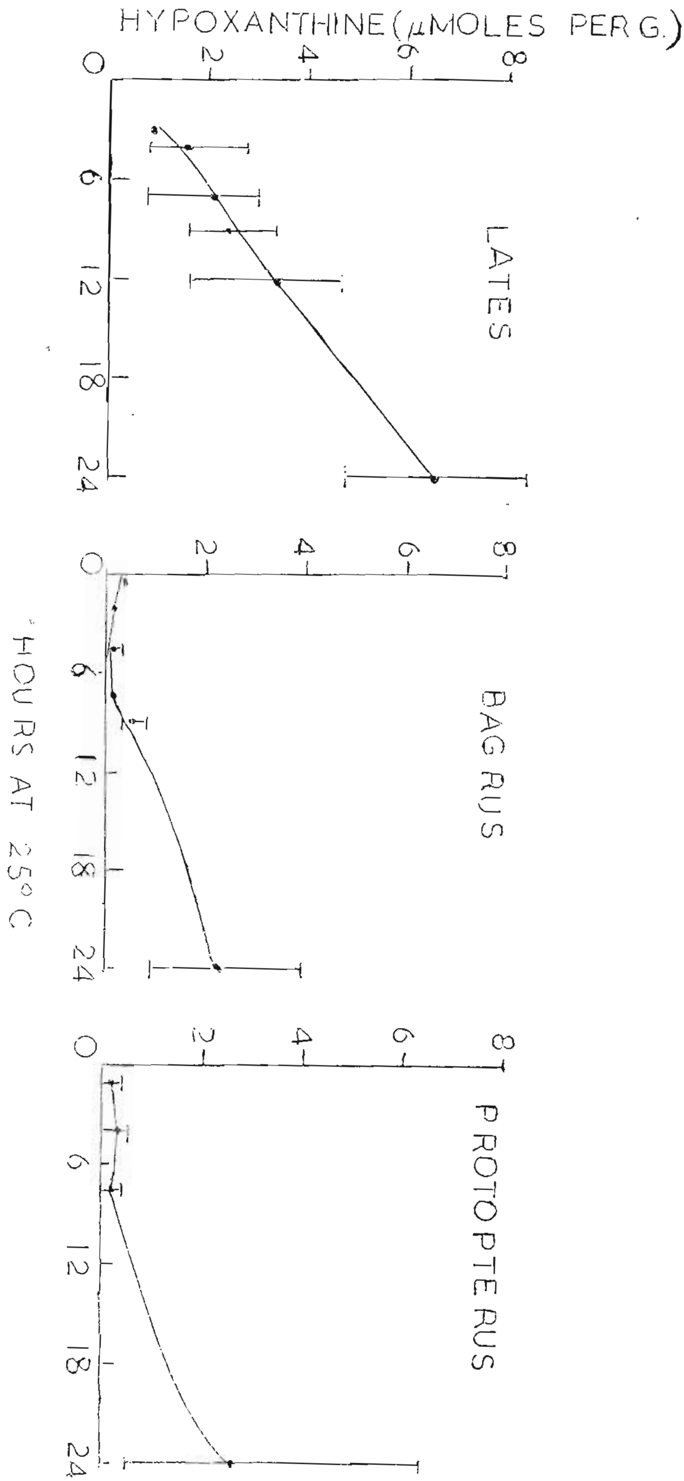


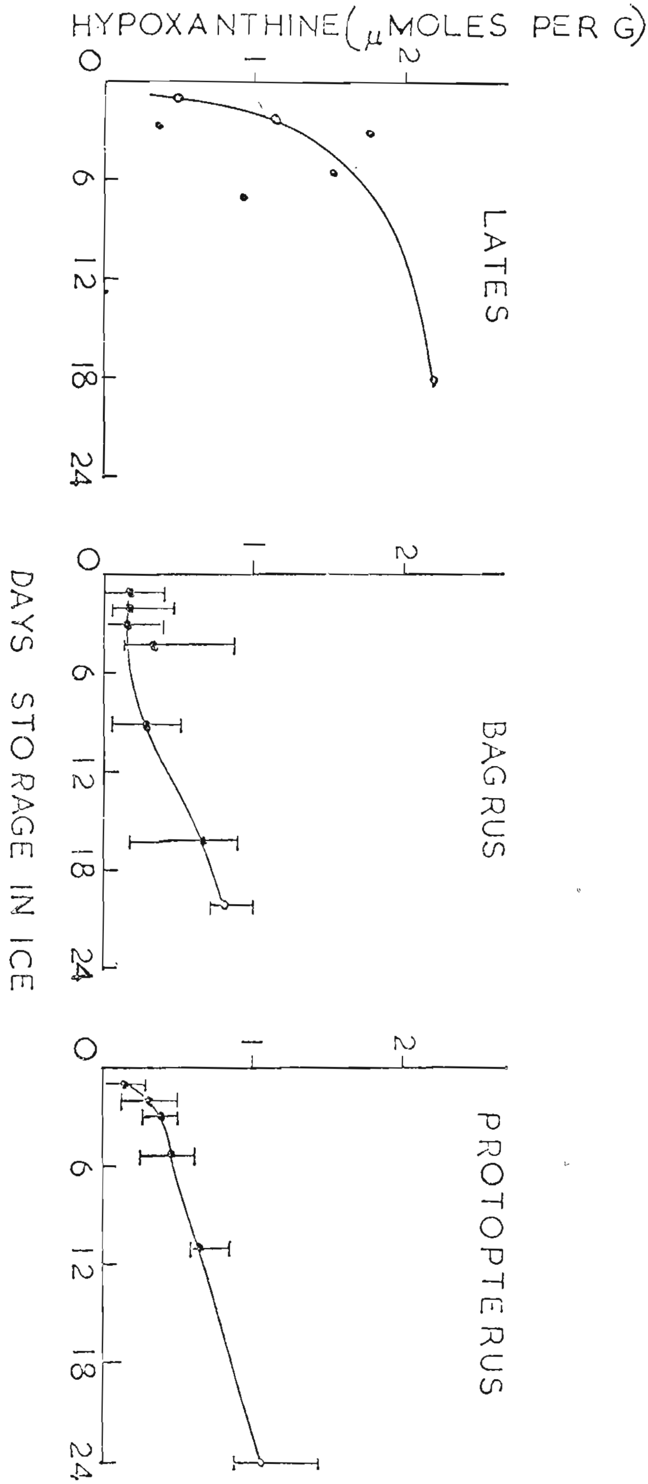
Figure 1. Tilapia esculenta and *T. nilotica*—Changes in Hypoxanthine.

× *T. esculenta.* Average of 4 fish, 2 gutted and 2 non-gutted.

○ *T. nilotica.* Average of 4 fish, 2 gutted and 2 non-gutted, except on day 22 when only 2 fish (1 gutted and 1 non-gutted) were used.



Changes in Hypoxanthine
Figure 2a. Storage at ambient temperature, 3 non-gutted fish.



Changes in Hypoxanthine
Figure 2b. Storage in ice.
Lates. 1 non-gutted fish.
Bagrus. Average of 6 fish, 3 gutted and 3 non-gutted.
Protopterus. Average of 6 fish, 3 gutted and 3 non-gutted.

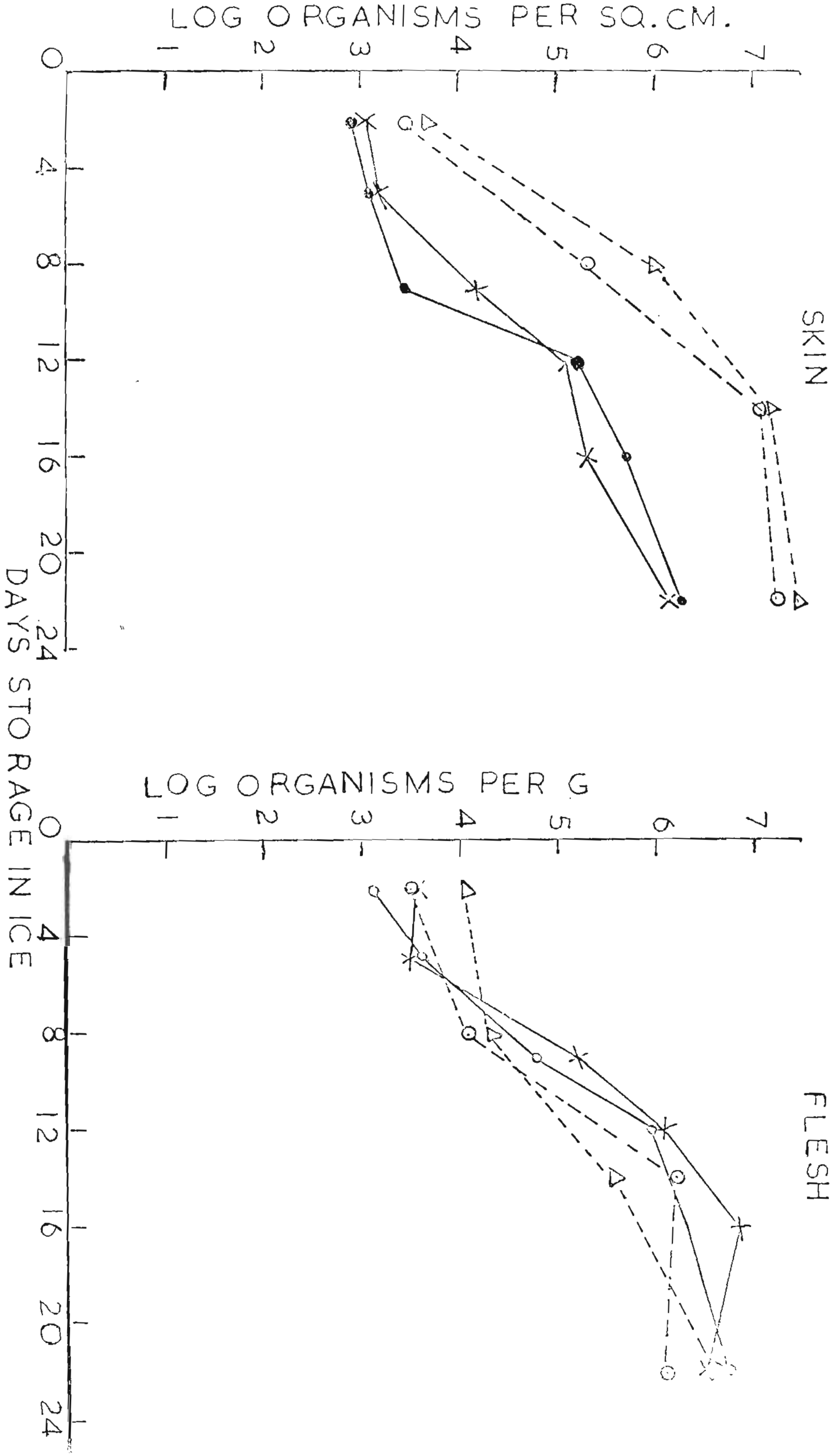


Figure 3. *Tilapia esculenta* and *Tilapia nilotica*—Changes in bacterial population (average of 2 fish).

x——x	*T. esculenta*, gutted
.——.	*T. esculenta*, non-gutted
O - - - -O	*T. nilotica*, gutted
Δ - - - - Δ	*T. nilotica*, non-gutted

fish eg. haddock appears to be adapted for survival at relatively low temperatures, while the microflora of East African lake fish is adapted to survive at temperatures of up to 30°C. If the microflora of the species examined in East Africa are less able to survive at the temperature of ice, this could account for the longer storage times observed in the present study. It has been suggested (WATANABE, 1965–66) that the flesh of *Tilapia* might contain compounds which retard bacterial growth. If so this could be an additional factor. There was no reduction in bacterial population in the gutted fish when compared with ungutted fish. This may explain why gutting produces only a slight improvement in the organoleptic quality of stored iced *Tilapia*. It has been observed that *Protopterus* is apparently more restistant to spoilage than the other three species investigated. This is perhaps due to its very tough skin which would present bacteria with a more effective barrier.

It was found that the bacterial count per 1 cm^2 of skin was higher than the count per 1 g of flesh when larger *Tilapia nilotica* of average weight of 450 g were examined, but the reverse was true for the smaller *T. esculenta*. The high skin to flesh ratio in these small fish which results in a greater proportional contamination of the flesh may be the cause. This finding is in agreement with the more general observation that smaller fish both tropical and otherwise tend to spoil more quickly.

The preliminary qualitative investigation carried out during these trials indicated that the initial microflora consisted largely of gram-negative rods such as pseudomonads, achromobacter and flavobacter. After a few days of storage in ice the population consisted entirely of gram-negative rods which were distinguishable only by colony morphology and colour. A more detailed qualitative investigation is planned to monitor bacterial spoilage in fish held for varying lengths of time at ambient temperature prior to the application of ice.

CONCLUSIONS

The early application of ice has been shown to give a storage life of three weeks or more for several of the more important food fish of East Africa. Previous work had shown this to be true for some species of *Tilapia* and the results presented here suggest that this is true of other fish species from Lake Victoria.

This long storage life has several important commercial implications for the fisheries of Lake Victoria.

The use of ice would permit the distribution of fresh iced fish to inland areas which at present rely on dried, smoked and salted fish products, it would permit the levelling off of supplies during periods of glut and shortage and would allow an extension of fishing operations on Lake Victoria.

It has been shown that gutting of fish neither reduces the bacterial population during iced and ambient storage nor does it significantly influence the shelf life of the species studied. Thus a wide scale introduction of gutting is not justified, particularly in East Africa where there is some consumer resistance to the gutted fish. However, the merits of gutting the very large *Lates* would require a separate study.

The preliminary investigation of the bacterial spoilage of iced *Tilapia* spp. indicates that the extended shelf life of the iced fish could be due to the effect of ice upon the temperature-senstitive microflora. It is suggested that iced storage has a similar effect in the case of *Bagrus*, *Lates* and *Protopterus*.

The possible use of hypoxanthine as a chemical quality control index was investigated. Further work is required but with the possible exception of *Lates* this test appears to be of limited value.

SUMMARY

Fish inspection and quality control will become important as the use of ice and refrigeration are introduced to the fishing industries in Africa. Work was carried out on some important food fish of Ugandan lakes in order to investigate their keeping qualities and to develop quality indices.

The experimental fish were obtained alive whenever possible. Part of the work was carried out in Jinja, Uganda, where fishes of 3 different species, gutted and entire, were stored in ice and at ambient temperatue. The fish were examined for visual and organoleptic changes. pH measurements and total volatile bases (TVB) estimations were made at different times and perchloric acid extracts were prepared and frozen for hypoxanthine estimations at the Tropical Products Institute, London.

The remainder of the practical work was carried out in London. Gutted and entire *Tilapia nilotica* and *T. esculenta* fish in ice were flown to London for a storage experiment. The fish were examined visually for signs of spoilage and *T. nilotica* was subjected to periodic taste panel assessments. Changes in the viable bacterial counts on the skin and in the flesh and of hypoxanthine levels were followed during iced storage.

It was shown that the early application of ice gave a storage life of three weeks or more for all species studied. Gutting apparently had no influence on shelf life. Bacterial counts for *Tilapia* in storage were found to be low, indicating that the extended shelf life of iced fish could be due to the effect of low temperature on the microflora. It was also shown that TVB and hypoxanthine were not suitable as chemical quality indices with the possible exception of hypoxanthine determinations in the case of *Lates*.

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SOMMAIRE

Les services de contrôle de la qualité des poissons auront une importance croissante à mesure qu'on introduit en Afrique l'emploi de la glace et la réfrigération dans les industries de pêche. On a examiné quelques poissons des lacs de l'Ouganda commercialisés en vue d'évaluer leurs qualités sous conservation et pour développer des normes de qualité.

On a obtenu des poissons vifs por l'expérimentation quand c'était possible. Une partie de la recherche état accomplie à Jinja, l'Ouganda, où trois espèces de poissons, vides et entiers étaient conservés en glace à une température ambiante. On a examiné les poissons pour évaluer les altérations visuelles et organoleptiques. Les mesures de

pH et estimations des bases volatiles totales ont été faits à diverses reprises et on a préparé des extraits en acide perchlorique et les a gelés pour faire une estimation d'hypoxanthine à l'Institut des Produits Tropicaux, Londres.

Le reste du travail expérimental a été fait à Londres. Des espèces de *Tilapia nilotica* et *T. esculenta* vidées et entières étaient transportées en avion à Londres pour expérimentation sur la conservation. On a examiné les poissons pour évidence de la détérioration et a subi *T. nilotica* à une appréciation de saveur par un groupe de personnes ("taste panel") périodiquement. On a observé des changements dans les comptes bactériens viables sur la peau et dans la chair, et les niveaux d'hypoxanthine étaient examinés pendant la conservation en glace.

On a observé que l'emploi de la glace au début a pour résultat une conservation de trois semaines ou plus pour toutes les espèces etudiées. Le vidage n'a pas évidemment influencé la durée limite. Les comptes bactériens pour *Tilapia* en conservation étaient bas, ce qu'indique que la durée limite prolongée des poissons glacés pouvait être due à l'influence d'une température basse sur la microflore. On a observé de plus que les BVT et hypoxanthine n'étaient pas convenables comme indices chimiques de contrôle de la qualité avec l'exception possible des dosages d'hypoxanthine pour *Lates*.

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