Food Safety and Quality Assurance for Green and Cooked Prawns:

Development and Evaluation of a Framework for the Validation of a Supply Chain Approach

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FISHERIES RESEARCH & DEVELOPMENT CORPORATION

December 2003

SPENCER GULF & WEST COAST PRAWN FISHERMEN'S ASSOC. INC.
Harvesting with Care in the State of South Australia

FRDC Project No: SIDF 2002/425

THE UNIVERSITY OF ADELAIDE AUSTRALIA

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OBJECTIVES:

1 Develop a framework designed to validate the Australian Prawn Industry Association (APIA) Code of Practice for food safety and shelf life across the supply chain for cooked prawns as a model for crustacean industries.
2 Benchmark industry performance criterion (i.e. quantify decrease/increase of indicator organisms) for cooking, freezing and preparation for retail (thawing, retail hygiene) processes.
3 Benchmark industry product criteria (i.e. acceptable levels of micro-organisms associated with food safety and shelf-life) across the supply chain to evaluate conformity with the FSANZ Food Standards Code.

NON-TECHNICAL SUMMARY

The principal objective of the project was to develop and pre-test a “water-to-waiter” approach/framework to evaluate the processing performance and resulting product criteria (compliance with food standards and market shelf-life requirements) of the Spencer Gulf West Coast Prawn Fishermen’s Association (SGWCPFA).

Participating boats were selected at random to obtain a cross-sectional profile of the fleet. Samples were comprised of composites collected across processing periods. Background information on boat hygiene procedures and dipping or cooking/cooling procedures were obtained for all participants. All samples were tested according to the Australian Standards at a NATA accredited laboratory (IMVS Food and Environmental Laboratory).

The principal findings of this work are:

1. Prawns harvested from the Spencer Gulf region of South Australia meet FSANZ food safety standards. Bacteria indicative of faecal contamination were absent and *Listeria monocytogenes* was not isolated from any sample tested. Processing procedures used on boat did not contribute additional contamination by micro-organisms of public health significance.
2. There was no evidence that seawater or other water used for washing or cooling of prawns introduced contaminants of public health significance.
3. The Standard Plate Count of cooked prawns was similar to, or greater than that of green prawns. Cooking would be expected to substantially reduce the microbial load of prawns significantly. This contrary result indicated the industry is performing below potential. The impact of failure to reduce microbial load is potential loss of shelf-life. This was indicated by retail shelf-life data.
4. The shelf-life of green prawns (dipped for the control of black spot), optimally thawed and stored at 4ºC, was greater than that of cooked prawns. This indicated opportunity to improve product shelf-life at the retail end through improved process control of cooked prawns. However, rapid development of black spot on stored prawns may have a greater effect on saleability in retail markets than microbiological status.
5. A survey of hygiene practices across boats in the fleet indicated little standardisation of the type and manner of use of sanitisers, hygiene and sanitation practices and policies to deal with involvement of sick crew in processing of prawns.

6. The anti-black spot agent, sodium metabisulphite, when used at concentrations that produce high residual levels of SO_2, was found to significantly reduce the development of this product defect. An added advantage of this chemical treatment is the reduction in the microbial load associated with green prawns.
7. Residual levels of SO_2 on green prawns sampled from different boats were strikingly variable. This indicated a failure, by the fleet, to adopt consistent dosing practices.

This project has developed and tested an approach/framework to evaluate the processing performance and resulting product criteria (food safety and shelf-life). In particular, the work has led to:

- Design of a "water-to-waiter" approach that targeted processing performance and food safety and shelf-life standards at retail and under controlled conditions
- Construction of a sampling framework that provided a randomised, cross-sectional evaluation of these criteria across the fleet (for both green and cooked prawns)
- Identification of considerable variability in:
    1. adoption of industry guidelines for boat hygiene
    2. achievement of appropriate levels of metabisulphite at retail
    3. the impact of cooking/cooling of prawns on total bacteria counts
- Identification of a potential pathway of contamination of ready-to-eat product by the marine pathogen, *V. parahaemolyticus*.

In particular, the project has identified (i.e. Planned Outcomes)

- Specific areas of the APIA Guide to *Handling Prawns at Sea* that require revision i.e. process monitoring, sampling, sample integrity.
- A need for targeted training to improve the uptake of APIA guidelines and consideration of targeted documentation to use for auditing purposes.
- Microbiological monitoring of cooked and cooled prawns (sampled before freezing) to provide an ongoing verification of the efficiency of cooking and cooling in microbial terms and potential shelf-life.
- A need to investigate cost-effective measures (eg UV in-line) to reduce potential contamination of disinfected surfaces and processed product by uncontrolled (untreated) water. Consultation with SGWCPFA identified this as a high industry priority.
- A need to adopt methods for correct use of, and capacity to monitor concentrations of, sodium metabisulphite or its alternatives. The routine, on-board use of sodium metabisulphite has important *Occupational and Health and Safety* implications. This agent is an irritant that can result in significant discomfort to the eyes, skin and airways. In some individuals it can result in skin sensitisation and allergic reactions, especially in situations where there is repeated exposure over long periods. Standard procedures for safe handling of sodium metabisulphite solid and solutions need to be established and documented in the APIA Guides to *Handling Prawns at Sea*. Appropriate training should be provided for all crew. The use of safer alternative agents should be examined.
- A need for a comparative investigation of the efficacy of sodium metabisulphite and alternative anti-black-spot agents, as antibacterial agents which could provide additional control of bacteria that impact on the public health status and shelf-life of green prawns.

In conclusion, the project has provided a baseline of industry hygiene, processing and product performance. For the SGWCPFA in particular, a set of strategies that may enhance product safety and shelf-life has been established. For the crustacea industries in general, the project provides principles and a framework to guide the evaluation of processes and product across the supply continuum.

KEYWORDS: Prawns, cooked prawns, food safety, quality assurance, microbiological analysis, Listeria monocytogenes, Vibrio, coliforms, sodium metabisulphite

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Background

Food Safety of Seafood

There is currently an international expectation that procedures to improve food safety must embrace the farm-to-plate or in this case “water-to-waiter” continuum to reduce the risk of food-borne disease (Anon., 2002). This is reflected nationally, through Food Standards Australia and New Zealand (FSANZ), by the development of a Primary Production and Processing Standard for Seafood that includes cooked crustacea.

The microbiological parameters of concern as listed by the Codex Alimentarius Commission (CAC) – Cooked Shrimp End Product Specifications are found in Appendix 1a (Codex Alimentarius Commission, 1995). In Australia, *Listeria monocytogenes* has been added to the FSANZ Food Standards Code for Cooked Crustacea (FSANZ, 2000). In addition, *Vibrio parahaemolyticus* has been identified as a pathogen of concern by SafeFood NSW (Anon., 2001).

There are many recorded instances of outbreaks of food-borne disease associated with prawns or shrimp throughout the world. Consumption of contaminated fish has resulted in potentially fatal illness and resulted in the major financial imposition of product recall, associated loss of revenue and damaged reputation.

For example:

- *Listeria monocytogenes* was identified as the causative agent in three sporadic cases of seafood-borne listeriosis in the USA in 1987 and has been suspected to be the causative agent in at least two other outbreaks involving seafood (Ben Embarek, 1994). *L. monocytogenes* has been isolated from several types of ready-to-eat seafood including cooked shrimp (McCarthy, 1997).
- *Listeria* contaminated shrimp resulted in seven recalls in the US in 1987 and affected in excess of 31,000 pounds of product (Elliot, 2000).
- Over the period 1990 to 1998, *Salmonella* spp have been isolated from 8.3% of 4440 raw crustacean samples from both domestic and foreign sources in the USA. *Salmonella* spp. have also been isolated from frozen and ready to eat shrimp in Florida (Heinitz, 2000).
- Pathogenic marine *Vibrios* are another group of bacterial pathogens that may be of concern. These bacteria may be indigenous to the prawns or could be present in the seawater used for processing the catch. They are ubiquitous in warm (>15°C) seawater environments (Huss, 1997). One species in particular, *V. parahaemolyticus*, has been identified as a medium risk for cooked prawns in Australia, and outbreaks of associated food poisoning have been reported (Sumner, 2002). The public health implications for *Vibrio* spp associated with green prawns are unknown.
- The European Economic Union and the USA banned imports of seafood (including shrimp) from Bangladesh as a result of the inspection of processing plants in 1997. This action resulted in the loss of nearly US$15 million in revenue for the Bangladesh industry (Cato, 1998).
- In Australia during the period 1990 – 2000, there were six reported outbreaks of food poisoning involving 159 cases that were attributed to bacterial pathogens in seafood (Table 1). While there may be under reporting of the order of 10 to 100 times, the relative occurrence of food-borne illness attributed to seafood is nevertheless low (Sumner, 2002).

Table 1: Outbreaks of food-borne infections and food-borne intoxications attributable to consumption of seafood in Australia during the period 1990-2000 (Sumner, 2002)

Causal Agent	Cases	Outbreaks
Ciguatera	612	9+
Histamine	28	10
Viruses in Oysters	1737	3
Bacterial pathogens	159	6
Bio-toxin in shellfish	102	3
Total	**2638**	**32+**

Microbiology of Prawns

The microbiology of prawns is known to be dependent on the environment from which they have been captured, their feeding habits, geography and degree of handling (Nicolaides, 2002). The greater the number of handling steps, the higher the number and variety of bacteria likely to contaminate the external surfaces of prawns. Poor hygiene practices during handling and preparation have the potential to introduce bacteria of public health significance such as faecal coliforms and pathogenic bacteria. Thus, even with the best quality raw product available, poor handling practices and temperature abuse can significantly degrade quality.

Initially, the bacterial flora of live prawns is representative of the environment from which they are caught. Thus, the microbial flora of prawns caught in tropical waters will differ from those caught in temperate waters and these differences are reflected in the types of bacteria that grow on refrigerated prawns. *Pseudomonas* spp, *Alcaligenes* spp, *Shewanella putrefaciens* and members of the *Acinetobacter-Moraxella* genera are the predominant spoilage bacteria associated with temperate water prawns. Spoilage is inevitable unless the prawns are frozen and stored at or below -18°C.

Cooking will eliminate the intrinsic bacteria associated with prawns and those added during any subsequent handling steps. However, good manufacturing practice is required to ensure that the product is not re-contaminated with bacteria from the processing environment or from workers. Provided boats use adequate sanitation methods to minimise contamination of work surfaces and use rinse water of good microbiological quality there should be minimal recontamination.

The principal human bacterial pathogens potentially associated with prawns and shrimp are *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio* spp, *Campylobacter* spp. and *Aeromonas* spp. With the exception of *Vibrio* spp such as *V. parahaemolyticus* and *V. vulnificus* which are natural inhabitants of marine environments, the other pathogens are not normally associated with crustaceans. For example, *Salmonella* is a mesophilic pathogen associated with warm-blooded animals and is usually only associated with prawns reared in intensive aquaculture or in waters contaminated by faeces.

Similarly, *S. aureus* is not a bacterium intrinsically associated with prawns. The primary source is the hands of processing workers. Healthy individuals (50-70%) for example, carry toxin-producing strains as part of the natural skin microflora. Thus, there is potential for transfer of these bacteria to prawns during handling.

Aeromonas sobria and *A. hydrophila* have not been confirmed to be causal agents of food-borne illness associated with consumption of prawns, even though the latter species has been reported to be isolated from prawns (Palumbo et al, 1985). *Campylobacter*, like *Salmonella* is likely to contaminate prawns by transfer from infected workers to prawns.

Non-biological hazards

An additional non-biological hazard associated with consumption of crustaceans is the sulphur dioxide liberated as a consequence of sodium metabisulphite dips used during processing of green prawns. Sulphur dioxide, in the form of sodium metabisulphite, prevents the formation of “black spot” during storage. Black spot is a harmless, but visually adverse discoloration of the surface of prawns that results from a series of enzymic reactions. The reactions result in deposition of melanin under the shell. This condition is not caused by excessive levels of bacteria that cause spoilage.

In sensitive individuals, sulphites can provoke asthma and other symptoms of an allergic response such as skin rashes and irritations (FSANZ, 2001). The maximum permitted level of sulphur dioxide in foods is prescribed in the Codex Standard for Quick Frozen Shrimps or Prawns (CAC, 1995) and the FSANZ Food Standards Code (FSANZ, 2000). For green and cooked prawns, the maximum allowable residual free sulphur dioxide concentrations are 100 ppm and 30 ppm respectively. Other compounds[1] are available to control development of black spot. Comparisons of efficacy of anti-black spot agents have been reported (Slattery *et al*, 1995), however there remains a need to determine the relative advantages and risks associated with use of alternative anti-black spot agents.

Needs

International

Developments in the global trade of food have exposed primary producers to a new set of opportunities and risks that are best managed with risk assessment. Estimation of ‘equivalence’ is the process that determines whether Australian products can penetrate foreign markets, and whether or not products produced abroad can penetrate Australian markets. This involves an appraisal of whether the imported product presents the same or lesser magnitude of human-health risk as posed by the domestic product. Under the guidelines produced by the World Trade Organisation (WTO) the assessment of equivalence demands the conduct of food safety risk assessments by the importing country. Countries may reject imported products that fail to meet equivalence standards. Thus, exporting nations require a pool of scientific expertise to conduct their own risk assessments and also to appraise the appropriateness of those produced by their trading partners.

National

At the domestic level, state food safety legislation and food standards are increasingly based on the risk assessment approach and demonstration of equivalence with national standards. It is timely, therefore, for industry to (develop and) validate an integrated supply chain approach to food safety that has international standing as a basis for meeting public health and trade access requirements.

The project developed a framework for an objective, transparent and scientifically robust basis for the management of food-borne hazards and shelf-life in the prawn industry. It conformed to the internationally accepted approach for the conduct of food safety risk assessment that is promulgated by CODEX, FAO and WHO (Anon., 2002).

[1] The principal agent used by South Australia prawn fishermen is sodium metabisulphite. However, Everfresh (4 hexyl resorcinol) is in use on at least one boat in the Spencer Gulf fleet.

Industry

The project provided an opportunity to work at all levels of the supply chain to ensure that the good reputation enjoyed by SGWCPFA prawns, was confirmed and maintained by validated quality systems. The project developed and pre-tested a framework to validate the production of cooked prawns produced under the APIA Code. It has provided industry with applied recommendations on the value of routine microbiological monitoring to support other audit verification processes.

Aims of the Project

Seafood Services Australia (SSA) in conjunction with the FRDC developed a Code of Practice (Carney, 2002) which is in wide use in the prawn industry. The Spencer Gulf & West Coast Prawn Fishermen's Association (SGWCPFA) utilise this Code and in 2002, approached the SARDI Food Safety Research Alliance to develop a framework which would enable validation of the production of cooked prawns under this program and to provide recommendations on the value of routine microbiological monitoring to support other audit verification processes.

The project aimed to develop an approach that validates this Code of Practice across the supply chain for green and cooked prawns caught within the Spencer Gulf & West Coast Prawn fishery. In particular, the project attempted to benchmark industry performance criteria (i.e. quantify decrease/increase of indicator organisms) for cooking/dipping, freezing and preparation (thawing, retail hygiene) for retail processes. Benchmarks for industry product criteria (i.e. acceptable levels of micro-organisms important for product safety and shelf-life) across the supply chain were evaluated for conformity with the FSANZ Food Standards Code (2000). The approach was intended to provide other crustacean supply chains with a generic framework for developing and/or validating food safety quality assurance programs.

Planned Outcomes

The project aimed to provide the following outcomes:

1. Framework/principles/guidelines for other crustacea industries to adapt/adopt.
2. Identify areas in need of further development to improve food safety and shelf-life.
3. Update the APIA Code of Practice in relation to hygiene, processing and cold chain as appropriate.
4. Benchmark the shelf-life and requisite food safety parameters to underpin and develop marketing opportunities.
5. A sound scientific basis on which processors can make recommendations on the correct storage and handling of the prawns.
6. Confidence for the fleet that the prawns processed on board the vessels are safe for consumption when they leave the vessel for distribution, thus safeguarding the reputation of the region's product.

Objectives

4 Develop a framework designed to validate the Australian Prawn Industry Association (APIA) Code of Practice across the supply chain for cooked prawns as a model for crustacean industries.

5 Benchmark industry performance criterion (i.e. quantify decrease/increase of indicator organisms) for cooking, freezing and preparation for retail (thawing, retail hygiene) processes.

6 Benchmark industry product criteria (i.e. acceptable levels of micro-organisms associated with food safety and shelf-life) across the supply chain to evaluate conformity with the FSANZ Food Standards Code.

Variation of Contract

While the contract was to conduct work on cooked prawns, the research group had also applied the same approach/framework to green prawns leading up to the start of this project (SIDF 2002/425). SSA accepted inclusion of data from the green prawn study in response to milestone reports.

Due to time constraints, Module D (Supply Chain Integrity) was not completed as originally described in the application for project funding. Module D was to have included monitoring of product temperature history during transport, as well as wholesale and retail level storage. For the purpose of this project, a more limited assessment of the supply chain performance has been achieved by analysis of data collected on-board, during storage trials and from retail sampling. This approach identified several points of failure associated with on-board processing, and wholesale/retail distribution and storage. Supply chain integrity monitoring would be better addressed by a full project covering the use of data loggers over a broad range of distribution options. This would create a more robust and meaningful set of temperature histories enabling better interpretation of shelf-life of retail product.

Methods

Overview of the Approach/Framework

In summary, the project was designed to evaluate the efficiency of key on-boat processing procedures and establish product criteria at retail. In addition, product from the randomly selected boats was evaluated for shelf-life under optimally controlled conditions. Unfortunately, product from the randomly selected boats (for both green and cooked prawns) could not be traced to retail. Consequently the retail data can only be related to the SGWCPFA fleet, not to processing efficiency and product criteria (at the point of freezing) achieved on the specific boats studied.

Such an approach enables not only evaluation of the effectiveness of important procedures, but also a comparison of product criteria across the supply continuum to identify failures/ areas for improvement.

The approach/framework also utilised the random selection of boats to provide a cross-sectional evaluation of the processing of prawns on-boat.

Sampling and Testing

The project involved microbiological analysis of surface swabs, water and prawn samples from the “water to waiter” supply chain. Levels of sulphur dioxide associated with processed (cooked or dipped) prawns were also monitored. The framework used for through chain validation and the microbiological tests are outlined in Figure 2 and Appendix 5. All testing was carried out according to the Australian Standard for Food Microbiology (AS1766) (or equivalent). The sampling framework was in accordance with that agreed to between SSA and FSANZ.

From an operational view, the project consisted of four key modules:

A. Assessment of on-board processing hygiene
B. Assessment of product shelf-life
C. Food safety study
D. Supply chain integrity

Guidelines used in training programs for sample collection/storage are provided in Appendices 2a and 2b, respectively.

The Microbiological Limits for Foods described by the Codex Alimentarius Commission and FSANZ (Appendices 3a and 3b) were used to evaluate microbiological data obtained.

The Spencer Gulf & West Coast Prawn Fishery

The Western King Prawn (*Penaeus latisulcatus*) is found around most of coastal Australia with South Australia and Western Australia having the main commercial fisheries for this species. This report focuses on the Western King Prawns that are caught and processed in South Australian waters by the vessels of the Spencer Gulf and West Coast Prawn Fishermen’s Association.

Spencer Gulf is a large triangular embayment situated from latitude 32°30' to 35° south, and centred in longitude at around 137° east. The major part of the fishery is situated between 33°30' south and 34°30' south, in depths greater than 10 metres (Figure 1). There is heavy industry nearby at Port Pirie and Whyalla. The region has no run-off from large rivers and it is a hyper saline environment with salinity ranging from 36‰ (parts per thousand) in the south to over 50‰ in the north. Water temperatures (at 10 to15 metres depth) range from 20-25°C in summer to 12 14°C in the winter. The main nursery areas for juvenile prawns are

situated in shallow water (<10 metres depth) on bare, sandy, mud substrates north of 33°40' south and in the vicinity of Franklin Harbour (latitude 33°45' south).

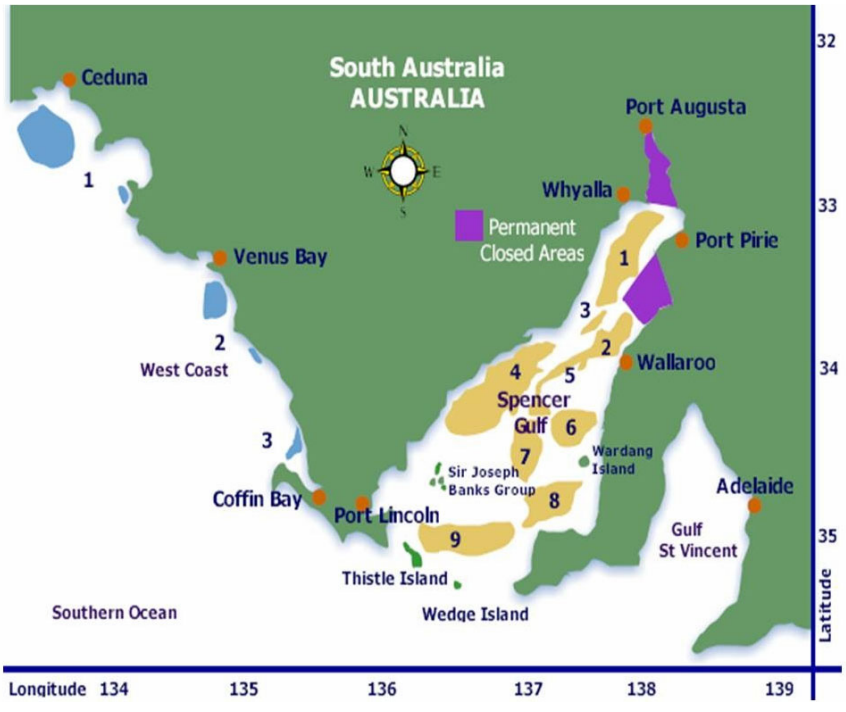
The fishing season is generally from November to June with the vessels at sea a few days either side of the new moon each month. Trawling takes place at night for a duration of 9 to 13 hours. Daylight trawling is prohibited. The average duration of each trawl is 50 mins. Fishing is limited to approximately 75 nights per year.

After each trawl, the catch is processed as shown in Figure 2. Prawns are emptied onto a sorting table, sorted, graded and are either cooked or, in the case of green prawns, dipped in a chemical solution to delay development of “black spot” (melanosis). Immediately after either of these operations, the prawns are snap frozen to less than -30°C. Prawns in excess of the immediate handling capacity of the freezer and crew, are stored in refrigerated brine (seawater) until they can be processed.

Snap frozen prawns are stored on board, typically at -25°C, in large freezers below deck. At the conclusion of each trip, frozen prawns are unloaded at ports adjacent to the fishing grounds and transferred to processing plants and distributors by refrigerated road transport.

In 2002, the Spencer Gulf and West Coast fisheries had an average catch of approximately 55 tonnes per vessel with an approximate value to the fleet of $39M (pers. comm. SGWCPFA, 2002). The catch is confined to a limit of 42 licence holders. Individual vessels of the fleet are registered with the Australian Quarantine and Inspection Service as (Export) Food Processing establishments and are thus subject to annual inspection.

This project was an opportunity to work at all levels of the supply chain to ensure that the safe reputation that SGWCPFA prawns enjoy is confirmed and maintained by validated quality systems.



The nine trawl regions in Spencer Gulf.

1. Northern Area (Middle Bank, Channel, Yarraville Basin, Stones & Musgrave Shoal)
2. Wallaroo
3. Shoalwater
4. Cowell, Arno and Western Gutter
5. Main Gutter
6. Wardang
7. Southern Gutter
8. Corny Point
9. Thistle Island and Rosalind Shoal

The three main trawl regions on the West Coast:

1. Ceduna
2. Venus Bay
3. Coffins

Figure 1: Trawl regions for the Spencer Gulf and West Coast Prawn Fishermen’s Association

A. Assessment of On-Board Processing Hygiene.

Based on the process diagram shown in Figure 2, the following samples were subjected to microbiological analysis to characterise the potential contribution of boat hygiene practices to the microbiological quality of green and cooked prawns.

A1 Swab samples of product contact surfaces during product handling and after cleaning on-board 24 randomly selected boats

A2 Samples of processing water (Appendices 5b and 6b)

A3 Prawns sampled before and after processing (cooking or dipping). The microbiological data obtained was used as a means of process validation. (Appendices 5c, 6c)

In addition to the microbiological sampling, a survey of on board sanitation practices was carried out.

A4 Survey form completed by most crews of the boats involved in the survey (Appendix 4).

B. Assessment of Product Shelf-life

This module examined the microbiological counts of commercial quantities of green and cooked prawns, taken directly from the vessels and stored for approximately 90 days at less than -21°C.The prawns were then thawed and stored under optimal conditions (less than 5°C) and sampled periodically. This work established a benchmark against which prawns sampled at retail could be compared. Unfortunately the prawns could only be compared to the fleet not those from the optimal storage study.

The module involved; (Figure 2)

B1 A survey of microbiological quality of retail product and comparison with data obtained from on-board processing (A3 above).

B2 Benchmarking of the microbiological shelf-life of optimally thawed and stored prawns. This provides the baseline against which the retail product are compared.

C. Food Safety Study

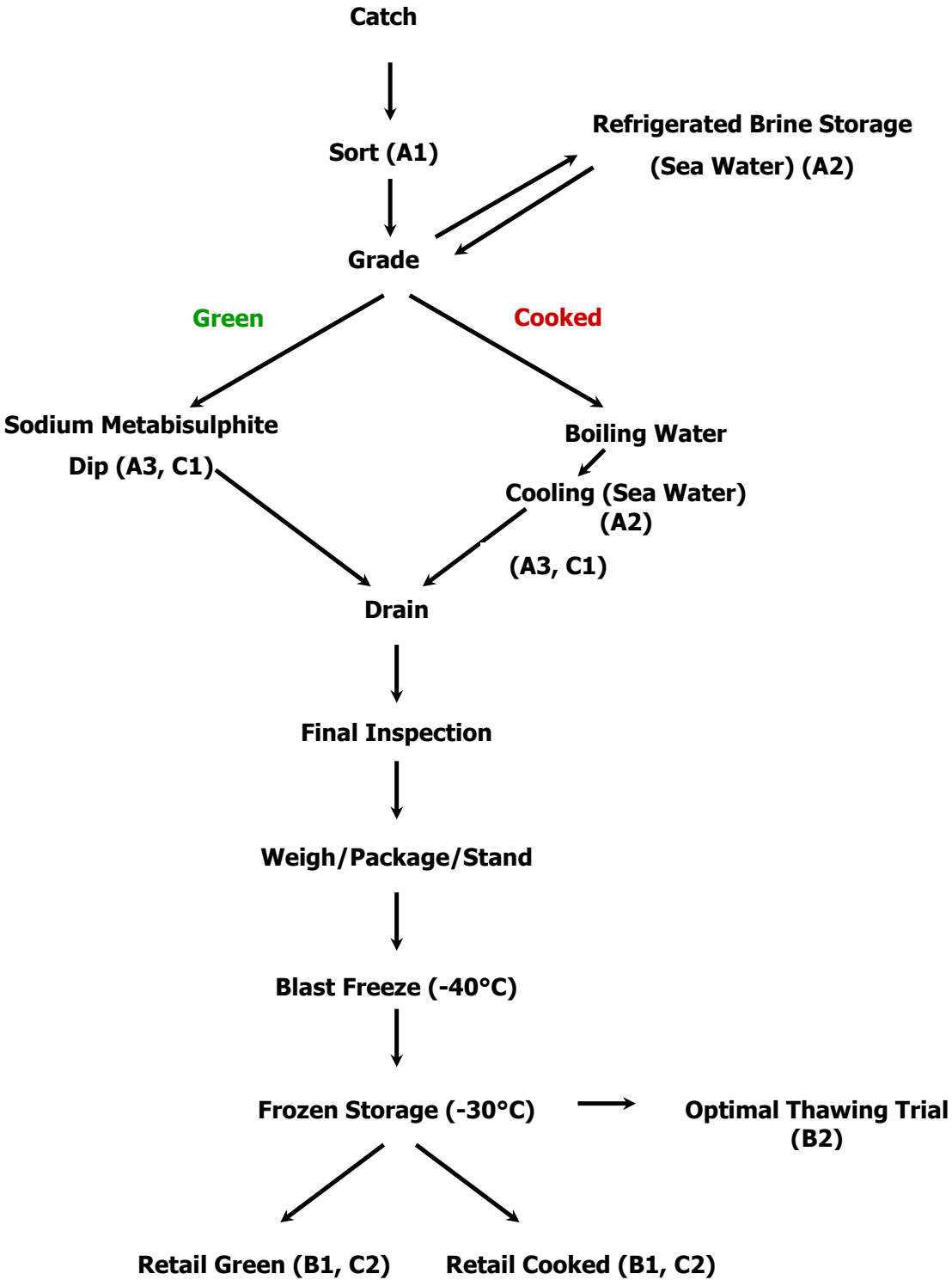
This module examined green and cooked prawns for the presence of microbiological and non-biological hazards as stipulated by the Food Standards Code (FSANZ, 2000). In addition, the prawns were sampled and examined for the presence of marine *Vibrio* spp. and indicators of faecal contamination. This part of the project allowed us to determine whether poor food handling throughout the supply chain contributes to the presence of microbiological hazards.

The study used samples of: (Figure 2)

C1 Cooked or green prawns taken on board for the presence of pathogenic bacteria prior to freezing (using data from A3 above)

C2 Cooked or green prawns purchased in the retail market (typical of those purchased by the consumer).

Figure 2: Flow Diagram of Prawn On-Board Processing



D. Supply Chain Integrity

The Standard Plate Count data collected from the on-board, storage trial and retail survey was analysed to assess the microbiological status of prawns throughout the supply chain continuum. This includes establishment of processing and product criterion.

Operational and Sampling Issues

For sampling purposes, prawn fishing vessels were selected at random by assigning unique numbers to boats followed by use of a random number chart to assign boat identification numbers for the survey. Twelve boats were selected for the survey of green prawns and 12 for the cooked prawn survey. In the event that a selected boat was unable to take part in the sampling, another was selected to replace it.

To further de-identify the data, new identification codes were assigned for reporting purposes. Data from boats were assigned as follows:

G1 to G12	Green prawn survey
C1 to C12	Cooked prawn survey
RG1 to RG8	Green retail prawn survey
RC1 to RC12	Cooked retail prawn survey

Due to the nature of the fishing process and for practical reasons, on-board sampling was carried out by a crew member from each vessel (Appendices 2a, 2b). Training sessions were held and detailed instructions were provided for all sampling procedures. All on-board sampling was carried out on the last night of fishing. SARDI personnel carried out other sampling. Prawns used for the storage trials were sampled by staff of the testing laboratory

Pre-labelled sampling kits, including detailed written instructions were supplied to boats prior to sailing. All samples, except for the frozen cartons of prawns were to be kept under refrigeration (0-5°C) while on board and packed in insulated containers with ice bricks for transport to the testing laboratory. The completed sample kits were transported by refrigerated transport from Port Lincoln to Adelaide where they were collected from the transport depot by car to the testing laboratory. Alternatively, the sample kits were collected direct from the vessels at the nearby ports of Wallaroo or Port Adelaide and transferred directly to the testing laboratory by car.

As the project included both boat hygiene and shelf-life/food safety benchmarking it was intended that all vessels would be sampled on the same voyage. This enabled consistent and meaningful interpretation of shelf-life and food safety data for green or cooked, pre-frozen product in the context of the boat hygiene.

All samples were processed according to Australian Standards for Microbiological Analysis. Details of testing methods and standards used are listed in Appendix 1.

Data Analysis

The data obtained in this study was used to develop the outcomes described in the background. In particular, we determined whether the microbiological data was consistent with the standards described by FSANZ and the CAC. The results obtained will allow the development of a framework for validation of the supply chain for Spencer Gulf and West Coast prawns.

To compare SPC levels of pre and post processing (dipping and cooking) of prawns and pre and post freezing of prawns, linear mixed effects models were fitted to the logarithm (base 10) of the green and cooked SPC data in turn. This logarithm was chosen to better satisfy

statistical assumptions. In addition, it also provides meaningful biological interpretations. The models allowed for different variability at each processing / storage trial / retail level. Tests of significance (using successive difference contrasts; Venables & Ripley, 2002) were then performed to investigate differences between pre and post processing and between pre and post freezing. All statistical models were fitted using the software package R (R Development Core Team, 2003).

Survey of On-Boat Hygiene Practices

A survey of on-board hygiene practices used on participating boats was carried out. A copy of the survey form is listed in Appendix 4. Each boat captain was asked to complete a survey form.

Selection of Retail Outlets

Retail outlets were chosen by type of store, supermarket or specialist seafood outlet, and by location, with an attempt made to sample across the Adelaide metropolitan area. Of the 20 outlets sampled 4 were supermarkets, 1 was a mixed gourmet store and 15 were specialist seafood retailers.

Samples were taken after midday so as to have the prawns exposed to the retail display cabinets for an extended period. Samples (1 kg) were purchased and the temperature recorded either in the display cabinet or immediately on return to the vehicle. Some retailers would not allow the temperature of the prawns to be taken in the store. The temperature was recorded with a non-contact laser thermometer.

The carton that the prawns were from was sighted and any relevant details were recorded to confirm supply from the SGWCPFA members. Unfortunately it was not possible to directly relate the retail samples to the boats from this study.

Results and Discussion

Introduction

The proposed approach/framework consisted of the following components:

- Coverage of the supply chain continuum – "water–to-waiter" – food safety and shelf-life
- Evaluation of the effectiveness of processing (control and critical control points) and bench-marking of processing criteria
- Benchmarking of the product criteria (ready-to-eat product)
- A random, cross-sectional sampling framework
- Scope
    - to meet FSANZ Food Safety Standards
    - to evaluate product performance in the marketplace (shelf-life, black spot)

The investigators wish to note the limited number of samples processed and the constraints on the confidence of the conclusions that may be drawn from the data. The project aimed to establish a framework for evaluation of processing standards and product criteria across a supply chain continuum for both food safety hazards and shelf-life indicator organisms. Consequently, with such a complex design, funding constrained the number of samples that could be analysed. Further fleet monitoring as a result of this work will lead to a larger pool of systematic data for industry to better evaluate performance.

A. On-Board Processing Hygiene

Processing Surfaces

To assess on-boat hygiene, we swab sampled crates, tables, weighing tubs, funnels, carton fillers and other items used during the handling and on-board processing of the prawn catch were swabbed. Counts of bacteria retained by swabs and those present in wash/rinse water were determined using standard microbiological methods.(Appendix 1)

Swab sampling showed that on-board hygiene practices resulted in a significant reduction in counts of bacteria associated with work surfaces (Standard Plate Counts (SPC) at 25°C) (see Appendices 4a and 4b). In general, the SPC of swabbed surfaces on boats before cleaning were low ($<10^3$ per 100 cm^2). However, the SPC of swabbed surfaces on several boats were very high prior to cleaning. *Listeria monocytogenes* was not isolated from any swab samples tested.

Wash and Rinse Water used On-board

Wash/rinse water used on board was of a high microbiological quality (see Appendices 5b and 6b). Bacterial indicators associated with faecal contamination (faecal coliforms and faecal streptococci) were found in only 2 of 44 samples of water used on-boat. Furthermore, *Listeria monocytogenes* was not detected in any water sample tested. As expected we consistently isolated marine vibrios from several water samples but these were present in low numbers (<100 per 100mL water).

Green and Cooked Prawns

Freshly harvested prawns were in general of a high microbiological quality (Appendices 5c and 6c). SPCs (25°C) of most samples were low (ca. 2.8 x 10^3 per gram ± 1.8 x 10^3). Furthermore, the SPC at 4°C mirrored the SPC at 25°C. Of particular interest was the observation that the SPC of prawn samples obtained from some boats was very high (eg. G4 1.1 x 10^5 per gram ± 4.9 x 10^4). However, high prawn SPCs did not correlate with high counts of bacteria associated with handling surfaces or water used for washing or rinsing.

Cooking was expected to significantly reduce the total aerobic plate count of green prawns. It is noteworthy, however, that only 3 of 12 boats demonstrated reduction in counts of bacteria on all 5 of 5 separate samples of prawns after cooking. The effect of cooking on the SPC (25°C) of prawns shown in Figure 3. The data shown represents the mean of groups of 5 samples taken from boats on a single day of fishing. Considerable boat to boat variation in SPC was noted. For many samples the SPC of bacteria on cooked prawns was greater than counts for samples of green prawns taken on the same day. These results may have been the result of:

- re-contamination by cooling/rinse water (Boat skippers confirmed the frequent use of unchilled seawater for cooling cooked prawns. Prawns were held in cooling water for undefined periods of time)
- a potential for inappropriate handling and storage of samples set aside for microbiological analysis by crew responsible.
- a cool chain failure that occurred following cooking. This may be a result of:
  - Extended holding of prawns partly cooled in untreated seawater
  - Inefficient heat loss from cooked prawns during the freezing process.

In any event, high counts associated with cooked prawns would ultimately impact on product shelf-life and safety. There is evidence for this conclusion based on observations that the microbiological shelf-life of cooked prawns is less than that of green prawns (see Figures 3, 4 and 5). Nevertheless, the work has identified that crews will need to develop consistent processing and sampling practices in order to allow the industry to be confident that a quality assurance program will have long term benefits and assure market advantage.

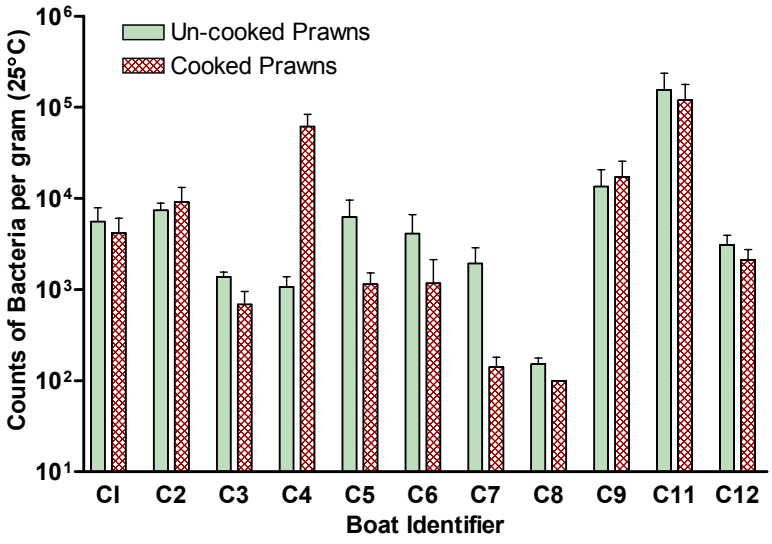


Figure 3: Effect of cooking on the SPC of prawns sampled from different boats. Error bars represent the standard deviation about the mean. Data from Appendix 6c.

Treatment of Green Prawns with Metabisulphite

All boats used chemical agents to control black spot in/on prawns harvested for sale raw. Most boats used sodium metabisulphite as the source of the anti-black spot agent, SO_2 (although one boat operator used 4 hexyl resorcinol as an alternative agent to prevent this defect). The recommended residual free concentration of SO_2 on green prawns is 100 ppm (100 mg per kg prawns). However, the residual concentration on green prawn samples from different boats varied from <10 ppm to 140 ppm (Table 2). This variation in SO_2 levels noted impacts directly on product quality in two ways:

1. Residual free SO_2 levels have a significant impact on development of black spot in prawns stored at 4°C (See Results and Discussion: Product Shelf-life). High levels of this agent delay development of black spot in the tail fans, head and carapace compared to prawns that have a low level of residual sodium metabisulphite. Early onset of black spot devalues the product from a consumer perspective.
2. One advantage of sodium metabisulphite dips is significant reduction in SPC of freshly harvested green prawns (Figure 4). Any reduction in the growth of bacteria capable of growth at refrigeration temperatures, for example, will lead to an increase in the effective shelf-life of the product.

The variation in free residual SO_2 levels associated with green prawns sampled immediately after harvest is an indication that dosing practices used on boats need to be standardised. For the reasons outlined above, there are important product quality issues that can be overcome by effective sodium metabisulphite dosing treatments.

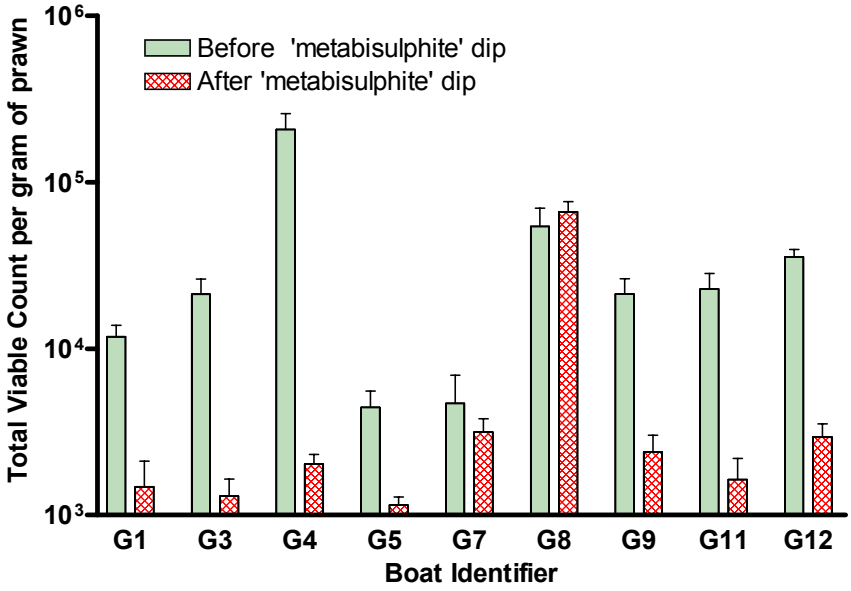


Figure 4: Effect of sodium metabisulphite dips on the SPC of green prawns. Error bars represent standard deviation about the mean. Data from Appendix 5c.

Note that low levels of residual free SO_2 were detected in samples of retail green prawns (usually <50 ppm) (see Appendix 5f). Nevertheless, black spot was evident on most retail green prawns sampled at point of sale. The high SPC on some samples of retail prawns is of concern and may represent cool chain failures at the retail level.

In consultation with the SGWCPFA, the issue of black spot development/aesthetic acceptance was highlighted. In response, product was photographed on sampling days during the storage trials. While objective criteria for aesthetic acceptance are not agreed, the researchers observed emergence of substantial black spot at the anterior and posterior ends of the carapace, the margins of the tail shell segments and on tail fans. Blackening was particularly evident on prawns thawed and held at 4°C for more than 4 days (ie. prior to development of numbers of bacteria sufficient to cause spoilage).

Table 2: Residual free SO_2 levels associated with green prawns sampled from different boats immediately after dipping in sodium metabisulphite solution. Data from Appendix 5c

Boat ID	SO_2 Level
G1	32.6
G3	no sample
G4	***107***
G5	<10
G7	***140***
G8	40
G9	10
G11	not detected
G12	<10

FSANZ standard: for green prawns: 100ppm. (Food Standards Code 1.3.1)

Hygiene Survey

A survey was conducted to determine on-board hygiene practices used by 17 of the 42 boats in the fleet (see Appendix 4). The aim of the survey was to determine whether standard practices were in place across all boats in the fleet. The results of the survey are summarised in Tables 3 and 4. The main outcomes of the survey were:

1. A combination of salt and/or fresh water was used for all processing and sanitation practices.
2. A variety of different sanitisers are used. Furthermore, sanitisers were used inconsistently as part of on-board hygiene practice. For example, not all boats sanitised work surfaces prior to processing, nor was there any standardisation for sanitising after processing had been completed. These inconsistent practices could have an important impact on the microbiological status of processed prawns.
3. Green and cooked prawn catches may be processed on the same night and in no specific order. Ideally, cooked prawns should be processed and packed prior to green prawns to minimise cross contamination by bacteria.
4. Temperature of water used to cook prawns was monitored by visual inspection only. Standard practice in the majority of food processing environments requires maintenance of temperature records as a component of good manufacturing practice.
5. There was no standard procedure for dealing with sick crewmembers. Sick crew should be quarantined from the processing and handling of prawns as part of good manufacturing practice (Carney, 2002).

The survey results indicated there are important failures in quality control that need to be addressed.

Table 3: Responses to survey of hygiene practices for green prawn catches (see Appendix 4)

Boat ID	Cleaning Water			When do you sanitise?			Ha	
	Outside	Inside	Sanitiser	Before	(Only) After	Between Green and Cooked	Green and Cooked Together	
G1	sea	sea/fresh	Domestos	no	yes	no	yes	
G4	sea	na	Sani-det	yes	no	yes	yes	
G5	sea	na	Lem Sol	no	yes	no	yes	
G10	sea	fresh	Bromosan/Century 2	yes	no	no	yes	
G11	sea	fresh	Teepol	yes	no	yes	yes	
G12	sea/fresh	sea/fresh	Dobatex	no	yes	no	yes	

Note: only 6 boats of 12 selected, responded to the survey.

Table 4: Responses to survey of hygiene practices for cooked prawn catches (see Appendix 4)

Boat ID	Cleaning Water			When do you sanitise?			Handling			
	Outside	Inside	Sanitiser	Before	(Only) After	Between Green and Cooked	Green and Cooked Together	Order	Gloves	Bo W Cl
C1	sea	fresh	Bromosan	yes	yes	yes	yes	none	yes	vi
C2	sea/fresh	sea/fresh	hot water during trip. Teepol, Marine Clean after trip	yes	no	yes	yes	cooked then green	yes	vi
C3	sea	sea	Teepol	yes	yes	no	yes	none	yes	vi
C4	sea	sea	Bromosan	no	yes	no	yes	none	yes	vi
C5	sea	fresh	Bromosan	no	yes	yes	yes	c then g	yes	vi
C6	sea	fresh	Teepol	yes	no	yes	yes	c then g	yes	vi
C7	sea/fresh	fresh	Lemon Sol	no	yes	yes	yes	none	yes	vi
C9	sea/fresh	fresh	Teepol	no	yes	yes	no	none	yes	vi
C10	sea	sea	Power Chlor	nc	yes	yes	yes	none	yes	vi
C11	sea	fresh	LCC-HYFoam	yes	no	yes	yes	none	yes	vi
C12	sea	fresh	Jasol	yes	yes	yes	yes	none	yes	vi

Note: 11 boats of 12 selected, responded to the survey.
nr – not recorded

B. Product Shelf-life

To assess the shelf-life of green and cooked prawns, sample cartons of frozen prawns were thawed and stored for up to 12 days at 4°C. At regular intervals, sub-samples of prawns were taken to determine the SPC at 4°C and 25°C. In addition, the samples were tested for presence or absence of *Listeria monocytogenes*.

The results of the storage trials for green and cooked prawns are shown in Figure 5. The plots comprise counts for samples derived from different boats. Interestingly, at any given storage interval, the SPC of cooked prawns was greater than that of green prawns. Nevertheless, given that organoleptic spoilage of seafood is generally regarded to occur when the SPC exceeds 10^6 per gram, the maximum shelf-life for frozen green or cooked prawns when stored at 4°C should be no more than 5 to 6 days.

Listeria monocytogenes was either not present or was at undetectable levels in green and cooked prawn samples taken from the storage trial (see Appendices 5e and 6e).

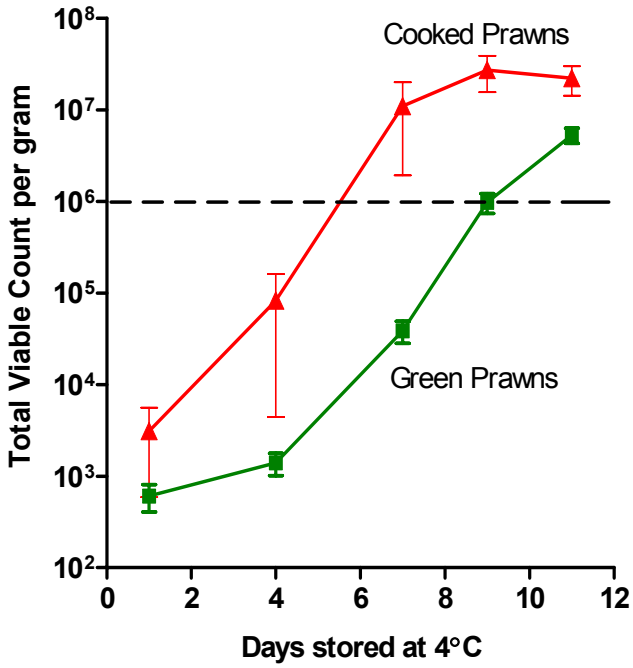


Figure 5: SPC of frozen green and cooked prawns stored at 4°C. Horizontal dashed line represents an arbitrary SPC at which organoleptic spoilage is evident. Data points at each sampling interval represent SPC for samples of prawns derived from separate boats. Error bars represent standard deviation about the mean. Data from Appendices 5e and 6e.

Development of Black Spot

For green prawns, acceptable shelf-life may be dependent on the rate of development of black spot rather than purely on microbiological grounds. As expected, the level of residual concentrations of SO_2 had a significant impact on development of black spot. Prawn samples with low levels of residual SO_2 developed black spot more rapidly than samples with high levels of residual SO_2 (Figure 6). Typically, green prawns developed significant black spot after about 4 days of storage at 4°C if residual SO_2 levels were low (<10 ppm), compared with about 8 to 9 days for samples with high residual SO_2 levels.

Black spot developed in a consistent and predictable manner during storage of thawed prawns at 4ºC (Figure 7). Blackening developed first at the anterior and posterior ends of the carapace. After 9 days of storage, the extent and intensity of blackening at these sites had spread. In addition, blackening was observed in the tail fans and at the margins of the tail segments. These changes are consistent with those reported by Slattery *et al* (1995).

Fresh Thawed

8 Days at 4°C

High
SO_2

Low
SO_2

Figure 6: Development of black spot on sodium metabisulphite treated green prawns during storage at 4°C. Prawns with high and low level residual SO_2 are shown at thaw and after 8 days storage at 4°C. Note the presence of black spot on prawns with low residual SO_2 at thaw.

DAY 1

DAY 3

DAY 4

DAY 9

Figure 7: Development of black spot on green prawns thawed and stored at 4°C for 1, 3, 4 and 9 days.

C. Product Food Safety

Table 5 represents a summary of all microbiological tests for food safety parameters. Although faecal coliforms were detected in ca. 2% of samples, coagulase positive staphylococci, salmonellae and *L. monocytogenes* were not detected in any sample tested. Marine vibrios, were detected in a number of samples. This data indicate that prawns harvested from the Spencer Gulf and West Coast fishery are of good microbiological quality.

Processing Water

Table 8 represents a summary of results of tests for food safety parameters for boat processing water. Processing water was only tested for presence of faecal coliforms and *L. monocytogenes*. Of 46 water samples tested, 2 samples were positive for faecal coliforms, and 12 contained >100 marine vibrio cells per 100mL. *L. monocytogenes* was not detected in any water sample tested.

Fresh and Stored Prawns

Tables 6 and 7 represent a summary of all microbiological data obtained which defined the food safety of prawns sampled on boat and during storage at refrigeration temperatures. None of the samples failed current FSANZ and CAC standards for cooked or green prawns. *L. monocytogenes* was not isolated from any sample tested. Numbers of marine vibrios were below the detection limits of the test used.

Retail Prawns

Tables 9 and 10 list the results of microbiological testing of green and cooked prawns obtained from retail outlets in South Australia. For confidentiality reasons, information about the retail outlets has been de-identified. Samples of prawns were either frozen or had been placed under refrigeration on ice. The temperature of all samples is recorded.

For retail green prawns, faecal coliforms, coagulase +ve staphylococci and *Salmonella* were either absent or present at undetectable levels. Counts of marine vibrios were below the level of detection of the sampling procedures used. However, SPC (25°C) varied from <1000 per gram of sample, to >60,000 per gram. SPC at 4°C mirrored counts the SPC at 25°C. None of the green prawns sampled from retail environments failed current FSANZ standards. High SPC did not correlate with sample temperature at point of sale. Some of the highest counts obtained were for samples maintained at the lowest temperature.

All retail cooked prawns sampled satisfied current FSANZ and CAC microbiological standards for relevant food-borne pathogens as described in Table 10. Faecal coliforms, faecal streptococci, *Salmonella* spp and coagulase positive staphylococci were found to be absent or below the levels of detection using the standard methods used for microbiological analysis. *Listeria monocytogenes* was not detected in any of the samples tested. Counts of marine *Vibrio* spp were <100 per gram of sample for all retail cooked prawns tested. This result contrasts with data obtained for freshly cooked prawns sampled on-boat (see Table 7) and suggested that freezing cooked (and green prawns – see Table 6) resulted in reduction in numbers of marine vibrios to acceptable levels.

SPC (25°C) of <100 to $>10^6$ per gram of sample were obtained for prawn samples. Samples of cooked prawns taken from retail outlets RC3, RC4, and RC8 all had SPC in excess of 5 x 10^5 cells per gram of sample (products above this level are deemed unacceptable). Samples from one outlet (RC12) had SPC that were marginally acceptable. Samples from all other retail outlets, plated on standard plate count medium and incubated at 4°C, had counts that

mirrored the SPC (25°C) data. Overall these observations match those obtained for green prawns obtained from various retail outlets (Table 6).

The sample of cooked prawns with high SPC from retail outlets RC3, RC4 and RC8 are of concern (Table 7). These high counts do not consistently correlate with higher retail storage temperatures. For example, some samples with high counts were from retail environments where cooked prawns were stored at below 0°C. This apparent anomaly of high counts may be a result of extended storage close to 0°C, exposure to several freeze-thaw cycles, temperature abuse during transport from the fishery to wholesale and retail environments or poor temperature control on boat. Unfortunately because samples were not tracked from boat to retail market, the cause for these high counts cannot be determined unequivocally.

Nevertheless, samples with SPC of $>10^6$ per gram can be considered to be close to, or at, incipient spoilage and as such will have little residual consumer shelf-life even if stored at optimal refrigeration temperatures (0 - 4°C). For these reasons the industry should, in addition to ensuring the microbiological safety of prawns, strive to ensure that prawns with low SPC are offered for sale to consumers.

Marine vibrios

The presence of marine vibrios is an issue of concern. Randomly selected isolates of presumptive *Vibrio* species were subjected to phenotypic characterisation using Microbact 24E identification test kits. This analysis indicated *V. alginolyticus*, *V. parahaemolyticus* as well as other marine *Vibrio* species are associated with prawns. *V. parahaemolyticus* isolates were not tested for Kanagawa reaction (a key pathogenicity indicator).

While some species of *Vibrio* are well known food-borne pathogens, estimates of numbers of marine vibrios do not provide information about potential to cause disease. In fact, the ability of many marine vibrios to cause disease in humans is unknown. Consequently, for the purpose of this study, 100 *Vibrio* cells per sample was deemed acceptable. Higher numbers might indicate a need for rejection of a sample or batch of prawns.

Table 5: Summary table of the proportion of samples meeting tested food safety parameters.

Sample Type	Numbers of Samples meeting tested Food Safety Parameters*											
	Faecal indicators		Coagulase +ve Staphylococci		Salmonellae		*Listeria*		*Vibrio* species		Sodium metabisulphite levels	
	detected	not detected	≥100	<100	detected	not detected	detected	not detected	≥100	<100	unacceptable	acceptable
All Tests	2(2.1%)	92(97.9%)	0	132	0	132	0	207	22(13%)	149(87%)	3(11.5%)	23(88.5%)

Note: Data includes results for surface swabs, water and all prawn samples. Not all tests could be completed on all samples. Consequently there are differences in numbers of tests performed.

Data summarised from Appendices 5 and 6

*Microbiological criteria as per Appendices 3a and 3b.

Table 6: Number of green prawn samples which meet tested food safety parameters. Samples were taken from prawn fishing boats following cooking, after storage at 4°C and from retail environments.

Sample Type	Numbers of Samples meeting tested Food Safety Parameters*											
	Faecal indicators		Coagulase. +ve Staphylococci		Salmonellae		*Listeria*		*Vibrio* species		Sodium metabisulphite levels	
	detected	not detected	≥100	<100	detected	not detected	detected	not detected	≥100	<100	unacceptable	acceptable
Green prawns - boat	0	17	0	57	0	57	not tested		0	57	2	8
Green prawns - storage trial	not tested		not tested		not tested		0	11	not tested		not tested	
Green prawns - retail	0	8	0	8	0	8	not tested		0	8	1	8

Note: Not all tests could be completed on all samples. Consequently there are differences in numbers of tests performed.
Data summarised from Appendices 5c, 5d, 5e and 5f.

*Microbiological criteria as per Appendices 3a and 3b.

Table 7: Number of cooked prawn samples which meet tested food safety parameters. Samples were taken from prawn fishing boats following cooking, after storage at 4°C and from retail environments.

Sample Type	Numbers of Samples meeting tested Food Safety Parameters*											
	Faecal indicators		Coagulase +ve Staphylococci		Salmonellae		*Listeria*		*Vibrio* species		Sodium metabisulphite levels	
	detected	not detected	≥100	<100	detected	not detected	detected	not detected	≥100	<100	unacceptable	acceptable
Cooked prawns – on boat	0	11	0	55	0	55	0	55	10	45	0	7
Cooked prawns – storage trial	not tested		not tested		not tested		0	45	not tested		not tested	
Cooked prawns – at retail level	0	12	0	12	0	12	0	12	0	12	0	4

Note: Not all tests could be completed on all samples. Consequently there are differences in numbers of tests performed.

Data summarised from Appendices 6c, 6d, 6e and 63f.

*Microbiological criteria as per Appendices 3a and 3b.

Table 8: Number of prawn boat processing water samples meeting tested food safety parameters.

Sample Type	Numbers of Samples meeting tested Food Safety Parameters											
	Faecal indicators		Coagulase +ve Staphylococci		Salmonellae		*Listeria*		*Vibrio* species		Sodium metabisulphite levels	
	detected	not detected	≥100	<100	detected	not detected	detected	not detected	≥100	<100	unacceptable	acceptable
Process water – seawater & fresh	2	44	not tested		not tested		0	49	12 detected	35 not detected	not tested	
Boat Processing Hygiene - surfaces	not tested		not tested		not tested		0	80	not tested		not tested	

Note: Not all tests could be completed on all samples. Consequently there are differences in numbers of tests performed.

Data summarised from Appendices 5b and 6b.

Table 9: Microbiological testing of green prawns obtained from 8 South Australian retail outlets. Data from Appendix 5f.

Sample Details				Microbiological tests						
Retail Outlet	Sample Date	Sample Time	Sample Temp	SPC/g at 25°C	SPC/g at 4°C*	Coagulase +ve staphylococci (/g)	*Salmonella* (/25g)	*Vibrio* (/g)	Faecal coliforms (/g)	SO_2 (mg/Kg)
RG1	20/08/2002	15:00	-22	26,000	6,600	<100	not detected	<100	<0.3	<10
RG2	20/08/2002	15:15	+1	1,000	100	<100	not detected	<100	<0.3	<10
RG3	20/08/2002	14:05	-23	1,700	<100	<100	not detected	<100	<0.3	33.4
RG4	20/08/2002	14:40	-7	720	300	<100	not detected	<100	<0.3	<10
RG5	02/09/2002	14:30	+16	23,000	50,000	<100	not detected	<100	<0.3	41.7
RG6	14/10/2002	15:30	-22	62,000	800	<100	not detected	<100	<0.3	<10
RG7	23/10/2002	13:15	-17	1,400	300	<100	not detected	<100	<0.3	148
RG8	23/10/2002	15:00	-13	52,000	14,000	<100	not detected	<100	<0.3	<10

* SPC at 4°C. An estimate of the number of Psychrotrophic bacteria capable of growth at refrigeration temperatures.

Table 10: Microbiological testing of cooked prawns obtained from 12 South Australian retail outlets (Data from Appendix 6f).

Sample Details				Microbiological tests						
Retail Outlet	**Sample Date**	**Sample Time**	**Sample Temp**	**SPC/g at 25°C**	**SPC/g at 4°C**	**Coagulase +ve staphylococci (/g)**	***Salmonella* (/25g)**	***Vibrio* (/g)**	**Faecal coliforms (/g)**	***Listeria monocytogenes* (/25g)**
RC1	4/06/2003	12:00	-2.0	8,700	2,600	<100/g	Nd/25g	<100/g	<0.3/g	nd
RC2	4/06/2003	12:20	-21.0	60	<100	<100	nd	<100	<0.3	nd
RC3	11/06/2003	13:50	-5.0	510,000	400,000	<100	nd	<100	<0.3	nd
RC4	11/06/2003	14:15	+7.0	1,700,000	890,000	<100	nd	<100	<0.3 (colif = 2.3)	nd
RC5	18/06/2003	14:30	+8.0	1,600	1,300	<100	nd	<100	<0.3	nd
RC6	18/06/2003	15:05	-20.0	3,300	900	<100	nd	<100	<0.3	nd
RC7	18/06/2003	16:00	+2.0	1,900	300	<100	nd	<100	<0.3	nd
RC8	24/06/2003	13:20	+4.0	1,600,000	1,500,000	<100	nd	<100	<0.3 (colif = 0.4)	nd
RC9	24/06/2003	14:05	+1.0	1,200	1,800	<100	nd	<100	<0.3 (colif = 0.4)	nd
RC10	24/06/2003	14:55	+5.0	2,500	4,300	<100	nd	<100	<0.3	nd
RC11	1/07/2003	15:30	+5.0	200	190	<100	nd	<100	<0.3	nd
RC12	1/07/2003	16:00	-5.0	420,000	390,000	<100	nd	<100	<0.3	nd

Supply Chain Integrity

Previous sections of the Results and Discussion focussed on data obtained for the separate modules of this project. In this section, Standard Plate Count data are presented in a manner that allows examination on a "water to waiter" basis. This approach allows identification of processing failures and identification of critical processing control points. The approach also enables comparison of quality ex-boat against its shelf-life under optimal conditions and shelf-life when presented at retail.

Through Chain Analysis of Green Prawns

As a means of examining the impact of processing and storage on the microbiology of green prawns, data described previously in this report was presented in a box-whisker plot format[2]. This approach allows direct comparison of disparate groups of data. In addition it illustrates any central tendency and spread.

Figure 8 is a box-whisker plot of SPC (25°C) data across the supply chain continuum obtained for green prawns. The data sets covered freshly caught green prawns, prawns after dipping in sodium metabisulphite, prawns from the optimal storage trial and retail prawns. The horizontal dashed line represents an arbitrary estimate of counts of bacteria above which, product is deemed organoleptically spoiled (ie. > 10^6 bacteria per gram). Metabisulphite dips result in a significant reduction of counts of bacteria (P-value<0.001). Freezing further reduced the SPC of prawns significantly (P-value<0.001). As expected, counts increase during controlled thawing and storage at 4C. By day 9 at 4°C, product is spoiled and unacceptable for sale.

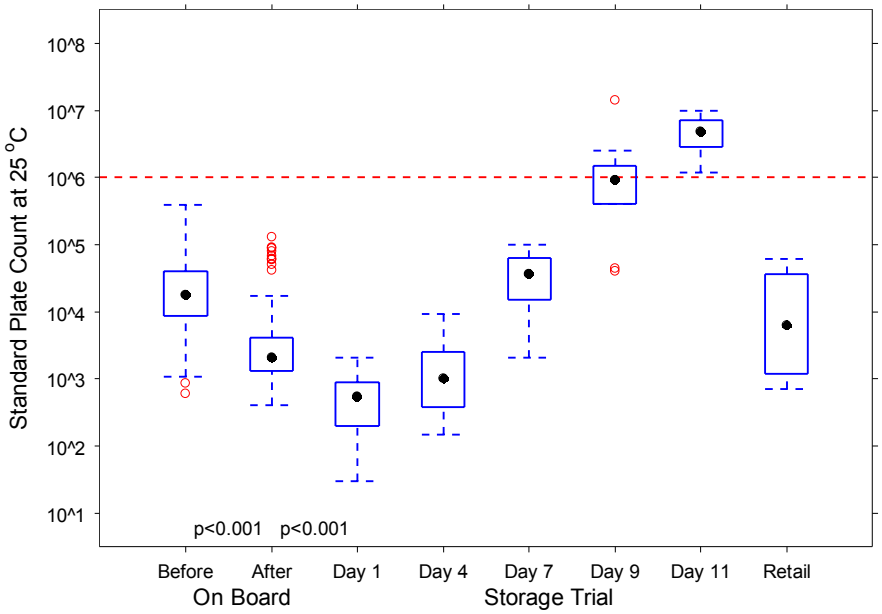


Figure 8: Boxplot of standard plate counts (25°) "through chain" for green prawns

[2] The boxplot, or box-whisker plot, illustrates the central tendency and spread of the data and is a useful tool when comparing data from several groups (Moore and McCabe, 2003). Central tendency is indicated by the median, the solid black dot within each box. 50% of the data fall below the median and 50% above. The spread of the data is indicated by the box, which has lower and upper bounds at the first and third quartile. These are the points, which have 25% and 75% of the data fall below and 75% and 25% above, respectively. The difference between these two quartiles, which is known as the inter-quartile range (IQR) is used in the calculation of the length of the whiskers. Observations falling far outside the extent of the whiskers may not fit the general pattern of the data and are likely to be different in some aspect.

If retail samples of green prawns were maintained at temperatures below 0°C, SPC should be comparable to that of freshly caught and treated frozen prawns. The box and whisker plot for retail green prawns clearly indicated that counts for these samples are of the order of 10 times greater than that of the freshly frozen samples. This suggested that retail prawns have been subjected to temperature abuse (involving freeze-thaw cycles or poor temperature control) at the retail level.

Through Chain Analysis of Cooked Prawns

Figure 9 is a box-whisker plot of SPC (25°C) data across the supply chain continuum obtained for cooked prawns. The data sets cover freshly caught prawns, prawns after cooking, prawns from the optimal storage trial and retail cooked prawns. As for Figure 8, the horizontal dashed line represents an arbitrary estimate of counts of bacteria above which, product is deemed organoleptically spoiled (ie. > 10^6 bacteria per gram).

Under ideal conditions, cooking would be expected to reduce the counts for cooked prawns to almost zero. However, cooking did not result in a significant change in the SPC of prawns (P-value=0.121), which is graphically confirmed by the box-whisker plot. Reasons for this observation may either be temperature abuse of samples and/or poor post cooking control of cooling that allowed growth of bacterial populations contaminating cooked/cooled prawns. Data obtained for day 1 of the storage trial support the latter contention (Appendix 3e).

A significant reduction in SPC occurred as a result of freezing (P-value=0.046) and, as expected, SPC increased during controlled thawing and storage at 4°C. Notably the data obtained for retail prawns showed large spread and in some cases counts were above those indicative of incipient spoilage.

As is the case for green prawns, counts for cooked prawn samples was variable and did not correlate with storage temperature at point of sale. In general counts for retail frozen prawns were consistently higher than those obtained for freshly frozen product.

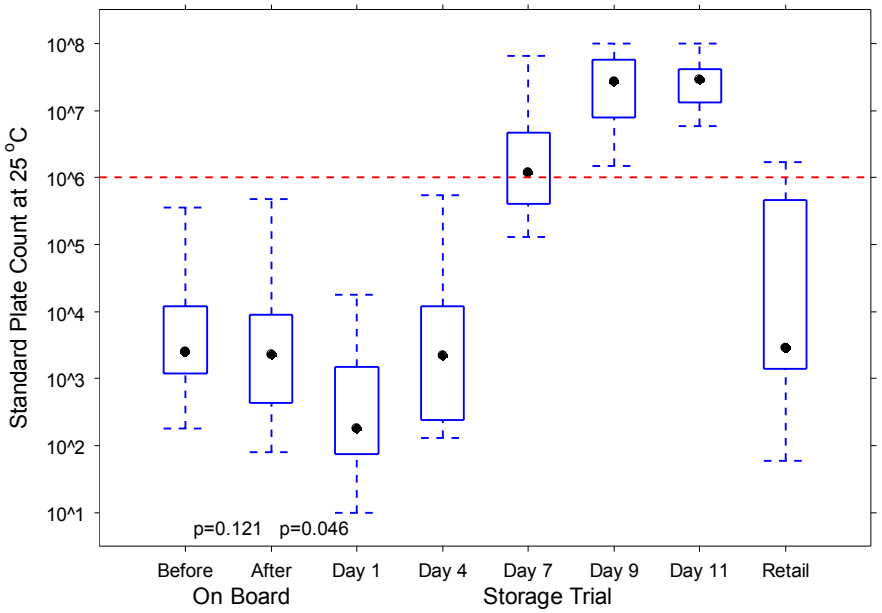


Figure 9: Boxplot of standard plate counts (25°) "through chain" for cooked prawns

Outcomes of the Through Chain Analysis

The through chain analysis identified several points of failure for processing and storage. Of particular concern was the observation that the cooking/cooling process failed to result in a significant reduction in the SPC of prawns (samples were taken just prior to freezing). As pointed out in the section devoted to On-Board Processing and Hygiene, this could have been a result of recontamination via cooling water, poor sampling practices or a cool chain failure following cooking. This point of failure has resulted in higher counts of bacteria, that in turn would be expected to have a negative impact on the shelf-life of this product.

Interestingly, the SPC of cooked prawns were similar to the SPC of metabisulphite dipped green prawns, yet the shelf-life of these two products were different. The shorter shelf-life of cooked prawns may be due to differences in the microbial flora of the two products. Recontamination of cooked prawns during cooling, for example, could introduce bacteria better suited to growth on prawns stored at refrigeration temperatures. Conversely, the impact of residual SO_2 on growth inhibition of spoilage bacteria that contaminate green prawns cannot be discounted as a significant factor contributing to the longer shelf-life of this product.

An additional point of failure is the observation that counts associated with retail prawns (green and cooked) are in the order of one log greater than those obtained for frozen fresh product. This observation is all the more interesting in view of the fact that most samples obtained from point of sale retail environments were in fact frozen. Assuming that prawns supplied to retailers were of similar quality to frozen fresh prawns sampled off boat, this paradoxical observation is an indication that retail prawns are probably subjected to temperature abuse arising from repeated freeze-thaw cycles.

The prawn industry will need to address the issue of failures related to process and temperature control if it is to ensure that product of a consistent high quality is to be presented to local, national and export markets. These could be addressed through education, attention to consistency of process application, inclusion of thawing and storage instructions on packaging, and monitoring of product temperature history. For example, one approach that would allow a detailed analysis of storage chain temperature histories, would be the inclusion of tamper-proof, retrievable temperature data loggers in cartons of frozen prawns, prior to wholesale distribution. A more detailed set of recommendations arising from this work is addressed in later sections of the report that deal with “Benefits and Outcomes” and “Further Development”.

Benefits and Adoption

1. Prawn Industry

The approach/framework developed to establish processing performance and product criteria provides a generic model for other prawn industry sectors to evaluate their industry performance and processes. It is noted that the framework and range of hazards should be reviewed prior to application to other sectors as the work was conducted in Mediterranean waters. Where estuary and warmer tropical waters are used a review of hazards and samples tested is advised.

While the number/frequency of testing was constrained by the available budget, sufficient data variability and non-compliance with existing standards was detected to indicate several processing steps require further attention. For example:

- decontamination agents – time of use, type of product, preparation of decontamination solution (Tables 3 and 4)
- sodium metabisulphite solution dipping of green prawns to control black spot
- processing, including cooling of cooked prawns
- use of untreated seawater for cleaning and cooling of cooked product
- review state/federal legislation to support implementation of QA programs to take up the R&D.

2. Other Crustacea Industries

While the framework may only provide a very general approach/framework that may be considered for evaluating other crustacea industries, some of the generic issues (hygiene and training) may be relevant to other industries. It is, therefore, recommended that the report be made available to those industries. It may be possible to develop training programs with common elements for all crustacea industries.

3. Food Safety Regulatory Agencies

While only preliminary data has been produced it provides initial baseline information on industry performance (and product safety). The systematic approach to sampling provides the necessary background information to enable confidence in inferences drawn from the results. The random and cross-sectional sampling of the industry underpins interpretation of the results.

The approach, together with the preliminary data, might be provided to SA public health regulators (Department of Human Services and PIRSA) to demonstrate:

- general product criteria compliance
- a commitment to improvement of processing, and
- underpinning of a risk-based food safety quality system that meets the requirements of the SA Food Act and pending legislation for the primary sector.

While product criteria data on retail product is limited, the absence of the expanded FSANZ (Appendices 1a and1b) pathogens (including marine vibrios, faecal coliforms and *L. monocytogenes*) along the supply continuum provides a preliminary indication of product safety. This is supported by data from the SA Human Health Surveillance Program that indicates an absence of food safety illness attributable to green or cooked prawns.

SSA/FRDC are encouraged to consider providing these data to FSANZ as part of the Primary Production and Processing Standards development process. The preliminary data indicate a high safety standard of the retail product and practical areas where this may be strengthened.

4. Spencer Gulf West Coast Prawn Fishermen's Association

For the SGWCPFA the results:

- confirmed the high food safety status of prawns, but show the shelf-life status of the product is variable
- indicated areas for improvement in Good Manufacturing Practice (GMP) to reduce product variability including:
    - crew hygiene
    - pre-processing sanitising
    - cooking
    - cooling post cooking
    - dipping procedures
- provided initial data on the effectiveness of current processes to meet food standards
- provided a basis for development of on-going monitoring as part of QA verification
- provided a basis to meet pending SA food safety regulations for the primary sector.

Based on the results of this study, the APIA should consider whether revisions to the APIA publication *Guidelines for Handling Prawns at Sea* are required. Furthermore, the SGWCPFA should consider a training program that targets areas identified for improvement (eg performance criteria of processing, boat and personal hygiene, sampling and sample handling etc).

The APIA may consider targeting the use of shelf-life and food safety data as a price-leveraging tool in marketing negotiations. Additional data acquired as part of on-going monitoring arrangements will improve opportunities in this area.

Further Development

1. Chemical Dips to Control Black Spot

Three issues arise from the project in relation to the processing of green prawns for the control of black spot.

1. The variability in product levels of sodium metabisulphite pre-freezing and at sale, including non-compliance levels, indicate a need for greater standardisation of the use of metabisulphite. This may include targeted training to increase the uptake of guidelines in the APIA code of practice (Carney, 2002). It may also include the use of dip-stick methods for in-line monitoring of the levels of metabisulphite within a quality system.
2. The associated occupational, health, safety and welfare issues associated with the use of sodium metabisulphite (*pers. comm*., SGWCPFA) are of sufficient concern to warrant investigation/evaluation of alternative products. Constraints to change should be identified, along with cost-savings that may be achieved by central (bulk) purchasing of decontaminants by the association. Use of any alternative needs to be accompanied by training and the use of systems to monitor processing water and product levels.
3. Although there are OH&S concerns related to use of sodium metabisulphite dips, dipping has several important and positive outcomes. Dipping results in reduction of the numbers of bacteria present on green prawns, and therefore has potential to increase shelf-life of thawed prawns held at refrigeration temperatures. However, it is not known whether alternative anti-black spot dipping agents (eg. Everfresh) will have the same antibacterial effect. Consequently, before alternative dipping agents are more widely adopted, a comparative investigation of the antibacterial efficacy of sodium metabisulphite and alternative anti-black-spot agents should be undertaken. The industry should ensure that dipping agents, where possible, should provide additional control of bacteria that impact on the public health status and shelf-life of green prawns. Given the potential impact of marine vibrios on the public health status of green prawns, any comparative study should also examine whether these chemical agents can eliminate marine vibrios from green prawns.

2. Use of Untreated Processing Water

The use of untreated sea water for wash down after cleaning and for cooling cooked (ready-to-eat) prawns may compromise both food safety (ie. presence of the marine pathogen *V. parahaemolyticus*) and shelf-life (poor reduction in bacterial counts of cooked/cooled prawns – Figure 3).

The SGWCPFA should investigate opportunities to use potable water for cooling cooked prawns and cleaning in terms of meeting GMP for ready-to-eat cooked prawns. The use of relatively cheap decontamination technologies such as in-line UV may provide a cost-effective option for supply of good quality water. Elimination of possible post process contamination is critical to optimisation of shelf-life of prawns at refrigeration temperatures.

3. Update: *Guide for Handling Prawns at Sea*

The APIA should consider updating the Level 1 and Advanced editions of the APIA guides. ocedures used in this project. For example, a new section dedicated to the recommended

on-going microbiological monitoring program should be considered. Any new section, should provide details on sampling and sample holding/management to ensure sample integrity. Information that might be used for this section is provided in Appendices 2c and 3c of this report.

As recommended elsewhere, it is also emphasised that renewed effort be placed on training in the:

- preparation and use of “anti-black spot” chemical dipping agents (Level 1, p 30; Advanced, p31)
- on-board processing (cooking/cooling and dipping) and personal hygiene (Level 1, p5-10)

4. Public Health Significance of *Vibrio parahaemolyticus*

Further work is underway at the University of Adelaide in a PhD project aimed at characterising *Vibrio* spp. isolated from marine crustacea (rock lobster, prawns and blue swimmer crab) (PhD Candidate: Damian May B.Sc. (Hons), Supervisor: Dr C J Thomas, School of Molecular and Biomedical Science). This study aims to use molecular methods to identify isolates to species level, determine whether isolates of the same species from different crustacea are phenotypically and genotypically distinct, as well as determine the public health status of these isolates. Of particular relevance to the current FRDC funded project, is the aim to assess the potential virulence of *V. para-haemolyticus* and *V. vulnificus* isolates recovered from green and cooked prawns and gulf waters used for cleaning and cooling product.

As of November 2003, an analysis of the results obtained from a raft of biochemical tests used for taxonomic purposes has shown isolates of the same species of *Vibrio* from rock lobsters are significantly different from those isolated from prawns. However, of the *V. parahaemolyticus* isolates tested, all possessed DNA encoding the *trh* locus. The *trh* locus (thermostable related haemolysin), is a genetic marker associated with virulence. Virulent strains of *V. parahaemolyticus* all encode *trh* or the related marker *tdh* (thermostable direct haemolysin). In addition, all *V. parahaemolyticus* isolates were shown to be cytolytic for human tissue culture cells. None of the *V. vulnificus* or other *Vibrio* spp. isolated possess these markers of pathogenicity.

These observations imply that *V. parahaemolyticus* isolates from crustacea are likely to be pathogenic and therefore represent a public health risk. The principal risk associated with these bacteria is that of food-borne infection as a result of cross contamination. Handling green prawns followed by preparation of other foods without adequate hygiene practices to eliminate cross contamination of foods could lead to growth of these bacteria to levels sufficient to cause food-borne disease. However, cooking or other heat treatments should eliminate the risk of food-borne infection associated with ingestion of prawns.

5. On-Going Monitoring by SGWCPFA

The SGWCPFA currently tests frozen, dipped prawns to meet their EU Accreditation with AQIS. It is suggested that the SGWCPFA consider targeted monitoring of cooked (immediately post-cooling/prefreeze) prawns. Such an approach would provide information about the efficacy of boat hygiene, cooking and post-cooking cooling processes as part of GMP and CCP verification in a QA program. It would also provide ongoing data on optimal product shelf-life.

The (recommended) program for cooked, chilled and frozen prawns includes the following microbiological tests:

- total plate counts (25°C)
- faecal coliforms
- *Listeria monocytogenes*

These tests should assist prawn fishers to monitor processing efficiency and compliance with targeted food safety hazards across the fleet.

Planned Outcomes

1. Framework/Principles/Guidelines for Other Crustacea Industries to Adapt/Adopt

The design phase of the project included consultation with FSANZ (Sally Hassell) and the SGWCPFA to ensure both regulatory "interests" (the inclusion of *L. monocytogenes*) and industry issues (eg effectiveness of existing processing procedures and potential shelf-life) were addressed. The industry was interested in the need for and type of on going monitoring appropriate to ensure compliance of industry members to food standards and verification of quality systems.

In relation to achieving these objectives the data variability and occasional non-compliance determined indicated the approach/framework provides a systematic approach to evaluating areas of regulatory and commercial interest.

As such, the approach/framework (evaluation of control and critical control points) provides principles and processes that might be adapted/adopted by other crustacea industries.

2. Implementation of Level 1 and Advanced Guides

The survey data on hygiene procedures (Tables 3 and 4; Appendix 3) indicates the need to increase the uptake of existing GMP included in these guides. In addition, further training programs should include sampling and sample handling as well as reinforcement of hygiene protocols and practices.

The use of simple documentation processes (i.e. check list) should be considered to record compliance with QA requirements (eg cleaning surfaces prior to processing).

These results and recommendations have been provided to the SGWCPFA Steering Committee for consideration.

3. Review of APIA Guidelines

As detailed in Benefits and Adoption and Further Development, the results support the updating of existing Guidelines. Criteria used to optimise sampling and sample handling in this project provide a basis for this revision (Appendix 2a and 2b).

4. Benchmarking the Shelf-life and Requisite Food Safety Parameters to Underpin and Develop Market Opportunities

The controlled shelf-life studies identified the potential shelf-life achievable under optimal freezing and thawing conditions. Studies on retail product also indicated potential temperature abuse of some product (thaw/re-freeze) during transport storage.

The SGWCPFA should consider how this preliminary data, with on-going monitoring data, may be used as a price-leveraging tool, or basis for promotion of premium (verifiable) product.

Further useful information may be obtained by a systematic evaluation of the cool chain (i.e. data loggers to retail).

5. Recommendations on Correct Storage and Handling of Prawns

Industry is urged to add storage and thawing instructions as part of labelling on retail-ready product to foster maintenance of product integrity.

6. Confidence in Food Safety of Boat Processed Prawns

While the microbiological analysis of ready-to-eat prawns indicated the product is safe, product shelf-life was shown to be highly variable. This report has identified processing procedures which require improvement if shelf-life is to be extended.

An on-going training and monitoring program that targets processing procedures that require improvement and verifies important processes should be implemented. Such a program should be seen by industry members as a means of tracking industry performance, particularly in relation to quantifying benefits derived from greater uptake of APIA Guidelines, QA enhancements and training identified by this project.

Conclusion

The proposed approach/framework consists of the following components

- Evaluation of the food safety and shelf-life of prawns across the supply chain continuum – “water–to-waiter”.
- Evaluation of the effectiveness of processing (control and critical control points), and benchmarking of processing criteria.
- Benchmarking of product criteria (ready-to-eat product)
- A random,cross-sectional sampling framework
- Scope
    - to meet FSANZ Food Safety Standards
    - to evaluate product performance in the marketplace (shelf-life, black spot etc)

Technical Conclusion

1. Prawns harvested from the Spencer Gulf region of South Australia meet FSANZ food safety standards. Bacteria indicative of faecal contamination were absent and *Listeria monocytogenes* was not isolated from any sample tested. Processing procedures did not contribute additional contamination by micro-organisms of public health significance.
2. No evidence was found that seawater or other water used for washing or cooling of prawns introduced microbial contaminants of public health significance.
3. The SPC of cooked prawns was found to be similar to or greater than that of green prawns. Cooking was expected to reduce the SPC of prawns significantly. These contrary results indicated the industry is performing below potential.
4. The shelf-life of green prawns (dipped for the control of black spot) optimally thawed and stored at 4ºC was greater than that of cooked prawns. There is opportunity to improve product shelf-life at the retail end through better process control. However, rapid development of black spot on stored prawns may have a greater effect on saleability in retail markets than microbiological status.
5. A survey of hygiene practices across boats in the fleet indicated there is little standardisation of the type and manner of use of sanitisers, hygiene and sanitation practices, and policies to deal with involvement of sick crew in processing of prawns.
6. The anti-black spot agent, sodium metabisulphite, when used at concentrations that produce high residual levels of SO_2 can significantly reduce the development of this product defect. An added advantage for the use of this chemical agent is the associated reduction in microbial load of green prawns.
7. The residual level of SO_2 on green prawns sampled from different boats is quite variable. This observation indicates that sodium metabisulphite treatment practices should be reviewed to ensure a consistent approach across all boats in the fleet.

Implications for Industry

In terms of meeting the objective of developing and pre-testing an approach/framework to evaluate the processing performance and resulting product criteria (food safety and shelf-life), the project met this by:

- Design of a through-chain approach that targeted processing performance (before and after levels) and food safety and shelf-life standards at retail and under controlled conditions.
- Construction of a sampling framework that provided a randomised, cross-sectional evaluation of these criteria across the fleet (for both green and cooked prawns).
- Identification of considerable variability (on limited sample numbers) in:
    1. adoption of industry guidelines for boat hygiene.
    2. achievement of appropriate levels of metabisulphite on processed prawns.
    3. the impact of cooking/cooling of prawns on total bacteria counts.
- Identification of the likely pathway of contamination of *V. parahaemolyticus* in ready-to-eat product (untreated sea water used for cooling cooked prawns).

While the number of samples across the supply-continuum was limited by budget constraints the project identified:

- Specific areas of the APIA *Guide to Handling Prawns at Sea* requiring revision eg: sampling and sample handling for monitoring purposes (Appendices 2a, 2b).
- The need for targeted training to improve the uptake of APIA guidelines and consideration of targeted documentation to use for auditing purposes.
- The need to investigate cost-effective measures (eg UV in-line) to reduce potential contamination of disinfected surfaces and processed product by uncontrolled (untreated) water. Consultation with SGWCPFA identified this as a high industry priority.
- A need to adopt methods for correct use of, and capacity to monitor concentrations of, sodium metabisulphite or its alternatives. The routine, on-board use of sodium metabisulphite has important *Occupational and Health and Safety* implications. This agent is an irritant that can result in significant discomfort to the eyes, skin and airways. In some individuals it can result in skin sensitisation and allergic reactions, especially in situations where there is repeated exposure over long periods. Standard procedures for safe handling of sodium metabisulphite solid and solutions need to established and documented in the APIA Guides to *Handling Prawns at Sea*. Appropriate training should be provided for all crew. The use of safer alternative agents shown to be effective in control of black spot (see Slattery *et al*, 1995) should be examined.
- The reduced numbers of bacteria associated with sodium metabisulphite treated green prawns compared with untreated green prawns indicates a need for a comparative investigation of the efficacy of sodium metabisulphite and alternative anti-black-spot agents, as antibacterial agents which could provide additional control of bacteria that impact on the public health status and shelf-life of green prawns.
- The need for an on-going microbiological testing program that monitors the impact of processing and hygiene procedures. It is recommended the monitoring program targets post-processed (dipped or cooked/cooled) prawns for total plate count (25°C), faecal coliforms and *L. monocytogenes*. Such testing would not provide rigorous information on a batch basis, but serve to remind industry of its responsibility to adhere to GMP as the basis of producing a consistently safe product with adequate shelf-life. Increased confidence in inferences regarding fleet performance would develop as consistent (good) results are achieved over time.

It is advised the approach can be used for other prawn sectors but it would require adaptation pending production conditions (eg aquaculture) and water temperature.

In terms of applying the approach to other crustacea it is advised that only the principles used in the epidemiological design and approaches to sampling and testing might be useful.

In terms of meeting the project outcomes, sufficient data was obtained to proceed as planned.

In relation to APIA Guides two activities are recommended.

1. A revision of the current guides in relation to sampling and sample handling as part of an on-going monitoring program.
2. Targeted training to increase the uptake of a standardised boat and personal hygiene and the preparation and use of “black-spot” dipping agents. In addition, training on sampling should be addressed.

While the limited sampling of retail ready (on boat processed) and prawns retail showed compliance with FSANZ food safety standards, non-compliance was detected at a low rate for metabisulphite and *V. parahaemolyticus*. Obtaining a better estimate of the extent of non-compliance may be considered for inclusion in the design of an on-going monitoring program, or further systematic surveillance work. In general, the food safety and shelf-life status of the product meets regulatory and market requirements. However, there is opportunity to improve the confidence of consistently meeting these standards.

In conclusion, the project provides a baseline of industry and product performance. For the SGWCPFA it establishes a set of strategies that may enhance product safety and shelf-life. For the crustacea industries the project provides principles and a framework to guide the evaluation of processes and product across the supply continuum.

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Appendix 1 Summary of testing methods and limits of detection.

Module	Sampling Area	Test	Method	Sample Size/Limit of Detection
A. Assessment of On-board Process Hygiene	Sanitation	Standard Plate Count	AS4709 – 2001	100cm^2
		Listeria spp	ELISA	100cm^2
	Water Quality	Faecal coliforms	AS4276.7 – 1995	100mL
		Faecal streptococci	AS4276.9 – 1995	100mL
		Vibrio spp	AS1766.1.5 – 1991	100mL
		Listeria spp	AS1766.1.5 – 1991	100mL
	Processing	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Faecal Coliforms	AS1766.2.3 – 1992	0.3 cfu/g
	Hygiene Practices	survey of hygiene and sanitation proctices.	Questionnaire	-
B. Assessment of product Shelf-life	Prawns**, pre-freezing	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Faecal Coliforms	AS1766.2.3 – 1992	0.3 cfu/g
	Prawns**, thawed commercial product	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Standard Plate Count - 4°C	AS1766.1.5 – 1991	100 cfu/g
		Listeria spp	ELISA	25g
		Listeria spp	AS1766.1.5 - 1991	100cfu/g
	Retail Prawns**	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Standard Plate Count - 4°C	AS1766.1.5 – 1991	100 cfu/g
C. Food Safety Study	Prawns**, pre-freezing	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Coagulase +ve staphylococci	AS1766.2.4 – 1994	100 cfu/g
		Faecal Coliforms	AS1766.2.3 – 1992	0.3 cfu/g
		Vibrio spp	AS1766.1.4 – 1991 AS1766.2.9 – 1991	100 cfu/g
		Listeria monocytogenes	ELISA*	5 x 25g
		Sodium metabisulphite	AOAC	10mg/Kg
	Retail prawns**	as for prawns pre-freezing		
D. Assessment of supply chain integrity	Prawns**	Standard Plate Count - 25°C	AS1766.1.5 – 1991	10 cfu/g
		Standard Plate Count - 4°C	AS1766.1.5 – 1991	100 cfu/g

*ELISA = Enzyme Linked Immunosorbent Assay
**Prawn samples prepared according to AS1776.3.5 – 1999

Appendix 2 Sampling Instructions

Appendix 2a Sampling Instructions for June '02 Survey – Green Prawns

SARDI Food Safety Group

Phone Geoff Holds – 0422 003 084

SAMPLING TO BE CARRIED OUT ON THE LAST NIGHT OF FISHING

All samples - except frozen product in cartons - to be stored refrigerated NOT FROZEN and the placed in the eskies with ice bricks on top of samples at the end of the trip.

Please mark the date and time of sampling on all samples.

1. Remove the ice bricks from the eskies and freeze ready for last night of fishing.
2. Unprocessed prawns: Take five samples of prawns (approx 200g) from the sorting table prior to dipping. Bags marked – 'green prawns pre process1,' 2,' 3,' 4', 5'. Record the date and time on the bag and refrigerate.
3. Processed prawns: Take one sample each (approx 200g) from six separate batches of dipped prawns. Bags marked – 'green prawns processed 1,' 2,' 3,' 4', 5' and sulphite'. Record the date and time on the bag and refrigerate.
4. Seawater – deck hose: Remove the submission form from the bottle. Remove the cap being careful not to touch the inside of either the cap or the bottle. From a deck hose that has been running for at least 1 minute and has not been lying on the deck, fill the water bottle leaving about a 2cm air space at the top. Replace the cap tightly and record the GPS reading, date, time and water temperature on the form. Place the form inside the adhesive pouch and attach the pouch to the bottle. Replace the bottle in the resealable bag and refrigerate.
5. Seawater – brine tank (if used): Remove the submission form from the bottle. Remove the cap being careful not to touch the inside of either the cap or the bottle. Using a fresh disposable glove fill the bottle by holding under the surface of the water until there is approximately 2cm air space at the top of the bottle. Replace the cap tightly and record the date and time information on the form. Place the form inside the adhesive pouch and attach the pouch to the bottle. Replace the bottle in the resealable bag and refrigerate.
6. Surface swabbing – PRIOR TO CLEANING – WHITE TUBES: Up to 4 sites that are in contact with the product after processing – (1) plastic crates, (2) inspection tables,

(3) weighing tubs, and (4) carton filling funnel will be sampled. The surfaces are to still be wet from handling the prawns.

7. Remove the blue plastic template from its plastic bag. Hold the template by the handle and place on the surface to be swabbed. Remove the swab stick from the tube and rub the fibre end across the space inside the template, rotating the stick as you move it. Then carry out the same action at right angles to the first strokes. Place the swab back in the tube being careful not to touch any other surface. Record the date and time on the label and place in the resealable bag and refrigerate.
8. Surface swabbing – PRIOR TO CLEANING – BLUE TUBES: Using the same template, on a similar area next to the one swabbed above carry out the same procedure using the BLUE “ENVIROSWABS”. Record the date and time on the label and place in the resealable bag and refrigerate.
9. Surface swabbing – AFTER CLEANING AND SANITIZING – WHITE TUBES: On the same areas as swabbed before cleaning, swab in the same manner. Record the date and time on the label and place in the resealable bag and refrigerate.
10. Frozen prawns in cartons: Select one carton of prawns from the last night of fishing and attach the adhesive label supplied. Record the date and time of packing. Freeze and store this carton as you would any other commercial product.

Appendix 2b Sampling Instructions for December '02 – Cooked Prawns

SARDI Food Safety Group

Phone Geoff Holds – 0422 003 084

SAMPLING TO BE CARRIED OUT ON THE LAST NIGHT OF FISHING

► All samples - except frozen product in cartons – are to be stored refrigerated NOT FROZEN and then at the end of the trip placed in eskies with ice bricks on top for transport to laboratory.

► Please mark the date and time of sampling on all samples.

1. Remove the ice bricks from the eskies and freeze ready for last night of fishing.
2. Uncooked prawns: Take one sample (approx 200g) from five separate batches prior to cooking. Place in bags marked – 'uncooked 1,' 2,' 3,' 4', 5'. Record the date and time on the bag and refrigerate.
3. Cooked prawns: Take one sample (approx 200g) from six separate batches of cooked and cooled prawns. Bags marked – 'cooked 1,' 2,' 3,' 4', 5' and sulphite'. Record the date and time on the bag and refrigerate.
4. Seawater – deck hose: Remove the submission form from the bottle. Remove the cap being careful not to touch the inside of either the cap or the bottle. From a deck hose that has been running for at least 1 minute and has not been lying on the deck, fill the water bottle leaving about a 2cm air space at the top. Replace the cap tightly and record the GPS reading, date, time and water temperature on the form. Place the form inside the adhesive pouch and attach the pouch to the bottle. Replace the bottle in the resealable bag and refrigerate.
5. Seawater – brine tank and cooling tank: Remove the submission form from the bottle. Remove the cap being careful not to touch the inside of either the cap or the bottle. Using a fresh disposable glove fill the bottle by holding under the surface of the water until there is approximately 2cm air space at the top of the bottle. Replace the cap tightly and record the date and time information on the form. Place the form inside the adhesive pouch and attach the pouch to the bottle. Replace the bottle in the resealable bag and refrigerate.
6. Surface swabbing – PRIOR TO CLEANING – WHITE TUBES: Up to 4 sites that are in contact with the product after processing will be sampled – (1) plastic crates, (2)

inspection tables, (3) weighing tubs, and (4) carton filling funnel. The surfaces are to still be wet from handling the prawns.

Remove the blue plastic template from its plastic bag. Hold the template by the handle and place on the surface to be swabbed. Remove the swab stick from the tube and rub the fibre end across the space inside the template, rotating the stick as you move it. Then carry out the same action at right angles to the first strokes. Place the swab back in the tube being careful not to touch any other surface. Record the date and time on the label and place in the resealable bag and refrigerate.

7. Surface swabbing – PRIOR TO CLEANING – BLUE TUBES: Using the same template, on a similar area next to the one swabbed above carry out the same procedure using the BLUE "ENVIROSWABS". Record the date and time on the label and place in the resealable bag and refrigerate.
8. Surface swabbing – AFTER CLEANING AND SANITIZING – WHITE TUBES: On the same areas as swabbed before cleaning, swab in the same manner. Record the date and time on the label and place in the resealable bag and refrigerate.
9. Frozen prawns in cartons: Select one carton of prawns from the last night of fishing and attach the adhesive label supplied. Record the date and time of packing. Freeze and store this carton as you would any other commercial product.
10. Questionnaire: Please fill out the questionnaire and return with the samples in the bag provided.

Appendix 3 Codex & FSANZ

Appendix 3a Codex Alimentarius Commission – Cooked Shrimp End product Specifications (Garret, 1997)

	n	**c**	**m**	**M**
Aerobic plate count	5	2	10^5	10^6
Staphylococcus aureus	5	2	500	5000
Salmonella spp	5	0	N/A	N/A

n, number of representative units.

c, maximum number of samples units that are allowed to exceed microbial level **m** but are less than or equal to **M** (for *Salmonella* spp., maximum allowable number of positive sample units).

m, microbiological numerical limit expected in products produced in plants operating under GMPs.

M, microbiological numerical limit that cannot be exceeded by any sample unit.

N/A, not applicable

Appendix 3b FSANZ Food Standards Code Part 1.6 – Microbiological and Processing Requirements

Standard 1.6.1 Microbiological Limits for Foods

Cooked crustacea

	n	**c**	**m**	**M**
Coagulase +ve staphylococci (per gram)	5	2	10^2	10^3
Listeria monocytogenes (in 25 grams)	5	2	0	0
Salmonella spp (in 25 grams)	5	0	0	0
Standard Plate Count (per gram)	5	2	10^5	10^6

n means the minimum number of sample units which must be examined from a lot of food.

c means the maximum allowable number of defective sample units**. A defective sample unit** means a sample unit in which a microorganism is detected in a sample unit of food at a level greater than **m.**

m means the acceptable microbiological level in a sample unit.

M means the level specified, when exceeded in one or more samples would cause the lot to be rejected.

Raw crustacea

	n	**c**	**m**	**M**
Coagulase +ve staphylococci (per gram)	5	2	10^2	10^3
Salmonella spp (in 25 grams)	5	0	0	0
Standard Plate Count (per gram)	5	2	5×10^5	5×10^6

n means the minimum number of sample units which must be examined from a lot of food.

c means the maximum allowable number of defective sample units**. A defective sample unit** means a sample unit in which a microorganism is detected in a sample unit of food at a level greater than **m.**

m means the acceptable microbiological level in a sample unit.

M means the level specified, when exceeded in one or more samples would cause the lot to be rejected.

Appendix 4 Hygiene Survey Questionnaire

Which water do you use to clean outside equipment? Sea ☐ Fresh ☐

Which water do you use to clean inside equipment? Sea ☐ Fresh ☐

Which sanitiser(s) do you use for cleaning the processing equipment?

_____

_____

How do you prepare your sanitiser solution?

_____

_____

_____

_____

Do you sanitise <u>before</u> product handling? Yes ☐ No ☐

Do you sanitise <u>only after</u> product handling? Yes ☐ No ☐

Do you handle/pack cooked and green prawns on the same night?

Yes ☐ No ☐

What is your order for handling/packing green and cooked prawns?

Cooked then green. ☐ No special order. ☐

Green then cooked. ☐

Do you sanitise between handling/packing green and cooked prawns?

Yes ☐ No ☐

Are gloves worn when handling the cooked prawns? Yes ☐ No ☐

Are the same gloves worn for handling the uncooked prawns? Yes ☐ No ☐

How do you know that the cooking water is hot enough?

Visual inspection ☐ Thermometer ☐

If by thermometer, at what temperature do you start cooking? ____°C

How do you know when the prawns are cooked?

Visual inspection ☐ Timer ☐

Other_____

Do you have a set procedure for dealing with crewmembers that may be suffering from a gastric illness that may involve vomiting and diarrhoea?

Yes ☐ No ☐

If yes, what procedure do you follow?

_____
_____
_____
_____

Outline any standard hygiene practices for crewmembers that are involved in processed prawn handling.

_____
_____
_____
_____
_____

Are there any other aspects of food handling hygiene you would like to let us know about or any questions you have?

_____
_____
_____
_____
_____

All information provided will be treated confidentially.

Appendix 5 Green Prawn Data

Appendix 5a – Green Prawn Data: On Board Hygiene Survey

Boat ID	Sampling			Date received at laboratory	Standard Plate Count per cm^2		Listeria per 100 cm^2 (before cleaning)
	Swab Site	Date	Time		Before Cleaning	After Cleaning	
G1	crate	11/05/02	5:40	13/05/02	130	0	nd
	table				18	0	nd
	tub				tntc	0	nd
	funnel				45	3	nd
G2	plastic basket	12/04/02	7:00	14/04/02	0	0	nd
	inspection table				0	0	nd
	weighing tub				0	0	nd
G3	crate	14/06/02	4:50	17/06/02	>250	0	nd
	table				60	0	nd
	weighing tub				>250	0	nd
	funnel				70	0	nd
G4	crate	14/06/02	1:15	17/06/02	120	0	nd
	table				0	0	nd
	weighing tub				0	0	nd
	funnel				0	0	nd
G5	crate	13/06/02	23:00	14/06/02	2	1	nd
	table				0	1	nd
	weighing tub				0	0	nd
	funnel				nr	nr	nd
	gloves				0	nr	nt
	under lip of table					tmtc	
G6	plastic crate	12/04/02	7:10	13/04/02	no result	no result	nd
	inspection table				1	0	nd
	weighing tub				no result	no result	nd
	funnel				2	0	nd
G7	crate	12/05/02	6:00	13/05/02	1	2	nd
	table	ns	ns			0	nd
	weighing tub	12/05/02	6:00		0	0	nd
G8	crate	17/05/02	2:30	22/05/02	0	0	nd
	table				0	0	nd
	tub				1	1	nd
	funnel				0	10	nd
G9	table	14/06/02	12:20	17/06/02	75	0	nd
	weighing tub				14	14	nd
	funnel				0	0	nd
G10	plastic basket	12/04/02	ns	13/04/02	0	0	nd
	inspection table				0	0	nd
G11	funnel	17/05/02	7:35	22/05/02	0	0	nd
G12	crate	14/06/02	6:00	17/06/02	0	0	nd
	table				0	0	nd
	weighing tub				0	1	nd
	funnel				0	0	nd

Appendix 5b – Green Prawn Data: Water Quality Survey

Boat ID	Sampling details					Tested	Faecal Coliforms	Faecal Streptococci	*E coli*	*Listeria* spp.	*Vibrio*.spp.
	Site	Date	Time	GPS	Temp.		per 100mL				
G1	hose	12/05/02	6:30	34°49.74S, 137°33.50E	19.5	13/05/02	<1	<1	<1	<1	<1
G2	hose	12/04/02	4:30	34°39.23S, 137°05.56E	19.8	13/04/02	<1	<1	<1	nd in 50mL	*V alginolyticus* = 68
G3	hose	14/06/02	4:35	33°44.68S, 137°33.08E	nr	17/06/02	<1	<1	<1	nd	<1
	br tank		4:30	na	nr		<1	<1	<1	nd	<1
G4	hose	14/06/02	1:00	34°46.42S, 137°34.97E	nr	17/06/02	<1	<1	<1	<1	<1
	br tank		1:00	na	nr		<1	<1	<1	<1	<1
G5	hose	13/06/02	20:30	33°48.05S, 137°32.90E	nr	14/06/02	<1	<1	<1	nd	<1
G6	hose	12/04/02	nr	34°56.60S, 136°15.80E	19.2	13/04/02	<1	<1	<1	nd in 50mL	*V vulnificus = 40*
	br tank		nr	na	nr		<1	<1	<1	nd in 50mL	<1
G7	hose	11/05/02	22:32	33°51.91S, 137°31.92E	19.4	13/05/02	<1	<1	<1	<1	*V alginolyticus = 33*
	fresh	12/05/02	7:30	na	nr		<1	<1	<1	<1/75mL	*<1*
G8	hose	17/05/02	20:00	33"39.00S,137"25.24E	18.8	22/05/02	<1	<1	<1	<1	<10 (100mL o/g)
	br tank		10:00	na	0		<1	<1	<1	<1	<1
G9	fresh	14/06/02	8:30	na	nr	17/06/02	<1	<1	<1	nd	<1
	hose	13/06/02	11:10	33°42.25S, 137°22.35E	nr		<1	<1	<1	nd	<1
G10	hose	12/04/02	5:00	34°45.04S, 137°04.53E	21.3	13/04/02	<1	<1	<1	nd in 50mL	*V alginolyticus* = 110
G11	hose	17/05/02	23:30	33"51.82S, 137"31.44E	18.5	22/05/02	<1	<1	<1	<1	*V species* = 20
G12	hose	14/06/02	6:30	33°40.58S, 134°24.72E	nr	17/06/02	<1	<1	<1	nd	<1

Appendix 5c – Green Prawn Data: Process Validation

Boat ID	Sampled	Tested	Faecal coliforms MPN per g	Standard Plate Count 25°C		Ave Standard Plate Count		Log reduction	SO_2 mg/kg	Comment
				pre-process	post process	pre-process	post process			
G1	12/05/02	13/05/02	<0.3	13,000	1,400	11,840	1,840	0.81	32.6	whole prawn tested
				16,000	490					
				14,000	1,200					
				12,000	410					
				4,200	3,900					
G2	12/04/02	13/04/02	no sample	no sample	1,220	no sample	1,220	no result	<10	tail only
G3	14/06/02	17/06/03	<0.3	24,000	no sample	21,200	1,300	1.22	no sample	
				9,000	no sample					
				16,000	1,900					
				39,000	700					
				18,000	1,300					
G4	14/06/02	17/06/03	<0.3	220,000	2,800	208,000	2,020	2.01	106.8	whole prawn tested
				100,000	2,500					
				390,000	2,000					
				130,000	1,600					
				200,000	1,200					
G5	13/06/02	14/06/03	<0.3	4,500	630	4,440	1,146	0.59	<10.0	whole prawn tested
				8,500	1,400					
				4,600	1,300					
				2,000	1,300					
				2,600	1,100					
G6	12/04/02	13/04/02	<0.3	230,000 (Only 1 sample)	17,000	230,000	70,800	0.51	<10	tail only
					79,000					
					130,000					
					67,000					
					61,000					

Appendix 5c Green Prawn Data: Process Validation cont.

Boat ID	Sampled	Tested	Faecal MPN per g	Standard Plate Count		Ave Standard Plate Count		Log reduction	SO2 mg/kg	Comment
				pre-process	post	pre-process	post			
G7	11/05/02	13/05/02	<0.3	5,400	5,100	4680	3,160	0.12	140	whole prawn tested
				3,300	1,700					
				600	2,100					
				13,000	2,800					
				1,100	4,100					
G8	17/02/02	22/05/02	<0.3	75,000	92,000	54,400	66,400	(+)0.12	40.3	whole prawn tested
				72,000	89,000					
				90,000	51,000					
				13,000	41,000					
				22,000	59,000					
G9	14/06/02	17/06/02	<0.3	12,000	no sample	21,340	2,400	0.95	10.1	whole prawn tested
				26,000	1,300					
				7,700	2,500					
				35,000	4,100					
				26,000	1,700					
G10	12/04/02	13/04/02	<0.3	850 (Only 1 sample)	2,700	850	3,158	(+)3.57	<10	tail only
					460					
					830					
					4,000					
					7,800					
G11	17/05/02	22/05/02	<0.3	42,000	3,400	22,800	1,634	1.15	no result	
				14,000	1,300					
				22,000	470					
				26,000	600					
				10,000	2,400					
G12	14/06/02	17/06/02	<0.3	34,000	5,000	35,600	2,960	1.08	<10	whole prawn tested
				25,000	1,900					
				30,000	2,700					
				47,000	1,900					
				42,000	3,300					

Appendix 5d – Green Prawn Data: Food Safety, On Board Sampling

Boat ID	Sampled	Tested	Standard Plate Count per gram				Coagulase +ve staph	Salmonellae	Vibrio spp	Faecal coliforms	SO2 mg/kg	Comments
			SPC 25°C		SPC 4°C							
			samples	ave	samples	ave						
G1	12/05/02	13/05/02	1,400	1,840	<100	<100	<100	nd	<100	<0.3	32.6	whole prawn
			490		<100		<100	nd	<100	<0.3		
			1,200		<100		<100	nd	<100	<0.3		
			410		<100		<100	nd	<100	<0.3		
			3,900		<100		<100	nd	<100	<0.3		
G2	12/04/02	13/04/02	710	1220	100	200	<100	nd	<100	nt	<10	tail only
			1100		300		<100	nd	<100	nt		
			490		100		<100	nd	<100	nt		
			2500		200		<100	nd	<100	nt		
			1300		300		<100	nd	<100	nt		
no sample	14/06/02	17/06/02	1,900	1,300	500	(567)	<100	nd	<100	<0.3	no sample	
			700		<100		<100	nd	<100	nt		
			1,300		1,100		<100	nd	<100	nt		
G4	14/06/02	17/06/02	2,800	2,020	1,200	(700)	<100	nd	<100	<0.3	106.8	whole prawns
			2,500		800		<100	nd	<100	nt		
			2,000		<100		<100	nd	<100	nt		high SO2
			1,600		<100		<100	nd	<100	nt		
			1,200		1,300		<100	nd	<100	nt		
G5	13/06/02	14/06/02	630	1,146	100	(100)	<100	nd	<100	<0.3	<10.0	whole prawns
			1,400		<100		<100	nd	<100	nt		
			1,300		100		<100	nd	<100	nt		Does not use sod met.
			1,300		100		<100	nd	<100	nt		
			1,100		<100		<100	nd	<100	nt		
G6	12/04/02	13/04/02	17,000	70,800	2,300	12,660	<100	nd	<100	nt	<10	tail only
			79,000		12,000		<100	nd	<100	nt		
			130,000		25,000		<100	nd	<100	nt		
			67,000		15,000		<100	nd	<100	nt		
			61,000		9,000		<100	nd	<100	nt		

Appendix 5d – Green Prawn Data: Food Safety, On Board Sampling cont.

G7	11/05/02	13/05/02	5,100	3,160	<100	<100	<100	nd	<100	<0.3	140	whole prawn
			1,700		100		<100	nd	<100	<0.3		
			2,100		<100		<100	nd	<100	<0.3		
			2,800		<100		<100	nd	<100	<0.3		
			4,100		<100		<100	nd	<100	<0.3		
G8	17/05/02	22/05/02	92,000	66,400	92,000	51,400	<100	nd	<100	<0.3	40.3	whole prawn
			89,000		82,000		<100	nd	<100	nt		
			51,000		45,000		<100	nd	<100	nt		
			41,000		22,000		<100	nd	<100	nt		
			59,000		16,000		<100	nd	<100	nt		
G9	14/06/02	17/06/02	1,300	2,400	200	1575	<100	nd	<100	<0.3	10.1	whole prawn
			2,500		1,800		<100	nd	<100	nt		
			4,100		3,300		<100	nd	<100	nt		
			1,700		1,000		<100	nd	<100	nt		
G10	12/04/02	13/04/02	2,700	3,158	700	(220)	<100	nd	<100	nt	<10	tail only
			460		<100		<100	nd	<100	nt		
			830		<100		<100	nd	<100	nt		
			4,000		<100		<100	nd	<100	nt		
			7,800		<100		<100	nd	<100	nt		
G11	17/05/02	22/05/02	3,400	1,634	1,900	(700)	<100	nd	<100	<0.3	no result	
			1,300		1,000		<100	nd	<100	nt		
			470		<100		<100	nd	<100	nt		
			600		400		<100	nd	<100	nt		
			2,400		<100		<100	nd	<100	nt		
G12	14/06/02	17/06/02	5,000	2,960	5,800	4,340	<100	nd	<100	<0.3	<10	whole prawns
			1,900		4,800		<100	nd	<100	nt		
			2,700		2,100		<100	nd	<100	nt		
			1,900		4,400		<100	nd	<100	nt		
			3,300		4,600		<100	nd	<100	nt		

Appendix 5e – Green Prawn Data: Shelf-life Study

Boat ID	Freezing date	Thawing date	Dav	Standard Plate Count		Listeria	Comment
				25°C	4°C		
G1	12/05/02	26/08/02	0	no test	no test	no test	
		27/08/02	1	580	<100	nd in 25g	
		30/08/02	4	300	300	no test	
		02/09/02	7	5,700	<100	<100	
		04/09/02	9	40,000	440,000	no test	
		06/09/02	11	10,000,000	>100,000,000	<100	
G2	12/04/02	29/07/02	0	no sample	no sample	no test	
G3	14/06/02	23/09/02	0	no test	no test	no test	SO2 = 30mg/Kg
		24/09/02	1	150	<100	nd in 25g	
		27/09/02	4	460	500	no test	
		30/09/02	7	53,000	350,000	<100	
		02/10/02	9	920,000	15,000,000	no test	
		04/10/02	11	5,500,000	96,000,000	<100	
G4	14/06/02	23/09/02	0	no test	no test	no test	SO2 = 40mg/Kg
		24/09/02	1	1,300	1,300	nd in 25g	
		27/09/02	4	9,400	8,600	no test	
		30/09/02	7	68,000	1,000,000	<100	
		02/10/02	9	14,000,000	13,000,000	no test	
		04/10/02	11	3,100,000	240,000,000	<100	
G5	13/06/02	23/09/02	0	no test	no test	no test	SO2 = <10mg/Kg
		24/09/02	1	380	<100	nd in 25g	
		27/09/02	4	800	300	no test	
		30/09/02	7	59,000	150,000	<100	
		02/10/02	9	2,000,000	5,400,000	no test	
		04/10/02	11	6,300,000	32,000,000	<100	
G6	12/04/02	29/07/02	0	no test	no test	no test	
		30/07/02	1	550	<100	nd in 25g	
		02/08/02	4	310	<100	no test	*Aerococcus and Moraxella spp.*
		05/08/02	7	11,000	1,100	<100	
		07/08/02	9	910,000	43,000	no test	
		09/08/02	11	9,500,000	1,900,000	<100g	
G7	11/05/02	26/08/02	0	no test	no test	no test	
		27/08/02	1	2,100	<100	nd in 25g	
		30/08/02	4	3,500	2,000	no test	
		02/09/02	7	21,000	2,400	<100	
		04/09/02	9	2,500,000	6,400,000	no test	
		06/09/02	11	4,800,000	app 46,000,000	<100	

Appendix 5e – Green Prawn Data: Shelf-life Study cont.

G8	17/05/02	26/08/02	0	no test	no test	no test	
		27/08/02	1	110	<100	nd in 25g	
		30/08/02	4	2,400	2,200	no test	
		02/09/02	7	78,000	8,400	<100	*Micrococcus* sp./ *Aeromonas* sp.
		04/09/02	9	1,100,000	11,000,000	no test	
		06/09/02	11	1,200,000	75,000,000	<100	
G9	14/06/02	23/09/02	0	no test	no test	no test	SO2 = <10mg/Kg
		24/09/02	1	600	<100	nd in 25g	
		27/09/02	4	2,600	1,200	no test	
		30/09/02	7	21,000	54,000	<100	
		02/10/02	9	400,000	1,900,000	no test	
		04/10/02	11	2,900,000	15,000,000	<100	
G10	12/04/02	29/07/02	0	no test	no test	no test	
		30/07/02	1	30	<100	nd in 25g	
		02/08/02	4	150	<100	no test	*Moraxella/Branhamella and Shewanella spp.*
		05/08/02	7	2,100	2,800	<100	
		07/08/02	9	410,000	150	no test	
		09/08/02	11	8,100,000	850,000	<100	*Micrococcus sp.*
G11	17/05/02	26/08/02	0	no test	no test	no test	
		27/08/02	1	1,300	<100	nd in 25g	
		30/08/02	4	2,400	1,900	no test	
		02/09/02	7	36,000	41,000	<100	*Micrococcus* sp./ *Aeromonas* sp.
		04/09/02	9	1,100,000	3,100,000	no test	
		06/09/02	11	2,800,000	est 20,000,000	<100	
G12	14/06/02	23/09/02	0	no test	no test	no test	SO2 = 40mg/Kg
		24/09/02	1	270	300	nd in 25g	
		27/09/02	4	1,000	<100	no test	
		30/09/02	7	100,000	330,000	<100	
		02/10/02	9	44,000	710,000	no test	
		04/10/02	11	2,000,000	19,000,000	<100	

Appendix 5f – Green Prawn Data: Retail Survey

ID	Sampling			Standard Plate Count per gram		Pathogens and Indicator Organisms				SO_2 mg/kg	Comment
	Date	Time	Temp	25°C	4°C	Coag +ve staph	Salmonellae	Vibrio spp	Faecal coliforms		
RG1	20/08/02	15:00	-22	26,000	6,600	<100	nd	<100	<0.3	<10	
RG2	20/08/02	15:15	1.0	1,000	100	<100	nd	<100	<0.3	<10	WA prawns sold as SA prawns
RG3	28/08/02	14:05	-23	1,700	<100	<100	nd 25g	<100	<0.3	33.4	Repacked in 1Kg bags
RG4	28/08/02	14:40	-7	720	300	<100	nd 25g	<100	<0.3	<10	display cabinet
RG5	02/09/02	14:30	16.0	23,000	50,000	<100	nd 25g	<100	<0.3	41.7	large pile on ice in display cabinet
RG6	14/10/02	15:30	-22	62,000	800	<100	nd 125g	<100	<0.3	<10	
RG7	23/10/02	13:15	-17	1,400	300	<100	nd 125g	<100	<0.3	148	violative SO_2
RG8	23/10/02	15:00	-13	52,000	14,000	<100	nd125g	<100	<0.3	<10	

Appendix 6 Cooked Prawn Data

Appendix 6a – Cooked Prawn Data: On Board Hygiene Survey

Boat	Sampling			Date	Standard Plate Count per		Listeria
ID	Swab Site	Date	Time	received at	Before	After	per 100
C1	plastic crate	11/11/02	10.35	13/11/02	4	0	nd
	inspection table		10.35		0	0	nd
	weighing tub		ns		0	0	nd
	carton filler		10.35		4	0	nd
C2	plastic crate	11/11/02	7:30	13/11/02	200	TNTC	nd
	inspection table	11/11/02	7:30		0	0	nd
	weighing tub	nr	nr		0	0	nd
	carton filler	nr	nr		0	0	nd
C3	plastic crate	nr	nr	13/12/02	190	2	nd
	inspection table				0	0	nd
	weighing tub	nr	nr	13/12/02	20	0	nd
	carton filler	nr	nr	13/12/02	3	0	nd
C4	plastic crate	nr	nr	13/12/02	0	0	nd
	inspection table	nr	nr		0	0	nd
	weighing tub	nr	nr		0	0	nd
	carton filler	nr	nr		0	0	nd
C5	plastic crate	11/12/02	nr	13/12/02	TNTC	0	nd
	inspection table		nr		160	0	nd
	weighing tub		nr		TNTC	0	nd
	carton filler		nr		30	0	nd
C6	plastic crate	nr	nr	13/11/02	600	110	nd
	inspection table	nr	nr	13/11/02	34	0	nd
	weighing tub	no sample					
	carton filler	no sample					
C7	plastic crate	nr	nr	11/11/02	TNTC	TNTC	nd
	inspection table	nr	nr		TNTC	64	nd
	weighing tub	nr	nr		TNTC	160	nd
	carton filler	nr	nr		37	60	nd
C8	plastic crate			13/12/02	50	0	nd
	inspection table	12/12/02	4:00		0	0	nd
	weighing tub				10	0	nd
	carton filler				10	0	nd
C9	plastic crate	02/12/02	nr	02/12/02	340	0	nd
	inspection table		nr		65	0	nd
	weighing tub		nr		>300	0	nd
	carton filler		nr		nr	10	nr
C10	plastic crate	02/12/02	nr	02/12/02	4	0	nd
	inspection table	02/12/02	nr	02/12/02	0	0	nd
	weighing tub	not done					
	carton filler	not done					
C11	plastic crate	11/11/02	3:20	13/11/02	0	450	nd
	inspection table	10/11/02	12:05		14	0	nd
	weighing tub	10/11/02	12:10		10	7	nd
	carton filler	10/11/02	1:30		26	34	nd
C12	plastic crate	nr	nr	13/11/02	90	nr	nd
	inspection table	nr	nr	13/11/02	18	0	nd
	weighing tub	nr	nr	nr	nr	nr	nr
	carton filler	nr	nr	nr	nr	nr	nr

Appendix 6b – Cooked Prawn Data: Process Water Survey

Boat ID	Sampling				Water Temp °C	Rec'd at lab	Faecal	*E coli*	Faecal strep	Listeria	Vibrio sp
	Site	Date	Tme	GPS			per 100mL				
C1	deck hose	10/11/02	9:00	33.47.916S	19.2	13/11/02	<1	<1	<1	<1	<1
				137.34.485E							
	brine tank	not sampled									
	cooling tank	10/11/02	21:00	33.48.285S	nr		<1	<1	<1	<1	<1
				137.34.132E							
C2	deck hose	11/11/02	1:30	33.33.14S	19.5	13/11/02	<1	<1	<1	<1	<1
				137.30.34							
	brine tank		2:00	nr	nr		12	12	30	<1	<1
	cooling tank		2:00	nr	nr		<1	<1	<1	<1	<1
C3	deck hose	11/12/02	21:55	33.53S	19	13/12/02	<1	<1	<1	<1	<1
				137.14E							
	brine tank		23:00	nr	2		<1	<1	<1	<1	<1
	cooling tank		23:05	nr	19.3		<1	<1	<1	<1	<1
C4	deck hose	11/12/02	20:45	33.54S	19.8	13/12/02	<1	<1	<1	<1	*V para* = 7
				137.02E							
	brine tank		21:00	nr	nr		<1	<1	<1	<1	<1
	cooling tank		21:00	nr	9		<1	<1	<1	<1	<1
C5	deck hose	11/12/02	9:00	33.52.2S	nr	13/12/02	<1	<1	<1	<1	<1
				137.06.09E							
	brine tank		9:00	nr	2		<10	<10	60	<1	no result
	cooling tank		11:00	nr	21		<2	<2	<1	<1	no result
C6	deck hose	11/11/02	22:30	33.46.67S	18.6	13/11/02	<1	<1	<1	<1	<1
				137.35.63E							
	brine tank	10/11/02	10:30	nr	nr		<1	<1	<1	<1	<1
	cooling tank	11/11/02	7:12	nr	nr		<1	<1	<1	<1	<1
C7	deck hose	11/11/02	7:10	33.44.87S	18.4	11/11/02	no result	no	<1	<1	V para = 3
				137.31.4E							
	brine tank	11/11/02	7:00	nr	nr		no result	no	<1	<1	V cincinatiensis = 4
	cooling tank	11/11/02	7:10	nr	nr		no result	no	<1	<1	<1

Appendix 6b – Cooked Prawn Data: Process Water Survey cont.

C8	deck hose	12/12/02	12:00	34.44.760S	18	13/12/02	<1	<1	<1	<1	<1
				137.04.160E							
	brine tank	not done									
	cooling tank	not done									
C9	deck hose	01/12/02	9:30	33.43.176S	21.1	02/12/02	<1	<1	<1	<1	V alginolyticus > 80
				137.33.604E							
	brine tank		9:30	nr	0		<1	<1	<1	<1	V alginolyticus > 80
	cooling tank		9:30	nr	21.1		<1	<1	<1	<1	V alginolyticus = 40
C10	deck hose	01/12/02	21:00	33.35S	20.6	02/12/02	<1	<1	<1	<1	<1
				137.30E							
	brine tank										
	cooling tank										
C11	deck hose	11/11/02	4:05	33.58S	18.5	13/11/02	<1	<1	<1	<1	<1
	brine tank		5:15	nr	nr		<1	<1	<1	<1	<1
	cooling tank		3:20	nr	nr		<1	<1	<1	<1	V sp = 12 V para <1
C12	deck hose	11/11/02	6:20	33.50.53S	18.8	13/11/02	<1	<1	<1	<1	<1
				137.35.64E							
	brine tank		6:30	nr	nr		<1	<1	<1	<1	<1
	cooling tank		6:30	nr	nr		<1	<1	<1	<1	<1

Appendix 6c – Cooked Prawn Data: Process Validation

Boat ID	Sampled	Tested	Faecal coliforms MPN per g	Standard Plate Count 25°C		Ave Standard Plate Count 25°C		Log reduction
				pre-process	post process	pre-process	post process	
C1	11/11/02	13/11/02	<0.3	1,800	440	5,560	4,188	0.12
				6,800	1,200			
				4,000	5,200			
				14,000	3,100			
				1,200	11,000			
C2	11/11/02	13/11/02	<0.3	5,300	1,800	7,400	9,140	-0.09
				9,700	13,0000			
				5,500	23,000			
				12,000	5,500			
				4,500	2,400			
C3	11/12/02	13/12/02	<0.3	1,000	130	1,380	684	0.30
	11/12/02			1,100	720			
	12/12/02			1,300	160			
	12/12/02			1,600	1,600			
	12/12/02			1,900	810			
C4	not recorded	13/12/02	<0.3	1,600	120,000	1066	61,340	-1.75
				380	98,000			
				810	7,700			
				2,000	15,000			
				540	66,000			
C5	11/12/02	13/12/02	<0.3	1,700	1,800	5,600	1,414	0.60
				16,000	1,800			
				4,600	420			
				2,800	550			
				no sample				
C6	10/11/02	13/11/02	<0.3	1,300	560	4,118	1,172	0.55
	10/11/02			1,700	90			
	11/11/02			390	100			
	11/11/02			3,200	110			
	11/11/02			14,000	5,000			

Boat ID	Sampled	Tested	Faecal coliforms MPN per g	Standard Plate Count 25°C		Ave Standard Plate Count 25°C		Log reduction
				pre-process	post process	pre-process	post process	
C7	11/11/02	11/11/02	<0.3	1,100	2,800	864	1,023	-0.35
				910	970			
				930	360			
				1,200	5,200			
				180	310			
C8	11/12/02	13/12/02	<0.3	2,600	230	4,018	152	1.42
	11/12/02			3,500	80			
	12/12/02			4,100	160			
	12/12/02			590	170			
	12/12/02			9,300	120			
C9	11/12/02	13/12/02	<0.3	1,400	3,900	1,800	13,460	-0.85
				1,200	2,800			
				2,400	39,000			
				2,200	20,000			
				2,300	1,700			

Appendix 6d – Cooked Prawn Data: Food Safety On Board Sampling

Boat ID	Sampling		Rec'd at lab	Standard Plate Count				*Salmonella*	Faecal Colif	Coag pos Staph	Vibrio sp.	Listeria	Sod met
				25°C		4°							
	Date	Time		25°C	Ave	4°	Ave	per 25g	MPN per g	per g	per g	per 25g	mg/Kg
C1	11/11/02	9:30	13/11/02	440	4,188	1,100	5700	nd	<0.3	<100	<100	nd	<10
		10:30		1,200		2,100		nd	nt	<100	<100	nd	
		11:45		5,200		6,000		nd	nt	<100	100	nd	
		12:40		3,100		6,300		nd	nt	<100	500	nd	
		13:45		11,000		13,000		nd	nt	<100	200	nd	
C2	11/11/02	5:30	13/11/02	1,800	9,140	2,100	4120	nd	<0.3	<100	<100	nd	<10
		4:15		13,000		3,500		nd	nt	<100	<100	nd	
		2:00		23,000		11,000		nd	nt	<100	100	nd	
		1:30		5,500		2,400		nd	nt	<100	100	nd	
		3:00		2,400		1,600		nd	nt	<100	<100	nd	
C3	12/12/02	3:15	13/12/02	130	684	100	(410)	nd	<0.3	<100	<100	nd	
				720		300		nd	nt	<100	<100	nd	
				160		<100		nd	nt	<100	<100	nd	
				1,600		<100		nd	nt	<100	<100	nd	
				810		<100		nd	nt	<100	<100	nd	
C4	not recorded		13/12/02	120,000	61,340	110,000	50400	nd	<0.3	<100	<100	nd	<10
				98,000		100,000		nd	nt	<100	<100	nd	
				7,700		10,000		nd	nt	<100	<100	nd	
				15,000		13,000		nd	nt	<100	<100	nd	
				66,000		19,000		nd	nt	<100	<100	nd	
C5	11/12/02	11:10	13/12/02	2,500	1,414	2,500	(1320)	nd	<0.3	<100	<100	nd	
				1,800		1,800		nd	nt	<100	<100	nd	
				1,800		1,800		nd	nt	<100	<100	nd	
				420		<100		nd	nt	<100	<100	nd	
				550		400		nd	nt	<100	<100	nd	

Appendix 6d – Cooked Prawn Data: Food Safety On Board Sampling cont.

C6	10/11/02	nr	13/11/02	560		700		nd	<0.3	<100	1,200	nd	<10
		2:00		90		<100		nd	nt	<100	100	nd	
		3:30		100	1,172	100	(1880)	nd	nt	<100	600	nd	
		5:30		110		<100		nd	nt	<100	<100	nd	
		6:45		5,000		8,400		nd	nt	<100	600	nd	
C7	11/11/02	7:30	11/11/02	2,800		<100		nd	<0.3	<100	<100	nd	no sample
		7:30		970		100		nd	nt	<100	<100	nd	
		7:30		360		<100		nd	nt	<100	<100	nd	
		7:30		5,200		300		nd	nt	<100	1,800	nd	
		7:30		310		<100		nd	nt	<100	<100	nd	
C8	11/12/02	23:00	13/12/02	230		<100		nd	<0.3	<100	<100	nd	
	11/12/02	0:00		80		100		nd	nt	<100	<100	nd	
	12/12/02	1:00		160		<100		nd	nt	<100	<100	nd	
	12/12/02	2:00		170		<100		nd	nt	<100	<100	nd	
	12/12/02	3:00		120		<100		nd	nt	<100	<100	nd	
C9	11/12/02	1:30	13/12/02	3,900		3,100		nd	<0.3	<100	<100	nd	<10
		1:30		2,800		3,100		nd	nt	<100	<100	nd	
		nr		39,000		47,000		nd	nt	<100	<100	nd	
		1:30		20,000		25,000		nd	nt	<100	<100	nd	
		1:30		1,700		7,400		nd	nt	<100	<100	nd	
C10	no samples												
C11	10/11/02	10:40	13/11/02	57,000		32,000		nd	<0.3	<100	<100	nd	<10
		11:50		470,000		340,000		nd	nt	<100	<100	nd	
		12:35		99,000		120,000		nd	nt	<100	<100	nd	
		2:15		140,000		80,000		nd	nt	<100	<100	nd	
		2:45		9,000		27,000		nd	nt	<100	<100	nd	
C12	11/11/02	nr	13/11/02	2,600		2,000		nd	<0.3	<100	<100	nd	<10
		nr		4,800		4,300		nd	nt	<100	<100	nd	
		nr		5,200		1,800		nd	nt	<100	<100	nd	
		nr		610		400		nd	nt	<100	<100	nd	
		nr		2,200		2,100		nd	nt	<100	<100	nd	

Appendix 6e – Cooked Prawn Data: Shelf-life Study

Boat	Date		Test Date	Day	Total Count 25°C	Total Count 4°C	Listeria
	Freeze	Thaw					
C1	11/11/02	24/02/03		0	no test	no test	no test
			25/02/03	1	50	<100	nd
			28/02/03	4	370	100	no test
			03/03/03	7	130,000	120,000	<100
			05/03/03	9	7,300,000	7,000,000	no test
			07/03/03	11	13,000,000	13,000,000	<100
C2	11/11/02	24/02/03		0	no test	no test	no test
			25/02/03	1	920	100	nd
			28/02/03	4	12,000	8,300	no test
			03/03/03	7	820,000	670,000	<100
			05/03/03	9	27,000,000	17,000,000	no test
			07/03/03	11	approx 29,000,000	19,000,000	<100
C3	11/11/02	24/02/03		0	no test	no test	no test
			25/02/03	1	10	<100	nd
			28/02/03	4	130	<100	no test
			03/03/03	7	1,200,000	940,000	<100
			05/03/03	9	8,400,000	5,100,000	no test
			07/03/03	11	5,900,000	7,300,000	<100
C4	11/11/02	24/02/03		0	no test	no test	no test
			25/02/03	1	18,000	5,400	nd
			28/02/03	4	550,000	340,000	no test
			03/03/03	7	65,000,000	36,000,000	<100
			05/03/03	9	>100,000,000	approx 290,000,000	no test
			07/03/03	11	>100,000,000	approx 720,000,000	<100
C5	11/11/02	24/02/03		0	no test	no test	no test
			25/02/03	1	2,400	200	nd
			28/02/03	4	12,000	9,300	no test
			03/03/03	7	8,000,000	3,800,000	<100
			05/03/03	9	43,000,000	44,000,000	no test
			07/03/03	11	41,000,000	31,000,000	<100
C6	11/12/02	24/03/03		0	no test	no test	no test
			25/03/03	1	110	<100	nd
			28/03/03	4	160	100	no test
			31/03/03	7	200,000	200,000	<100
			02/04/03	9	1,500,000	540,000	no test
			04/04/03	11	Testing halted after day 9		0.01g
C7	11/12/02	24/03/03		0	no test	no test	no test
			25/03/03	1	180	<100	nd
			28/03/03	4	2,200	1,300	no test
			31/03/03	7	2,700,000	2,300,000	<100
			02/04/03	9	77,000,000(est)	91,000,000	no test
			04/04/03	11	Testing halted after day 9		0.01g

Appendix 6f – Retail Survey – Cooked Prawn Section

Outlet ID	Sampling			Total Count 25°C		Coagulase +ve staphylococci	Salmonellae	*Vibrio* species	Faecal coliforms	*Listeria* species	SO_2 mg/kg	Comments
	Date	Time	Temp	25°C	4°C							
RC1	04/06/03	12:00	-2.0	8,700	2,600	<100	nd	<100	<0.3	nd	<10	Display cabinet
RC2	04/06/03	12:20	-21.0	60	<100	<100	nd	<100	<0.3	nd	<10	Pre-packed
RC3	11/06/03	13:50	-5.0	510,000	400,000	<100	nd	<100	<0.3	nd	nt	Packed with bare hands
RC4	11/06/03	13:15	+7.0	1,700,000	890,000	<100	nd	<100	<0.3 (colif = 2.3)	nd	nt	gloves worn
RC5	18/06/03	14:30	+8.0	1,600	1,300	<100	nd	<100	<0.3	nd	nt	display cabinet
RC6	18/06/03	15:05	-20.0	3,300	900	<100	nd	<100	<0.3	nd	nt	from freezer storage
RC7	18/06/03	16:00	+2.0	1,900	300	<100	nd	<100	<0.3	nd	nt	display cabinet
RC8	24/06/03	13:20	+4.0	1,600,000	1,500,000	<100	nd	<100	<0.3 (colif = 0.4)	nd	nt	packed with bare hands
RC9	24/06/03	14:05	+1.0	1,200	1,800	<100	nd	<100	<0.3 (colif = 0.4)	nd	nt	packed with tongs
RC10	24/06/03	14:55	+5.0	2,500	4,300	<100	nd	<100	<0.3	nd	nt	display cabinet
RC11	01/07/03	15:30	+5.0	200	190	<100	nd	<100	<0.3	nd	<10	gloves worn
RC12	01/07/03	16:00	-5.0	420,000	390,000	<100	nd	<100	<0.3	nd	<10	Packed with tongs