FISHING INDUSTRY RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

The effects of environmental factors on microbial growth in prepared seafood products and prediction of shelf life and safety

A full progress report was provided to FIRDC in 1989 when Dr JA Statham indicated her intention to resign her position as part-time Research Fellow on the project. A copy of this report is appended (Appendix 1).

Dr Statham was replaced by a full-time graduate research assistant in July 1989. From that time to completion of the project in June 1990, research concentrated on the development of a mathematical model to describe the effect of temperature and water activity (salt concentration) on the growth of *Staphylococcus aureus* in fisheries products. A full report of this aspect of the work, a list of publications arising and extension efforts with industry are provided below.

DEVELOPMENT OF A MODEL TO PREDICT GROWTH OF *STAPHYLOCOCCUS AUREUS*

Selection of Models

The general form of the model chosen was the square root model developed by Ratkowsky *et al.* (1982) *J Bacteriol* **149**: 105, to describe temperature effects and modified by McMeekin *et al.* (1987), *J appl Bacteriol* **62:** 543-550, to include the effect of water activity (a_W) adjusted by varying the salt concentration of the product.

The temperature model has the form

$$\sqrt{r} = b(T - T_{min}) \quad (1)$$

and the temperature/a_w model has the form

$$\sqrt{r} = b'\sqrt{(a_w - a_{w_{min}})}\,(T - T_{min}) \quad (2)$$

where r = rate of growth; a_w = water activity; $a_{w_{min}}$ = theoretical lower a_w limit for growth; T = temperature; T_{min} = theoretical lower temperature limit for growth; b, b' = scaling constants.

These general models were chosen as they meet the criteria of providing accurate predictions with ease of use.

Determination of Model Parameters

The general experimental protocol used was to evaluate the effects of temperature at close intervals (~1°C) and across the range of water activity values allowing growth of the organism.

Specifically, *Staphylococcus aureus* 3b, a wild strain originally isolated from prawns imported from Thailand was chosen as the test organism. A model for the growth of this organism in response to temperature and water activity (NaCl) was constructed by measuring optical density changes at approximately 1.5°C temperature intervals in Brain Heart Infusion Broth (BHIB) supplemented with sodium chloride to alter water activity. In other experiments growth rates in broths with various NaCl concentrations at approximately 1% relative humidity intervals, held at constant temperature, were determined. Temperatures ranged from 5°C to 35°C and water activities from 0.997 to 0.848. All data were combined to generate a model of the square root type. The validity of the model was then tested by inoculation of *S. aureus* 3b onto aseptically shelled and deveined tiger prawns (*Penaeus monodon*), commercially prepared filleted smoked Atlantic salmon and the same salmon product rebrined to achieve a lower water activity. Replicate samples of the inoculated products were incubated at closely controlled temperatures (±0.1°C). Duplicate samples were withdrawn at appropriate intervals and *S. aureus* enumerated and total viable count determined by spread plating on Baird-Parker agar and plate count agar, respectively. At the end of incubation, the pH of broths was 7.2 ± 0.2 and of the food was 7.0 ± 0.2.

RESULTS

As observed for other organisms, the value T_{min} was found to be constant for square root plots of generation time when water activity was varied, with a mean value of 7.4 ± 0.6 (SD) °C. The $a_{w_{min}}$ determined from the slopes of the individual square root plots is 0.86 and was confirmed by determining growth rates at a range of water activities at fixed temperatures. These data are summarised in Figures 1 and 2. The value of 'b' was fitted by substitution of data into the equation to give the model

$$\sqrt{r} = 0.21\sqrt{(a_w - 0.86)(T - 7.4)}$$

The T_{min} values for the growth rates on the three foods have an average T_{min} of 7.45 ± 0.13 (SD) °C and a mean $a_{w_{min}}$ of 0.87. Comparisons of the growth rates predicted from this model and those observed on food products are shown in Figures 1(a), (b) and (c) and in Table 1. The model predictions are generally in good agreement with the experimental observations. The much slower than predicted growth rate determined on the rebrined salmon (a_w = 0.92) at 12.6°C may be explained by the large numbers of other organisms competing with and inhibiting the growth of *S. aureus*. This was the only experiment in which the viable count greatly exceeded that of *S. aureus* at the end of the incubation. Overall, excluding the rebrined salmon result for 12.6°C for the reasons stated, the mean absolute percentage difference between predicted and observed results is 13.0%.

The significance of this work to date is that it truly assesses the predictive ability of the model used. Most other comparisons have tended to assess the ability of a model to fit a particular data set.

TECHNOLOGICAL APPLICATION

Temperature loggers record temperature at selected intervals and then analyse that temperature history with specific computer software to predict the extent of microbial growth over any time interval during the processing, storage or distribution of the product.

The models developed to describe the effect of temperature and water activity on the growth of *S. aureus* provide the basis of a specific application computer program to estimate growth of *S. aureus* in products with water activity levels from 0.92 - >0.99 stored at temperatures between 5°C and 35°C.

In an attempt to gauge the interest of the local fishing industry in this technology, several Delphi Temperature Loggers were purchased outside FIRDC funds. The loggers were offered to fishermen and fish processors as outlined in the attached information. Contact with industry was facilitated by Mr David Stone of the Tasmanian Fishing Industry Training Council (Inc) (Appendix 2). The possibility of writing a computer program comparable with the Delphi system to interpret temperature history data to predict growth of *S. aureus* is under investigation.

In more general terms, the importance of strict temperature control of product was emphasised in an article published in *Australian Fisheries* and reproduced in several State fishing industry journals. This elicited several enquiries, particularly from CA Olsen (Master Fisherman), Queensland, who was mainly concerned about the rate of spoilage of shark caught in nets.

A further popular article on temperature loggers, temperature integrators and other quality monitoring devices is in preparation.

PUBLICATIONS

McMEEKIN TA and ROSS T (1990) Temperatures can spoil the fresh seafood market. *Australian Fisheries* February 1990.

ROSS R and McMEEKIN TA Principles and potential of predictive microbiology. Submitted to *Food Australia* November 1990.

ROSS T Microbial quality evaluation of seafoods by temperature recording devices. In preparation for *Australian Fisheries*.

ROSS T and McMEEKIN TA Development and validation of a predictive model for the growth of *S. aureus* in response to water activity and temperature. In preparation for *Journal of Applied Bacteriology*.

Table 1

Comparison of predicted and observed growth rates for *Staphylococcus aureus* on some seafoods

Food Type	a_w	Temperature (°C)	Generation Time (min) Predicted	Observed	% Deviation from Prediction
Prawns	.995	12.5	646	540	16.4
	.995	17.5	165	135	18.2
	.995	20	106	118	-11.3
	.995	25	54	48	11.1
	.995	30	33	39	12.1
	.995	32.5	27	27	11.1
Smoked Atlantic	.966	12.5	822	704	14.4
Salmon	.966	17.5	210	247	-17.6
	.974	22.5	87	84	3.7
	.954	25.0	78	100	-28.2
	.974	27.5	49	55	-12.2
	.966	32.5	34	36	-5.8
	.954	35.0	32	30	6.3
Rebrined Smoked	.92	12.6	1398	2736	-95.7*
Salmon	.92	17.5	360	361	0.3
	.92	22.5	166	232	-39.8
	.92	27.5	94	97	-3.2
	.92	32.5	60	66	-10.0
Mean Absolute % Deviation					13.0

*Not included in mean error estimate (see text).

FIGURE 1. Square root plots for the growth of *Staphylococcus aureus* 3b on various seafood products, comparing the observed results to those predicted by the derived model

Fig. 1a: S. aureus on prawns (aw ≈ .995)

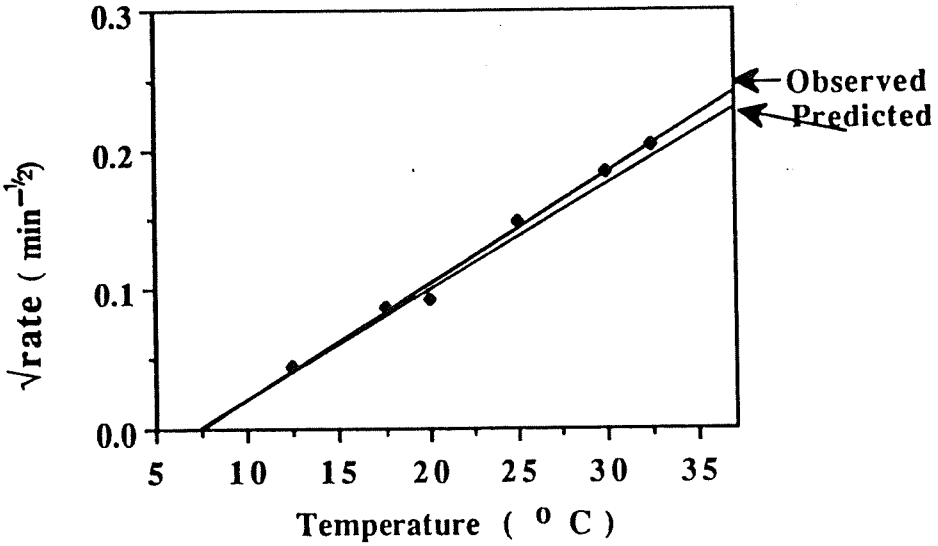


Fig. 1b: S.aureus on smoked salmon (aw ≈ .96)

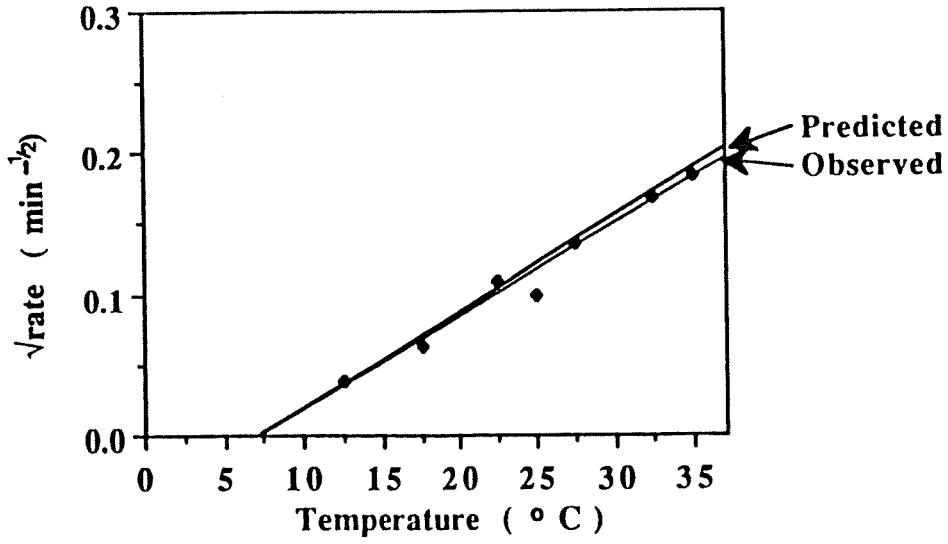
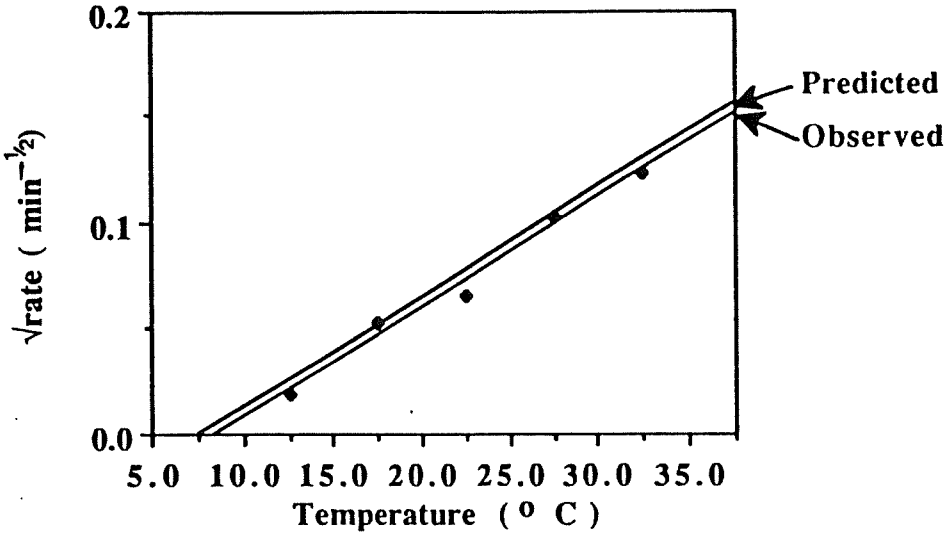


Fig.1c: S. aureus 3b on smoked salmon (aw ≈ 0.92)



APPENDIX 1

FISHING INDUSTRY RESEARCH AND DEVELOPMENT

TRUST FUND

APPLICATION FOR CONTINUING GRANT

1. PROJECT TITLE

The effects of environmental factors on microbial growth in prepared seafood products and prediction of shelf life and safety.

2. OBJECTIVES

1) To investigate the microbial status of value added "convenience" seafood products, in terms of shelf life and safety, during chilled storage.

2) To establish a model by which the microbial status of such products can be predicted under different conditions, of temperature, atmosphere, product formulation.

3. PROGRESS REPORT

The project commenced in July 1987 and at this time has reached approximately the half way stage. Work has been carried out with the cooperation of various sections of the fishing industry including

oyster growers and processors

salmonid farmers

and producers of value added products.

For each of the products investigated the basic research method has been to determine the microbial status of the product in conjunction with sensory evaluation. In this way a knowledge of the microbial ecology of each product under different storage conditions is built up and related to the functional characteristics (appearance,

odour, taste etc) that cause rejection of the product by the consumer.

Most fresh seafood products in chill storage deteriorate rapidly as a result of the growth of gram negative psychrotropic bacteria mainly **Pseudomonas** and **Alteromonas** (now **Shewanella**). These organisms produce putrid spoilage compounds such as sulphides, ammonia and some amines. Manipulation of the storage conditions (eg gaseous atmosphere) or formulation of the product (pH, water activity) may lead to replacement of the gram negative microbiota with gram positive bacteria. The latter organisms tend to grow less rapidly at chill temperatures and produce less obvious spoilage changes leading to a longer shelf life.

PRODUCTS SPOILED BY GRAM NEGATIVE BACTERIA

PACIFIC OYSTERS

At present chilled oysters shipped on the half shell have a shelf life limited to 3-4 days at the recommended storage temperature of 4°C. This was extended to 6-8 days at 4° by vacuum packing. CO_2 flushing of packs also delayed spoilage but was inappropriate because of massive gas uptake by the tissue leading to "fizzy" oysters.

Despite the shelf life advantage gained by vacuum packing the application of this technology to commercial oyster storage was considered to present potential public health risk if temperature abuse occurred. e.g. storage at > 10°C.

Oysters stored aerobically at chill temperature spoil as a result of the growth of typical gram negative psychrotropic bacteria. These organisms are known to respond to temperature in accordance with the square root model proposed by Ratkowsky, Olley, McMeekin and Ball 1982 (J. Bacteriol. **149**, 1-5). A relative rate function derived

from this model indicates that shelf life at 0°C will be approximately double that at 4°C i.e. the same advantage as that gained by vacuum packing. The prediction was tested experimentally and oyster meats on the half shell were shown to remain in good condition for 8-10 days at 0°C. Maintenance of distribution temperatures close to 0°C, which can be achieved using modern packing material, ice blankets etc would allow sufficient time to transport oysters to more distant markets by surface or air freight

CRAYFISH PATÉ

This product had a water activity of ∿1.00 and a pH value > 6. The predicted spoilage biota at 4° was confirmed to be 100% pseudomonads and a shelf life of 7 days at 4° was indicated. The critical control factor is storage temperature and predicted shelf lives at other temperatures are shown below.

Temperature	Relative spoilage rate	Shelf life (days)
0°	1.00	14
2°	1.44	9
4°	1.96	7
7°	1.89	4.5
10°	4.00	3.5

POACHED SALMON

This is a new product in the value added, ready prepared meal category on which preliminary work has been carried out. The water activity and pH of the product allowed gram negative bacteria to

proliferate and shelf life was estimated to be 7 days at 4°.

The microbial ecology of the product indicates that predictions similar to those of crayfish paté will be appropriate. As this is a high price product further detailed examination is planned.

CAVIAR

Caviar prepared from salmon and trout eggs have a shelf life of 14 days at 2°C if the pH is < 6.0. Reducing the pH prevents the growth of **Alteromonas putrefaciens** a potent producer of sulphydryl compounds and other noxious odours.

PRODUCTS SPOILED BY GRAM POSITIVE BACTERIA

When fresh seafood substrates are modified by reduction of water activity, and/or pH or stored in an atmosphere other than air, gram negative psychrotroph bacteria generally fail to compete with gram positive bacteria, including lactic acid bacteria, and yeasts.

SALMONID PATÉS

Smoked trout and smoked salmon patés with reduced water activity values (0.96 and 0.97) did not support the growth of gram negative spoilage bacteria, but development of lactic acid bacteria and yeasts. The growth of this population follows a relative rate function different from that of gram negative bacteria and has yet to be determined in detail. Using a criterion of time to reach 10^7 bacterial cells/g, patés stored at 5° had a shelf life of 5 days at 5°C and 21 days at 0°C. By organoleptic assessment the shelf life at 5°C was estimated to be 7 days and 14 days at 0°C. This suggests that non microbial factors such as enzymes or oxidative processes may also contribute to spoilage.

Some samples of pate in which the trout received a less severe smoking process had an a_w value $\sim$1.00 which would be expected to

allow growth of gram negative bacteria. However again only lactic acid bacteria and yeasts were recovered. Further samples of the product were deliberately inoculated with gram negative spoilage organisms which died out during storage. The pH of the product was 5.6 which by itself would not inhibit pseudomonad growth. The hypothesis under investigation is that low levels of lactic acid from cheese blended in the paté is the inhibitory factor. If this is correct the potential exists to control gram negative bacteria in seafood products by addition of small amounts of a permitted food additive rather than water activity reduction by addition of large quantities of NaCl.

SMOKED SALMON AND TROUT

Smoked salmon and trout products in vacuum packs have shelf lives estimated at 21 days at 2-3°C. The limit is set by product appearance rather than odour or flavour, in particular by development of small microbial colonies after 21 days. Water activity values were measured at 0.91-0.95 and at this level with vacuum packing controlled spoilage bacteria. The combination of inhibitory factors is important as bacteria grow rapidly under aerobic conditions on removal from the vacuum package. This is an important consideration requiring further investigation as it simulates product handling practices likely to occur in retail trade.

SUMMARY AND PROJECTIONS

At the half way stage of the project a range of products have been examined, their microbial ecology determined and their sensory characteristics evaluated. This has been time consuming work but has

provided essential background information for use in predictive studies of shelf life. Predictions on the shelf life of products spoiled by gram negative organisms were as expected and consistent with relative rates based on the square root model (Ratkowsky et al. 1982, J. Bacteriol. **149** 1-5). In the remaining time further predictive work will be carried out on shelf life estimation of products spoiled by gram positive bacteria (products in this category include many new to Australia that are "value added" and expensive); the role of lactic acid in control of gram negative bacteria will be investigated as it may offer an alternative preservation strategy; and challenge studies with organisms of public health significance will be carried out.

TRANSFER OF RESULTS TO INDUSTRY

Suppliers of product are advised directly of results and provided with an evaulation of shelf life.

General article(s) on shelf life evaluation and factors affecting microbial growth in seafood products will be submitted to Australian Fisheries. More detailed results will be published in scientific journals.

5. PREDICTED COMPLETION DATE

JUNE 1990

6. REQUESTED BUDGET. 1989/90

	ORIGINAL ESTIMATE	REVISED ESTIMATE
SALARIES	26316	25058
OPERATING EXPENSES	6500	4000
TRAVEL	1236	1300
CAPITAL ITEMS	Nil	
TOTAL	34052	30358

7. PREVIOUS YEARS ALLOCATION

YEAR	FUNDS ALLOCATED
1987/88	30644
1988/89	10216 interim payment
	20428 subsequent payment

8. FUNDS SOUGHT FROM OTHER SOURCES

NIL.

9. FINANCIAL CONTRIBUTION OF APPLICANT

Salary component for Dr T.A. McMeekin, Project Supervisor.

Fully equipped microbiological laboratory,

Administrative and ancillary support.

10. DETAILS OF FUNDS REQUESTED FOR 1989/90

Detailed Statement of Funds

	ORIGINAL ESTIMATE	REVISED ESTIMATE
SALARIES AND WAGES		
i) Research Fellow/Grad. Research Assist	20817	20773
ii) Payroll tax workers comp.	1499	2285
iii) Casual labour	4000	2000
OPERATING COSTS		
i) Product/Raw material Freight	2000	2000
ii) Chemicals & Consumables Plastics, Media, Glassware	2000	2000
iii) Repair and Maintenance To CSIRO	2500	Nil

TRAVEL

Airfare (based on Perth return i.e. maximum likely distance)	836	900
Living allowance (6 days @ 66.50)	400	400

11. ORGANISATION

UNIVERSITY OF TASMANIA

Head Responsible for Project

Dr J.A. Beattie

Department of Agricultural Science

University of Tasmania

GPO Box 252C

Hobart

Tasmania 7001

Telephone (002) 202620

Tax (002) 202186

Telex 58150 UNITAS

12. PROJECT SUPERVISOR

Dr T.A. McMeekin

Reader in Agricultural Microbiology

Address - as in 11 above

13. STAFF

Graduate Research Assistant to be appointed

Technical Assistant (part time) to be appointed

14. ADMINISTRATIVE CONTACT

Mrs J. Rush

Address, Fax & Telex as in 11 above

Telephone (002) 202032.

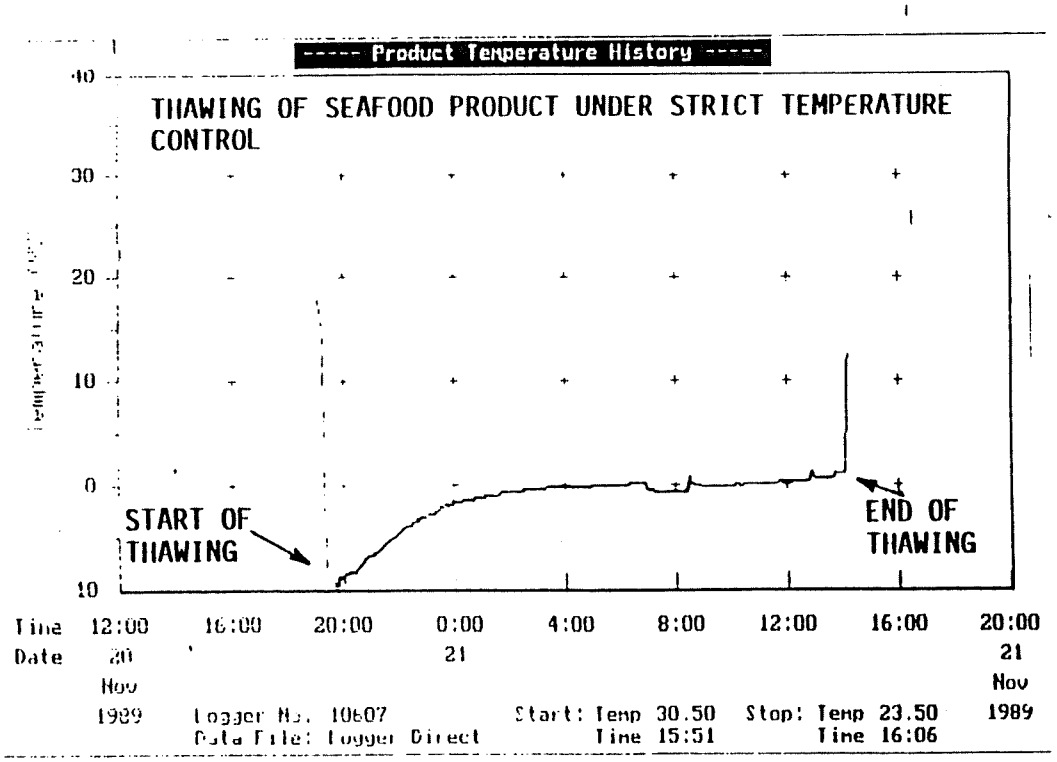
FIRDC/UNIVERSITY OF TASMANIA

SAFETY AND QUALITY OF SEAFOOD PRODUCTS

TEMPERATURE is the major factor controlling the rate of spoilage of seafood products during transport, processing and storage, but strict temperature control is often neglected.

To assist processors to optimise temperature control, the **Department of Agricultural Science, University of Tasmania,** has purchased **2 DELPHI TEMPERATURE LOGGERS** using FIRDC funds.

These devices record temperature at pre-set time intervals for several weeks. The information is retrieved and analysed using an IBM-compatible computer. The temperature history is printed out graphically as shown below.



The loggers, which operate in the range -20°C to +40°C, are available to processors for short periods to evaluate the hygienic efficiency of processing and storage systems.

Full instructions on use will be given. The logger will be interrogated on return to the University and the printed temperature profile and an interpretation provided. All information will be treated confidentially.

There is **NO CHARGE** for this service but users will be asked to guarantee safe return of the logger (value ≈$700).

Further information may be obtained from:-

TOM ROSS or TOM MCMEEKIN

Department of Agricultural Science
University of Tasmania
GPO Box 252C
Hobart
Tasmania 7001
Telephone (002) 202637 or 202620
Facsimile (002) 202186