Microbiological Risk Assessment of Meat and Seafoods

John Sumner

BSc (Hons) London; PhD Keele

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Declarations

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any other University, and that, to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by any other person than myself, except where due reference is made in the text, nor does the thesis contain any material which infringes copyright.

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The body of work submitted here comprises papers and reports of which I am the sole author of ca. 15%, the remainder in collaboration with ca. 150 co-authors. I have developed a spreadsheet which describes my contribution to three elements of each of the 148 publications used in this thesis: Conception, Data collection and Analysis/writing up. However, for the purposes of this declaration I summarise estimates of my contributions as follows:

- During the period 1969-1988 I worked at universities and my involvement with all three elements was around 80%.
- From 1988 - present I have worked as a member of large teams e.g. risk assessment where my contribution was around 20-50%, and with 1-2 co-authors where it was 50-60%. In general, where I was not first author, I was last author, being responsible for the paper's narrative and draft development.

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Acknowledgments

The publications listed in this thesis include around 150 co-authors and I acknowledge their collaboration, especially the University's Tom McMeekin and Tom Ross who pointed me in directions which were both innovative and of immense usefulness to the food industry. More recently I have benefitted greatly from my association with Ian Jenson (Meat and Livestock Australia) and with SARDI scientists Andreas Kiermeier and Jessica Jolley.

The University library unearthed many publications I had long forgotten or lost and, as well as filling gaps in my journey, also brought back memories which made the preparation of this thesis infinitely more pleasurable.

Acronyms

ACIAR	Australian Centre for International Agricultural Research
ANZFA	Australia New Zealand Food Authority
AQIS	Australian Quarantine and Inspection Service
ASIQAP	Australian Seafood Industry Quality Assurance Program
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CDC	Centers for Disease Control
DANIDA	Danish International Development Agency
EEC	European Economic Community
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FIMA	Federal Meat Inspection Act
FRDC	Fisheries Research and Development Corporation
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
FSKP	Food Safety Key Program
GTZ	Gesellschaft für Technische Zusammenarbeit (German Corporation for Technical Cooperation)
HACCP	Hazard Analysis Critical Control Point
HBI	Hot Boning Index
IAFI	International Association of Fish Inspectors
ICI	Imperial Chemical Industries
ISO	International Organisation for Standardisation
JEMRA	Joint Expert Microbiological Risk Assessment
MISP	Meat Industry Strategic Plan
MRA	Microbiological Risk Assessment
NEPPS	National Enteric Pathogens Surveillance Scheme
PHLS	Public Health Laboratory Service
PIRSA	Primary Industries and Regions South Australia
QA	Quality Assurance
QC	Quality Control
QMRA	Quantitative Microbiological Risk Assessment
RI	Refrigeration Index
RMIT	Royal Melbourne Institute of Technology
SARDI	South Australian Research and Development Institute
SPC	South Pacific Commission
UNEP	United Nations Environment Program
UNSW	University of New South Wales
VMA	Victorian Meat Authority
WHO	World Health Organisation

Chapter 1 Microbiological risk assessment (MRA) of meat and seafoods

1.1 Introduction and context

This thesis summarises elements of more than half a century of my work as an academic and as a consultant. It has been influenced by numerous events, responses and developments, to which I refer as footnotes. Of these, three developments have been pivotal: the Hazard Analysis Critical Control Point (HACCP) concept, Quality Assurance (QA) and Predictive Microbiology. These are key elements of the discipline of Risk Assessment, which is the subject of this thesis – my involvement with risk assessments for the seafood and meat industries. However, underpinning any credible risk estimate is knowledge of the food process, the supply chain and information about how pathogens behave through that chain. These are the "hard yards" of risk assessment, without which modelers must rely on assumptions which, as we saw with early estimates of Covid illness and death, may be orders of magnitude from reality.

My work as a member of risk assessment teams, is contained in two appendices which catalogue hazard identification, food safety, shelf life, quality assurance, HACCP and predictive tools in Appendix 1 for the meat industry and Appendix 2 for the seafood industry. The scope of these appendices merits some brief context.

1.1.1 Hazard Analysis Critical Control Point (HACCP)

It is often related how, in 1959, Howard Bauman (the "father" of HACCP) received a call from the Quartermaster Food and Container Institute of the US Armed Forces asking (to paraphrase): *"Can Pillsbury produce foods for consumption in the zero gravity of space capsules?"* Equally oft-told is that the need for HACCP was borne out of the cost of testing space foods *"In fact, a large part of the production …. had to be utilised for testing, leaving only a small portion, hence the enormous costs of production…."* (Bauman 1994)[i].

I first encountered HACCP in 1979 when Dr Florian Majorak (US Food and Drug Administration) presented the concept at the NZ Institute of Food Science and Technology Conference in Auckland. I was very impressed with the concept, discussed it with him afterwards and he kindly gave me his overhead projector slides. I immediately introduced it into my teaching at RMIT and into QA plans for various industry sectors.

1.1.2 Quality Assurance (QA)

QA became *de rigueur* in the 1980s as industries moved from just inspecting and measuring products during their manufacture (Quality Control, QC) to a system which embraces all the company's planned and systematic actions which provide confidence that its goods will satisfy the requirements of consumers and regulators (QA). Quality systems flourished, as did gurus,

[i] Bauman, H. (1994). The origin of the HACCP system and subsequent evolution. *Food Science and Technology Today*, 8, 66-72.

like W. Edwards Deming whose ideas gave the Japanese industry much-needed impetus after WW II. The lag by western countries in their approaches to quality led the Economist magazine in the early 1990s to blame "quality gurus", who they also rated. Notwithstanding that he received the highest score, the octogenarian Peter Drucker responded immediately, thanking the Economist for his rating but gently reminding them that they were called gurus only because "charlatan" was difficult to spell.

I introduced QA plans to processors in the meat industry in the late-1980s and the seafood industry in the 1990s, with food safety and HACCP their underpinning. These were innovations that gave early adopter domestic meat companies a key advantage of export to numerous countries; for many seafood processors in the Indo-Pacific, my QA systems enabled market access to the USA and EU.

1.1.3 Predictive microbiology

In *Shelf life of Australian red meat* (77) Tom Ross introduces the section on Predictive Microbiology by stating: *"The behaviour of spoilage bacteria is predictable, which serves as the foundation of a field of food microbiology called Predictive Microbiology. In this research discipline, predictive tools (models) are produced by measuring and understanding how quickly bacteria grow (or die) in different food environments. Once understood, the data are converted into mathematical equations, which are then translated into software tools that help food companies manage the growth or death of bacteria in food processing systems and supply chains. The benefits of validated tools include reduced reliance on microbiological tests and greater flexibility in meeting performance standards."*

In Australia, predictive microbiology had its foundation in modelling at CSIRO in the early-1970s to predict spoilage of fish (Olley & Ratkowsky 1973)[ii]. By the late-1980s, U Tas and CSIRO collaboration led to models for the dairy and seafood industries and publication of *Predictive microbiology: theory and application* (McMeekin *et al*. 1993[iii]).

My collaboration with researchers at U Tas on Predictive Microbiology is documented throughout this thesis and a personal highlight is the work we did with companies producing manufacturing meat destined for grinding in the USA by a technology called "hot boning". It is an efficient process because, about 30 minutes after slaughter the carcase has been broken down, packed into cartons, and is in the freezer. However, with a centre temperature around 30°C the potential for growth of pathogenic *E. coli* led the Australian regulator to begin withdrawing licences to export from hot honing establishments. I believed a cooling system based on predictive microbiology was more appropriate than the current "7°C within 15 hours" regulation. I obtained industry and regulatory approval to design and carry out a trial involving all five hot boning plants to supply information on potential growth of *E. coli* during chilling of hot boned meat. The University integrated the data into a predictive model called

[ii] Olley, J. & Ratkowsky, D. (1973). Temperature function integration and its importance in the storage and distribution of flesh foods above the freezing point. *Food Technology in Australia*, 25:66-73.
[iii] McMeekin T., Olley J., Ross T. & Ratkowsky D. (1993). *Predictive microbiology: theory and application*. Pub. John Wiley & Sons Inc. New York.

the Hot Boning Index which allowed hot boning operations to continue and which was so well received that it was extended to the entire meat industry as the Refrigeration Index (RI); more detail is contained in Chapter 4.8.2.

1.2 Career summary

My career began as a laboratory assistant at Akers Research Laboratories in Welwyn, Hertfordshire, a facility of ICI (Imperial Chemical Industries) housed in a manor house commandeered as a secret research establishment in the Second World War. Now, teams of chemists, physicists and plant biologists led by no fewer than three Fellows of the Royal Society worked in a series of huts scattered over 47 acres of gardens on basic research of fungal metabolites such as griseofulvin and plant hormones such as gibberellins. We lab assistants were granted one day off each week to study for a London degree at Hatfield Polytechnic receiving great encouragement from our bosses.

When financial pragmatism saw the site sold to Unilever and staff subsumed within ICI Pharmaceutical Division in Cheshire, the university atmosphere was lost and many sought careers elsewhere. Now a graduate, I aspired to an academic career and in 1965 I applied for a research fellowship at Keele University. I was interviewed by Prof Alan Gemmell, a household name in the UK because of his weekly appearance on BBC radio Gardeners' Question Time. In the interview he asked what I'd study - if I got the job that is. I said I was interested in moulds that grew at high temperatures (thermophiles) - an idea he liked.

I was able to isolate thermophilic *Mucor* and *Rhizopus* species from warm coal spoil tips, cooling towers and self-incubating hay bales. The isolates grew well around 50°C with maxima up to 60°C and I worked on their nutrition and later on the lipid composition of their membranes (1-3).

It was almost a rite of passage in the 1960s to do post-doctoral research in North America and I landed a Teaching Fellowship in the Department of Food Science at the University of Alberta in Edmonton. My boss was interested in the behaviour of *Salmonella* at sub zero, non-frozen temperatures – the organism was inactivated very rapidly. Unfortunately, he left soon after my arrival for a 12-month sabbatical in the UK and I was handed his lectures and graduate students, but not his lecture notes. Somewhat time-poor I was only able to collaborate with colleagues interested in the lipid composition of the single cell phycomycete (*Blastocladiella*), in formation of sclerotia from *Sclerotinia sclerotiorum* and in basidiomycetes (4-8).

As the end of my two-year fellowship approached I was fortunate to be able to begin an academic career at Massey University in New Zealand. My next twenty-five years presented great opportunities for teaching, research and extension to industry at Massey, Lincoln College (NZ), RMIT and Victoria University in Australia, as well as semester and shorter stints in universities in Turkey, The Philippines and Singapore.

From 1976 I have operated a consulting practice and worked with many food companies and with all Australian governments; the United Nations Food and Agriculture Organisation (FAO), World Health Organisation (WHO) and Environment Program (UNEP); the World Bank; German Corporation for Technical Cooperation (GTZ); Danish International Development Agency (DANIDA); Food Standards Australia New Zealand (FSANZ); the Australian Centre for

International Agricultural Research (ACIAR) and with a number of foreign governments. Much of the work has been in capability building for seafood industries in the Asia-Pacific, for which I was awarded Technologist of the Biennium in 2003 by the International Association of Fish Inspectors (IAFI).

In the late 1990s risk assessment of microbiological hazards in foods was identified as a priority by the Codex Alimentarius Commission (CAC), which published *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (CAC 1999)[iv]. In Australia the meat industry addressed burgeoning food safety issues by investing in a Food Safety Key Program (FSKP). I was commissioned to design its business plan for which I co-opted industry expert Chris Orr and the University's Tom McMeekin. We developed an R&D program of 32 projects that set the industry's agenda for the next decade, of which more detail is provided in Chapter 4.6.1.

Finally, I note that it is common for university staff to highlight their publications and I've selected six, five of which are concerned with MRA and are regularly cited plus one which is rarely cited but is easily the most widely read: two publications with Tom Ross on design of a predictor tool, Risk Ranger, and its use in a risk profile of the seafood industry (9,10); three large reviews with the Joint Expert Microbiological Risk Assessment (JEMRA) on *Vibrios in Seafoods* (11-13) and a paper in New Scientist on bias of cricket umpires which was picked up by newspapers in cricketing countries (14).

1.3 Current work

Every day, each export meat establishment is required to monitor the microbiological and visual attributes of its products against criteria that were set more than a quarter of a century ago. During this time the industry has progressed (see HACCP, QA and Predictive Microbiology, above) so that current monitoring is both costly and of little utility to the establishment. With colleagues at SARDI I have introduced changes in both microbiological (15) and visual monitoring (16) which are geared to a risk-based approach.

[iv] CAC (Codex Alimentarius Commission). (1999). Principles and Guidelines for the Conduct of Microbiological Risk Assessment. Codex Alimentarius Commission. CAC/GL–30.

Chapter 2 Seafood risk assessments

In the 1990s there had been food poisoning outbreaks involving meat and seafood products in Australia and, with U Tas colleagues McMeekin and Ross, I estimated rates of food poisoning in Australia and speculated on origins of illness (17,18). I also completed an assignment in Geneva with WHO in the start-up phase of their mammoth *Foodborne diseases: Global Burden*. I supplied data from Australian sources and published a rudimentary attribution of food categories causing salmonellosis in Australia (19).

Continuing in Geneva and Rome as part of the JEMRA team, my role was to provide details of food processes and to estimate prevalence and concentrations of pathogens through the production, processing and consumption phases. Serendipitously, Tom Ross (mainly) and I had developed Risk Ranger (9), a software tool which could give realistic risk estimates in a short timeframe. It proved ideal for industry-wide screening, the opportunity for which arose almost immediately when the FRDC commissioned a national seafood risk assessment. It has become the risk profiling tool of choice both nationally and globally. In the former we used it for the national seafood, meat and egg risk profiles and in the global context the FAO Guide to Ranking Food Safety Risks at the National Level (2020)[v] stated: *"Risk Ranger … was carefully developed and maintains the theoretical model of risk as defined by Codex, being an excellent choice if the goal is to focus on microbial hazards and the number of food categories is manageably".*

During 1999 we compiled the basic information to undertake a risk profile for the industry (20), which was completed in 2001 using Risk Ranger and which screened pathogen:product pairs into risk categories. Low risk pairs had a Risk Rating (RR) <32 e.g. mercury poisoning; medium risk pairs had a rating of 32-48 e.g. *Listeria monocytogenes* in cold smoked fish, and high risk had a rating >48 e.g. ciguatera from recreational fishing in susceptible areas, viruses in shellfish from contaminated waters and algal biotoxins from uncontrolled waters after an algal event (10). The ratings we estimated were included in the FSANZ seafood risk assessment during development of the Primary Production and Processing Standard (PPPS).

Much of global seafood exports originates in developing countries for which there was an expectation by importing countries that a risk assessment would be undertaken – even by the smallest, atoll countries. The FAO commissioned me to write a straightforward text to help nations produce a credible risk profile and, with Tom Ross I published "*Application of risk assessment in the fish industry*" (21).

Our JEMRA team on *Vibrios in seafoods* also provided risk assessments for *V. vulnificus* (11), *V. cholerae* (12, 22) and *V. parahaemolyticus* (13) in which Risk Ranger was used to generate qualitative risk assessments.

The Australian Seafood Co-operative Research Centre commissioned several risk-based investigations including assessments on prawns (domestic and imported) consumed in Australia (23), raw fish consumption and parasites (24), hazards in seafoods in general (25, 26) and in

[v] FAO Guide to Ranking Food Safety Risks at the National Level. (2020). *FAO Food Safety and Quality Series* (10), Rome, Italy.

South Australian oysters, in particular (27). Risks and benefits of seafood consumption were identified and estimated (28) and an exposure assessment made of methyl mercury in Australia seafoods (29). We also contributed a chapter on *Risk assessment to improve risk management* in *Seafood Processing: Technology, Quality and Safety* (30) and questioned whether regulators (risk managers) globally were hesitant to implement risk assessment findings they had commissioned (31); I expand on this contention later in Chapter 3.

In the case of Australia, the follow-through by regulators on risk assessments they had commissioned was commendable. The South Australian (32) and Tasmanian (33) governments commissioned me to undertake risk assessments of their regulatory frameworks as they pertained to food safety. In Victoria a series of investigations of the Shellfish Quality Assurance Program (34-36) culminated in my reviewing how the State manages risks associated with shellfish aquaculture (37). In Tasmania the government responded to a risk assessment following four listerioses (of which two proved fatal) from consumption of smoked salmon by gazetting risk mitigation changes for manufacturers (38).

Chapter 3 Meat risk assessments

In 2002, MLA commissioned a risk profile of the red meat, processed meat and ready-to-eat meat industries. Several groups were involved: industry, regulatory (AQIS and State food authorities), standards setters (FSANZ) and researchers. We managed this disparate group *via* an iterative consultation process between risk managers and assessors, which was at variance to the traditional view that they should remain at arm's length (39). We undertook a rigorous Hazard Identification phase (40) and used data from national baseline studies to complete the task (41). We presented the work at the 5th World Congress on Foodborne Infections and Intoxications in Berlin (42). The profile received recognition by Lammerding (2006)[vi]: "*A current drawback of QMRA is the length of time required to complete a comprehensive assessment, in many cases, multiple years. By contrast, the risk profiling of the Australian meat industry was completed within a 12-month period and provided timely answers to contemporary risk management questions*".

The integrated management approach of assessors and managers we used in the national meat industry risk assessment was also used in the national egg industry risk assessment (43) and we supplied a great deal of the background information to FSANZ risk assessments of raw and processed meats during the PPPS exercise (44,45).

In the national risk assessment *Listeria monocytogenes* and ready-to-eat meats were identified as a pathogen:product pair requiring a full QMRA. The assessment predicted that processed meats could be responsible for up to ~40% of listerioses in Australia, a level which aligned with available epidemiological data (46). The model included relatively novel aspects including the influence of lactic acid bacteria on the potential for growth of *L. monocytogenes*, the reduction of nitrite levels, plus product shelf life and its potential disposition prior to consumption (47). We published an industry document on risks and controls (48) and undertook awareness sessions in each State.

Beginning 2007, in the red meat industry the testing of manufacturing meat for presence of Shiga Toxigenic *Escherichia coli* (STEC) was gradually strengthened, leading to increased detection rates both at home and at Port of Entry (PoE) testing in the USA. We assessed the effect of changing from testing pieces of meat to the new, surface slice sampling method (49) and also assessed a rumoured intensification from N60 to N120 sampling whereby 120 x 5g surface slices would be tested. We found that while the current and suggested sampling plans were more likely to detect highly contaminated lots, they did not always do so, confirming that sampling and testing cannot be relied upon as a food safety measure (50).

A desirable aspect of Australian manufacturing meat is its low-fat content, coming as it does generally from cull dairy cows and allowing it to be blended in the USA with higher fat trim from feedlot cattle. We investigated the likelihood of "Australian" manufacturing meat causing STEC illness in the USA, a theoretical estimate because our meat is typically co-

[vi] Lammerding, A. (2006). Modelling and risk assessment for *Salmonella* in meat and poultry. *Journal of AOAC International*, 89:543-552.

mingled with meat from USA and other importing countries during blending and grinding. We estimated 50 STEC cases/annum in the USA from consumption of the 2.5 billion hamburgers made from "Aussie beef", with 99.9% of illnesses likely to occur in the home due to undercooking (51).

Australia is a major supplier of manufacturing meat to the McDonald's organisation in Japan and the company indicated its intention to undertake a risk assessment of our manufacturing meat. We suggested the work be done in Australia with a joint team. The Japanese team spent one week in Australia, seeing operations at their suppliers, and working with us on a modification of the USA risk model. The final estimate was that "Aussie beef" might be expected to cause a handful of deaths over a century timeframe from consumption of McDonald's hamburgers in Japan, a risk considered acceptable by that company.

The value of baseline studies was appreciated in some States which undertook intensive microbiological monitoring to assess performance of their coregulatory systems. I designed and undertook several surveys of products - in South Australia, for example, on products from meat (52), poultry (53,54), kangaroo (55) and turkey (56) operations, and in New South Wales on poultry (57) and red meat operations (58).

The response by risk managers in Victoria, South Australia, New South Wales and Tasmania to assessment findings may be seen as an outlier where globally, risk managers rarely accept the results of risk assessments they have commissioned, often preferring to rely on the Precautionary Principle (PP). For example, the European Union banned Asian shrimp imports because of the detection of chloramphenicol (CAP), the USA declared zero tolerance for *L. monocytogenes* in ready-to-eat foods and of STECs in raw meat intended for grinding.

The scientific fragility of the Precautionary Principle was exposed by Hanekamp *et al*. (2003)[vii] who cited the CAP issue in Europe as an example where system managers foster a culture that they alone must prevent all damage, irrespective of cost and reality. In so doing, the authors suggest, risk managers become "moral free riders". Perhaps this resonates with the responses of some State Premiers (risk managers) and Chief Health/Medical Officers (risk assessors) to Covid 19 prevalence?

Finally, sometimes the task of being a risk assessor is itself a risky business. In 2012 an Italian court convicted six scientists of manslaughter for underestimating the likely consequences of an earthquake which killed 309 people in L'Aquila. The risk assessors were sentenced to six years in jail and $10 million in costs and damages incurred - later overturned on appeal but likely to make would-be risk assessors think twice.

[vii] Hanekamp, J., Frapporti, G. & Olieman, K. (2003), Chloramphenicol, food safety and precautionary thinking in Europe. *Environmental Liability*, 6:209-221.

Chapter 4 Hazard identification, food safety, shelf life, quality assurance, HACCP and predictive tools for the meat industry

A significant part of my early career in New Zealand was with the meat industry: teaching the Diploma of Meat Technology at Massey University and the Diploma of Meat Inspection at Lincoln College. The meat industry of the early 1970s produced carcases which were broken up in butcher shops into cuts and trim, the latter for grinding into minced meat or emulsifying into sausages. Meat was exported as whole bodies (sheep) or as quarters (beef) usually frozen shrouded in muslin to prevent foreign matter contamination. At that time, while beef and sheep abattoirs had no reason to want to know the microbiological condition of their product an opportunity arose to monitor a new meat product - venison.

4.1 Feral and farmed deer

In the 1970s feral red deer (*Cervus elaphus*) populations were high in the Southern Alps and helicopter hunting was a means of simultaneously controlling population increase and allowing recovery of shot animals from areas virtually inaccessible to hunters on foot. Bodies were ferried immediately to a convenient point where the viscera were removed and the pelvis opened to facilitate chilling, before flying to a packing house or refrigerated field depot to await collection. A similar model is used for harvesting kangaroo carcases in Australia, with a specialised truck instead of a helicopter.

At five packing houses I sampled venison cuts and trim – the latter destined for grinding. Aerobic plate counts (APCs) were generally below $\log_{10}$ 6/g on both cuts and trim, though the latter ranged to $\log_{10}$ 8/g. Conditions of processing and storage were obviously less than optimal with all samples being positive for *E. coli* and *S. aureus* and some samples ranging to $\log_{10}$ 5/g and $\log_{10}$ 6/g, respectively (59). *Salmonella* was not detected on any of the 128 samples analysed, in distinction to a survey of farmed deer, an industry then in its infancy, where S. *St Paul* was isolated from 16/30 samples. The serovar was associated with the gecko (*Hoplodactylus pacificus*) in the South Island of New Zealand and was a possible source via grazing (60).

4.2 Microbiological standards and minced (ground) beef

In the mid 1970s there was a view that product hygiene could be regulated by setting standards. In 1973 in the UK, retailer Marks and Spencer Ltd formulated a set of microbiological criteria for suppliers (Goldenberg & Elliott 1973)[viii]. The criteria were

[viii] Goldenberg, N. & Elliott, D. (1973). The value of agreed non-legal specifications. In: *The Microbiological safety of food*. Eds B. Hobbs & J. Christian. Academic Press, London and New York.

revolutionary because they had all the rigour of a standard; at the same time they were considered unattainable by suppliers.

Standards at retail for minced meat were regulated in several American States. In Oregon for example an aerobic plate count (APC) >5x10^6/g or *E. coli* 50/g or presence of *Salmonella* became a criminal offence for which the supermarket manager or retail butcher could be imprisoned for up to one year and fined $1000 (Carl 1975)[ix]. When it was shown that it was often impossible to meet the standard and that the causes lay with stages upstream from the retailer (boning, trimming, grinding) the law was repealed, and the standard replaced by a guideline (Winslow 1975)[x].

Nonetheless, the situation re minced meat was an obvious area for investigation and, during 1977 to 1980, I worked in Turkey, New Zealand and Australia, where I surveyed the microbiological condition of minced meat at retail (61-63). In each country the position was basically the same: more than 30% of samples failed the APC criterion, 40-100% the *E. coli* criterion and in the Melbourne survey *Salmonella* was isolated from 7% of samples. The reasons why each country had high failure rates varied. In Turkey poor abattoir hygiene and temperature control was balanced by rapid turnover at butcher shops; In NZ and Australia carcase quality was higher but the practice of accumulating trim at ambient over long periods before grinding into mince led to increased counts. An Australian national survey of minced meat at retail in 2008 showed great improvements with the median APC now < log10^6/g, the count being influenced particularly by the length of time product had been in the supply chain (64).

4.3 The advent of flexible packaging

With the advent of flexible packaging during the 1970s the industry underwent a revolution with abattoirs building boning rooms which operated colder than 10°C in which prime cuts were vacuum packed and trimmings frozen for grinding in export markets.

CSIRO showed that chilled vacuum-packed meat cuts had an atmosphere around 20% carbon dioxide, which inhibited biochemically active, Gram-negative spoilers and, when beef of normal pH (5.4-5.8) was packed in a film of low oxygen permeability (<100cm^3/m^2/day at 25°C), the biochemically benign, Lactic Acid Bacteria (LAB) grew to dominate the microbiome (Egan 1983)[xi]; the result was the ability to export vacuum-packed meat to distant markets in refrigerated containers. The current impact of sea- and air freight shipments on shelf life is considered further in Chapter 4.5.

[ix] Carl, K. (1975). Oregon's experience with microbiological standards for meat. *Journal of Milk and Food Technology*, 38: 483.

[x] Winslow, R. (1975). A retailer's experience with the Oregon bacterial standards for meat. *Journal of Milk and Food Technology,* 38: 487.

[xi] Egan, A. (1983). Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek*, 49:327-336.

While vacuum packing has proven the most suitable packaging format for transporting primal cuts to distant markets, in the 1980s a format was introduced in which retail portions were packed in a bottom tray, surrounded by a gas atmosphere which preserved its red bloom and sealed with a clear lid. Termed Modified Atmosphere Packaging (MAP), with colleagues at RMIT I undertook an R&D project with the developers of form-fill machinery which won the 1987 Australian Institute of Food Science and Technology (AIFST) Innovation Award (65).

4.4 Shiga Toxigenic *E. coli*

To service the global expansion of hamburger restaurants, the industry began the export of trim (manufacturing meat) frozen in blocks for grinding. In 1982 an outbreak involving hamburgers in Oregon and Michigan was caused by "*a rare E. coli serotype O157:H7*" (Wells *et al.* 1983)[xii]. Then in 1992-93, outbreaks involved more than 400 people in the western United States revealed the risk of *E. coli* O157 illness from consumption of undercooked hamburgers (CDC 1993)[xiii]. The US Food Safety and Inspection Service (FSIS) responded by declaring *E. coli* O157 an adulterant for which there was zero tolerance and required processors to test meat destined for grinding.

In the early 1990s the industry knew very little of the microbiological quality of its meat products and in 1993, as consultant to the Meat Research Corporation I was part of a team which designed the first national baseline study. Beef and sheep carcases had mean $\log_{10}$ APCs of 3.2 and 3.9, respectively and *E. coli* prevalence was 9.2% and 55.5%, respectively (Vanderlinde *et al*. 1999a[xiv]; 1999b[xv]). Industry developments such as operator training, infrastructure improvements and the implementation of HACCP-based food safety plans were monitored in further national surveys in 1998, 2005 and 2011 (66-71) with the inclusion of specific cuts as well as carcases and the prevalence of Shiga Toxigenic *E. coli* (STEC). Compared with the first national baseline survey in 1993, subsequent surveys showed >90% reduction in APCs on carcases and primal cuts and significant reductions in prevalence and concentration of pathogens (*Listeria*, *Campylobacter* and *Salmonella* were rarely isolated).

A recurring problem in the first three surveys was the prevalence and concentration of Coagulase positive staphylococci, isolated from 23.4% to 32.7% of carcases and boneless meat samples, respectively, with positive samples having a mean log count of 0.93 cfu/cm^2 and 1.14 cfu/g, respectively. In a subsequent investigation (72), it was found that practices had changed

[xii] Wells J., Davis B., Wachsmuth K., Riley L., Remis R., Sokolow R. & Morris G. (1983). Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *Journal of Clinical Microbiology*, 1983, 18(3):512–520.

[xiii] CDC, Centers for Disease Control. (1993). Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – Western United States 1992-993. *Morbidity & Mortality Weekly*, 42:258-263.

[xiv] Vanderlinde, P., Shay, B. & Murray, J. (1999a). Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *Journal of Food Protection*, 61:437-443.

[xv] Vanderlinde, P., Shay, B. & Murray, J. (1999a). Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *Journal of Food Protection*, 61:437-443.

in beef plants with, for safety reasons, operators being required to wear cut-proof or chain mail gloves, which resulted in the elimination of coagulase positive *S. aureus.* In sheep processing however, where the practice was not followed, prevalence of *S. aureus* on meat carcases and cuts ranged between 20-80% at the three plants studied.

In 2005, we used survey data to set meaningful performance criteria for slaughter and dressing of beef animals (73), updated in 2012 (74) and 2020 (75) after abattoirs developed HACCP systems and after many had installed hot water (>85°C) cabinets as an intervention to reduce the prevalence of contamination in general and of STECs in particular.

Early studies indicated that pathogenic *E. coli* may be associated with dairy cattle and one processor of cull dairy cows supplying the USA hamburger market commissioned its own study in which faecal samples from 500 dairy cattle were examined. The prevalence of STEC 0157: H7 (0.2%) was lower than in studies in other countries using similar methodology but genes for Shiga-toxin production were isolated for non-O157 *E. coli* (76).

4.5 Shelf life and accessing distant markets

While food safety is clearly a *sine qua non* for any food industry, so is the ability to transport meat to markets with sufficient shelf life remaining for distribution through the retail sector. Australia has supplied meat to the most distant of markets (Europe) since 1880, first as frozen carcases and, from the 1970s, as chilled vacuum-packed primal cuts. For many years there was anecdotal evidence from the international trade that Australian vacuum-packed beef had a longer shelf life than that of its competitors, citing acceptable quality of 100 days at 0°C. This claim was more than borne-out when vacuum packed beef from six abattoirs located from Tasmania to North Queensland was found to have a shelf life of 160 days when stored at a mean 0°C (77).

We gathered background material on Australian meat quality (physical, sensory and microbiological) into a text *Shelf life of Australian red meat* (78). We also wrote chapters in *Safety of meat and meat products in the twenty-first century*, in *Practical Food Safety: Contemporary Issues and Future Directions* (79) and in *Food Safety and Quality-Based Shelf Life of Perishable Foods* (80). We published a monograph on *Microbiological safety and storage life of Australian red meat*, containing 240 publications and reports chronicling Australia's evolution as a meat exporting country starting with the inaugural shipments aboard the SS Strathleven voyage to London in 1880 (81).

While the microbiological condition of meat at packing is an important determinant of shelf life, storage temperature was shown to be the key determinant by Gill and others who defined the optimum temperature for storage of VP primals as -1.5±0.5°C (Gill *et al.* 1988a)[xvi]. The same workers also established that small rises in temperature reduce shelf life significantly – at temperatures of 0, 2 or 5°C, the storage life was reduced by about 30, 50 or

[xvi] Gill. C., Phillips, D. & Harrison, J. (1988a). Product temperature criteria for shipment of chilled meats to distant markets. In: *Proceedings: 1st International Refrigeration Conference*: *Refrigeration for Food and People*, 1988, Brisbane p 40-47.

70%, respectively, compared with storage at -1.5°C (Gill *et al.* 1988b)[xvii]. We showed the effect of storage temperature on vacuum-packed lamb primals stored in three abattoirs, the cool rooms of which averaged 0°C, -0.5°C and -2.4°C. At the former temperature APC reached $\log_{10}$ 7 cfu/cm^2 after 80 days, while at the other two plants the 85-day APCs were $\log_{10}$ 5 cfu/cm^2 at -0.5°C and $\log_{10}$ 2 cfu/cm^2 at -2.4°C (82).

The effect of temperature on shelf life is most pronounced in transport to distant markets and I was able to utilise the fact that many companies now include data loggers in cartons of meat shipped in a refrigerated container and so can record product temperatures from door-to-door. Serendipitously, Tom Ross had developed a predictive tool which, with inputs of starting bacterial level and temperature:time data was able to predict the remaining shelf life of vacuum-packed beef and lamb primals when the consignment enters the customer's warehouse (83).

I obtained 125 data loggers of sea shipments to North America, Europe, Middle East, Japan and China and 25 air freight shipments to Europe, Japan and Middle East. High oil prices since the financial crisis beginning 2009 have resulted in "slow steaming" whereby some shipments from Asia to Europe currently take almost as long as those by the tea clippers in the 1880s. Slow steaming and longshoremen's strikes in California notwithstanding the U Tas tool predicted that there would be sufficient time for product to complete the supply chain with sufficient remaining shelf life. A possible exception was China where in-country storage and transport did not always provide a secure supply chain (84).

This thesis is being completed in late-2021 when there are difficulties for Australian exporters because of reduced shipping calling at Australian ports and commensurate shortage of containers. As well, delays in North American and European ports are prolonging unloading times by 3-4 weeks, reducing significantly the remaining shelf life. The U Tas predictor is proving to be a tool for the times.

4.6 Quality assurance

Quality Assurance (QA) is a system which embraces all the company's planned and systematic actions which provide confidence that its goods will satisfy requirements of consumers and regulators. By contrast, Quality Control (QC) is confined to inspections and measurements of products during their manufacture and was the *modus operandi* of the meat industry in the 1970s via a system of command-and-control by veterinary and inspection staff.

Meat has traditionally been targeted as a product posing risks to human health and therefore in need of government intervention by way of regulation. In 1905, Upton Sinclair infiltrated the Chicago meat industry and published *The Jungle*[xviii], exposing deficiencies in workplace safety and product hygiene. The result was the Federal Meat

[xvii] Gill, C., Phillips, D. & Loeffen, M. (1988b). A computer program for assessing the remaining storage life of chilled red meats from product temperature history. In: *Proceedings: 1st International Refrigeration Conference: Refrigeration for Food and People*, 1988, Brisbane p 35-39.

[xviii] Sinclair, U. (1905). *The Jungle*. Penguin Books Inc, USA.

Inspection Act (FMIA) 1906 regulating four major areas: livestock (*ante-mortem*) inspection, *post-mortem* inspection, sanitary standards for meat, plus on-plant inspection and monitoring. Globally, abattoir operations were placed under veterinary control, where they remained for almost a century until traditional veterinary inspection of meat animals was questioned. Blackmore (1983)[xix], Hathaway & McKenzie (1991)[xx] and Gill (1995)[xxi] were prominent in pointing out the changed emphasis of excluding diseased animals from the supply chain to one of containing enteric diseases associated with meat and that alternative approaches were needed to improving its hygienic quality.

4.6.1 The case for change

In Australia, a process of uncoupling the nexus between veterinary meat inspection and meat safety began in 1990 when then-Treasurer, the Hon. Paul Keating, instituted a user pays policy for meat inspection. For the first time, establishments were required to meet full cost recovery of veterinary meat inspection provided by governments: State, Territory and Federal. The costs were onerous and representations by industry led to the setting up of a trial to assess whether government meat inspectors could be replaced by company meat inspectors, all under an overarching Quality Assurance agenda.

I designed and managed that trial at three domestic establishments in Victoria, Tasmania and New South Wales for each of which I developed a HACCP-based QA system, with full work instructions for each unit operation from stockyards to product load-out – the first HACCP system for a meat processing establishment in any country. Trial design was protracted because of resistance from meat inspector unions fearful that their members would become redundant and from consumer groups worried that diseased meat would flood supermarket shelves. Eventually a simple “before and after” trial was accepted by all parties in which data would be gathered under the established system (government supervised) and under an alternative system where company-employed inspectors undertook both online meat inspection of carcases and offal plus new duties such as microbiological monitoring of product and the abattoir environment. Veterinarians from the Australian Quarantine Inspection Service (AQIS) and union meat inspectors undertook announced and unannounced audits of both phases of the trial while I undertook microbiological testing of carcases under the established and alternative systems. The experimental work indicated that domestic abattoirs were capable of operating with company-employed QC staff to at least the same standard as abattoirs supervised by traditional means (85-89).

The new system where company-employed QC Officers operated within a QA team allowed substantial reductions in inspection costs. As an example, at one of the trial plants 12

[xix] Blackmore, D. (1983). A new approach to meat inspection. *Nordisk Veterinaermedicin*, 35:184-189.

[xx] Hathaway, S. & McKenzie, A. (1991). *Postmortem* meat inspection programs; separating science and tradition. *Journal of Food Protection*, 54:471-475.

[xxi] Gill, C. (1995). Current and emerging approaches to assuring the hygienic condition of red meats. *Canadian Journal of Animal Health*, 75:1-13.

government inspectors each costing $80k/annum had been required, however only six company employed staff at $40k/annum were needed to properly manage the same operation. Many domestic plants switched to the new system especially when negotiation with foreign governments allowed them (as Tier 1 plants) to export to certain markets without on-plant veterinary control.

The trial changed the face of the Australian meat industry and I presented the work as *The quality challenge for the Australian food industry* to the influential National Agricultural and Resources Outlook 1996 conference (90). Quality assurance gained impetus across the industry. As indicated earlier, State regulators embraced a new paradigm of "co-regulation" to assess the QA system operated in plants by QC officers. The industry embarked on an intensive training program in QA and I published *A Guide to Food Quality Assurance* (91) as a basic text.

In 1995 the Meat Industry Strategic Plan (MISP) listed six strategic imperatives of which "Guaranteed Food Safety" was one. The explicit industry goal was that all enterprises from producer to retail would operate in accordance with QA systems based on HACCP principles by 1999. Charged with undertaking the food safety element the MRC decided on a Food Safety Key Program (FSKP), the business plan for which I was charged with developing. With industry expert Chris Orr and the University's Tom McMeekin, I developed an R&D program of 32 projects costed at $8.8 million over three years, with Risk Analysis as its underpinning (92).

4.7 HACCP and the MegaReg

The industry commitment to install QA systems based on HACCP stemmed from the Jack-in-the-Box outbreaks of STEC illness in USA in 1992-93. In 1996, authorities in the United States implemented a series of important regulations surrounding the hygiene of meat destined for grinding. These included the Pathogen Reduction Final Rule (known as "the MegaReg") and the declaration of *Escherichia coli* O157 as an adulterant for which there is zero tolerance. As a major supplier of manufacturing meat to the USA for grinding, Australia was required to adhere to testing requirements which, over time became more stringent, culminating in so-called N60 ("robust") testing and inclusion of six non-O157 serotypes.

The scientific bases behind the MegaReg were heavily criticised by meat scientists: *E. coli* was a commensal in the intestines of animals, so how could it be an adulterant merely because some of the organisms had specific somatic or flagellar antigens? As well, the principle of testing for a pathogen was also criticised by a panel of thirty-five international experts assembled by the American Meat Science Association. The panel established that, if *E. coli* O157 were present, say at 0.1% prevalence, the numbers of samples from a contaminated lot needed to detect the pathogen with probabilities of 0.90, 0.95 and 0.99 were 2,303, 2,996 and 4,605, respectively (Anon. 1999)[xxii]. A subsequent gathering of meat safety experts, under the *aegis* of the International Livestock Congress, concurred with the AMSA findings and

[xxii] Anonymous. (1999). The role of microbiological testing in beef food safety programs: the scientific perspective. *Consensus of the 1999 symposium under the aegis of the American meat Science Association*, Kansas City, Missouri, January 1999.

concluded that testing programs should focus on enumerating indicator organisms, particularly when pathogens are present at low concentration (93). We pursued this proposition by interrogating data from the 2006 national baseline, concluding that enumeration of coliforms is a useful adjunct to enumeration of *E. coli* biotype I or *Enterobacteriaceae* in red meat (94).

We also challenged the effectiveness of the methodologies used in the government-overseen *E. coli Salmonella* Monitoring (ESAM) program, both the consistency of the sponge-swab removal of bacteria from meat surfaces (95) and the effect on the final count of the temperature at which cultures were incubated (96). We also challenged the temperature at which knives and other equipment were sanitised between carcases, finding scant justification for the regulated 82°C, other than it was the conversion from 180°F originating in the USA. Water availability and energy costs have become important aspects of meat slaughter and dressing and we showed how, by increasing the time the non-use knife was held in the "steriliser", an equivalent reduction of bacteria on the knife blade could be achieved at 70°C to a momentary dip in 82°C water (97,98).

The adoption of "co-regulation" in some States led to the study of whether changes in meat and poultry regulation in Australia had affected the prevalence of *Salmonella* in products or of salmonellosis in the community (99). The study concluded that regulatory change aligned with improvements in microbiological quality of red meat and poultry but that these improvements had not carried through to reduced case-rates for salmonellosis, possibly due to a lack of control in the food processing, food service and home sectors. A parallel paper (19) attempted serovar matching between food and human isolates from the National Enteric Pathogens Surveillance Scheme (NEPPS) with only the broadest of ascriptions being possible: poultry and egg products (35% of salmonellosis), meat 31%, plant foods 12% and 22% in "other" categories. Taken together the two papers served to indicate how difficult it then was, in an era before genomics, to ascribe food borne illness to food categories and to assess the effectiveness of regulatory changes.

4.8 Predictive microbiology

In the mid-1990s, MRC began funding the development of models at U Tas, a process continued by MLA, which has led to tools which are used regularly in the red meat and RTE meat sectors. A group of post-graduate students laid the foundations including Presser *et al*.

(1997)[xxiii]; Krist *et al*. (1998)[xxiv]; Salter *et al*. (2000)[xxv]; Shadbolt *et al*. (1999)[xxvi] and Mellefont *et al*. (2003)[xxvii]. By 2000, at the 3rd International Conference on Predictive Modelling in Foods, we were able to illustrate to an audience of mathematicians how the use of modelling and software tools in Australia was progressing to everyday use (100). In 2006 we published an updated list of predictive microbiology tools in use in Australia (101).

4.8.1 The salami predictor

The monograph *Predicting E. coli inactivation in uncooked comminuted fermented meat products (UCFM)* by Ross & Shadbolt (2001)[xxviii] came at an important time for the sector which, in the aftermath of the Garibaldi outbreak, had been charged with demonstrating that its processes would inactivate at least 99.9% of *E. coli* which might be present in each gram of in-going raw meat, the so-called '3-log kill'. Challenge testing was the only alternative and was beyond the resources (technical and financial) of all but the largest companies. In 2001, the UTas team developed the *E. coli* Inactivation Predictor, a tool which required monitoring of only two parameters in the UCFM process: temperature and time. I was charged by MLA with rolling out the tool to industry and regulators in every State and the Predictor is still used extensively by establishments and regulators in a cooperative manner to verify the extent that each UCFM process can inactivate *E. coli*.

4.8.2 The Refrigeration Index (RI)

In 1999 I received a call from the owner of a hot-boning establishment, its licence just suspended because it could not meet the 15-hour time limit specified to reach 7°C at the slowest cooling point (centre) of the carton. I led an approach to the regulator (AQIS) to allow an industry trial based on the potential growth of *E. coli.* I worked with the five hot-boning plants to gather chilling rates at the centre of the carton, plus the APC and *E. coli* prevalence before and after freezing. The data were used by U Tas to develop a predictive model. Criteria

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[xxiii] Presser, K., Ratkowsky, D. & Ross, T. (1997). Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Applied Environmental Microbiology*, 63:2355-2360.

[xxiv] Krist, K., Ross, T. & McMeekin, T. (1998). Final optical density and growth rate; effects of temperature and NaCl differ from acidity. *International Journal of Food Microbiology*, 43:195 –203.

[xxv] Salter, M. Ratkowsky, D. Ross, T. & McMeekin, T. (2000). Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using generalised nonlinear regression. *International Journal of Food Microbiology*, 61:159-167.

[xxvi] Shadbolt, C., Ross, T. & McMeekin, T. (1999). Nonthermal death of *Escherichia coli*. *International Journal of Food Microbiology*, 49:129-138.

[xxvii] Mellefont, L., McMeekin, T. & Ross, T. (2003). Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *Escherichia coli*. *International Journal of Food Microbiology*, 82**:**45-58.

[xxviii] Ross, T. & Shadbolt, C. (2001). Predicting *E. coli* inactivation in uncooked comminuted fermented meat products. *Meat and Livestock Australia* (July 2001), North Sydney, Australia.

for assessing chilling regimes, based on predicted growth of *E. coli* were approved by the regulator (AQIS) as a Hot Boning Index (HBI) and establishments were able to verify conformance with the criteria merely by observing temperatures of active chilling. So successful was the predictive tool that the HBI was subsumed within a regime for all meat establishments (hot- and conventional boning), the Refrigeration Index (RI). The tool is used every day at every export plant in Australia to verify chilling rates. It has also proved invaluable in assisting companies to evaluate the effects of a loss of refrigeration, most notably when Cyclone Yazi caused loss of power at a plant for 48 hours. The entire day's production, valued around A$500,000, was saved from condemnation by using the RI Predictor. We published the work as a chapter on how the Australian industry was using predictive tools in *Case studies in food safety and authenticity* (102).

4.8.3 The *Listeria* predictor

In the late 1990s there were large outbreaks of listeriosis globally with a range of foods implicated, including RTE meats. MLA commissioned U Tas to undertake a range of studies on *Listeria monocytogenes* in RTE meats, particularly the effect of including organic acids in the formulation to prevent growth, together with a quantitative risk assessment (46).

The UTas team also cooperated in a global project led by Danish researchers to develop a spreadsheet tool to predict growth of *L. monocytogenes* in RTE meats (Mejlholm *et al*. 2010)[xxix]. The tool led to regulation in Australia being amended to distinguish between products which could or could not support growth of *L. monocytogenes* during refrigerated storage.

4.8.4 The shelf-life predictor

The University developed a tool to predict shelf life of vacuum-packed beef and sheep meats for which the establishment needs only know the Total Bacterial Count on primals as they are packed, together with the temperature:time history, and the tool will predict remaining shelf life. The model has been validated by industry trials (103) and, as this thesis is being completed (late-2021), is in regular use by companies experiencing unexpected, prolonged delays in shipping to distant markets.

4.8.5 Process control predictors

We surveyed the unit operations in beef and sheep plants to develop tools to enable each operation to assess the efficacy of their operation by equating with the challenge they faced with incoming livestock (104, 105).

[xxix] Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D. & Dalgaard, P. (2010). Predicting growth rates and growth boundary of *Listeria monocytogenes* - an international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology*, 141:137-150.

Chapter 5 Hazard identification, food safety, shelf life, quality assurance, HACCP and predictive tools for the seafood industry

In New Zealand, working in seafood plants in the early 1970s proved very different from my previous ten years ensconced in a laboratory. Now there was an emphasis on applying microbiology to the food process and, since the New Zealand Fishing Industry Board had invested in the Massey's Food Technology Department our focus was on New Zealand's major seafood export earners of the time: rock lobsters and live eels.

After New Zealand I began work at RMIT and in the Indo-Pacific region, the result was a series of student and staff exchanges between my department and researchers in Sri Lanka, The Philippines, India, Malaysia and Indonesia. While the focus remained on process hygiene, a "purer" topic was investigating the proposal that tropical fish have a longer shelf life in ice than fish from temperate waters.

A major export from the region was (and is) prawns and many supplying countries encountered Port of Entry (PoE) difficulties, particularly in USA and Europe; I was able to work with local scientists in several countries to enhance process control and reduce levels of rejection.

The fishing industry generates a significant proportion of waste via by-catch and disposal of non-marketable portions (heads, frames, guts); I worked on the possibility of ensiling these wastes in several countries.

5.1 New Zealand seafoods

In the early 1970s rock lobster were relatively plentiful in New Zealand waters and around the Chatham Islands. Because of distance from port, a significant proportion of the catch was tailed and frozen at sea, then thawed, repacked and refrozen on land. We studied whether a double freeze/thaw cycle was detrimental, finding an increase in bacteriological count and decrease in sensory quality at some plants, but not at others. From this we were able to identify key unit operations and prepare a processing guide for the lobster industry (106-108), as we were for processing and handling finfish in general (109-111).

Close to Lincoln College was Lake Ellesmere, from which *Anguilla australis* (short-finned) and *A. dieffenbachii* (long-finned) eels were harvested and air freighted live to Europe. The major quality criterion was the lipid content which we found to be lower (8-10%) than in European eels (25-30%). We set up a pilot scale fattening facility and increased the lipid content to 25% over a three-month period (112-115). In Australia, we were able to monitor short-finned eels from the Victorian industry (*A. australis australis*) and found them to have higher lipid contents that the New Zealand equivalent (116).

5.2 Australia and the Asia-Pacific

In the early 1980s ACIAR funded a large seafood project involving four entities: U Tas, CSIRO (Hobart), UNSW and RMIT. Fish drying was a major focus with U Tas developing cheap,

effective tent dryers and isolating *Staphylococcus xylosus*, an extreme halophile which grew well on salted fish and in the brines which never seemed to be changed.

The RMIT focus was on seafood processing, food safety and shelf life. I and some of my staff taught and researched at the College of Fisheries in Manila and we were at pains to get our findings published in journals in the ASEAN region (117-119). We were also looking in Melbourne at consumer expectations for finfish (120) and at water addition to scallops when it suddenly became industry practice to add 10L of water to 10kg of scallops in a plastic box, store overnight and find 20kg of scallops (121). Fish substitution was rife, as we found when gel electrophoresis indicated all the "quality" species (Snapper, John Dory, King George Whiting) were being substituted by Orange Roughy from New Zealand (122).

5.3 Fish silage

In 1976 I received a telex from FAO in Rome asking whether I would undertake a six-week consultancy to evaluate the potential for fish silage in the Indo-Pacific region. It was the first of scores of consultancies for United Nations agencies, each time learning more about processes and utilisation of seafoods in the region. I travelled to Rome for briefing and injections then to Thailand, Malaysia, Singapore and Indonesia to identify volumes of waste streams and institutions that might be able to work with industry. There were vast quantities of potential waste as by-catch from the wild caught shrimp and pelagic fish sectors – usually juveniles which were trapped because of the small net sizes. My report, together with updates, was presented at conferences in USA (123), The Philippines (124) and Singapore (125). At RMIT my students, Mike Phillips and Nick Brown made sufficient silage by acidification and natural fermentation to carry out feeding trials on pigs and poultry (126-128). In Australia however, commercial production of fish silage has never progressed to use in livestock formulations, being used only in garden fertilisers.

5.4 Prawn processing

Leaving Lincoln College in New Zealand for RMIT in Melbourne in 1978 I took a five-month consultancy with FAO and DANIDA at the newly finished Institute of Fish Technology in Colombo, Sri Lanka. It was a time when prawn imports were under pressure in Europe where rejections of Malaysian prawns were averaging 19%, and in the USA where more than 5200t from Asia had just been rejected over a four-month period, 70% for presence of *Salmonella*.

We decided to monitor the Sri Lankan industry, working at 15 processing plants. The industry was operating in premises which weren't designed for the task, often converted residences with working surfaces which were difficult to clean and with inadequate chilling and freezing capacity. A further problem was the microbiological condition of incoming raw materials: APC (100% > $\log_{10}$ 6/g); *S. aureus* (49% >100/g) and *E. coli* (72% positive) – challenging enough, but cooked, peeled product had similar bacterial loadings. We investigated the hands of workers finding 137/262 (52%) carried *S. aureus* on their fingers, ranging from 22% to 92% between individual companies. We published our findings as a trilogy in the local journal (129-131) and internationally (132, 133) and Institute staff gave on-site training sessions to 600 process workers.

In 1980 I challenged what I saw as discrimination against Asian prawns with the setting of standards for raw product much more rigorous than those for imported raw meat and poultry. I abstracted data from Communicable Disease reports of the PHLS in the UK which showed that isolations of salmonellae in foods in England and Wales during 1977-1980 were poultry (48%), red meat (14%), smallgoods (21%), compared with prawns 4% (134). Among the reasons given for the stringent standards for Asian prawns was that salmonellae were not found in prawns in their natural environment, while in the meat and poultry industries, salmonellae were commensals. This proposition was debunked by Reilly and Twiddy (1992)[xxx] who confirmed *Salmonella* and *Vibrio* as natural components of the gut microflora of cultured prawns.

There have been significant improvements in shrimp production and processing, most notably in properly designed plants that can be cleaned and sanitised, together with much improved microbiological condition of incoming raw material predominantly from aquaculture. Processing is still undertaken by hand though temperature control during processing prevents multiplication of *S. aureus* (135).

5.5 Shelf life of fish from warm and temperate waters

In the 1970s, knowledge of fish spoilage was based on research done on cold-water species, particularly cod (*Gadus morhua*) for which a shelf life of 12 days in ice was established by Shewan and others, who suggested that psychrophilic spoilers, such as *Pseudomonas* were prevalent in their gut and gills (Shewan 1976)[xxxi]. However, spoilage of finfish became a controversial topic for discussion following a report by Disney *et al*. (1971)[xxxii] that *Tilapia* kept 30 days in ice - the proposition being that fish from warm waters had fewer psychrotrophic bacteria so took longer to spoil.

I began investigating this proposition in New Zealand on fish taken from waters around 15°C, with spoilage occurring around 15 days in ice, commensurate with an upsurge in Trimethylamine (TMA), a recognised indicator of spoilage (136). We were able to investigate further in Australia, comparing mullet (*Mugil cephalus*) caught off Brisbane in waters at 23°C with the same species caught in Gippsland (Victoria) waters ca. 10°C; the former kept 24 days in ice compared with 15 and 19 days for the latter. We also compared the shelf life in ice of Rainbow trout (*Salmo gairdnerii*) harvested from the same farm in northern Victoria in summer (shelf life 18 days from water of 5-22°C) and winter (shelf life 14 days, water 2-8°C). At the time of rejection by taste panels, product in all trials had an APC $\log_{10}$ 8 cfu/g,

[xxx] Reilly, A. & Twiddy, D. (1992). Prevalence of *Vibrio cholerae* and *Salmonella* in a major shrimp production area in Thailand. *International Journal of Food Microbiology*, 28:101-113.

[xxxi] Shewan, J. 1976). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. *Proceedings of the Tropical Institute Conference on the Handling, processing and Marketing of Tropical Fish* (pp 51-66). London, UK.

[xxxii] Disney, J., Cameron, J., Hoffman, A. & Jones, N. (1971). Quality assessment in *Tilapia* species. Ed: Kreuzer, R. *Fish Inspection and Quality Control*. London Fish News (Books).

dominated by sulphide producers, mainly *Alteromonas putrefaciens* (now known as *Shewanella putrefaciens*) (137).

We also simulated spoilage typical of tropical fisheries where product might be taken from waters close to 30°C and held in the mid-30s for much of the day, so-called mesophilic spoilage. Rainbow trout exhibited flat-sour odours and soft texture after 8h storage at 35°C. The bacterial flora was dominated by colonies on Violet Red Bile Glucose (VRBG) agar at $\log_{10}$ 7 cfu/g; H_2S producers were $\log_{10}$ 5 cfu/g from which 88% of isolates of *Aeromonas hydrophila* produced flat-sour odours and 44% of *Enterobacteriaceae* producing sweaty-fruity odours (138).

Work in collaboration with the College of Fisheries in The Philippines offered unique opportunities to compare shelf life of cold- and warm-water fish. Mullets (*Valamugil seheli* and *Liza subviridis*) taken from waters in Luzon Province at 26-28°C were stored in ice, with the latter keeping for 29 days and H_2S producers were $\log_{10}$ 5-8 cfu/g. *Valamugil seheli* had a shelf life of only 21-22 days perhaps influenced by its size (50g on average) raising the possibility that surface area:volume ratio may also be a factor (139). By comparison, mullets taken from Australian waters at 14°C kept for 24 days but were larger (1000g on average). We also investigated *Tilapia* (*Oreochromis niloticus*) grown in the mountain province (Banaue) in waters of 16°C and at Munoz (28°C) with shelf lives of 21 and 27 days, respectively (140).

Taken together, work done in Australia and The Philippines provided considerable circumstantial evidence that warm-water fish keep longer than cold-water fish when stored in ice. The work also confirmed the sulphide producer, *A. putrefaciens* as a major spoiler (141).

5.6 The Australian Seafood Industry Quality Program (ASIQAP)

Following the success of the domestic meat industry in implementing QA systems the Australian government launched its Food Quality program calling tenders for the installation of through-chain programs and stipulating ISO 9002 as the quality system of choice. I was the successful tenderer for the seafood element and during 1995-97 facilitated 22 operations to ISO 9002 certification: five prawn trawlers, two spanner crab boats, two reef fishing boats, six prawn farms, six processing plants and one beach seine operation (142-143). An Innovation was the incorporation of a HACCP-based food safety plan into the Process Control element of each operation's ISO protocol, for the first time including hazard management as a component of the ISO 9000 system.

The work: *Developing and implementing quality systems for the seafood industry* was a second presentation at the influential National Agricultural and Resources Outlook 1996 conference (144). The ASIQAP showed for the first time how diverse seafood production and processing operations could be managed under a quality umbrella rather than a command-and-control government system (145).

5.7 HACCP for Pacific seafood companies

The success of the ASIQAP was picked up by the FAO and the South Pacific Commission (SPC) to assist seafood companies as a matter of urgency as they faced a deadline to install and

operate a HACCP-based food safety plan as a regulatory prerequisite for export to the USA. Over the period 1995-98 I worked with every exporter in Fiji, Tuvalu, Vanuatu, Samoa, Tonga, Federated States of Micronesia, Marshall Islands, New Caledonia and Palau to install a HACCP plan and to certify their competence (146-147).

5.8 The *Listeria* predictor

A spreadsheet tool to predict growth of *L. monocytogenes* (Mejlholm *et al*. 2010)[xxxiii] has been developed progressively to include predicting growth of the pathogen in seafoods and proved invaluable in the gazetting of *Guidelines for the safe manufacture of smoked fish: focus on Listeria management* by Biosecurity Tasmania (38).

5.9 FSANZ Food Standards Code

In 2020 I led a review of Standard 1.6.1 and the associated Schedule 27: Microbiological Limits in Food of the Australia, New Zealand Food Standards Code (the Code), in consultation with SafeFish and Seafood New Zealand. The Australia New Zealand Food Standards Code still contains criteria for raw and cooked crustacea which were reviewed to ensure they were fit for purpose considering the current risk-based preventive approach to food safety management. I provided background material, directed online workshops with participants in Australia and New Zealand and developed final recommendations to FSANZ, which recommended that all criteria (for *Salmonella*, *S. aureus* and Standard Plate Count) be removed from the Code (148).

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Chapter 6 Selected publications and conference proceedings

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