1972/8.

MICROBIOLOGICAL CONSIDERATIONS IN SHARK HANDLING

BY

MR. A.S.J. YAP*

SOUTH AUSTRALIAN FISHERMEN'S CO-OPERATIVE LIMITED

30-46 Wright Street,

Adelaide, South Australia, 5000

* Present Address:

Roseworthy Agricultural College,
Roseworthy, South Australia, 5371

INTRODUCTION

The spoilage flora of shark flesh has been studied by Wood (1950). He and others (James & Olley, 1971) attributed the spoilage of shark to urease-producing bacteria.

The purpose of this study was to determine bacterial numbers and flora of shark and of the environment in which it is caught, processed and stored. It was hoped that the data would be of value in the handling and processing of this fish.

MATERIALS

The School Shark used in Experiment 1 was obtained from the Adelaide Fish Market. The shark, which had been held in iced storage for 3 days after gutting, was in good condition.

Sharks for Experiments 4 and 5 were caught during a fishing trip off the coast of Streaky Bay. All materials were sent to Adelaide in an iced container and plated within 24 hours of sampling.

Bacto Marine Agar 2216 (Difco) was used to isolate, purify and enumerate bacteria in Experiments 1, 3, 4 and 5. Nutrient Agar (BBL) was used in Experiment 2. Inoculated plates and slants were incubated at 20-22°C for 5 days. 0.1% peptone water was used for preparing serial decimal dilutions.

METHODS

Quantitative Assessment

Method I: Using a sterile double-bladed scalpel, 5 equal portions of flesh measuring 6.4 x 6.4 x 2 mm were excised from the

internal abdominal walls. All 5 portions were homogenised together in 100 ml 0.1% peptone water. Serial one tenth dilutions in duplicate were made to determine total counts.

Method II: An area 7.1 cm^2 was swabbed using a sterile cotton wool swab and an aluminium foil template, 100 x 100 mm with a central hole 30 mm in diameter. Each swab was immediately placed into 5 ml of 0.1% peptone water. Serial one tenth dilutions in duplicate were made to determine total counts.

pH Measurement.

pH measurements of the flesh in Experiment 1 were carried out using a pH meter.

Isolation and Identification of Bacteria.

20 colonies from plates carrying 100-300 colonies were randomly picked using Random Number Tables. After purification, the isolates were generically identified where possible using the identification scheme of Shewan et al, (1960a, 1960b) for screening gram-negative bacteria.

For non-motile bacteria previously grouped as *Achromobacter/ Alcaligenes* the cytochrome oxidase test was carried out to differentiate *Moraxella* & *Acinetobacter* (Baumann et al, 1968a, 1968b). Determination and confirmation of other isolates were carried out using the techniques suggested by Breed et al (1957), Skerman (1967) and Cowan and Steel (1965).

Bacteriology.

The following experiments were carried out.

1. Changes in Bacterial Numbers and Flora of Shark in Iced Storage.

   The fresh school shark was packed in ice and stored in a chiller at $0^{o}C$. Ice was not placed inside the abdominal cavity. Flesh was removed from the abdominal walls on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12. pH of the flesh was also determined. A total of 100 isolates from days 1, 3, 5, 8 and 10 was obtained.

2. Total Count and Bacterial Flora of Ice.

   Three samples of fresh ice used for icing the shark in Experiment 1 were tested.

3. Bacterial Numbers and Flora on the Deck and Well of the Boat.

   The surfaces of the deck and well of the boat were swabbed during a shark fishing trip off the coast of Streaky Bay. The wooden deck was swabbed before and after cleaning. Cleaning was effected by scrubbing with a chlorinated detergent and hosing down with sea water.

4. Bacterial Numbers on Skin, Mouth and Gill and Flora of Skin of Freshly Caught Shark.

   Two sharks were swabbed as soon as they were taken out of the water. Five places from each of the head, mid-body and tail regions were swabbed.

5. Bacterial Numbers and Flora on Abdominal Walls of Freshly Gutted Shark before and after Icing.

   Immediately after gutting and washing, flesh from the abdominal walls of two sharks was removed. One shark was iced externally, i.e. as in Experiment 1. Ice was placed

inside the abdominal cavity as well as externally in the other. After 22 hours, further flesh samples were collected from the abdominal walls of both sharks.

RESULTS

Experiment 1

The total aerobic counts for flesh of shark stored at $0^{o}C$ is shown in Figure 1. The initial bacterial count was 2.7×10^{3} per cm^{2}. Bacterial numbers did not increase significantly over the next 3 days of storage. The pH was 6.5 from day 1 to 5. Bacterial numbers rose sharply from 2.8×10^{4} per cm^{2} on day 5 to 1.5×10^{6} per cm^{2} on day 12. Correspondingly, pH also increased. Very faint ammonia odour was detectable on day 6, however on day 10 the odour was very strong.

The 100 isolates from the flesh were broadly classified into 10 groups, as shown in Table 1. Of the initial flora, the Micrococcaceae group comprised 30%, gram-negative cocci 40%, *Achromobacter/Alcaligenes* 5%, *Flavobacterium/Cytophaga* 10%, gram-positive rods 10% and *Pseudomonas* 5%. *Moraxella* and *Acinetobacter* were isolated from day 3 onwards. The *Pseudomonas* group showed an increasing predominance during spoilage, reaching 70% of the total organisms present after 8 days. The second prominent group was *Moraxella*. The percentage of urease-producing bacteria increased from an initial 5% to 25% after 8 days. Of the urease-producing bacteria, 80% belong to the *Pseudomonas* group and the remainder to the *Moraxella* group.

Experiment 2

The average total aerobic count of ice was 2.1×10^{2} per ml. The 20 isolates were classified into 5 bacterial groups (Table 1).

They were *Pseudomonas* 10%, gram-negative cocci 5%, Micrococcaceae 35%, *Moraxella* 35% and *Acinetobacter* 10%. One isolate was not identified. 30% of the isolates were urease-producing, made up of 15% Micrococcaceae group, 10% *Moraxella* group and 5% of which were not identified.

Experiment 3.

There was a drastic reduction in numbers of bacteria on the wooden deck from 1.3×10^4 per cm^2 before cleaning to 89 per cm^2 after cleaning. The well had 1.6×10^3 aerobic bacteria per cm^2.

The bacterial flora on the deck and well was classified into 7 groups (Table 2). The *Achromobacter/Alcaligenes* group (40%) showed a predominance on the deck. The other groups were *Pseudomonas* 20%, gram-negative cocci 15%, Micrococcaceae 10%, *Moraxella* 10% and gram-positive rods 5%. Of the viable isolates from the well, *Moraxella* was the only group identified. All the other isolates were not viable on sub-culture. None of the isolates from the deck and well produced urease.

Experiment 4

Bacterial numbers on the skin of the shark varied from 310 per cm^2 at the tail to 1.9×10^3 per cm^2 at the head regions (Table 3). The average bacterial loads in the mouth and gills were 22 per cm^2 and 390 per cm^2 respectively. Of the flora on the skin, the *Pseudomonas* group 40%, Micrococcaceae 30% and *Moraxella* 15% made up the majority (Table 2).

Experiment 5

Bacterial numbers on the flesh of both sharks were significantly lower before icing than after icing for 22 hours (Table 3). Table 2 shows the groups present before and after icing. The predominant

groups were *Pseudomonas* and *Moraxella*. 35% of the isolates from iced shark were found to produce urease.

DISCUSSION

Spoilage of shark flesh during ice storage had occurred when counts reached 3.9 x 10^4 per cm^2. At this level very faint ammonia odour was detectable. Increasing pH and bacterial numbers were associated with increasing spoilage. The *Pseudomonas* group began to predominate the flora by the fifth day and by the eighth day formed over 80% of the total flora (Table 1). The mesophilic Micrococcaceae group was suppressed with duration of storage and so were the gram-negative cocci group. Only a small percentage (5%) of the spoilage group *Achromobacter/Alcaligenes* was present. *Moraxella* was the second largest group, next to *Pseudomonas*. The urease-producing bacteria belong to the *Pseudomonas* (80%) and *Moraxella* (20%) groups. *Pseudomonas* has been shown to be an important group responsible for spoilage of fish (Adams et al, 1964; Cox and Lovell, 1973; Shewan, 1961).

Ice used to chill the shark during storage carried a bacterial load of 2.1 x 10^2 per ml melt water. This ice was tested 2 hours after it had left the factory. The counts are likely to increase when kept over a longer period due to the natural growth of psychrophiles. Thus, stored ice with high bacterial load would contribute to the load on the skin and flesh of freshly caught shark during icing (Table 3). The *Pseudomonas* group comprised 10% of the flora in ice (Table 1). Urease-producing bacteria comprised 30% of those present in the ice melt water of which 50% belong to the Micrococcaceae group, 33% to the *Moraxella* group and 17% were not identified. The *Achromobacter* group, shown by Liston (1960) and Shewan (1961) to be important, was not found in ice. It is therefore desirable to use good quality ice with an extremely low bacterial load. This would reduce the initial

level of contamination and delay the onset of spoilage during storage.

The deck was found to carry a high bacterial load (1.3×10^4 per cm^2 of surface). Cleaning removed more than 99% of the surface load. The most important spoilage groups *Pseudomonas* and *Achromobacter* made up 60% of the total flora. The walls of the well also carried a high load (1.6×10^3 per cm^2). As 55% of the isolates were not viable on sub-culture, the distribution of groups is not known. It is interesting to note that only the *Moraxella* group was viable.

Sharks which have been caught are normally placed in the well after their "tails" have been cut to allow bleeding. They are later removed from the well for butchering on the deck. The bacteria present in the well and deck are potential sources of contamination. Thus frequent cleaning of the deck and well would lower the level of bacterial load and more importantly, the number of spoilage bacteria.

Flesh of newly caught fish is generally considered to be sterile (Shewan, 1961). The presence of bacteria on the flesh of shark immediately after heading, evisceration and washing is due to contamination by entrails and their contents and sea water. It is expected that the knife incision would also introduce large numbers of bacteria. Further increase in bacterial load occurs when ice causes contamination of the cavity wall during storage. Bacterial numbers on the exposed abdominal walls increased 400 fold in 22 hours where ice was placed inside the abdominal cavity compared to a 60 fold increase where fish was only iced externally (Table 3).

Slight changes in the bacterial flora on the flesh of shark occurred after being in ice for 22 hours (Table 2). However, there was a

drastic increase of urease-producing bacteria from 0% in the uniced shark to 35% in the iced shark. It is possible that their presence is due to contamination by ice, which has previously been shown to contain a fairly high proportion (30%) of urease-producing bacteria (Table 1).

The *Pseudomonas* and *Achromobacter* groups have been shown by several researchers (Adams et al, 1964; Cox and Lovell, 1973; Shewan, 1961) to be responsible for spoilage of fish.

The data reported in this study show that *Pseudomonas* was the predominant group in spoiled shark and was present on the deck of the catching vessel, and the skin of freshly caught shark and in ice. The *Achromobacter/Alcaligenes* group was isolated from the deck and the flesh of the shark. The Micrococcaceae and *Moraxella* roups are also prominent and along with other groups probably contribute to spoilage to a lesser extent. It was found that some isolates from these groups produce urease.

SUMMARY

Changes in bacterial numbers and composition during ice storage of school shark (*Galeorhinus australis*) were studied. Spoilage of shark stored in ice($0^{o}C$) had occurred when counts reached 3.9×10^4 per cm^2. *Pseudomonas* redominated during spoilage, reaching 70% of the total organisms present after eight days storage. Bacterial numbers on the skin of freshly caught shark varied from 310 per cm^2 to 1.9×10^3 per cm^2 for tail and head regions respectively. Main groups on the skin were *Pseudomonas* 40%, Micrococcaceae 30% and *Moraxella* 15%. 30% of the isolates from ice produced urease. It is possible that urease-producing bacteria isolated from the flesh of shark are contaminants from ice. The deck and well surfaces carried high

bacterial loads.

Pseudomonas and *Achromobacter/Alcaligenes* made up 60% of the total flora of the deck. *Pseudomonas*, which is an important spoilage bacteria, was predominant in shark, ice and the boat environment.

ACKNOWLEDGEMENTS

This work was funded by the Fishing Industry Trust.

The author wishes to thank his colleagues at Safcol for their assistance in collecting samples and helpful discussion and Dr. Olley, CSIRO, for her helpful suggestions.

REFERENCES

Adams, R., Farber, L. and Lerke, P. (1964). Bacteriology of spoilage of fish muscle. 2. Incidence of spoilers during spoilage. Appl. Microbiol. 12:277

Baumann, P., Doudoroff, M., and Stanier, R.Y. (1968a). A study of the *Moraxella* and *Neisseria catarrhalis* group. J. Bact. 95:58

- ibid - (1968b) A study of the *Moraxella* group. II. Oxidative-negative species (genus *Acinetobacter*). J. Bact. 95:1520

Breed, R.S. Murray, E.G.D. and Smith, R. (1957). "Bergey's Manual of Determinative Bacteriology". 7th Edition. The Williams and Wilkins Co., Baltimore.

Cowan, S.T. and Steel, K.J. (1965). "Manual for the Identification of Medical Bacteria". Cambridge University Press.

Cox, N.A. and Lovell, R.T. (1973). Identification and ahacterisation of the microflora and spoilage bacteria in freshwater crayfish. J.Fd.Sci. 38:679

James, D.G. and Olley, J. (1971). Spoilage of shark. Aust. Fish. 30(4):11

Liston, J. (1960). The bacterial flora of fish caught in the Pacific. J. Appl. Bact. 23:469

Shewan, J.M., Hobbs, G., and W. Hodgkiss (1960a). A determinative scheme of the identification of certain genera of gram-negative bacteria with special reference to the Pseudomonadaceae. J. Appl. Bact. 23:379

- ibid - (1960b). The *Pseudomonas* and *Achromobacter* groups of bacteria in the spoilage of marine white fish. J. Appl. Bact. 23:463

Shewan, J.M. (1961). In "Fish as Food" Vol. 1 (Ed. G. Borgstrom). pp 487 - Academic Press Inc., New York and London.

Skerman, N.B.D. (1967). "A guide to the identification of the Genera of Bacteria". 2nd Edition. The Williams and Wilkins Co., Baltimore.

Wood, E.J.F. (1950). Bacteriology of Shark Spoilage. Aust.J.Mar. Freshw.Res. 1:29

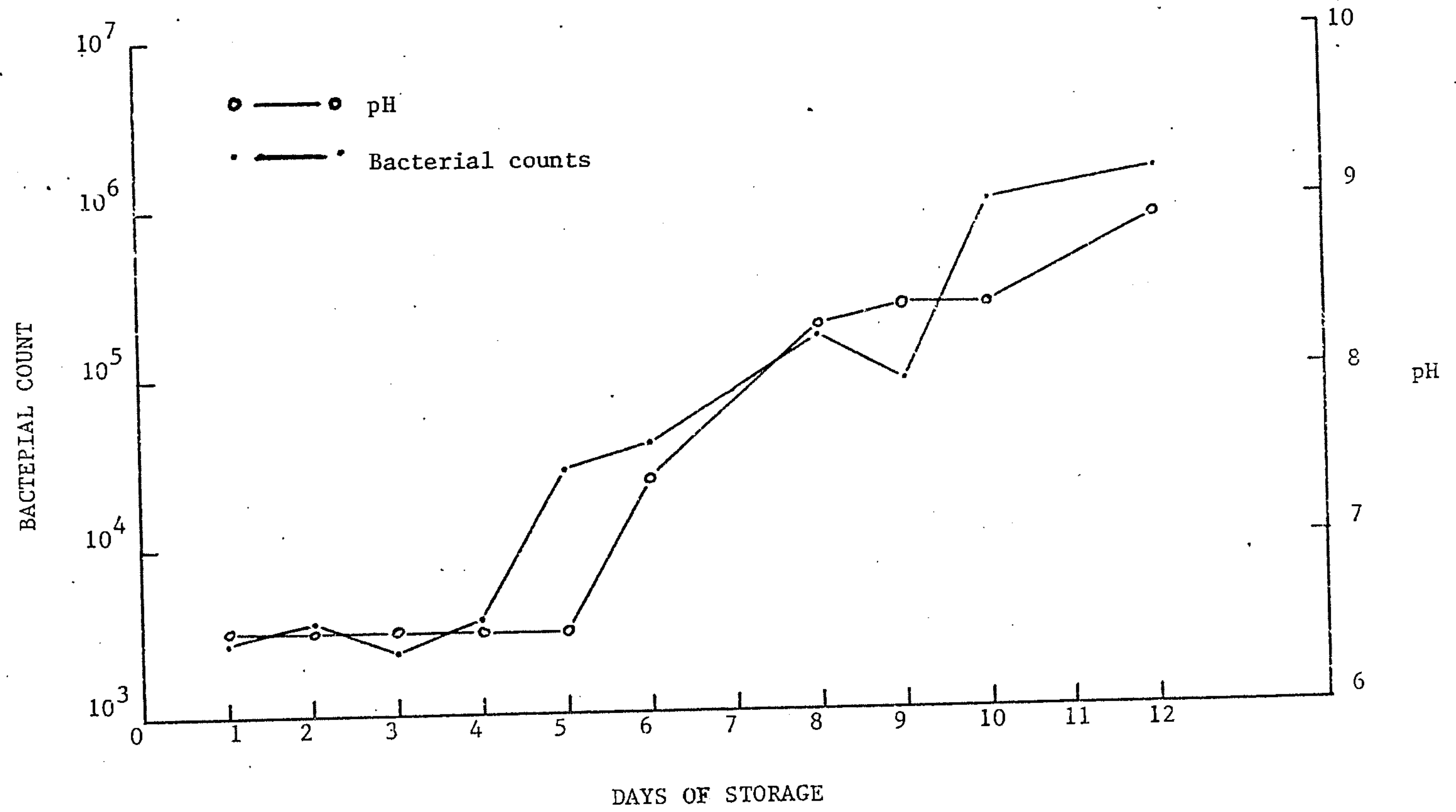

FIG. 1

TOTAL AEROBIC COUNTS OF FLESH FROM THE INTERNAL ABDOMINAL WALL AND pH OF SHARK BODIES STORED AT 0°C IN ICE.

TABLE 1.

COMPOSITION OF THE BACTERIAL FLORA OF ICE ON THE INTERNAL ABDOMINAL WALL OF SHARK STORED AT 0^oC.

Genera/Groups	Percent of twenty isolates: Internal abdominal wall, Days Storage 1	3	5	8	10	Ice
Achromobacter/Alcaligenes	5	0	5	0	0	0
Acinetobacter	0	5	15	5	5	10
Aeromonas	0	0	0	5	0	0
Flavobacterium/Cytophaga	10	0	10	5	0	0
Gram-negative cocci	40	25	5	0	15	5
Gram-positive rods	10	0	0	0	0	0
Micrococcaceae	30	30	5	5	5	35
Moraxella	0	30	20	10	25	35
Pseudomonas	5	10	40	70	45	10
Unclassified	0	0	0	0	5	5
Urease-producing isolates	5	15	5	25	20	30

TABLE 2.

COMPOSITION OF BACTERIAL FLORA ON DECK, WELL, SKIN AND FLESH OF SHARK

	Percent of twenty isolates				
				Internal abdominal wall	
	Deck	Well	Skin	Before Icing	In contact with ice for 22 hours
Achromobacter/Alcaligenes	40	0	0	10	0
Acinetobacter	0	0	0	5	0
Aeromonas	0	0	0	0	5
Flavobacterium/Cytophaga	0	0	0	5	5
Gram-negative cocci	15	0	5	10	10
Gram-positive rods	5	0	5	0	15
Lactobacillaceae	0	0	0	0	5
Micrococcaceae	10	0	30	10	10
Moraxella	10	45	20	15	30
Pseudomonas	20	0	40	15	15
Unclassified	0	55[a]	0	36[a]	5
Urease-producing isolates	0	0	10	0	35

a = Primary isolates not viable on sub-culture

TABLE 3.

TOTAL AEROBIC COUNTS OF MOUTH, GILL, SKIN AND FLESH OF SHARK

Surfaces Swabbed	Average No. bacteria per cm^2
Mouth	22
Gill	390
Skin (head region)	1,900
Skin (mid-body region)	1,400
Skin (tail region)	310
Flesh of abdominal wall before icing	140
" " " " after icing for 22 hours[a]	9,200
Flesh of abdominal wall before icing	130
" " " " after icing for 22 hours[b]	55,000

a = Shark iced externally

b = Ice placed externally and inside abdominal cavity

SPOILAGE AND SPOILAGE INDICATORS IN SHARK HELD IN ICE

BY

MR. P.F. WALLER*

SOUTH AUSTRALIAN FISHERMEN'S CO-OPERATIVE LIMITED

30-46 Wright Street,

Adelaide, South Australia, 5000

* Present Address:

Engineering and Water Supply Department,
Bolivar, South Australia, 5110

INTRODUCTION

In recent years, the shark fishery has been one of Australia's most important fisheries with an annual value of approximately $2.5 million (Muir, 1973). Despite the decline in catch as a result of the Victorian Government ban on the landing of school shark over 1.041 metres in length because of their high mercury content, consumption of shark (flake) is likely to remain high because of its excellent eating qualities.

The quality of shark flesh deteriorates rapidly if the fish is not handled correctly, with the development of a strong ammonia smell, particularly after cooking. Various spoilage tests were applied to shark bodies of known quality to determine which would be most applicable in assessing shark quality. The ultimate aim of the work was to find a simple, quick, unambiguous test that could be applied to the bodies at the time of purchase.

MATERIALS

The vast majority of shark landed in Australia is either school shark (*Galeorhinus australis* (Macleay)) or gummy shark (*Mustelus antarcticus* (Guenther)), and it was with these two species that this work was carried out. The shark used were either purchased from the Adelaide Fish Market or obtained at the time of catching and killing on board the fishing vessels. All shark used had been netted and were between 0.9 and 1.15 metres in dressed length. The netted shark used in these studies was alive when landed unless otherwise stated.

METHODS

A. PHYSICAL TESTS

Flesh Electrical Conductivity

The electrical conductivity of the cell tissue was measured

using an Intelectron Fish Tester V and a Torry Fish Tester.

B. CHEMICAL ANALYSES

i) Hypoxanthine.

Muscle extract preparation and hypoxanthine estimation were performed by the use of xanthine oxidase as outlined by Jones et al (1964).

ii) Ammonia.

Ammonia determinations were carried out by the accelerated microdiffusion method as described by Vyncke (1968).

iii) pH.

Flesh pH was measured with a glass electrode on a flesh and water homogenate (25 gms flesh to 50 ml water). Surface pH was estimated with Whatman BDH narrow range (pH 6-8) indicator paper.

iv) Urea.

Urea concentrations were determined by an adaptation of the Technicon automated method utilizing diacetyl monoxime. Urea when heated with diacetyl monoxime in acid solution produces a yellow colour which is read on a spectrophotometer at 520 nm.

These chemical analyses were performed on surface flesh and deep flesh (Fig. 1), from different sites along the length of the carcass (Fig. 2).

C. BACTERIOLOGY

Bacterial counts were made from the surface of the abdominal wall. A double-bladed knife was used to remove a constant area of flesh (1 cm^2). The top 2 mm of this excised flesh was removed and agitated in 4 ml of 0.5% peptone water. Serial one tenth dilutions in duplicate were made from this and 1 ml of the

appropriate dilutions plated with nutrient agar and/or with urea agar (Christensen, 1946).

D. ORGANOLEPTIC ASSESSMENT

Taste tests were carried out using an untrained panel of tasters. The shark meat used in the taste testing was cooked by steaming for a period of up to 10 minutes.

RESULTS

A. FLESH ELECTRICAL CONDUCTIVITY

Measurements were made daily on iced school and gummy shark held in a cool room. The results were very erratic, although by all other tests the shark were still of good quality.

B. CHEMICAL ANALYSES

i) Hypoxanthine.

Hypoxanthine increased with increased storage time in ice for both school and gummy shark. Fig. 3 shows the normal pattern of hypoxanthine formation. However the daily increase in hypoxanthine is very small and even after 13 days, when the flesh was ammoniated, the level was still less than 1.0 μmole/g.

The hypoxanthine concentration at any one time was found to be uniform along the length of the carcass for both surface and deep flesh.

ii) Ammonia.

The level of ammonia varies considerably throughout the shark carcass, depending on the depth within the flesh and the position along the length of the shark.

Initially there was no appreciable difference in the ammonia concentration of the surface and deep flesh. After day 5 and 6 there was a rapid rise in ammonia concentration in the surface flesh while the deep flesh ammonia concentration did not increase until day 10 (Fig. 4). After day 8 there was a very slight smell of ammonia at the head end of the dressed shark and this became considerably stronger after days 10 to 12 and could then be detected over the whole surface of the abdominal wall.

The ammonia levels along the length of a shark carcass in the surface and deep flesh after 8 and 12 days storage in ice are shown in Figs. 5 and 6. The surface ammonia concentration was higher than the deep flesh concentration by an amount depending on the length of time in ice. In all cases the flesh from the head of the carcass above the gills had the highest ammonia concentration. The surface ammonia concentration increased consistently in going from a position just behind the gills to the anal region.

iii) pH.

Flesh pH: The daily pH change of shark flesh using a flesh and water homogenate is shown in Fig. 7. Although the pH gradually rises, the magnitude of the increase is small even by the time the shark begins to smell of ammonia after 11 to 14 days.

Surface pH: Determinations of the surface pH using narrow range pH paper have been carried out over the whole surface of the shark. Consistent daily changes were recorded on remnants of brachial and cranial nerves

exposed by the beheading of the shark. A typical result is shown in Fig. 8.

iv) Urea.

In fresh shark the urea concentration was uniform along the length of the shark but the surface urea concentration was generally lower than the deep flesh concentration (Table 1). As the shark aged in ice the urea concentration decreased and the decrease was greatest where the ammonia concentration was highest.

C. BACTERIOLOGY

The increase in bacterial counts with time of storage was found to be typically exponential as shown in Fig. 9. The bacterial population was uniformly distributed over the abdominal wall with no significant difference in numbers along the length of the carcass.

The percentage of urease to total bacteria followed a similar pattern in all shark tested (Fig. 10). After 6 to 9 days of storage of the carcass in ice the percentage of urease bacteria had increased from an initial 5-10% to a maximum of greater than 50% of the total bacteria. This high percentage corresponded to the time at which ammonia could first be smelt at the head of the shark.

D. ORGANOLEPTIC

The untrained panel of tasters were able to distinguish very fresh from old shark, but shark over three days old could not be reliably distinguished from 10 day old shark.

DISCUSSION

After death and during storage, the cell walls of natural tissue become increasingly permeable due to enzymatic breakdown of the

protein, and consequently gradually lose their capacitance. Thus the differences in the impedances measured in fresh tissue become less and less during storage and finally disappear, so that these differences would seem to be correlated with freshness and therefore could be used as indices of freshness. Castell (1965) and Wittfogel and Schlegel (1965), however, have found that although the Intelectron Fish Tester readings tend to decrease with decreasing quality they are easily affected by physical defects and any treatment that tends to disrupt the coherency of the muscle cells, e.g. rough handling, pressing, bruising, etc.

Shark bodies are relatively large and when in rigor are extremely fragile so that muscle structure is easily torn. This could explain the erratic results obtained in this investigation with conductivity testing equipment.

Following the death of any animal adenosine triphosphate (ATP) is degraded and one of the breakdown products is hypoxanthine. In a number of scale fish the hypoxanthine levels have been found to correlate closely with the number of days of iced storage and is therefore useful as a quality index (Spinelli et al (1964); Jones et al (1964)). Thomson et al (1974) have also found that hypoxanthine concentration shows good promise as a spoilage indicator in queen scallops (*Chlamys opercularío*) held in ice. In shark there is a general, consistent increase in muscle hypoxanthine concentration with storage time in ice, although the daily change is usually very small and can only be detected using sophisticated laboratory equipment.

Spoilage of shark is frequently brought about by formation of ammonia in the flesh. Although ammonia is formed in other animal tissues due to the breakdown of nucleic bases, proteins, amino

acids and the oxidation of amines, the characteristically high urea content of shark (up to 2% by weight (Simidu 1961)), renders it particularly susceptible to this form of spoilage. Under the action of urease, urea is converted into CO_2 and ammonia. Vyncke (1967), working with dogfish, showed that volatile ammonia determinations proved to be of real value for quality assessment. The results of this investigation have also shown the usefulness of ammonia determinations as a quality indicator, provided care is taken in selecting the samples. This care is necessary because of the pattern of ammoniation that occurs in shark.

Ammoniation is initially a surface phenomenon brought about by the urease bacteria living along the abdominal wall. As the shark ages in ice, the bacteria penetrate below the surface bringing about the development of the ammoniation found later in the deeper flesh.

The differential ammoniation in any one day along the length of the shark appears to be related to the condition of the flesh surface, the surface population of bacteria and the temperature. At the head end of the shark (the area above the gills) there are many cut surfaces and exposed blood vessels and nerves that can harbour a large population of bacteria and rapidly give rise to a very high ammonia level. It is here that the odour of ammonia can first be detected on the body. The abdominal wall along the length of the shark provides a marked contrast to the head end in that it is a flat, uncut surface of minimum area. The results obtained in this investigation have shown that in fresh shark there is no variation in either urea concentration or bacterial numbers along the length of this area of the carcass and therefore it is clear that the differential ammoniation along the shark from just behind the gills to the anal region is not due to a corresponding differential in

either urea concentration or numbers of bacteria. It appears that the increased ammoniation in the anal region is due to the slightly higher temperatures in this region which are possibly related to the way the shark are stored in ice. It has been found that a warm air pocket is formed by the closeness of the flaps in the anal region and it appears that the small increase in temperature (0.5°C) produces the increased ammoniation encountered here. As the tail region lacks any surface readily accessible to bacteria, the flesh here is low in ammonia even after the flesh in other areas has become strongly ammoniated.

As shark ages in ice, there is a significant increase in the percentage of urease-producing bacteria to total bacteria over all parts of the shark. Eventually this high percentage declines. The selection for urease-producing bacteria may be due to the urea content of the flesh. As the urea is hydrolysed and ammonia produced, the percentage of urease bacteria decreases.

Despite the many physical and chemical changes taking place as shark ages in ice, it was thought that a simple measurement such as pH could be used to monitor these changes. The flesh pH of shark does rise consistently during iced storage although the rise is only small. The consistency of the rise, however, coupled with the knowledge that ammonia formation is initially a surface phenomenon, led to the surface pH test using narrow range pH paper. A consistent pattern has been found. In good quality shark, the remnants of the brachial and cranial nerves, exposed where the head was severed, have a pH of 6. On occasions, shark of apparently good quality have shown a pH of 7 to 8 in this region. These shark have been found to become ammoniated after only 4 to 6 days storage in ice, compared to 10 to 12 days for shark with an initial pH of 6. The surface pH is, therefore, more definitive in assessing the

shark quality than general appearance along.

SUMMARY

In the search for a rapid, non subjective test for shark quality during storage in ice, several physical and chemical tests have been evaluated. The physical tests which utilized measurement of the electrical conductivity of the cell tissue by an Intelectron Fish Tester V or a Torry Fish Tester showed little promise. Of the chemical methods investigated, the measurement of hypoxanthine and/or ammonia concentration do provide a useful laboratory monitor of spoilage, but the estimation of surface pH using narrow range pH paper holds the most promise as a rapid method of assessment of large quantities of shark.

ACKNOWLEDGEMENTS

This work was financed by funds from the Fishing Industry Research Trust Account.

The author wishes to express his gratitude to Mr. B. Wilson for technical assistance, Mr. R. Gallasch for preparing the manuscript, and the staff of Safcol Research and Development Division and Mr. James, CSIRO, for their help and advice during the course of the work.

REFERENCES:

Castell, C.H. (1965) "Preliminary Studies of Quality Assessment with the Intelectron Fish Tester V" in the Technology of Fish Utilization, p 158. Fishing News (Books) Ltd., London.

Christensen, W.B. (1946). J.Bact. 52 : 461 - 466

Jones, N.R., J. Murray and (in part) Mrs. E.I. Livingston and C.K. Murray (1964). J.Sci.Fd.Agric., 15; 763

Muir, T. Fisheries of Australia 1973 p 4. Aust. Gov. Publishing Service, Canberra, A.C.T.

Simidu, W. (1961) in Fish as Food Vol. 1 p 353. Academic Press, New York.

Spinelli, J.M. Eklund, and D. Miyauchi (1964), J.Fd.Sci. 29; 710

Thomson, A.B., H.K. Davis, J.C. Early, and J.R. Burt (1974), J.Fd.Technol. 9; 381

Vyncke, W. (1967) Fishing News International, 6(4); 39

Vyncke, W. (1968) Fishing News International, 7(7); 49

Wittfogel, H., and H.L. Schlegel (1965) in The Technology of Fish Utilization 162. Fishing News (Books) Ltd., London.

TABLE 1.

UREA CONCENTRATION % IN SURFACE AND DEEP FLESH

Position	Surface Flesh Sample	Deep Flesh Sample
2	1.15%	1.94%
3	1.31%	1.74%
4	1.31%	1.77%
5	1.48%	1.72%
6	1.66%	1.66%

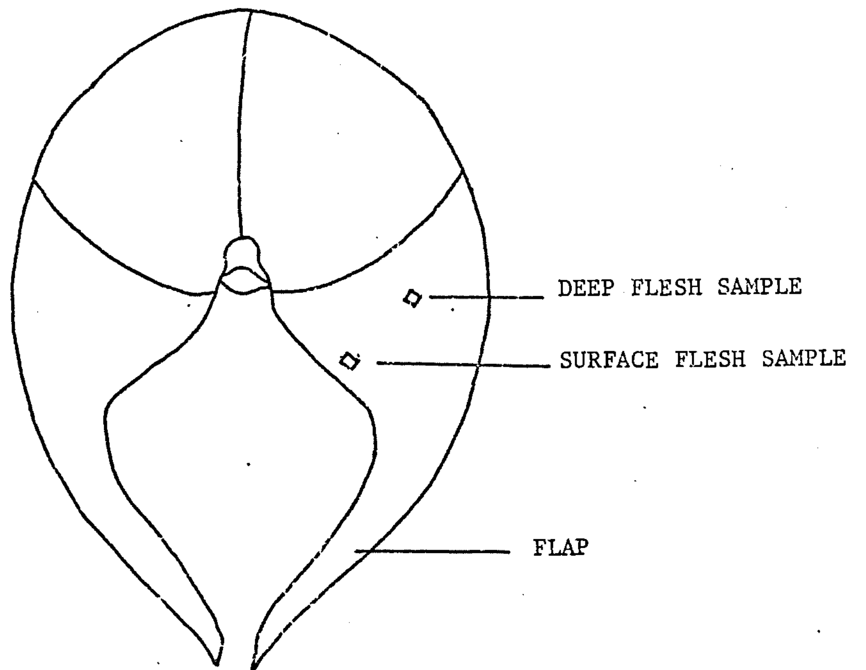

FIG. 1

CROSS SECTION OF GUTTED SHARK SHOWING
POSITION OF SURFACE AND DEEP FLESH SAMPLES.

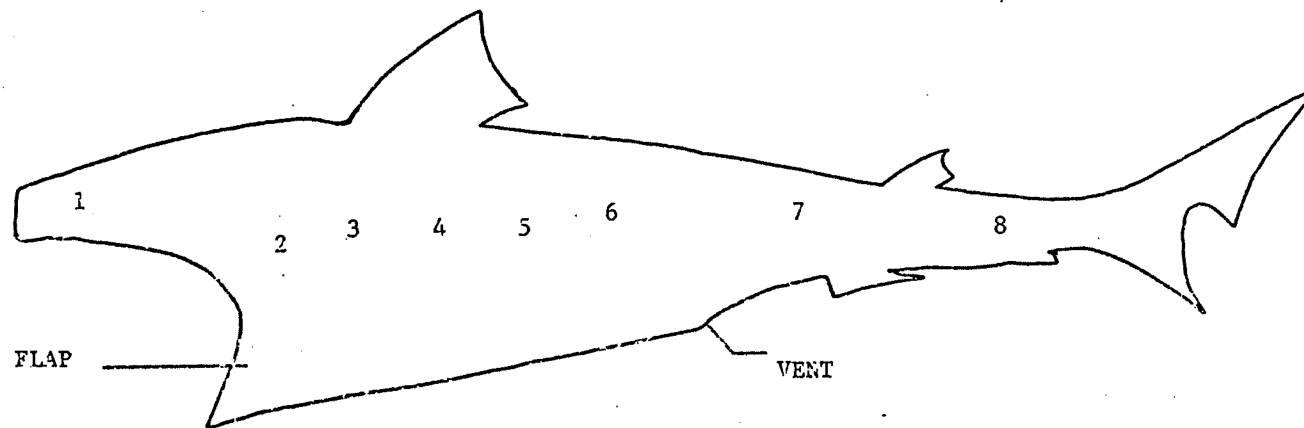

FIG. 2

POSITION OF SAMPLES TAKEN ALONG THE LENGTH OF THE SHARK.

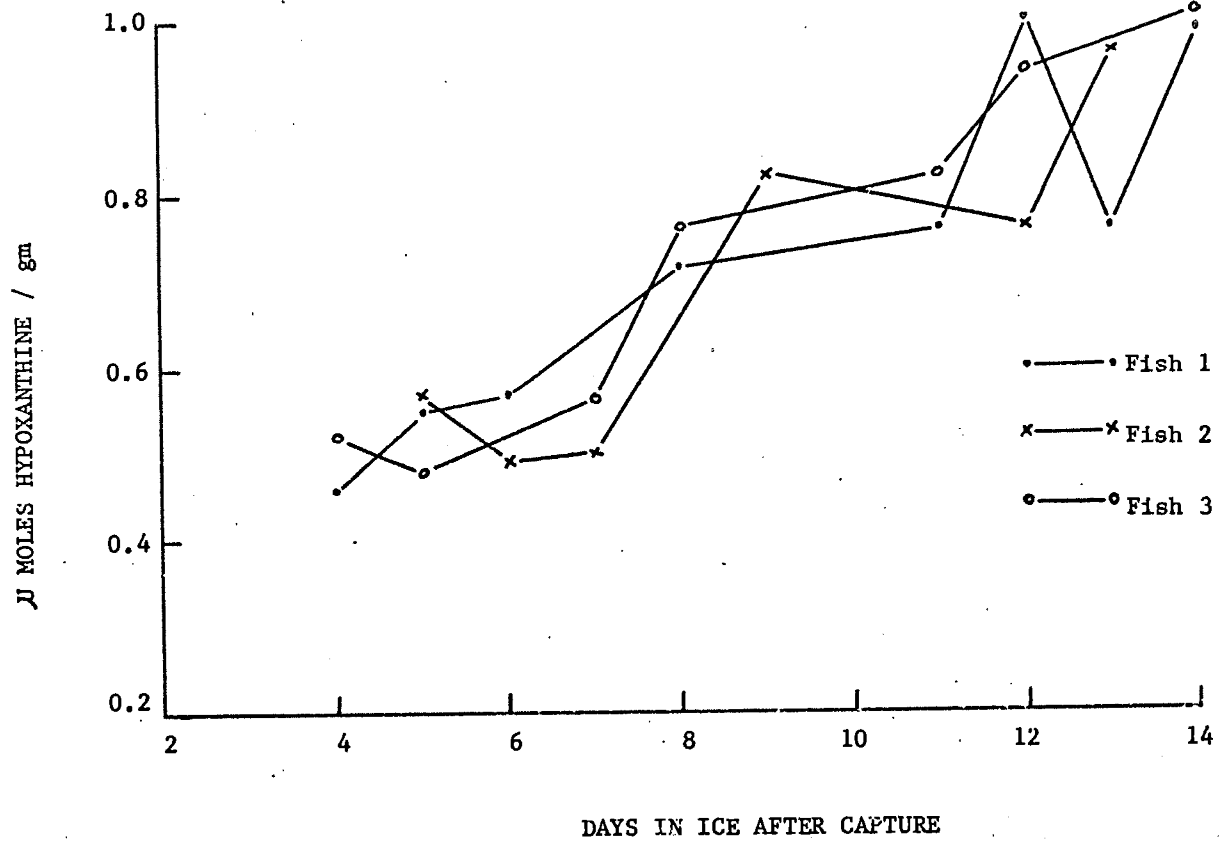

FIG. 3

HYPOXANTHINE FORMATION IN GUMMY SHARK

DURING STORAGE IN ICE.

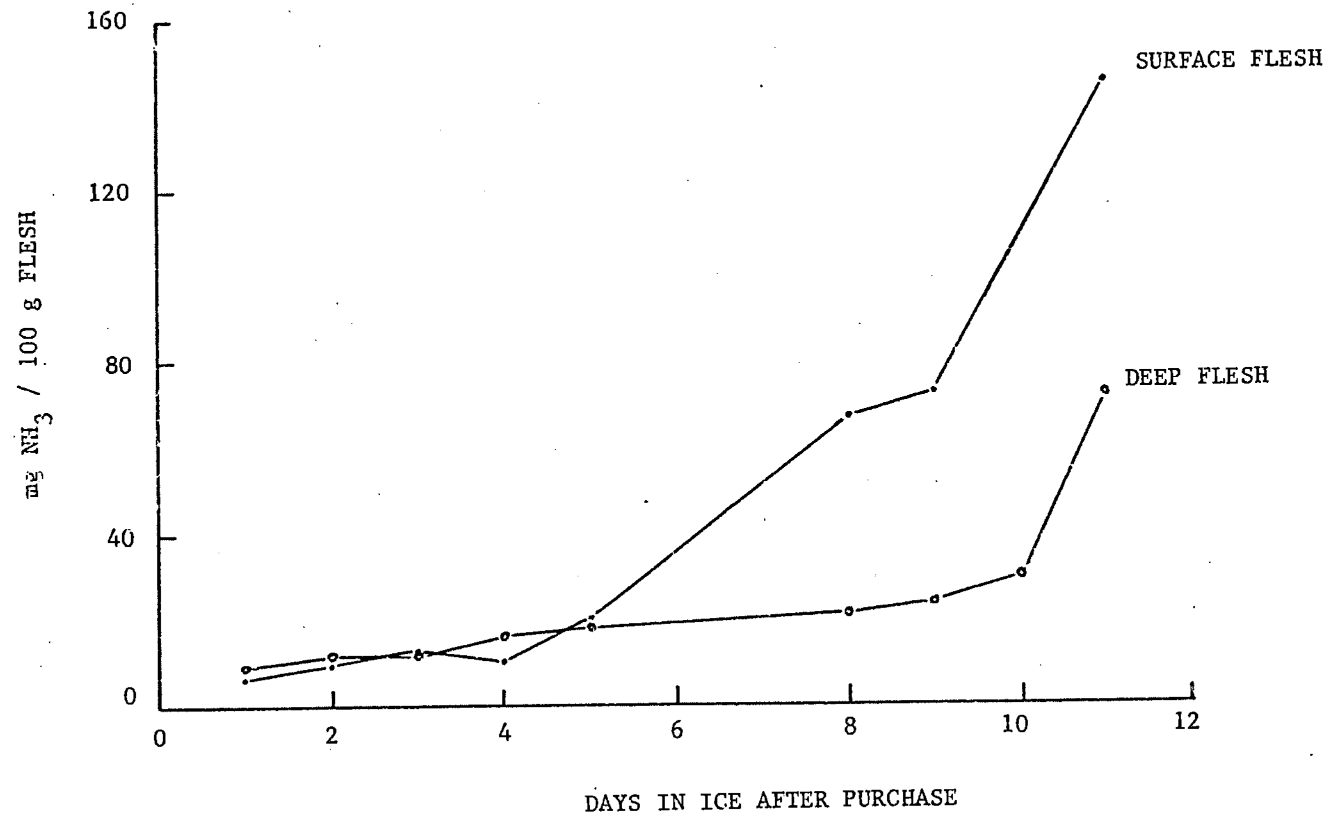

FIG. 4

AMMONIA FORMATION IN SURFACE AND DEEP FLESH SAMPLES DURING STORAGE IN ICE.

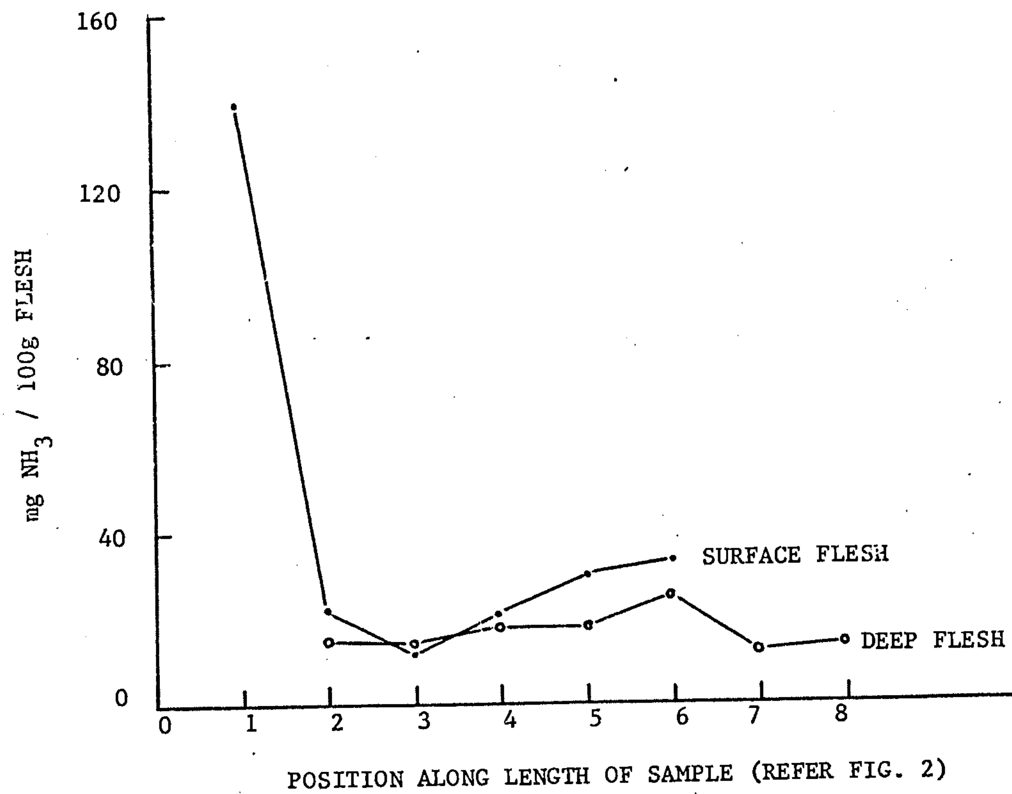

FIG. 5

AMMONIA CONCENTRATION OF SURFACE AND DEEP FLESH SAMPLES FROM DIFFERENT POSITIONS ALONG THE LENGTH OF THE SHARK CARCASS AFTER 8 DAYS' STORAGE IN ICE.

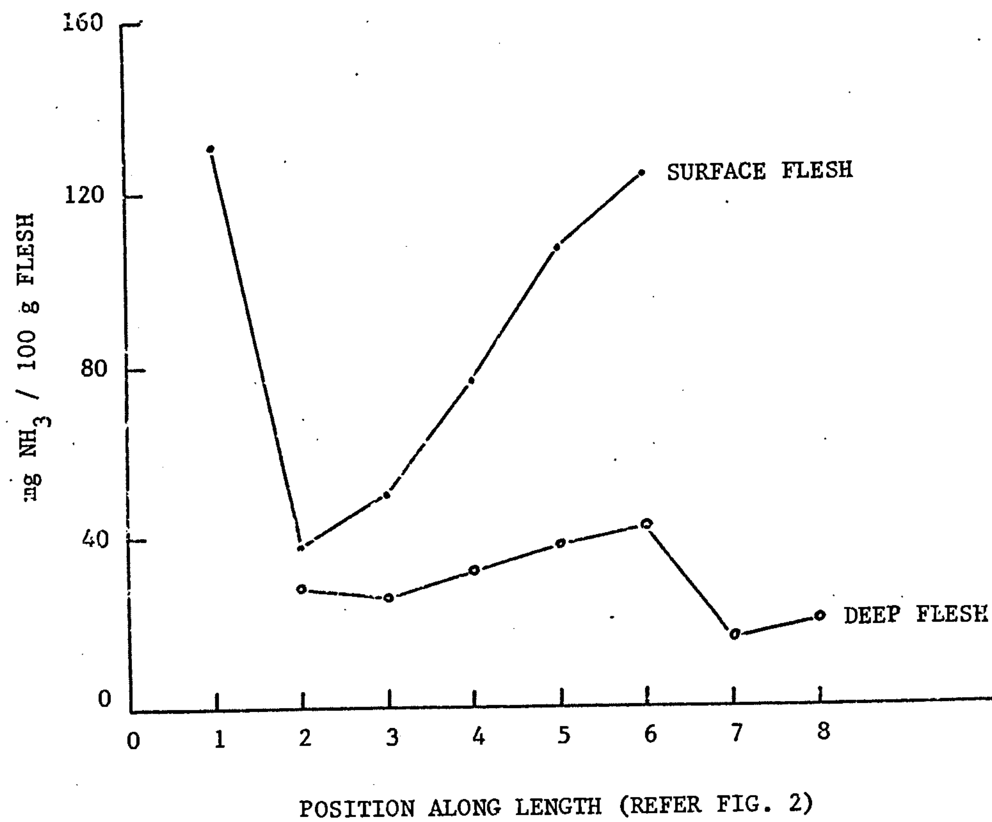

FIG. 6

AMMONIA CONCENTRATION OF SURFACE AND DEEP FLESH SAMPLES FROM DIFFERENT POSITIONS ALONG THE LENGTH OF THE SHARK CARCASS AFTER 12 DAYS' STORAGE IN ICE.

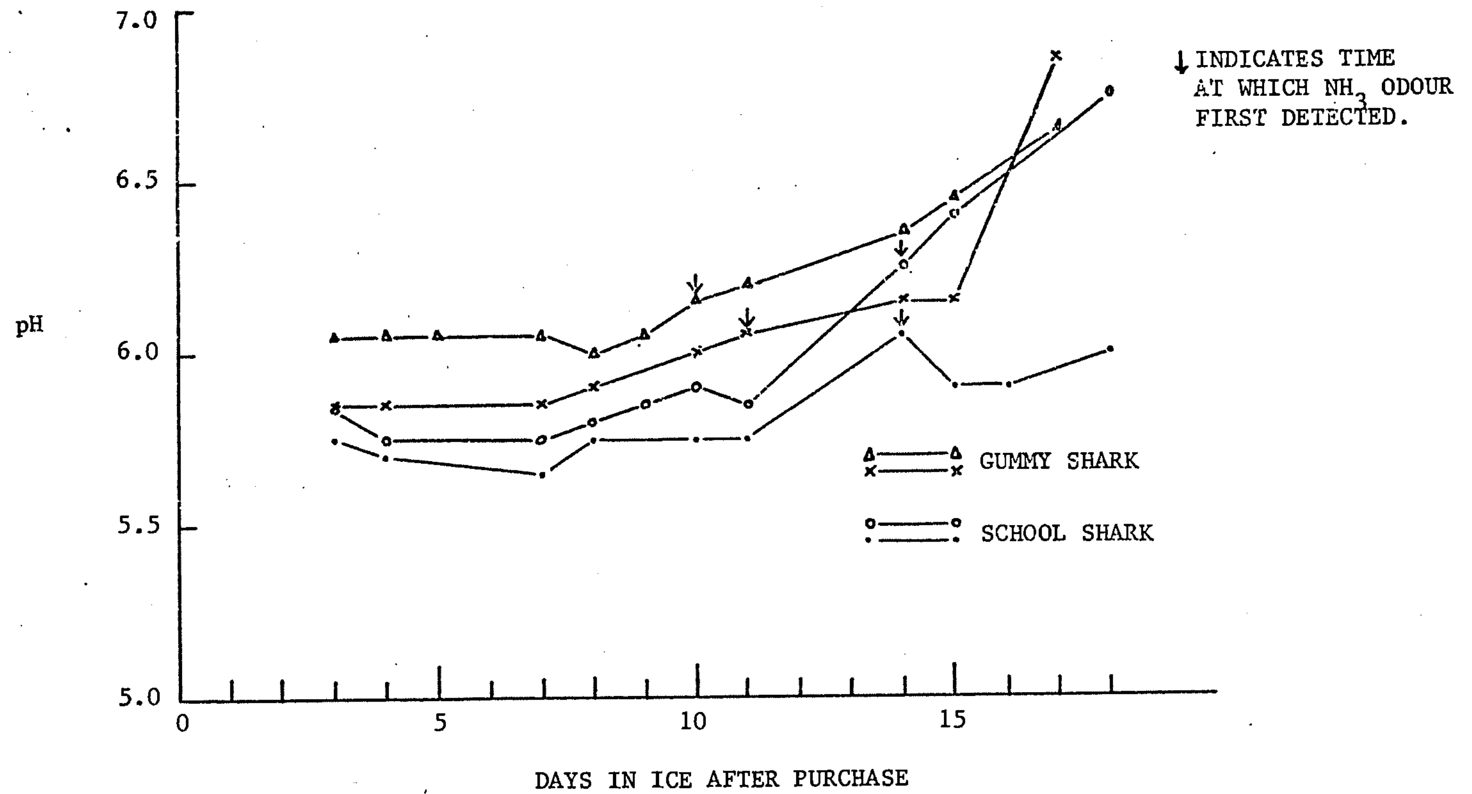

FIG. 7

CHANGE IN FLESH pH WITH TIME OF STORAGE IN ICE.

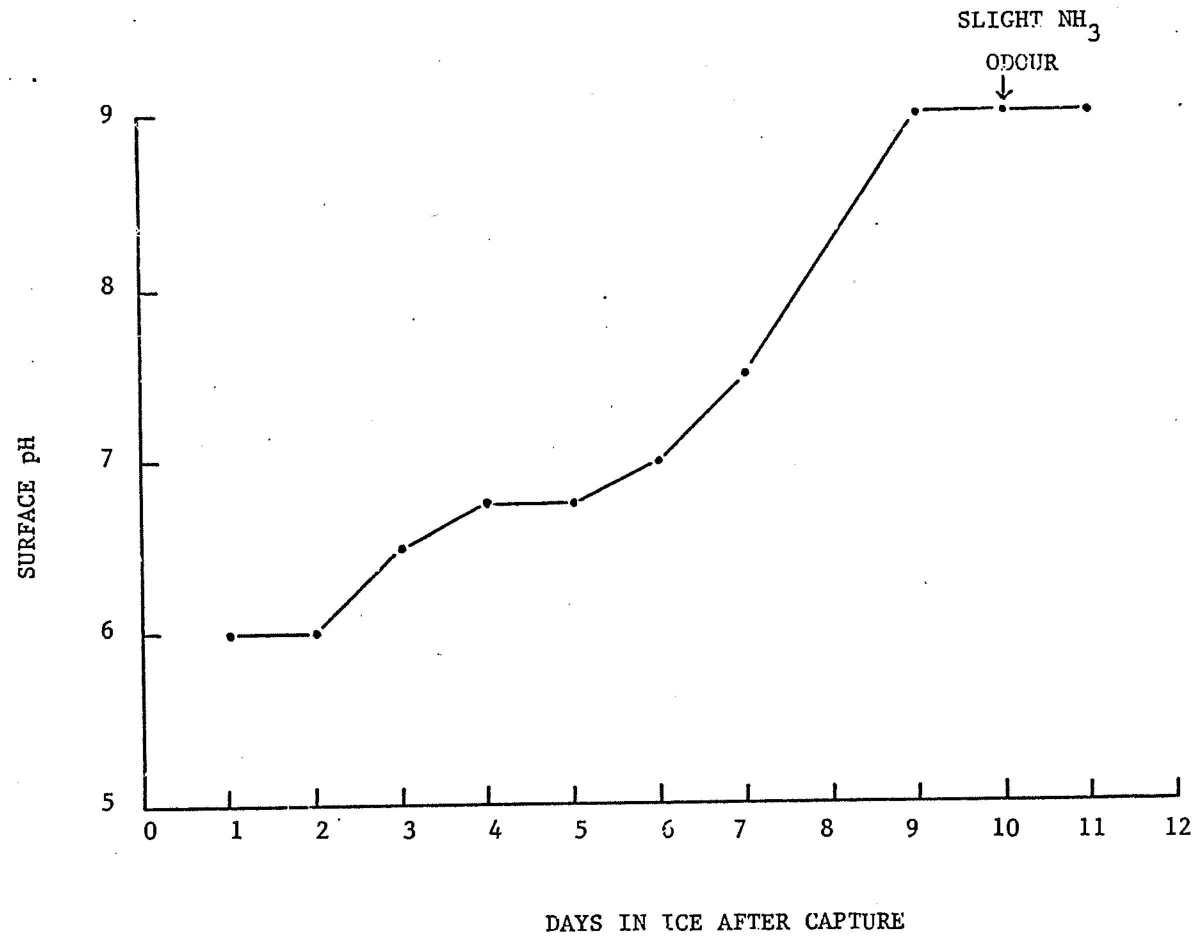

FIG. 8

CHANGE IN SURFACE pH WITH
TIME OF STORAGE IN ICE.

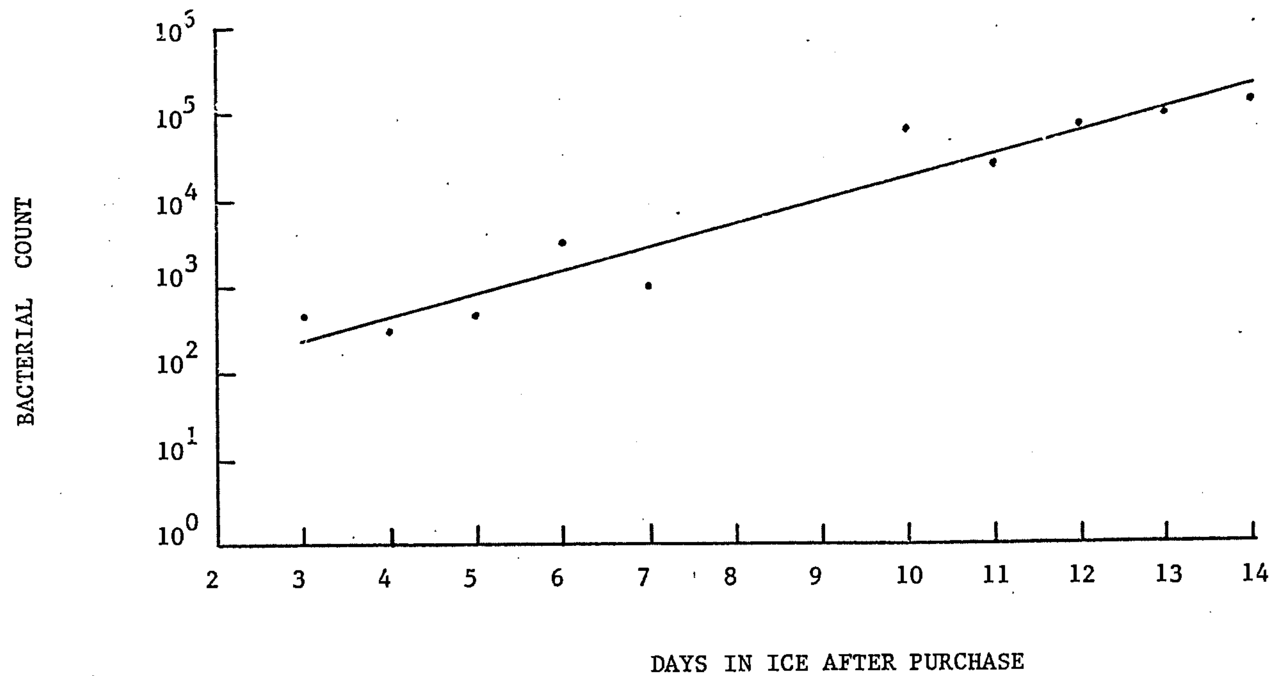

FIG. 9

THE RELATIONSHIP BETWEEN BACTERIAL COUNT AND DURATION OF STORAGE IN ICE.

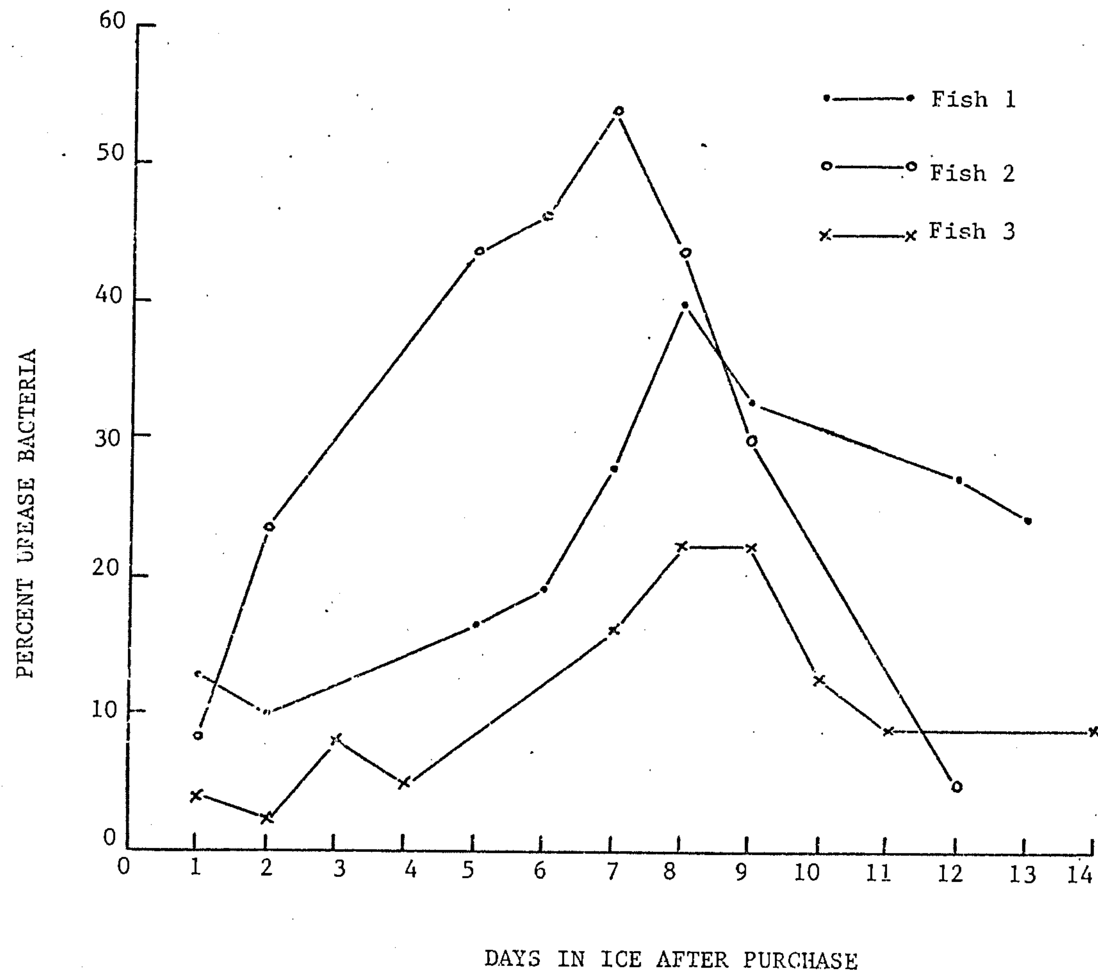

FIG. 10

CHANGE IN THE PERCENTAGE UREASE BACTERIA ON THE SURFACE OF SHARK BODIES DURING STORAGE IN ICE.

SPOILAGE AND SPOILAGE INDICATORS IN FROZEN SHARK

BY

MR. P.F. WALLER*

SOUTH AUSTRALIAN FISHERMEN'S CO-OPERATIVE LIMITED

30-46 Wright Street,

Adelaide, South Australia, 5000

* Present address:

Engineering and Water Supply Department,
Bolivar, South Australia, 5110

INTRODUCTION

Preservation of food by freezing is an important method of preservation and one which provides the closest approach which has yet been attained to maintaining the fresh quality of the food for extended periods. Freezing preservation is especially important in its application to fish because:

- i) Fish is by nature highly perishable;
- ii) Fish may be captured at regions remote from the place of consumption;
- iii) Fish catches can be of an irregular or seasonal occurrence.

Nevertheless, quality deterioration of the frozen product does occur. The most common method of following these quality changes in frozen fish is by some form of organoleptic evaluation; but because of the subjective (and hence variable) nature of this type of evaluation an objective test, or tests, that could be correlated with the subjective taste panel results would be of great benefit to the industry, both in maintaining and improving the quality of their product.

The aim of this study has been:

- i) To follow a number of biochemical and physical changes taking place during freezing and frozen storage of shark fillets and to evaluate their correlation with changes in quality attributes judged subjectively;
- ii) To evaluate the effects of:
  - a) freezing rate;
  - b) duration of frozen storage;
  - c) initial pre-freezing quality

on the final eating quality of shark fillets.

MATERIALS AND METHODS

The vast majority of shark landed in Australia is either school shark (*Galeorhinus australis* (Macleay)) or gummy shark (*Mustelus antarcticus* (Guenther)), and it was with these two species that this work was carried out. The shark used were obtained at the Adelaide Fish Market. All shark used had been netted and were between 0.9 and 1.15 metres in dressed length.

The biochemical, physical or organoleptic criteria used to assess quality were:

1. Organoleptic Assessment:

Taste tests were carried out using an untrained panel of tasters who were asked to rate the acceptability of the samples in consumer terms. The shark meat was sliced into fillets approximately 1 cm thick and then soaked in a 10% salt solution for 7 minutes. These fillets were then drained and rolled in flour prior to cooking in a deep frier at 380°C for 2-3 minutes.

2. Extractable Protein:

The extractable protein was determined by a modification of the method of Dagbjartsson and Solberg (1973). 15 g of minced flesh were homogenised with 150 ml of ice-cold $KCl/NaHCO_3$ buffer (0.6M KCl + 0.01M $NaHCO_3$: pH = 7.2) in a plastic container with corrugated sides using a glass or teflon pestle. Initially the fish was blended with only a little of the 150 ml until an homogeneous slurry was formed, and the remainder of the 150 ml was then added with stirring. The solution was allowed to stand at 0-4°C overnight. This solution was centrifuged at 14,000 x G for 20 minutes at 0°C. 2 ml of the supernatant were digested in a digestion mixture containing 18N H_2SO_4, 0.5% SeO_2 and 0.5% $CuSO_4$.

Nitrogen was estimated using a micro-kjeldhal technique.

Total nitrogen was determined by digesting 60-100 mg of wet flesh in the digestion mixture, and determining nitrogen by the micro-kjeldhal method.

Non-Protein Nitrogen: The supernatant from the total extractable nitrogen estimation was poured into a beaker and the pH was adjusted to 4 with ice-cold 10% trichloroacetic acid (T.C.A.). The precipitated proteins were removed by filtering through a Whatman 42 filter paper. 2 ml of the filtrate were digested with 2 ml of the digestion mixture and the nitrogen was determined by the micro-kjeldhal method.

The soluble protein nitrogen results were expressed as a percentage of total protein nitrogen following the formula of Cowie and Little (1966).

$$\%\ \text{extractable protein nitrogen} = \frac{\text{Extractable N} - \text{Non Protein N}}{\text{Total N} - \text{Non Protein N}} \times 100$$

3. <u>Ammonia Levels</u>:

Estimation of ammonia levels was carried out by the accelerated micro-diffusion method as described by Vyncke (1968).

4. <u>Surface pH</u>:

This was estimated with Whatman BDH narrow range (pH 6-8) indicator paper.

5. <u>Flesh pH</u>:

This was measured by a method similar to that of Kelly (1969). 25 g of muscle were macerated with 50 g of water and the pH of the macerate measured at room temperature using a glass electrode.

6. Moisture Content:

A known weight of sample was dried in an oven under standardised conditions (95-100°C for 48 hours) and the percentage weight loss of water was calculated.

7. Histological Structure of Muscle:

1 cm square sections, 0.5 mm thick, were cut from a fixed location in the shark fillets. These sections were processed, embedded, cut and stained by R.A. Sandison, South Australian Institute of Technology.

8. Thaw Drip:

i) % Drip Loss:

The frozen fillets were removed from the storage freezer, frozen weight recorded and the fillets were then placed in a refrigerator at 2-5°C overnight. The following morning they were placed at room temperature until thawed completely. The loss in weight of the fillet after thawing was used as a measure of the drip loss.

ii) pH:

The drip from the thawing fillets was collected and the pH of the undiluted drip was measured using a glass electrode.

iii) Ammonia Concentration:

The drip liquid was diluted 1:20 with distilled water and the ammonia levels estimated by the method of Vyncke (1968).

iv) Protein Nitrogen:

2 ml of the diluted drip liquid were digested with 2 ml of digestion mix, and the nitrogen was determined by the micro-kjeldhal method.

9. Freezing and Thawing:

Shark fillets were frozen to -10°C in 15 minutes using a nitrogen freezer at -140°C, or to -10°C in 12 hours using a freezer at -20°C. To assess the quality of the fillets, the rapidly frozen fillets were thawed in a plastic bag under running water (these conditions representing the optimum conditions possible) and the slowly frozen fillets were thawed in still air.

10. Storage:

Fillets of good and poor quality school and gummy sharks were frozen in freezers representing fast commercial freezing and slow commercial freezing. The frozen fillets were stored under commercial cold storage conditions (-20°C). They were assessed using the above criteria before freezing, after storage for one week, one month, 3 months and 6 months.

RESULTS

Rate of Freezing.

The chemical, physical and organoleptic criteria listed above were used to assess the quality of the fillets after different freezing and thawing regimes. All tests gave identical results for both sets of fillets frozen at the different rates.

Storage Trial.

The good quality shark had a surface pH of 6 and had all the characteristics of good quality shark as described previously (Waller in press).

The poor quality shark had the characteristics of 12 day old fish as described previously (Waller in press) viz., the odour of ammonia could be detected but was not strongly apparent and the

surface pH was 9.

The following observations were made:

i) Organoleptic Assessment:

Fillets of initial good quality remained of acceptable (although declining) quality throughout this trial. The reports from the inexperienced taste panel, which assessed the quality in terms of consumer acceptability, indicated the flesh became drier and tougher, with less taste, after extended periods of frozen storage. Fillets from the poor quality shark showed a slight improvement in acceptability after being frozen for one week, but were judged unacceptable after storage for one month. No differences in quality could be detected as a result of the different rates of freezing.

ii) Extractable Protein:

The levels of extractable protein were much lower than expected. The maximum level observed for good quality shark was 50%, with most samples having only 22-35% of the protein extractable. In most cases there was no correlation between the extractable protein and the storage time.

iii) Ammonia Concentration:

The flesh ammonia concentration in fillets from good quality shark was independent of the storage time and the type of freezing. The mean flesh ammonia concentration in the 28 samples analysed was 13.67 ± 0.54 mg of ammonia per 100 g flesh.

The poor quality school shark fillets showed considerable variation in ammonia concentration after different periods of storage. The initial level of 66 mg of ammonia per 100 g fell to less than half this level after frozen storage for one week, then progressively increased with increased storage time, returning to the initial level after storage for six months.

iv) Surface pH:

The surface pH from good quality shark (gummy and school) remained at pH 6.0 over the six months period of storage.

Fillets from poor quality shark initially had a surface pH of 7.0 or higher. This fell to 6.0-6.5 after one week of frozen storage and thereafter increased to 7.5-8.0 after six months storage.

v) Homogenised Flesh pH:

The pH of the homogenised flesh of fillets from good quality shark remained within the range of pH 5.5-5.8 and was unaffected by storage times or freezing conditions.

The pH of the homogenised flesh of poor quality fillets was 6.3 and also was unaffected by storage time or freezing conditions.

vi) Moisture Content:

The mean moisture content of the 34 samples tested was 76.5 ± 0.21%. The moisture content decreased with increased storage time but the magnitude of the decrease over the six month storage period was smaller than the variation between initial levels of moisture.

vii) Histology:

The histological structure of the tissues showed that the structural characteristics of the shark flesh were unaffected by freezing rates, or fish quality, while prolonged storage resulted in increased distortion up to a maximum of 30%. Crystallization occurring during freezing took place in the connective tissue displacing and distorting the muscle fibre pattern, but the muscle fibres remained intact.

viii) Drip:

a) Volume:

Despite the controlled conditions used to measure the drip volume, the thaw loss was variable for the one sample and there was no correlation between drip loss on thawing and quality (as estimated subjectively), freezing time or time of storage. The mean drip loss for the 30 samples tested was 8.5 ± 0.5%.

b) pH:

The pH of the drip from fillets of good quality shark remained constant at pH 5.88 ± 0.3 (m = 23) and was independent of the rate of freezing or length of storage. The pH of the drip from fillets of poor quality shark also remained constant but was higher than that from good quality shark (pH 6.47 ± 0.11).

c) Ammonia Concentration:

The ammonia concentration of the drip from all fillets increased as the time of storage increased. The initial concentration of the drip from fillets of good quality shark was 13.60 ± 0.69 mg of NH_3 per 100 ml of drip and this increased during storage period to 20.14 ± 0.69 mg of NH_3 per 100 of drip (the increase being 4 to 7 mg

of ammonia per 100 ml of drip for the six month storage period). The drip from fillets of poor quality shark initially contained in excess of 20 mg of ammonia per 100 ml of drip and this figure rose to levels in excess of 60 mg of ammonia per 100 ml of drip after six months storage.

d) Protein Content:

There was a great deal of variability in the protein content of the drip even from individual fish. The variability could not be correlated with fish quality, freezing conditions or time of storage. The protein content of the drip was 10.81 ± 0.58% (m = 26).

DISCUSSION AND CONCLUSION

When fish is frozen and held in frozen storage, deteriorative changes occur which are quite different from those that occur in wet fish. Quality deterioration in fresh fish is generally related to the production of spoilage flavours and odours, whereas in frozen fish the primary change appears to be increased toughness and dryness of the cooked fish. Flavours and odours characteristic of frozen storage do develop but normally in lean fish the textural quality deterioration predominates. The taste panel reports of this study indicated a similar pattern of deterioration.

Several investigators have reported that the decrease during frozen storage in taste panel acceptability, especially texture, is paralleled by a decrease in the level of protein extractable from the frozen muscle (Dyer,(1951), Dyer and Dingle (1961), Awad et al (1969)). Other studies have indicated that this total extractable protein-tenderness relationship does not always apply. Temperature of storage (Luijpen (1957), Cowie and Little (1966),

(1967)) appear to be complicating factors.

No correlation between extractable protein and storage time or subjective quality was found in this investigation. The maximum levels of extractable protein (i.e. 50%) recorded in the shark flesh were generally much lower than expected. The levels of extractable proteins reported in the literature for good quality fish, frozen at ordinary commercial rates and immediately thawed, are in the range 70-90% (Cowie and Little (1966), (1967) and Cowie and Mackie (1968)), although an initial value of 60% salt soluble protein has been recorded in skate, another non-oily elasmobranch (Olley et al, (1967)). Cowie and Mackie (1968) and Ravesi and Anderson (1969) have demonstrated the care that is needed in determining protein extractability in order to use it as a measure of cold storage protein denaturation. Reproducibility of results is only obtained if identical extraction procedures are used. In addition, the absolute values of protein extractability depend on such factors as degree of subdivision of the cells during homogenisation, extractant solution, physical state of sample (frozen or thawed) and blending time. The extended thawing time prior to the extraction may have had some effect (Dyer and Dingle (1961)) on the protein extractability.

Spoilage of fresh or iced shark is frequently brought about by the formation of ammonia in the flesh as a result of the action of bacterial enzymes on the urea in the flesh. Although bacterial growth will be prevented under frozen storage, it was thought that the presence of the bacterial ureases (and possibly endogenous ureases) might allow the production of ammonia to continue. The results from this investigation demonstrate that if the shark flesh was showing incipient spoilage at the time of freezing, ammonia production continues during frozen storage. The formation of

ammonia during storage is insignificant in shark fillets of initial good quality.

Waller (in press) indicated that as the ammonia level increases during the spoilage of iced shark, there is a concomitant increase in flesh pH, particularly surface pH. The results reported here indicate that, in frozen shark, a similar relationship exists between ammonia production and surface pH, but not the pH of a flesh homogenate. This difference appears to result from the surface nature of ammonia formation due to the presence there of bacterial ureases.

Clearly, the ammonia concentration and/or pH of frozen stored shark fillets is primarily dependent on the pre-freezing quality and cannot be used as a spoilage indicator for frozen shark of initial good quality.

Another property of fish muscle which has been investigated in relation to textural changes in frozen muscle is drip formation. Free and/or press thaw drip has been used as a quality test for frozen fish (Heen and Karsti (1965)), but Dyer (1968) has concluded that, although some increase, especially of free thaw drip, may occur on storage, the results are generally too variable to be of use as a quality indicator. Our results are in agreement with this conclusion. Pawar and Magar (1966) and Awad et al (1969) have reported that the pH of drip exuded from sardines and fresh water whitefish respectively, increased steadily during storage. In this investigation the drip pH was found to be more dependent on pre-freezing quality than on length of frozen storage.

The other criteria investigated, namely moisture content, ammonia concentration of drip, and protein content of drip, did not show

sufficient, consistent variation during the period of storage to suggest that they would be of use as an objective measure for assessing eating quality.

In this investigation, the relative effects of three primary variables on frozen quality have been studied:

i) rate of freezing,

ii) duration of cold storage,

iii) initial quality of fillets.

The results clearly indicate that the rates of freezing (at least within the limits studied) have little detectable effect on the eating quality. The initial pre-freezing quality of the shark fillet, however, has considerable effect on the eating quality of the product and appears to be of greater importance than frozen storage deterioration (determined by length and condition of storage) in determining the quality of the final product at consumer level. This finding is at variance with the conclusion of Dyer (1968), based on the results of Dyer et al.(1964) that, in cod, frozen storage deterioration is at least as important a factor as pre-freezing quality in determining final eating quality. This difference would be related to the susceptibility of shark flesh to ammoniacal spoilage which is particularly noticeable and objectionable to the consumer.

None of the physical or chemical criteria used in this study to assess the quality of the shark fillets could be correlated with their quality as judged by the taste panel. These criteria cannot, therefore, be used for rapid, non-subjective assessment of the quality of frozen shark fillets.

The results do show, however, that the quality of the frozen shark

fillets is determined by the quality of the fillet prior to freezing. Every effort must, therefore, be made by those catching the fish and those processing the fish to see that the shark is rapidly chilled and rapidly processed to ensure an optimum final quality on the market.

SUMMARY

Attempts were made to correlate taste panel assessment of shark quality after extended frozen storage periods with some chemical or physical parameter. The parameters tested were surface pH, homogenised flesh pH, ammonia levels in flesh and drip, extractable protein, moisture content, histological structure of the muscle, drip loss on thawing, pH of thaw drip and protein nitrogen of thaw drip.

Tests were also conducted on the effects of fast and slow freezing and thawing comparing nitrogen freezing and water thawing with slow freezing and still air thawing, and flesh quality before freezing with quality after storage periods.

None of the physical or chemical parameters could be correlated with shark quality after storage. It was found that poor quality shark deteriorated during storage far more than good quality. It is therefore important for everyone involved in the catching and processing of shark to ensure that good handling and processing practices are used so that an optimum quality product is supplied to the trade.

ACKNOWLEDGEMENTS

This work was funded by the Fishing Industry Trust Account. The author wishes to thank Mr. James, CSIRO Division of Food Research, Hobart, for his help in initiating this study and his interest throughout the work, Mr. Sanderson, South Australian

Institute of Technology for performing the histological examination, Mr. Wilson for technical assistance, his colleagues in Safcol Research and Development Division for their assistance during the work and Mr. Gallasch for preparing the manuscript.

REFERENCES

Awad, A., W.D. Powrie and O. Fennema (1969) J.Fd.Sci. 34; 1

Cowie, W.P. and W.T. Little (1966) J.Fd.Technol. 1; 335

Cowie, W.P. and W.T. Little (1967) J.Fd.Technol. 2; 217

Cowie, W.P. and I.M. Mackie (1968) J.Sci.Fd.Agric. 19; 696

Dagbjartsson, B. and M. Solberg (1973) J.Fd.Sci. 38; 242

Dyer, W.J. (1951) Fd.Res. 16; 522

Dyer, W.J. (1968) Recent Advances in Food Science Vol. 4. p 333

Dyer, W.J. and J.R. Dingle (1961) in "Fish as Food" Vol. 1. p 275, Academic Press, N.Y.

Dyer, W.J., D.I. Fraser, R.G. MacIntosh and M. Myer (1964) J.Fish.Res.Bd.Canada 21; 577

Heen, E. and O. Karsti (1965) in "Fish as Food" Vol. 4. p 355 Academic Press, N.Y.

Kelly, T.R. (1969) J.Fd.Technol. 4; 95

Luijpen, A.F.M.G. (1957) Nature 180; 1422

Olley, J., E. Stephen, J. Farmer and I. Robertson (1967) J.Fd.Technol. 2; 207

Pawar, S.S. and N.G. Magar (1966) J.Fd.Sci. 31; 87

Ravesi, E.M., and M.L. Anderson (1969) Fish.Ind.Res. 5 (4); 175

Vyncke, W. (1968) Fishing News International 7 (7); 49

THE EFFECT OF HANDLING ON SHARK QUALITY

BY

MR. P.F. WALLER*

SOUTH AUSTRALIAN FISHERMEN'S CO-OPERATIVE LIMITED

30-46 Wright Street

Adelaide, South Australia, 5000

* Present Address:

Engineering and Water Supply Department,
Bolivar, South Australia, 5110

THE EFFECT OF HANDLING ON SHARK QUALITY

The object of an efficient fishery is to land high quality fish which the consumer will be prepared to buy at a price which will give satisfaction to the buyer and a profit to the producer.

A study of shark handling and processing methods with the aim of improving the quality of both fresh and frozen shark on the wholesale market has been carried out under a grant from the Fishing Industry Research Trust. This article briefly reports the findings of the study as related to the quality of fresh shark.

At present, the quality of shark is assessed by its appearance and smell. Good quality shark has a sea-fresh smell, the belly flaps and body are firm and the flesh beneath the skin is elastic to the touch. Well kept shark is still in rigor and quite stiff for up to 50 hours after capture, whereas unchilled and poorly handled shark can become ammoniated and limp in less than 10 hours. Although an experienced person can assess shark quality on appearance, such assessments are necessarily subjective and unsatisfactory for improving and maintaining uniform quality standards.

The quality of fresh shark on the market could be improved if a rapid objective test could be devised. The fishermen could be paid according to quality and thus given incentive to upgrade the catch. The work done under this grant has therefore been directed towards determining suitable means of assessing quality of fresh shark.

As soon as a fish dies, spoilage begins. Spoilage is the result of a whole series of complicated changes brought about in the fish

tissue by its own enzymes and by the action of bacteria. These changes generally follow a definite pattern for a particular species. A knowledge of the changes occurring may allow a quality test to be devised on an estimation of the quantities of some of the chemicals produced as a result of the bacterial and enzymic activity on the fish flesh.

One of the most obvious features of a spoiling shark is usually its strong smell of ammonia. This ammonia is produced by the action of an enzyme (urease), released by bacteria, on urea, a substance which is present in shark blood and flesh to maintain osmotic pressures. While the ammonia smell alone does not render the fish inedible, it is one of the first signs of spoilage and flesh so spoiled can no longer be considered good quality. As the production of ammonia in the shark flesh occurs at a relatively early stage in spoilage, it therefore seemed that it should be possible to base a reliable assessment of shark quality on the ammonia content in the flesh.

Unfortunately, we have found that the level of ammonia in the flesh of a shark is not the same throughout the whole of the shark body at any one time. Not only can there be a variation through the cross-section of the body, but there is also a variation along the length of the shark. Ammoniation is initially a surface phenomenon brought about by the breakdown of urea caused by the action of urease produced by some of the bacteria living along the abdominal wall. As the shark ages in ice, the bacteria penetrate below the surface bringing about the ammoniation found later in the deeper flesh. The difference in ammonia level along the length of the shark on any one day appears to be related to the condition of the flesh surface, the surface population of bacteria and the temperature. At the very front end of the dressed shark there are many cut

surfaces and exposed blood vessels and nerves that can harbour a large population of bacteria and this area of the body is often not iced down very well. It is here that the odour of ammonia can first be detected on the carcass. The abdominal wall along the length of the shark provides a marked contrast to the front end in that it is a flat, uncut surface. It appears that the higher levels of ammonia found toward the vent region of the shark are due to the slightly higher temperatures which have been found in this area, even in well-iced shark, possibly due to the insulating effect of the pocket of air formed by the closeness of the flaps in the vent region. The importance of temperature of storage to ammoniation of shark has been shown by the reduction in the time to ammoniation from 12 days or longer at $-1^{\circ}C$ to $-2^{\circ}C$ to only two days when held at $1^{\circ}C$ to $2^{\circ}C$.

Measurement of the ammonia level can be used as an indicator of the shark flesh quality but sophisticated laboratory equipment is necessary to get accurate measurements and great care must be taken in selecting the samples of flesh from the body for the ammonia analysis.

One characteristic of ammonia is its high pH value (approximately 11). This suggested that despite the many physical and chemical changes taking place as shark ages in ice it might be possible to use a simple measurement like pH as an indicator of the changes taking place. Shark, when it is freshly caught, has a pH of approximately 6. As spoilage commences and ammonia is formed, the surface pH of the shark flesh increases to a value of 9 (or greater) at which time the flesh is strongly ammoniated. This increase in pH follows a consistent pattern particularly when the measurements are carried out on remnants of brachial and cranial nerves exposed where the head was severed.

Surface pH can be easily assessed by the use of narrow range indicator paper which measures pH in the range 6-8. In good quality shark, the remnants of the nerves have a pH of 6. On occasions shark of apparently good quality have shown a pH of 7-8 in this region. These shark have been found to be ammoniated after only 4-6 days storage in ice compared with 10-12 days for shark with an initial pH of 6. This pH test is therefore more definitive in assessing the shark quality than general appearance.

Beside ammoniation, broken and split fillets are a major cause of down-grading the final product. Broken and split fillets are produced by poor physical handling, particularly when the fish is in rigor. Poor handling results from the tendency of many in the fishing industry to regard and treat shark as a non-perishable commodity rather than a highly perishable foodstuff. Figures 1 and 2 show the effect that rough handling has on shark. Figure 1 shows the normal appearance of a shark in rigor while figure 2 shows the result of poor handling. This shark was deliberately mishandled by dropping it on to the floor from waist height a total of six times which is a much milder treatment than is often found in practice (Figure 3).

The fillets produced from the mishandled shark were split and of poor quality, whereas the fillets from the shark which had been handled with reasonable care were in excellent condition.

A system of quality assessment can be set up employing the use of surface pH measurement and physical appearance. This is illustrated in Table 1.

TABLE 1.

EFFECTS OF AGE ON THE PHYSICAL APPEARANCE, AND pH OF SHARK AFTER OPTIMUM STORAGE CONDITIONS IN ICE.

pH	Physical Appearance		Age in Ice after killing
5.5-6	Stiff (in rigor)	(see Figure 1)	0-4 days
6.5-7.5	Not stiff (post rigor)	(see Figure 2)	6 days
7.5-8	Not stiff (post rigor)	(see Figure 2)	9 days
9	Ammonia evident	(see Figure 2)	12 days

Most shark fishing trips are less than three days, therefore well-handled shark will have the characteristics of 0-4 day old shark when received at the factory. If they have been poorly handled, the pH will be characteristic of 0-4 day old shark but the appearance will be that of a 6-12 day old shark. The latter shark will yield split fillets.

To ensure that top quality shark is produced, the following basic precautions should be observed:

i) Keep the shark well iced as soon after capture as possible.

ii) Keep all areas which the shark will come in contact with as clean as possible so that the bacterial contamination of the shark is kept to a minimum.

iii) Handle the shark as a top quality, highly perishable foodstuff.

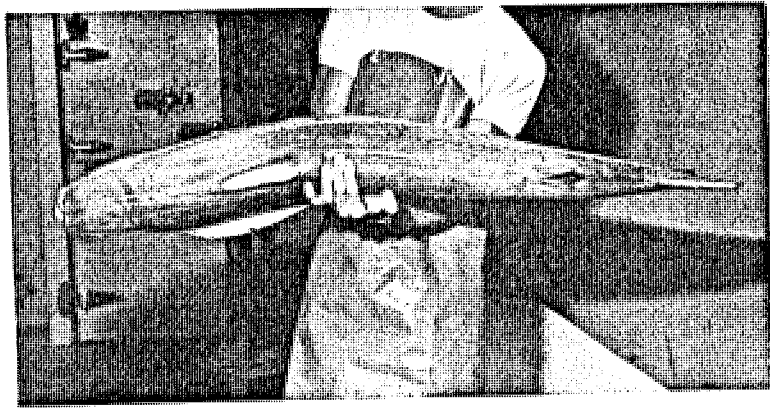

Figure 1. Good quality shark in rigor.

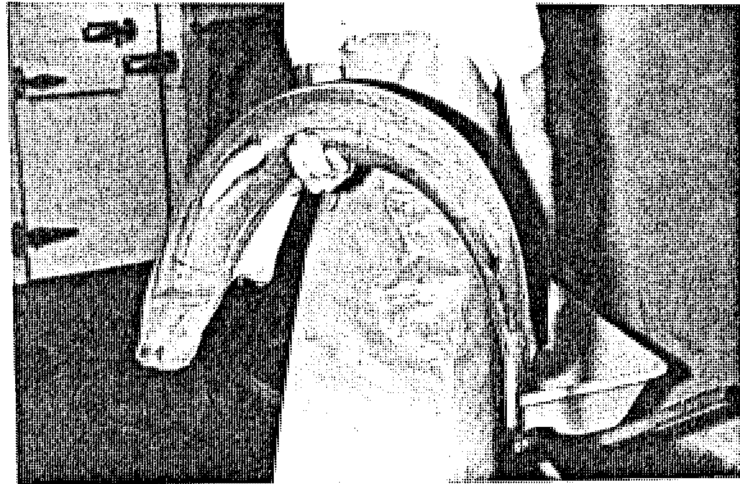

Figure 2. Results of dropping a good quality shark in rigor from the waist 6 times. This will yield split fillets on filleting.

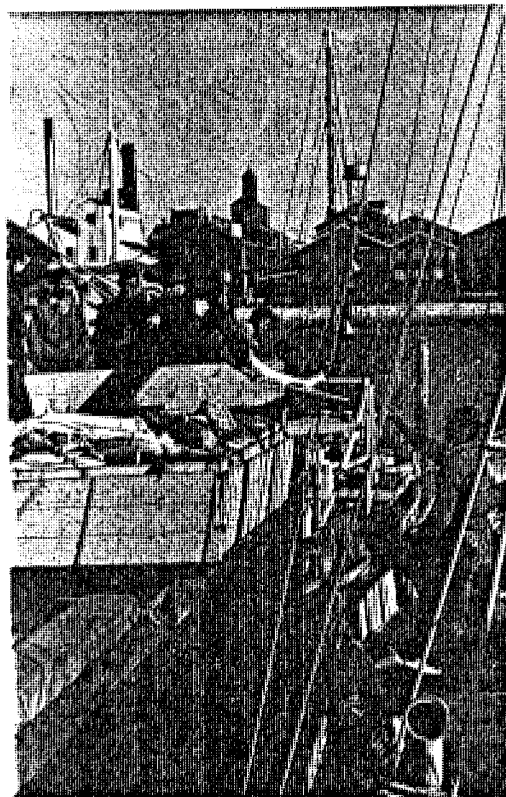

Figure 3. Example of poor commercial shark handling. Shark are being thrown from the boat to the truck.

ACKNOWLEDGEMENTS

This work was financed by funds from the Fishing Industry Research Trust Account.

The author wishes to express his gratitude to Mr. B. Wilson for technical assistance, Mr. R. Gallasch for preparing the manuscript and the staff of Safcol Research and Development Division and Mr. James, CSIRO, for their help and advice during the course of the work.

THE EFFECT OF FROZEN STORAGE ON SHARK QUALITY

Fishermen, fish processors and fish retailers are all in the food industry and their object should be to provide a top quality product which is attractive to the consumer. Two important aspects in the marketing of fishery products are:

i) fish, by nature, is highly perishable;

ii) the availability of fish supplies can be an irregular or seasonal occurrence.

Since fish is highly perishable, proper care must be exercised at every stage in the progression of the fish from the sea to the consumer's plate. Some method of preservation is needed to ensure a constant supply throughout the year. Preservation by freezing provides the closest approach yet achieved to maintaining the fresh quality of food for extended periods.

A study of shark handling and processing methods with the aim of improving the quality of both fresh and frozen shark on the wholesale market has been carried out under a grant from the Fishing Industry Research Trust. The aim of this study was two-fold:

i) to evaluate the effect of slow and fast freezing on the quality of shark fillets;

ii) to study the physical and biochemical changes taking place during freezing and storage in order to define some rapid, non subjective assessment of quality (or time of storage).

The biochemical, physical and organoleptic criteria used to assess quality were decided upon in consultation with Mr. D. James, CSIRO Division of Food Research, Hobart, Tasmania. The parameters

selected were surface pH, homogenised flesh pH, ammonia levels, extractable protein, moisture content, histological structure of muscle and analysis of thaw drip for percentage drip loss, pH, ammonia concentration and protein nitrogen. These parameters were compared with taste panel assessment of quality.

Shark fillets were either frozen to $-10^{\circ}C$ in 15 minutes using a nitrogen freezer at $-140^{\circ}C$, or frozen to $-10^{\circ}C$ in 12 hours using a conventional blast freezer operating at $-20^{\circ}C$.

The biochemical, physical and organoleptic tests mentioned earlier were used to assess the quality of the fillets. Identical results were obtained for both fast and slow freezing. Clearly, the extremely fast freezing rate offers no advantage over the freezing rate more typical of commercial practice.

Fillets of good and poor quality school and gummy sharks were frozen in freezers representing fast commercial freezing and slow commercial freezing. The frozen fillets were then stored under commercial freezer conditions ($-20^{\circ}C$). The fillets were assessed for quality by the above tests before freezing and after various periods of frozen storage of up to 6 months.

The fillets initially of good quality remained of acceptable (although declining) quality throughout the trial with the flesh becoming drier and tougher with less taste after extended periods of storage.

The fillets from the poor quality shark showed a slight improvement in acceptability after being frozen for one week, but were judged unacceptable after one month's storage. The overall rate of deterioration of poor quality fillets was far greater than that of

good quality fillets, possibly due to the activity of the bacterial urease enzymes still active on the tissue.

None of the physical or biochemical criteria used to assess the quality of the fillets could be correlated with the quality as judged by the taste panel. These criteria cannot, therefore, be used as the basis for a rapid, non subjective assessment of the quality of frozen shark fillets.

It is clear, however, that the quality of the frozen shark fillets is determined largely by the quality of the fillets prior to freezing. Every effort must therefore be made by the fishermen and processors to see that the shark is rapidly chilled and rapidly processed to ensure optimum quality of the final product on the market.

MACHINES SUITABLE FOR SHARK SKINNING

Shark skinning is a very arduous task if done manually and bad skinning can result in a large trimming effort or low fillet recoveries.

In order to overcome these problems, we examined the possibility of mechanising the skinning operation. The machine selected was a Townsend Model 600 membrane skinner.

This machine, unlike those described by Davis (1974) operates by gripping the skin and thereby dragging the fillet over a stationary knife. The blade is spring loaded so that the depth of cut is determined by the blade length and the skin thickness.

A Townsend Model 600 membrane skinner was purchased with funds from the Fishing Industry Trust Account for trials in the production area. It was found that the machine had two problems; it required a skilled operator on each side of the machine and the blades quickly dulled, making the initial feed difficult. However, in the hands of skilled operators and with proper attention to the blades, the skinning machine was quite successful in reducing the skinning effort.

D.E. BOTTRILL

Reference:

Davis, P. (1974) Skinning and scaling machines.
Aust. Fisheries 3.3 : 29