QUALITY CHANGES IN FISH FROM THE SOUTH CHINA SEA: ICED STORAGE OF CHUB MACKEREL, GROUPER AND FUSILIER

by

R.G. Poulter, Linda Nicolaides and D.A. Hector
Tropical Products Institute
London. U.K.

Abstract

Quality changes during storage in ice were investigated in three commercially important fish from the South China Sea. Physical and organoleptic examination showed that chub mackerel (*Rastrelliger brachysoma*) and fusilier *(Caesio cuning)* were acceptable for up to two and a half weeks and grouper *(Plectropoma maculatum)* for four weeks. Measurement of nucleotide and total volatile base concentrations largely confirmed these finds but are unlikely, on their own, to be reliable quality indices. At the limit of acceptability, the bacterial counts of the flesh and skin (10^6-10^7 organisms per cm^2 skin or g of flesh) were lower than would be expected for temperate species.

INTRODUCTION

Ice is very widely used in the more developed parts of the world to maintain the quality of fresh fish. The increase in shelf life that is brought about by storing cold- and temperate-water fish in ice has been well researched, documented and publicized (Bramsnaes, 1965). Furthermore, much research effort has been expended in developing methods for assessing the quality of these fish (Connel, 1975).

In the developing tropical countries the situation is very different. Only relatively recently has the use of ice or the application of ice or the application of refrigeration been introduce and neither is widespread. The losses that occur through not using ice are difficult to quantify but they must be extremely high. It is ironic that in many cases the greatest losses occur in countries with the lowest *per caput* intake of protein.

The use of ice is possibly the simplest method of preserving the fish catch and benefits in the tropics are perhaps even greater than in the colder zones. Stored at ambient temperatures, the acceptable shelf life of fish in the tropics is shorter than for fish from cooler climates but the available evidence suggests that the shelf life of tropical fish, stored in ice, may be up to twice as long as that for cold- or temperate-water fish (Disney, 1976).

It is only in recent years that fish technologists have realized that the technology developed for use in cold- and temperate-water fisheries may not be appropriate for use in the tropics (Disney, 1976). The development of appropriate technology has also been hampered by the dearth of basic information on the spoilage characteristics of tropical fish.

The purpose of the present study was to determine the shelf life of three commercially important fish species from the South China Sea and to evaluate the usefulness, for tropical fish, of several objective quality tests which have been developed for cold- and temperate-water fish. This study complements similar studies carried out on Indian and West African marine species (Velankar and Kamasastri, 1956; Aldrin *et al.*, 1970; Nair *et al.*, 1971, 1974; Govindan, 1971; Amu and Disney, 1973).

MATERIALS

Chub mackerel *(Rastrelliger brachysoma* Bleeker*)*, grouper (coral cod, *Pletropoma maculatum*, Block) and fusilier (yellow-tailed fusilier, *Caesio cuning* Block) were caught in the South China Sea off the coast of Brunei. The chub mackerel were caught by gillnet on 21 February 1976 and the grouper and fusilier in pots at a depth of 36 m on 30 June 1977. The fish were placed in ice as soon as possible after capture, re-iced in insulated boxes on landing at Bandar Seri Begawan by the Brunei Fisheries Department and sent by air freight to London. On arrival at the Tropical Products Institute (three days after capture) the fish were again re-iced (approxi-

mately 1:1 fish to ice) and packed in insulated containers with provision for drainage of melt-water. The ice was topped up daily and renewed once a week. At two- to three-day intervals over a period of one month, fish (two specimens each of grouper and fusilier and five specimens of chub mackerel) were removed from the ice and samples were taken for bacterial counts. The fish were then filleted and suitable portions of fillet were put to one side for organoleptic analysis. After the dark muscle, skin and large pieces of connective tissue had been removed, the remaining fish was finally chopped with a scalpel and scissors. This finely chopped white muscle was maintained at 0^{o} C until analyzed.

METHODS

Crude protein

This was determined on 2 g samples of fish muscle using the micro-Kjeldahl method (Bradstreet, 1965); total nitrogen content was converted to crude protein content by multiplying by a factor of 6.25 (Pearson, 1970).

Total lipid and peroxide values

The lipids contained in 20 g of flesh were extracted by the Blight and Dyer method (1959) using methanol and chloroform. The peroxide content of the extracted lipid was determined using potassium iodide and sodium thiosulphate (AOA, 1965) and expressed as milli-equivalents/kg lipid.

Thiobarbituric acid values (TBA)

The TBA values of duplicate 10 g samples of fish muscle were determined by the malonaldehyde distillation method of Tarladgis *et al* . (1960). The optical density of the final solution was determined at 538 nm using an SP 500 spectrophotometer. The values were expressed as mg/kg sample.

Water content

Approximately 10 g portion of fish muscle were placed in a convection oven maintained at 105^{o}C and heated for 12 h. The weight loss was presumed to be entirely due to evaporation of water.

Ash

Fish muscle (3 g) was placed in a silica dish and charred using a bunsen burner. The charred sample was ashed in a muffle furnace at 525^{o}C.

Total volatile bases (TVB)

Perchloric acid extracts were prepared from duplicate 3 g samples of muscle (Jones *et al.*, 1965). The TVB contents were estimated by a procedure based on the micro-diffusuion method of Conway (Beatty and Gibbons, 1937) using sulphuric acid to absorb the liberated bases.

Nucleotides

Duplicate samples of fish muscle (2 g) were extracted with 20 ml of perchloric acid and hypoxanthine and other nucleotides were estimated using the TLC method of Norman *et al.* (1974).

Assessment of bacteriological load

Using a sterile template a representative area of skin was aseptically removed (25 cm^2 – fusilier and grouper cm^2 – chub mackerel) and placed inside a sterile stomacher bag. Peptone water (100 ml – fusilier and

grouper; 50 ml – chub mackerel) was added and the contents were macerated in a Colworth stomacher for min. This suspension constituted the initial dilution.

Samples of flesh were removed (5 g – chub mackerel; 10 g – fusilier; 25 g – grouper) and placed in a sterile stomacher bag together with a quantity of peptone water which resulted in a 10^{-1} dilution (45 ml – chub mackerel; 90 ml – fusilier; 450 ml – grouper). This suspension, after being 'stomached' for 1 min, constituted the initial dilution.

Tenfold dilution series were made from the initial dilutions, in sterile universal containers, using 9 ml quantities of peptone water as the diluent. Two sets of pre-poured plates (plate count agar) were inoculated with 0.1 ml of the appropriate dilution. Duplicate plates of each dilution were prepared for each set. One set of plates was incubated at 4°C and the other at 27°C. The number of colonies which had grown after 3 (27°C) and 14 (4°C) was recorded.

pH

Fish muscle (2 g) was homogenized in 10 ml of neutralized 0.005 M iodoacetic acid. The pH was measured at room temperature with a Radiometer 26 pH meter using a glass electrode. Each determination was performed in duplicate.

Taste panel

Portions of skinless fillet weighing approximately 25 g were sealed in nylon pouches and cooked in boiling water for 30 min. The taste panel, consisting of four to six experienced judges, was asked to give the samples an overall acceptability score; 10 being good, 1 being bad, 4 being just acceptable.

Physical examination

At intervals during the storage period each fish species was examined for signs of quality deterioration. Particular attention was paid to the appearance of the eyes and skin, the colour and odour of the gills and the firmness of the flesh.

Objective physical quality assessment

A GR-Torry meter was used (Gr-International Electronics Ltd., Camberley, England). The dielectric properties of fish tissue change progressively during post rigor storage. The Torry meter responds to the dialectric changes and is claimed to provide an accurate measurement of the freshness of fish. For each assessment, four fish were taken and four readings made of each fish. The 16 readings thus obtained were averaged electronically by the instrument and read off directly.

RESULTS AND DISCUSSION

The weight of the fish and their proximate composition are given in Table 1. The weight of the fish, particularly the grouper and fusilier, was quite variable (i.e., a factor of 3 between the largest and smallest) but it is inevitable that there will be size variation when working with fish caught by small-scale artisanal fishermen.

Despite the fact that chub mackerel is termed a fatty fish the lipid content of the flesh was relatively low. The lipid and water contents of fatty fish vary inversely throughout the year and the mackerel studied here must be considered to be 'lean' specimens. Unlike chub mackerel, which is a pelagic fish, grouper and fusilier are demersal fish and their flesh lipid contents were less than one percent.

The quality of the fish was assessed at intervals during iced storage by physically examining the whole fish

and by tasting portions of cooked fillet. Although both methods are subjective in nature, they are in the long run perhaps the most important methods of determining quality since it is on the basis of such assessments that the consumer accepts or rejects the fish. The results (physical examination, Table 2; taste panel, Fig. 1) indicate that grouper remained acceptable for about four weeks whereas fusilier and chub mackerel remained acceptable for only two and a half weeks. For comparable cold-water fish species, e.g., cod (*Gadus morhua)* a large demersal fish and mackerel *(Scomber Scombrus)* a small pelagic fish, the acceptability life in melting ice would be about two and one week respectively (Bramsnaes, 1965; Stansby and Lemon, 1941). The results therefore confirm the conclusion of other investigators (Disney, 1976) that tropical fish have a much longer shelf life in ice than cold-water species. It is not clear why the fusilier and grouper, two demersal fish of similar fat content, should have such different shelf live. It may be that the size of the fish is important (Hoffman *et al.*, 1974) but no systematic study has been made.

As fish spoil, there is a progressive breakdown of the flesh constituents as a result of the action of endogenous enzymes and the growth of bacteria. The study showed that the bacterial population on the skin increased gradually during the first two weeks of storage (Fig. 2 and 3), from 10^3/cm^2 skin (27°C) to 10^5 bacteria/cm^2 skin (27°C) on chub mackerel and fusilier and to 10^6 bacteria/cm^2 (27°C) on grouper. After this the counts reached a plateau around 10^7 bacteria/cm^2 skin (27°C) for the three species examined. This is probably due to a washing effect of the melting ice which removed a proportion of the bacteria from the skin surface.

Invasion and growth of bacteria in the muscle tissue of tropical fish is slow (Fig. 2 and 3). After one week's storage in ice the flesh was found to be virtually sterile in all three species. Then followed a steady increase to between 10^6 and 10^7 bacteria/g after 3-4 weeks. This is very different from results obtained with ice-stored temperate fish where a bacterial load of around 10^8/g is reached within 10 to 14 days (Shewan, 1971).

At the beginning of the storage trial, the number of bacteria able to grow on plates incubated at 4°C was lower than the number of bacteria which grew on plates incubated at 27°C. After six days' storage in ice the number of bacteria monitored at 27°C showed a slight decrease. At this point it is probable that the species which were present in the original skin flora could not tolerate the low storage temperatures (0°C) and were eliminated. After about one and a half weeks' storage in ice, counts monitored at both 4°C and 27°C were virtually the same. This shows that psychrophilic bacteria were now predominant.

Various chemical and physical tests have been suggested as objective means of assessing fish quality but, as with many other aspects of fisheries research, most of them have been developed for use with cold- or temperate-water species. Several potential quality control dices were investigated in the present study.

The degradation of fish flesh during spoilage results in a gradual increase in volatile bases, mainly di- and trimethylamine and ammonia. TVB values of 30-40 mg percent have been suggested as the limit of acceptability for temperate- and cold-water fish (Connel, 1975). The TVB results for the three species studied here are shown in Fig. 4. For goruper and fusilier the values varied between 10 and 35 mg percent but there appeared to be no upward trend during storage. For chub mackerel, on the other hand, the values started at 15-20 mg percent and gradually rose to 55 mg percent after four weeks. It is interesting to note that the 30 mg percent level was reached after two and a half weeks of storage. e.e., at the point of incipient spoilage. If the TVB results of the three species studied here are considered with other studies carried out by the Tropical Products Institute (Amu and Disney, 1973; Hoffman et al., 1974; Maynell, in pres), the results for 11 species are available. Of these, measurement of TVB appeared to be a useful index of quality in only four species. The reason for the absence of any significant change in TVB values in the majority of tropical fish species examined is not clear.

The changes in pH during iced storage of the three fish species under investigation in the present study are shown in Fig. 5. The pH of grouper rose by approximately 0.5 of a pH unit, that of fusilier by 1 pH unit and that of chub mackerel by 1.5 pH units. Although the pH of fish muscle can be affected by a great many post mortem biochemical reactions, it is surprising that such relatively large pH increases in grouper and fusilier were recorded without a concomitant rise in TVB. Although the initial post rigor pH of fish muscle varies considerably, depending on the nutritional state of the fish, degree of struggling prior to death, etc., the changes in the muscle

pH of the three species studied here correlated with the taste panel scores more closely than any of the other objective quality assessment methods.

The estimation of hypoxanthine (Hx) has also been proposed as a measure of fish freshness (Jones *et al.*, 1964). Hypoxanthine is formed in fish muscle mainly as a result of autolytic degradation of adenosine triphosphate (ATP). However, the last step in the pathway, inosine to hypoxanthine can be brought about by both autolytic and bacterial enzymes (Jones, 1966). Fig. 6 shows the inosine monophosphate, inosine and Hx levels in grouper, fusilier and chub mackerel at invervals during one month's storage in ice. The Hx content of chub mackerel increased in a regular manner during the first three weeks of storage but dropped slightly in the last week. Grouper and fusilier, on the other hand, had much lower initial Hx levels but they showed a slight upward trend during storage. At the point of incipient spoilage, the Hx levels were between 1.2 and 1.5 moles/g for grouper, 1.0 and 1.3 moles/g for fusilier and 9.3 and 10.3 moles/g for chub mackerel. In grouper and chub mackerel, this point also corresponds to the maximum level found during storage. The levels of Hx have been found to differ widely between four West African marine species (Amu and Disney, 1973). These authors also found that different sized fish of the same species had different Hx contents but concluded that the measurement of hypoxanthine was potentially useful as a quality test. The results of the present study, shown in Fig. 6, indicate a different mode of ATP degradation in grouper and fusilier compared with chub mackerel but further work is required to determine whether this difference is real and what effect it would have on the usefulness of Hx measurement as a means of assessing fish quality.

An estimate of the degree of rancidity in the fish during storage in ice was obtained by measuring the malonaldehyde content (TBA value) for grouper and fusilier and the peroxide value for chub mackerel (Table 3). Grouper and fusilier are lean fish and, although the TBA values showed a high degree of variation, they were generally low and did not reach the level of 1.80 mg/kg which has been designated the 'just spoiling' value for beef (Pearson, 1968). The peroxide values for chub mackerel are more difficult to evaluate. First, peroxides are intermediate lipid degradation products which build up initially during storage and then decline. This also means that very rancid materials may have low peroxide values. Second, peroxide values are always quoted as milliequivalents/kg fat. Just spoiling values of 10-20 m equivalents/kg fat, depending on the type of fat, have been suggested (Pearson, 1970). This is the range of values at which the fat itself tastes rancid. It does not mean that when distributed throughout the flesh of fish at a concentration of 1 or 2 percent, as in chub mackerel, the flesh itself will taste rancid. However, during the first 2-3 weeks of storage in ice, there was a steady build-up of peroxides in the chub mackerel flesh and this must be a factor contributing to the decrease in taste acceptability.

The Torry meter readings for the three species are shown in Fig. 7. In grouper the readings fell progressively from a value of 14 after three days in ice to a value of 6 at the point of incipient spoilage, i.e., after 28 days in ice. This corresponds fairly well to the taste panel's results and, although the time scale is different, the range in values is similar to that obtained for cod (Cheyne, 1975). Amu and Disney (1973) also found that the Torry meter worked well for sea bream, a West African marine species. For the other two species studied in the present work, the change in values with time was very small and, particularly for chub mackerel, the readings were very low. Compared with other objective methods of measuring fish quality the Torry meter has the advantage of being simple to operate, providing a rapid result and the sampling is non-destructive. The present results indicate that many more data, particularly with tropical species, must be collected before the true worth of the Torry meter can be assessed as a reliable means of evaluating fish quality.

CONCLUSIONS

Storage in ice under laboratory conditions has been found to give a storage life for 3 fish species from the South China Sea of between two and a half and four weeks. Although this period would be reduced under commercial conditions it should still be sufficient to permit capture, storage and transport, over considerable distances. This, of course, requires ice to be widely available which, unfortunately, is often not the case. Nonetheless, this and other similar studies indicate the particular benefits to be gained from storing tropical fish in ice and, hopefully, will encourage the introduction of more ice plants and refrigerated holding facilities in the developing world.

A number of subjective and objective quality assessment tests have been considered. Simple tests to determine changes in taste acceptability and physical appearance were found useful in determining quality and would be particularly suitable for routine quality assessment in developing countries. Of the objective methods studied, none was found to correlate perfectly with the taste panel results but several showed promise.

The bacterial numbers increased in a fairly regular manner during the storage period but the dis-advantage of using total viable bacterial counts as a quality index is that by the time the results are available (3 or 4 days after sampling) the quality of the fish will have significantly changed. The results of microbiological examinations made in this study are particularly interesting; however, since they support earlier suggestions (Disney, 1976; Shewan, 1977) that the extended shelf life of tropical fish in ice, compared with temperate- or cold-water species, is due to the fact that the naturally occurring bacterial flora contain only a low proportion of psychrophilic bacteria.

Of the other objective methods, measurement of total volatile bases gave very variable results and, although the pH of the flesh of all three species increased in a fairly regular fashion, variation in initial pH between fish of the same species would probably made it unsuitable for use as a quality index. Measurement of rancidity is important in fatty fish such as chub mackerel but it has been shown that if the peroxide method is used the interpretation of single values must be treated with care. However, the TBA method did not prove to be a substantially better method of measuring rancidity in iced fish since the results obtained with grouper and fusilier were quite variable.

The Torry meter worked extremely well with grouper but, possibly because of their smaller size, not so well with the other two species. Experiments with this instrument should be undertaken on a wide range of tropical fish types and on different sizes of the same species. If it can be shown to be accurate and reliable it could provide a good method for assessing the quality of wet fish.

Hypoxanthine levels were found to correlate very well with taste panel results in one of the species examined but not so well in the other two. Again data for a much larger range of fish are required before definite conclusions can be drawn on the usefulness of Hx as an index of fish quality.

This investigation has added three more species to the list of tropical fish which have been the subject of iced storage trials. In relation to the number of commercially important fish species in the tropics, this list, though growing, is still extremely small. Furthermore, there is almost no published work on the effect of season of capture, plane of nutrition, etc., on the iced storage characteristics of tropical fish. This paper therefore ends with a plea to institutes involved in fishery research in the tropics to study the iced storage characteristics of fish which are commercially important in their countries and above all to publish the results.

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Table 1

Weight and proximate composition of grouper, fusilier and chub mackerel

GROUPER	Mean	Range	Number of replicates
Weight (g)	2,600	1,200-4,200	16
Crude protein (% w/w)	20.0	19.2-20.7	4
Total lipid (%w/w)	0.9	0.6-1.2	4
Moisture (% w/w)	78.5	77.5-78.8	4
Ash (% w/w)	1.1	0.9-1.4	4
FUSILLER			
Weight (g)	400	250-700	16
Crude protein (% w/w)	17.4	16.1-18.7	4
Total lipid (% w/w)	0.7	0.5-0.9	4
Moisture (% w/w)	79.2	78.4-80.2	4
Ash (% w/w)	1.0	0.8-1.3	4
CHUB MACKEREL			
Weight (g)	110	80-140	40
Crude protein (% w/w)	8.0	16.7-19-1	8
Total lipid (% w/w)	1.9	0.9-3.3	22
Moisture (% w/w)	78.2	76.5-18.2	8
Ash (% w/w)	1.2	1.1-1.4	4

Table 2

Physical changes in grouper, fusilier and chub mackerel during iced storage

Days in ice		Grouper	Fusilier	Chub mackerel
13/14	Eyes	Translucent	Sunken, opaque	Slightly opaque
	Gills	Pink, neutral odour, white mucus	Red, neutral odour white mucus	Pink-brown, neutral odour
	Flesh	Firm	Firm	Firm
	Skin	Bright	Bright	Bright
6	Eyes	Slightly sunken and opaque	Sunken, opaque	Slightly sunken and opaque
	Gills	Pink, neutral odour, white mucus	Red, neutral odour cream mucus	Red-brown, neutral odour
	Flesh	Firm	Firm	Firm
	Skin	Bright	Slightly dull	Slightly dull
10	Eyes	Slightly sunken, opaque	Sunken, white spot	Slightly sunken and opaque
	Gills	Red, neutral odour, some mucus	Red, neutral odour, clear mucus	Red-brown, fruity odour, some mucus
	Flesh	Firm	Firm	Firm
	Skin	Bright	Slightly dull	Dull
13/14	Eyes	Slightly sunken, opaque	Sunken, white spot	Sunken
	Gills	Red, slightly stale odour, mucus	Red, slightly stale odour, mucus	Light brown, strong fruity odour, mucus
	Flesh	Firm	Fairly firm	Slightly soft
	Skin	Bright	Dull, loose scales	Dull, loose scales
17/18	Eyes	Slightly sunken, opaque	Sunken, white spot	Sunken, opaque
	Gills	Red, sweet odour, mucus	Purple-red, strong sweet odour, mucus	Light brown, strong fruity odour, mucus
	Flesh	Fairly firm	Fairly firm	Soft
	Skin	Bright	Dull, slimy, loose scales	Discoloured, slimy, loose scales
20/21	Eyes	Slightly sunken, white spot	Sunken, white spot	Sunken, opaque
	Gills	Red, aromatic odour, mucus	Dark red, sweet odour, mucus	Light brown, putrid odour, mucus
	Flesh	Slightly soft	Slightly soft	Soft
	Skin	Fairly bright	Discoloured, loose scales	Discoloured, slimy, loose scales

Days in ice		Grouper	Fusilier	Chub mackerel
24/25	Eyes	Slightly sunken, white spot	Sunken, mucus	Sunken, opaque, splitting
	Gills	Red, putrid odour, mucus	Dark red, putrid odour, mucus	Brown, strong putrid odour, mucus
	Flesh	Slightly soft	Soft	Soft
	Skin	Dull, few loose scales	Discoloured, loose scales	Discoloured, slimy, loose scales
27/28	Eyes	Sunken, completely opaque	Swollen, yellow	Swollen, split
	Gills	Red, putrid odour, mucus	Dark red, putrid odour, mucus	Brown, strong putrid odour, mucus
	Flesh	Slightly soft	Soft	Soft
	Skin	Slightly discoloured, few loose scales	Discoloured, slimy, loose scales	Discoloured, slimy, loose scales

Table 3

Effect of storage in ice on the peroxide values of chub mackerel and TBA values of grouper and fusilier

Days in Ice	Peroxide values (milli equivalent/ kg fat)		TBA (mg/kg)			
	Chub mackerel		Grouper		Fusilier	
	Mean	Range	Mean	Range	Mean	Range
3			0.48	0.43-0.53	0.42	0.41-0.44
4	33.3	25.0-41.8				
6	27.6	20.7-34.4	0.82	0.78-0.86	0.74	0.62-0.87
10	39.2	29.4-48.9				
11			0.32	0.27-0.39	1.10	0.82-1.37
13	52.1	49.9-54.3				
14			0.17	0.16-0.18	0.20	0.18-0.22
17	31.4	29.4-33.7				
18			0.15	0.13-0.18	0.26	0.23-0.30
20	57.0	46.4-67.5				
21			0.66	0.59-0.73	0.96	0.94-1.01
24	23.2	17.9-28.5				
25			0.47	0.46-0.48	0.61	0.60-0.62
27	20.6	20.4-20.7				
28			0.27	0.27-0.27	0.74	0.73-0.75

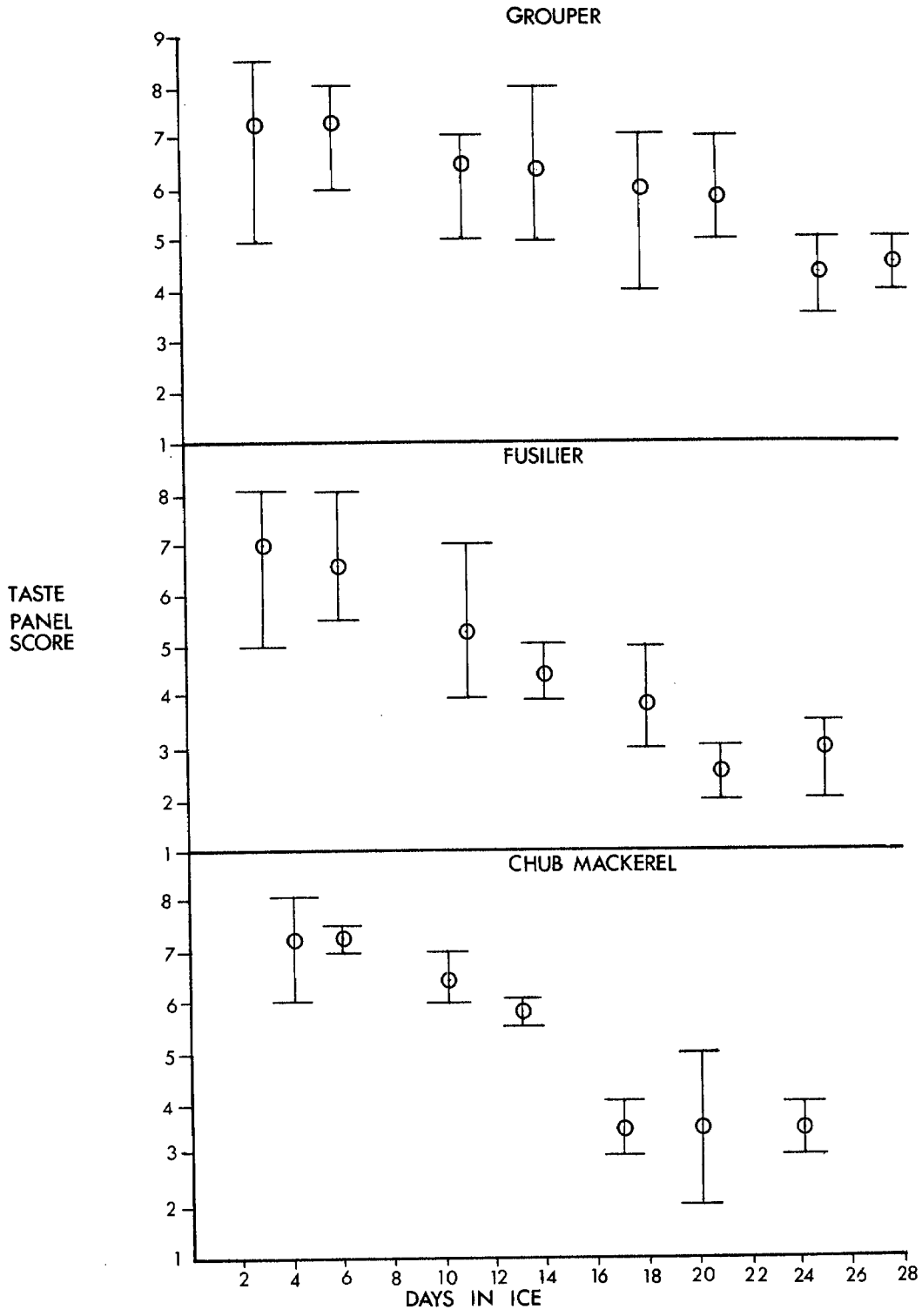

Fig. 1 – The effect of storage in ice on overall taste panel scores.

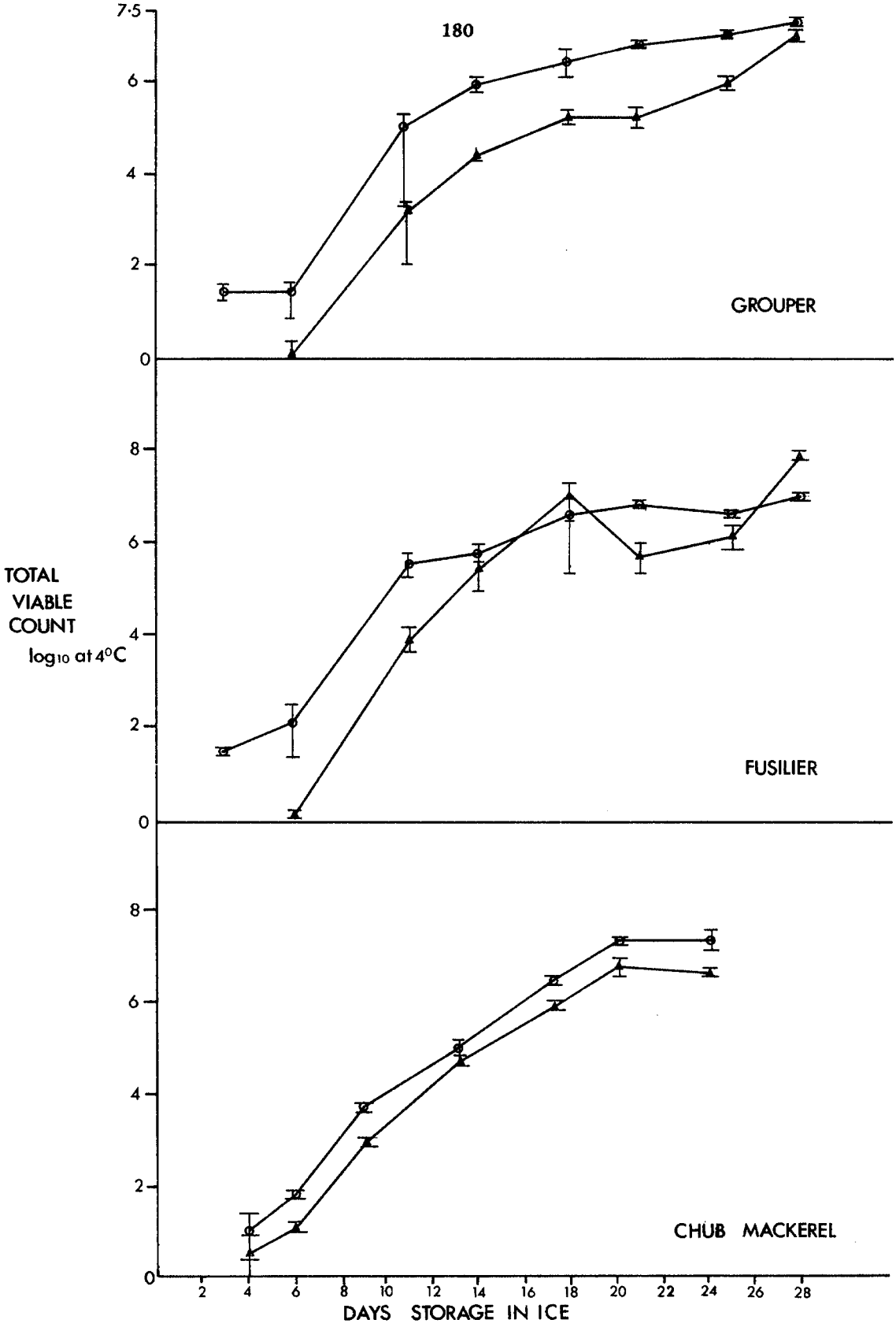

Fig. 2 – The effect of storage in ice on total viable counts of skin and flesh (4°C).

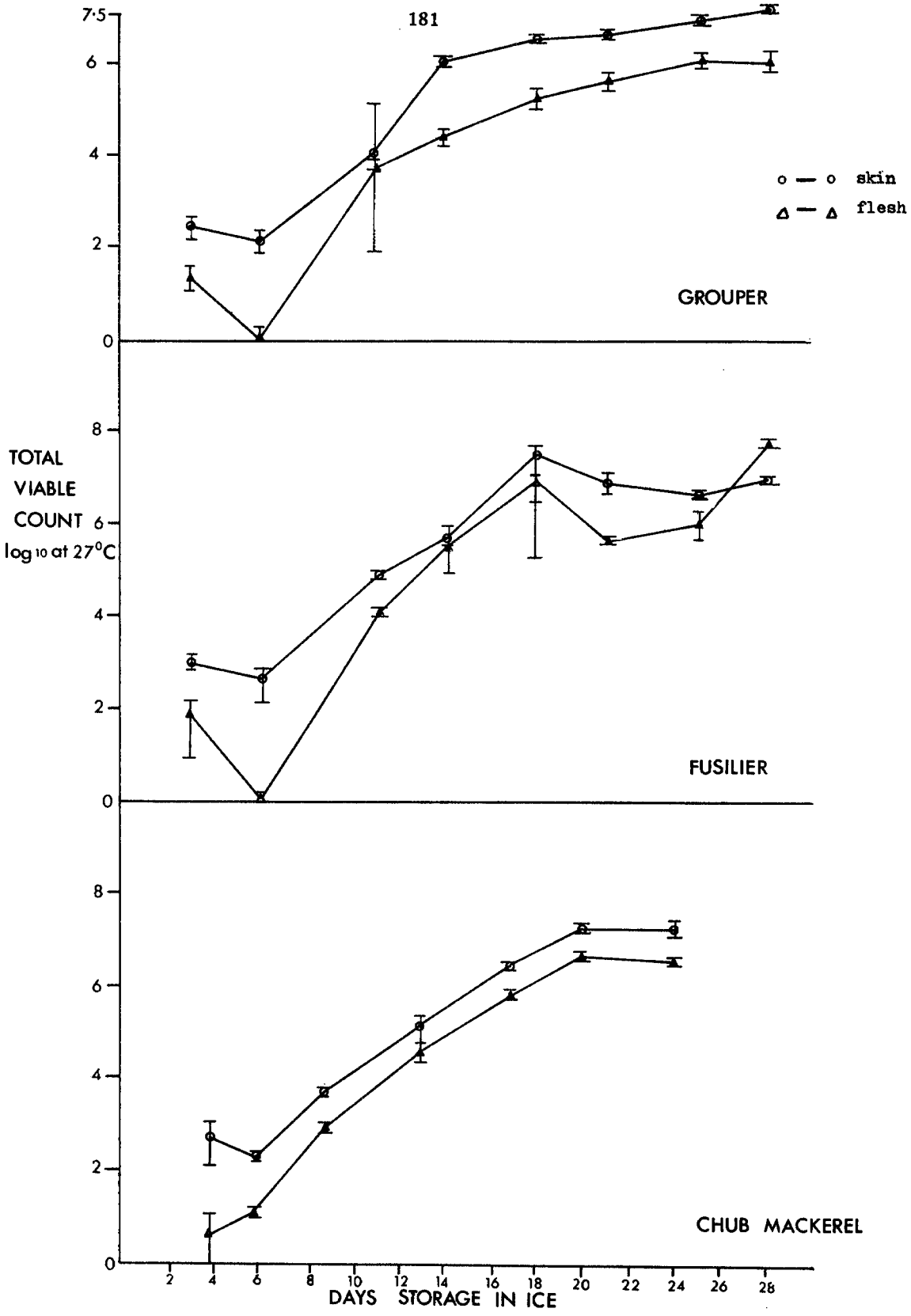

Fig. 3 — The effect of storage in ice on total viable counts of skin and flesh (27°C).

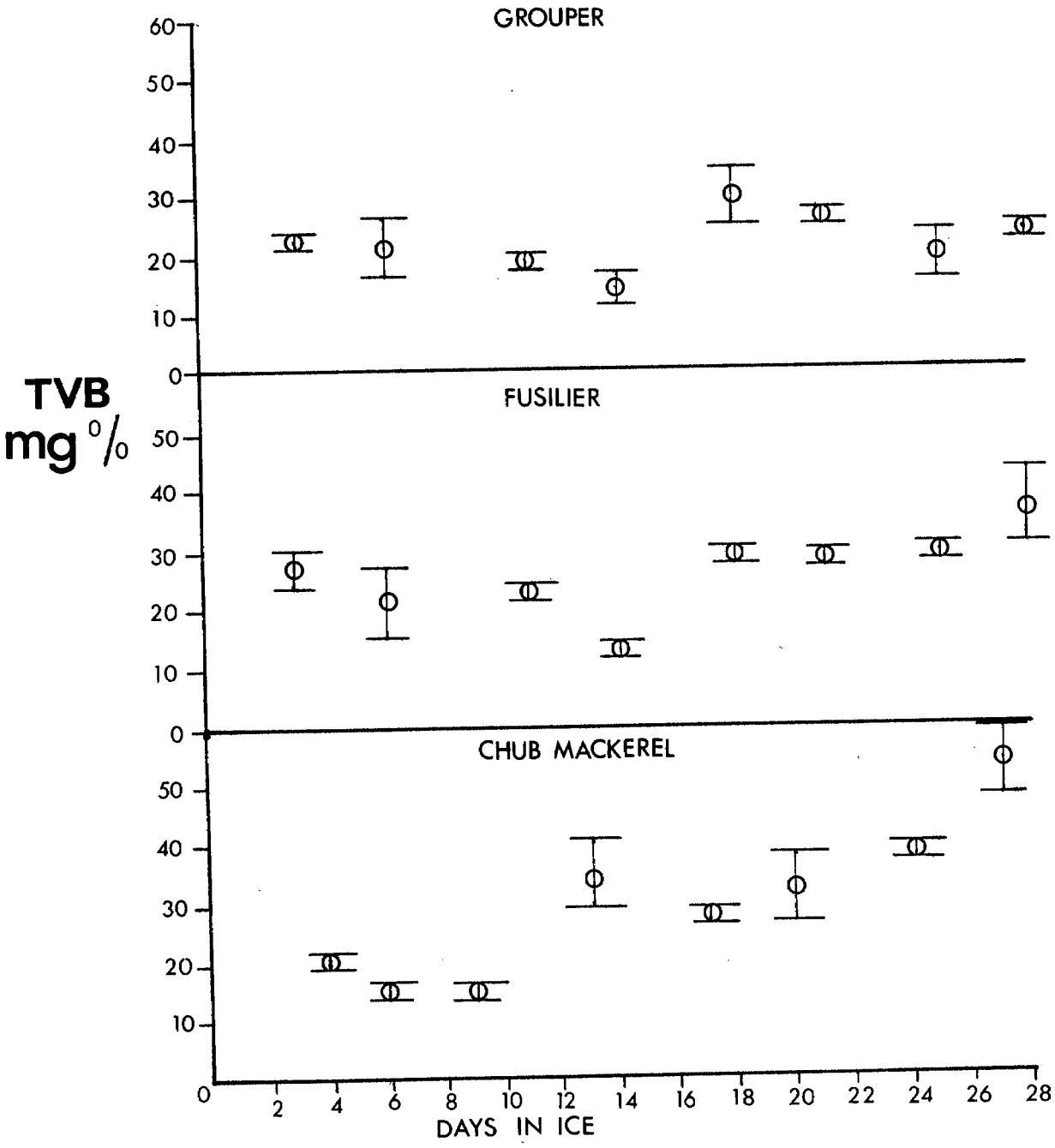

Fig. 4 – The effect of storage in ice on total volatile base (TVB).

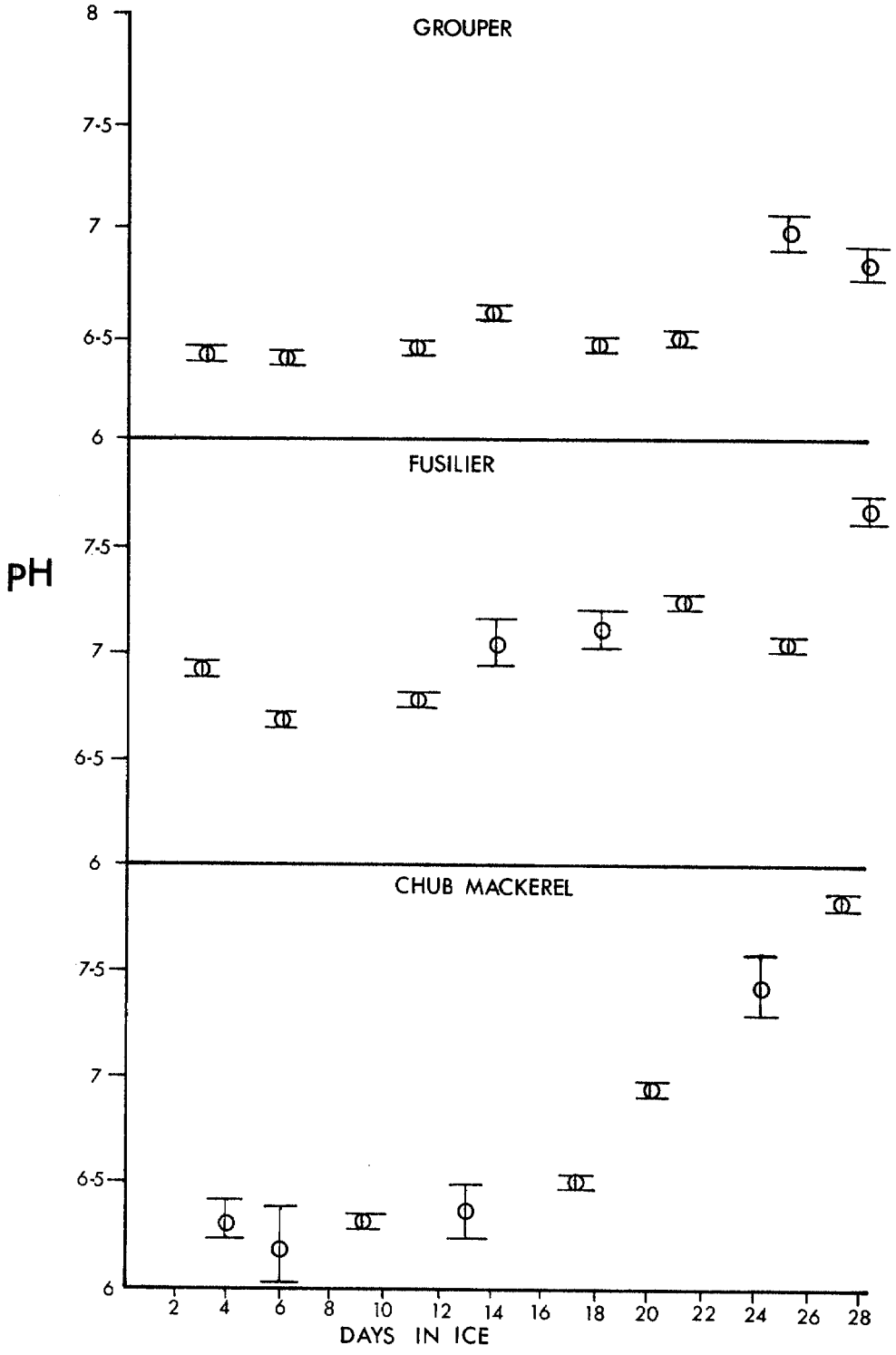

Fig. 5 – The effect of storage in ice on p^{h}.

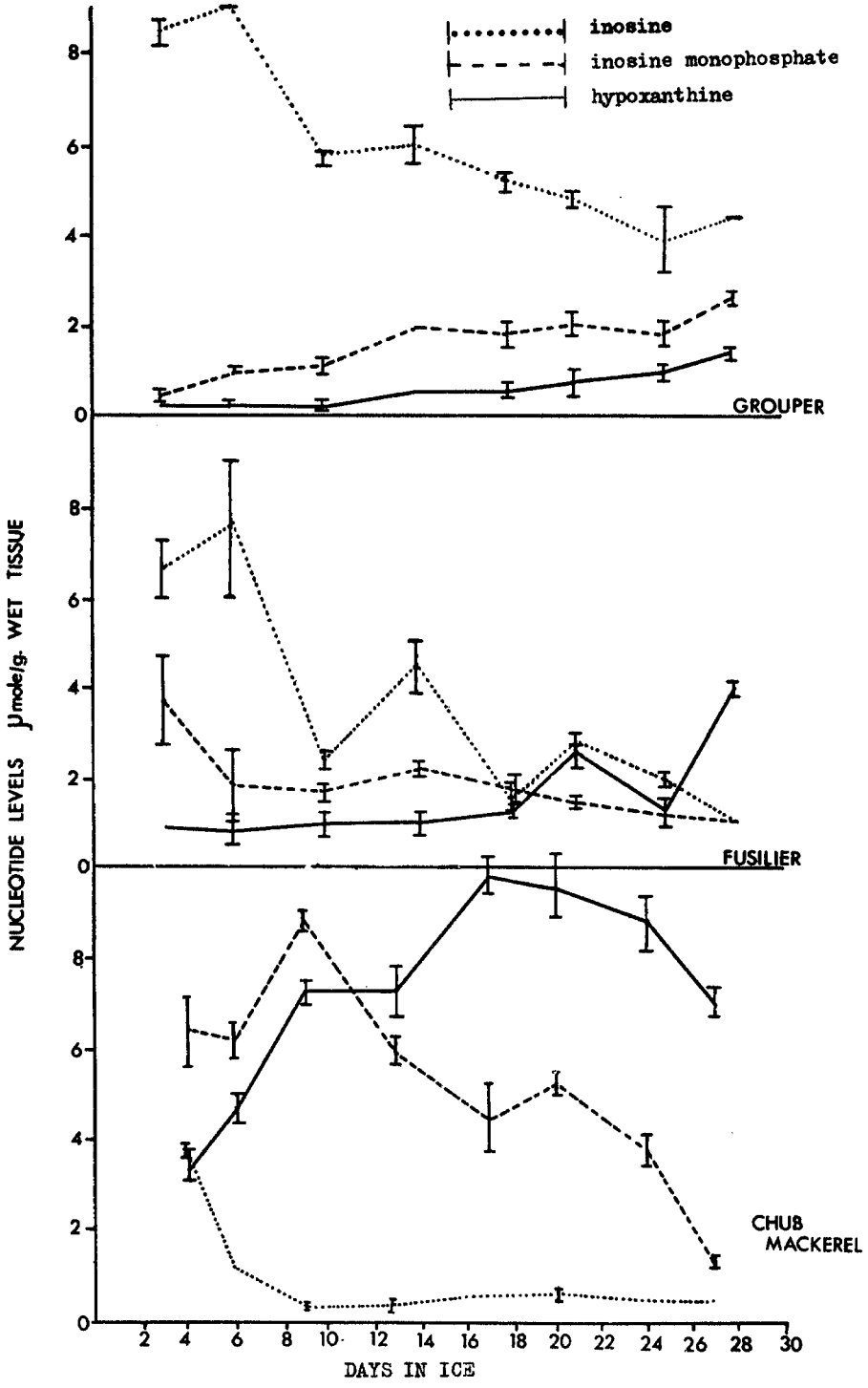

Fig. 6 — The effect of storage in ice on nucleotide levels.

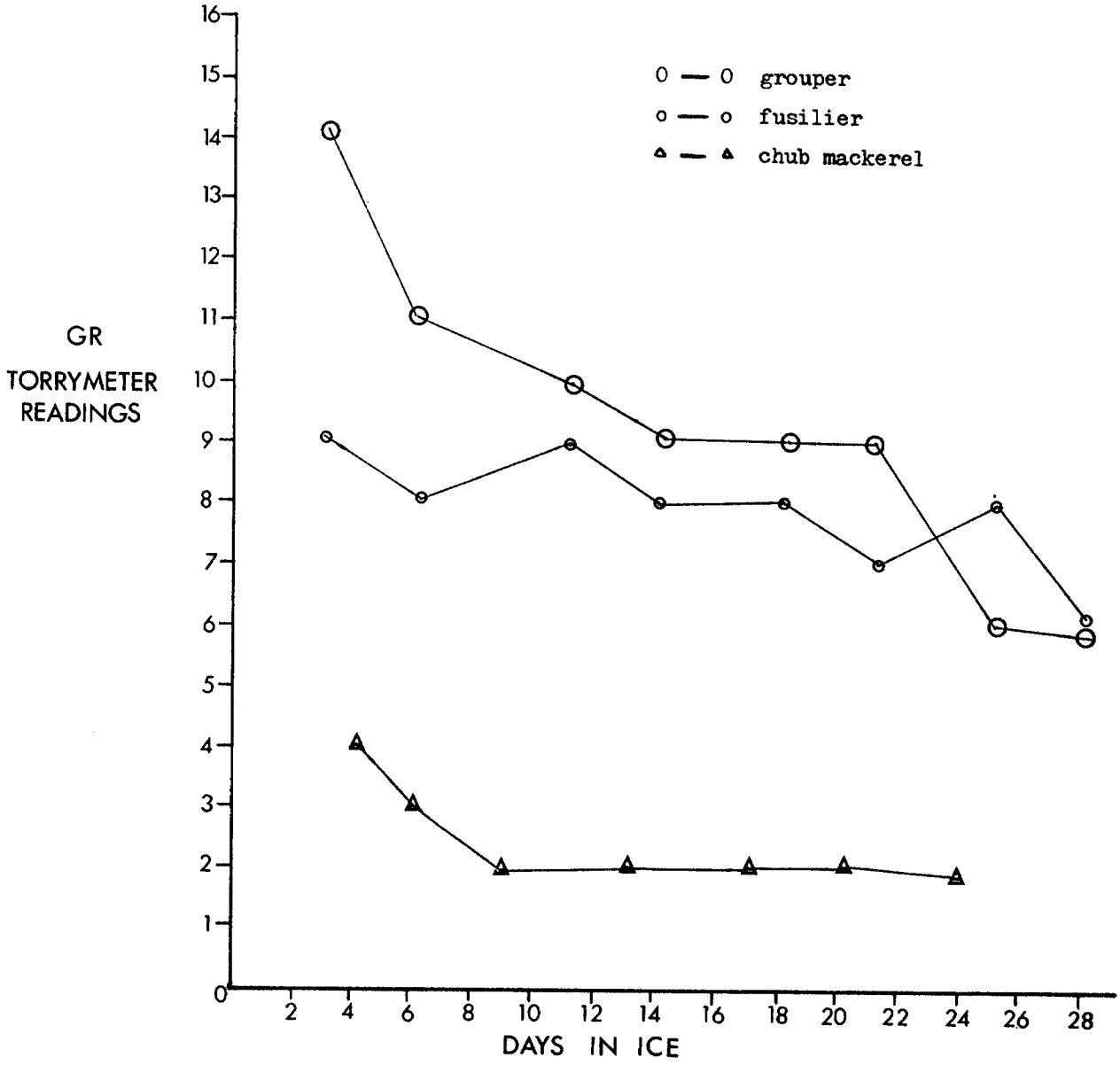

Fig. 7 -- The effect of storage in ice on GR Torrymeter readings.