Prawn Market Access Defenders

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Contents

Non-Technical Summary

Prawn Market Access Defenders, Seafood CRC Project No. 2009/787

This project comprises three key components, which aim to provide:

a. A recognised capacity to assist with potential health and trade issues related to *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in Australian prawns.

b. A scientific risk assessment on cadmium in prawns to underpin future trade negotiations.

c. Assess and rank the risks (food safety and trade risk) to the prawn industry and prioritise key areas of concern to target risk reduction initiatives in the future.

Component 1: Vibrios

Vibrio are naturally occurring marine bacteria. Some species of this genus are capable of causing human illness and are responsible for food safety interventions that impact on seafood trading practices. The *Vibrio* species most commonly detected in trade border control measures and human health outbreaks are *V. cholerae, V. parahaemolyticus* and *V. vulnificus*. These species were responsible for 50% of border rejections for crustaceans into the European Union (EU) market between 2008 and 2013. Increased international attention on these species has led to the Codex Committee on Food Hygiene (CCFH) advancing international standards for *Vibrio* species in seafood. The importance of these pathogens for prawns in international trade is reflected by Australia setting import requirements for *V. cholerae*. Further, some major retailers are setting microbiological requirements for specific *Vibrio* species and these requirements are becoming more stringent.

The changing international and domestic dynamics may create business risks for the Australian prawn industry through increased testing costs to comply with regulations and the potential for positive testing results for *Vibrio* species. The latter is a significant concern as increased international attention is likely to mean heightened market testing requirements for vibrios. However, current commercially available methods in Australia are not capable of discerning pathogenicity (i.e. they determine the presence of vibrios at a species level and cannot discern between virulent and non-virulent strains). Considering the absence of vibrio-related illness outbreaks associated with consumption of Australian prawns, there is some potential that appropriate methodologies may help differentiate Australian prawns in the international market place as being of low risk for pathogenic *Vibrio* species. This work aimed to review all the available methods to identify the most appropriate for determining the presence of pathogenic *Vibrio* species in Australian prawns.

Based on a review of available methodologies, four methods for detecting these pathogens were chosen for further analysis. The main considerations when selecting a method is to assess if they are specific, robust, repeatable and rapid. These aspects were considered by establishing and undertaking a comparative assessment of four separate techniques. The methods trialed were:

- Most Probable Number (MPN) - Real-Time Polymerase Chain Reaction (PCR);
- MPN-conventional PCR;
- MPN-Loop-Mediated Isothermal Amplification (LAMP) DNA hybridisation;
- Colony picks from thiosulfate-citrate-bile salts-sucrose (TCBS) agar followed by DNA hybridisation.

Real-Time PCR methods fulfilled most of the key method performance criteria and were found to be fit for purpose, whereas the LAMP and DNA hybridization methods did not perform well. Conventional PCR fulfilled most of the performance criteria, however the Real-Time PCR method was more advantageous as it includes an internal amplification control (IAC). The IAC ensures there is no inhibition of amplification from the sample, which is a common problem with seafood samples. For these reasons, Real-Time PCR was selected as the most appropriate method for confirming the presence of *V. cholerae*, *V. parahaemolyticus* and *V.*

vulnificus in prawn samples, and also for confirming the presence of virulence-associated genotypes of these species. This method was used for the final stages of the project.

Following the initial comparative trials, studies to investigate the specificity, robustness, sensitivity and linearity of the Real-Time PCR methods were undertaken. In brief, to assess the specificity of the Real-Time PCR methods selected, a library of known *Vibrio* strains was developed. This included both Australian and international *Vibrio* strains from both target and non-target species of *Vibrio*. Each duplex Real-Time PCR method was assessed against DNA extracts from 96 representative bacterial strains, including both target and non-target species, selected from the library which is comprised of n = 197 Australian and international *Vibrio* strains. Each method was demonstrated to be 100% specific for the target species. This provides a high level of confidence for future assessment of prawn samples using these methods where a wide range of *Vibrio* species may be present.

Following the specificity assessment, the robustness of the methods was assessed. Firstly, the duplex methods targeting species-specific genes and the IAC were assessed against either mixed cultures (*V. parahaemolyticus* and *V. vulnificus*) or mixtures of extracted DNA from the mixed culture with pre-prepared target DNA (*V. cholerae*) to assess robustness. All methods correctly identified the target species. To demonstrate that the selected methods showed linearity with increasing concentrations of the target species when the MPN technique was employed, green prawn meat samples were contaminated in triplicate with known amounts of either *V. parahaemolyticus* and *V. vulnificus* (*V. cholerae* could not be assessed due to legal limitations on laboratory cultures in Australia). An MPN-Real-Time PCR method was also found to be able to reliably detect 0.3 organisms per gram of prawn tissue. In the case of *V. cholerae*, the same method can be used with a 25 g prawn enrichment in 225 mL of Alkaline Peptone Water (APW) to detect 1 organism in 25 g as per many national and international requirements. These results demonstrate that the methods identified and assessed as part of this work are specific, sensitive and robust and are appropriate for use in assessing the presence or absence of these species in Australian prawns.

A small survey of Australian prawns was undertaken. This survey evaluated 29 samples collected from around Australia for the presence of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae.* This work identified a very low prevalence of these *Vibrio* species in the prawns assessed. All samples were below the level of detection (0.3 MPN/g) except one sample that was positive for *V. parahaemolyticus* (present at the level of detection). No virulence genes were present in this sample indicating that the strain present was unlikely to cause human illness, particularly at this very low concentration.

The methods developed in this study are intended to support Australian prawn industry enterprises should standard methods currently used by commercial testing providers show positive results for *Vibrio* species. These new methods provide an opportunity to determine whether *Vibrio* species detected as part of end product testing requirements are of human health concern (e.g. pathogenic *Vibrio* spp). Results from this study, which show low prevalence of pathogenic *Vibrio* species in a small subset of Australian wild caught prawn samples, may also contribute to future risk assessments on which market entry requirements could be negotiated.

Component 2: Cadmium and selenium

Cadmium is a toxic metal that can contaminate marine waters and biota, including commercially important seafood products such as prawns. Cadmium in food items is regulated by food safety agencies around the world; some agencies – notably the European Commission – prescribe safe limits for cadmium in crustaceans. Some shipments of Australian export

prawns have in the past been subject to cadmium compliance failures detected by European food import analytical testing; such interventions cause economic disruption to the export prawn industry and have the potential to damage the reputation of Australian prawns as a safe, nutritious and quality product.

There have been previous suggestions that the limits for cadmium prescribed for prawns are 'over-precautionary' and this component of the work was undertaken to evaluate the risk of cadmium in Australian prawns and the efficacy of the regulatory limit prescribed by the European Commission.

An initial scan of the literature suggested that there is enhanced bioavailability of cadmium from foods in experimental animals that are deficient in Zn, Fe and Ca, and that there was potentially increased Cd absorption in humans with iron-deficiency status. This led to the initial proposal to undertake a risk-benefit assessment taking into consideration the presence and status of a range of beneficial and detrimental nutrients. However, detailed investigation revealed that the scientific basis for this was flawed and would not support a risk-benefit approach. Therefore, a traditional risk assessment has been undertaken. The rationale for this change in the project direction is discussed in more detail in Part 1: Introduction and Background – Component 2 – Cadmium.

To support a comprehensive risk assessment of cadmium in Australian wild-caught prawns it was necessary to undertake testing of a representative portion of prawns for cadmium. While a large amount of data for cadmium in prawns was sourced and collated from government and industry, this historical dataset (1991 – 2010) was not representative of production and was generated using a range of different (and unknown) analytical methods. Therefore, the historical dataset was not considered appropriate to support the risk assessment and a comprehensive cadmium survey was undertaken in addition to the risk assessment. The survey component of the risk assessment has provided useful insights into the key parameters that influence the cadmium level in prawns. Some of these provide options for industry consideration in terms of reducing the risk of non-compliant export products.

Selenium is a metalloid element that is an essential micronutrient; selenium-poor soils in some parts of the world result in selenium deficiency states in particular countries (although not in Australia). Seafood products are generally viewed as good sources of dietary selenium.

The survey was designed to sample and test prawns from Australia's principal wild-harvest prawn fisheries. Cadmium and selenium concentrations were measured in 140 prawn samples collected from the Northern Prawn Fishery, both within and beyond the Gulf of Carpentaria, the Queensland East Coast Prawn Fishery, Shark Bay and Exmouth Gulf in Western Australia, and the Spencer Gulf Western King Prawn Fishery in South Australia.

The majority of prawn samples (95.7%) were found to contain cadmium levels in prawn muscle tissue below the EC regulatory limit of 0.5 mg Cd per kg prawn muscle tissue. Six of the 140 prawn samples (4.3%) were found to have cadmium levels in prawn muscle tissue that exceeded the EC regulatory level. Two of these samples were *Metapenaeopsis crassissima*, the principal species marketed as coral prawns, sampled from Shark Bay. *M. crassissima* is known to be a high-risk species for bioaccumulation of excessive concentrations of cadmium. The other four samples came from within the Gulf of Carpentaria: three were endeavour prawns (*Metapenaeus endeavouri*) and one brown tiger prawn (*Penaeus esculentus*).

Statistical inference testing revealed that prawn species was the most important predictor of variability in cadmium concentration, with secondary-level influence of geographic location (i.e. fishery). *M. crassissima* had high cadmium levels, but were not included in statistical considerations because they were sampled in low numbers (2 of 2 samples with high cadmium). Endeavour prawns were found to accumulate more cadmium than other prawn species; western king prawns and banana prawns (*P. merguiensis*) had the lowest cadmium levels. Prawns from Spencer Gulf were particularly low in cadmium.

Selenium levels were less variable than cadmium concentrations in prawn muscle. Again, some species differences were revealed through statistical hypothesis testing. Endeavour prawns and western king prawns had the highest selenium levels; prawns from Spencer Gulf were particularly rich in selenium. Selenium concentrations in all prawns sampled in the survey were sufficiently high to meet the formal requirements of Australia's food standard on nutritional claims for Australian wild-caught prawns to be described and marketed as a "good source" of dietary selenium.

Prawn hepatopancreas tissues were found to have higher levels of cadmium than were seen in muscle tissues. This finding is in line with reports from elsewhere in the world.

Prawn species and, to a lesser extent, prawn fishery thus represent the most effective variables that can be targeted by industry-wide strategies to address market-access challenges raised by excess cadmium in export prawns. The differential distribution of cadmium in prawn tissues may also be a matter for consideration by industry-initiated laboratory testing of prawn product. We recommend that industry-commissioned testing of prawns for cadmium and other contaminants specify which tissues should be analysed, depending on the reasons for conducting the sampling and testing.

The scientific risk assessment demonstrated that the 0.5 mg/kg maximum level (ML) stipulated by the EC may be an ineffective risk minimisation strategy for prawns and could result in the inappropriate rejection of prawn consignments. For typical (median-level) consumers of prawns in Europe, cadmium intake from prawn muscle tissue is minor. Prawns may be a substantial source of cadmium intake for a small proportion of the population (1-2%) who are high prawn consumers, however the dietary consumption patterns in this group are not adequately characterised. The risk assessment provides robust scientific evidence that the application of the European regulatory limit does not reduce dietary intake of cadmium significantly, and could be used to support future negotiations on market access standards for cadmium.

Component 3: Risk Ranking

This component has been published as the report *Food safety risks associated with prawns consumed in Australia* by John Sumner, September 2011. A project steering group comprising industry, government food safety and researcher representatives oversaw the work at various stages of the process. The steering group and external peer reviewers are listed in Appendix 3.

The report scientifically evaluated the human health impact of chemical and microbial hazards associated with prawns. Risk ratings indicate a very low risk of human illness associated with the consumption of prawns produced domestically, imported prawns and exported prawns. This finding is consistent with the public health record which shows few reports of illness related to the consumption of prawns that have been handled appropriately. Work undertaken for component one (vibrios) and component two (cadmium) aims to 'ground truth' these findings. The scientific findings contained in the report may assist negotiations for improved trade access conditions into domestic and overseas markets, and risk-commensurate testing requirements for retail outlets. The report has been utilised by the industry to support safety declarations.

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PROJECT OBJECTIVES:

This project comprises three key components which aim to provide:

- Component 1 - A recognised capacity to assist with potential public health and trade issues related to *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in prawns.
- Component 2 - A scientific risk assessment on cadmium in prawns to underpin future trade negotiations and support the Codex position that a standard for cadmium in crustaceans is not required.
- Component 3 - Assess and rank the risks (food safety and trade risks) to the prawn industry and prioritise key areas of concern to target risk reduction initiatives in the future

OUTCOMES ACHIEVED

Component 1: *Vibrio* Method Development

1. Developed a library consisting of 197 Australian and international *Vibrio* strains to support future research initiatives and to assist other laboratories (including commercial) in Australia to validate testing methods.
2. Developed appropriate capability in three main areas including:
    - The development and validation of a methodology which distinguishes virulent and non-virulent strains of *V. parahaemolyticus* and *V. cholerae* in prawns
    - Increased Australian profile in the international network of *Vibrio* researchers.
    - Increased skills and knowledge of Australian *Vibrio* researchers.
3. Appropriate tools are now available to support trade access requirements for the Australian prawn industry. These tools can be accessed to confirm pathogenicity of any samples that are found to be positive through required testing regimes (such as that required for supermarkets).
4. The methods developed are critical for any future risk assessments on pathogenic *Vibrio* species in Australian prawns, which will support the industry by ensuring trade requirements for these species in prawns are appropriate in scale (risk-commensurate).

Component 2: Cadmium and selenium

1. A systematic review of the peer-reviewed literature on cadmium in edible prawns. This review comprises over 140 papers and reports, and has been summarised in tabular form (Appendix 1 within the *2012-13 survey of Australian wild-caught prawns for analysis of cadmium and selenium*). This is likely the largest and most comprehensive review of this topic conducted anywhere in the world to date.
2. Developed a random sampling program weighted for past fishery productivity. This tool addresses the potential for bias inherent in sampling seafood for analysis

of nutritional and/or harmful trace elements when convenience sampling or non-random sampling methods are adopted.

3. An up-to-date understanding of cadmium and selenium levels in Australian prawns through the results of a large survey comprising 140 prawn samples, with analyses conducted by a single laboratory using defined methods and reporting of quality assurance results.
4. An understanding of the distribution of cadmium in prawn alimentary tracts compared to muscle cadmium levels.
5. An assessment of the food safety risks for cadmium in Australian prawns, including assessment of the impact of the EC regulatory limit, and identification of information gaps.
6. Potential for improved standing of Australian toxicology and risk assessment capabilities. This should result in enhanced seafood industry support for research investigating food safety-based market access challenges (contingent on release of data for peer-reviewed publication).

Component 3: Risk Ranking

Determination of risk ratings indicates a very low risk of human illness associated with the consumption of prawns produced domestically, imported prawns and exported prawns. This outcome is consistent with the public health record which shows few reports of illness related to the consumption of prawns that have been handled appropriately. This report has been utilised by the wild caught prawn sector to underpin trade and market access needs and support marketing initiatives.

LIST OF OUTPUTS PRODUCED

Component 1: Vibrios

1. A written review of the literature focused on methods of detection and quantification of pathogenic *Vibrio* species in seafood, including comment on international standards and guidelines for method validation (see report *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*)
2. A *Vibrio* strain library has been established (currently containing 197 strains from Australia, Japan, Germany, USA and the UK). These strains are systematically maintained at SARDI and are available to other Australian researchers on request.
3. Laboratory bench protocols for the detection of pathogenic *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (see attached report *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*)
4. A report detailing method comparisons and validation of Real-Time PCR methods for pathogenic *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (see attached report *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*).

Component 2: Cadmium and Selenium

1. Major report on a survey of cadmium and selenium in Australian wild-caught prawns, incorporating a systematic review of cadmium in edible prawns (attached

as report *2012-13 survey of Australian wild-caught prawns for analysis of cadmium and selenium*).

2. Risk assessment of cadmium in Australian wild-caught prawns (attached as report *Risk assessment of cadmium in Australian wild-caught prawn muscle tissue*). Stand-alone document that can be utilised to support trade standard negotiations for cadmium.

Component 3: Risk Ranking

1. A comprehensive review of hazards in Australian prawns (published as ASCRC report *Food safety risks associated with prawns consumed in Australia*). The report has been used by the prawn sector in discussions with trade negotiators, food safety regulators and to support marketing initiatives.

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1. Introduction and Background

Background
There have been several recorded instances of food-borne illness associated with the consumption of prawns in Australia, including two separate outbreaks of *Vibrio parahaemolyticus* involving >50 cases associated with the consumption of imported prawns. The development of the global food trade has resulted in heightened food safety and import requirements for prawns in many countries throughout the world. Because of these heightened requirements there have been trade failures related to the detection of potential hazards in Australian prawns during 'port of entry' testing. These trade failures have occurred in several different countries including: China, Japan, the European Union and New Zealand. The majority of the trade failures were related to the presence of *Vibrios* and cadmium.

This project has three components which aim to:
1. Establish a recognised capacity to assist industry with domestic and international trade issues/failures related to pathogenic *Vibrio* species in prawns.
2. Provide a scientific risk assessment on cadmium in prawns to support the existing Codex position on cadmium in prawns and future multilateral trade negotiations.
3. Assess and rank the risks (food safety and trade risk) to the prawn industry and prioritise key areas of concern to target risk reduction initiatives in the future.

Component 1 - **Vibrios**:
The Codex Committee on Food Hygiene have finalised an international standard for *Vibrio* species in seafood, and retail requirements for pathogenic *Vibrio* species are becoming more stringent (e.g. the Woolworths supermarket chain currently require *V. cholerae* testing of prawns by suppliers). The species most commonly detected in trade border control measures and human health outbreaks are *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*, and international efforts to introduce *Vibrio* standards through Codex focus on these three species. The impost of these international and domestic standards is a significant risk for the Australian prawn industry as it may result in a larger proportion of product detentions and rejections for *Vibrio* species in prawns, and increased costs to industry in compliance testing.

Currently accredited methods for pathogenic *Vibrio* species in Australia are based on standard microbiological techniques. These methods are time consuming and cannot differentiate between disease-causing and non-disease-causing strains, so they over-score (false positives). The lack of a rapid capability that distinguishes pathogenic and non-pathogenic strains means that Australia is not able to effectively or efficiently dispute *Vibrio*- related prawn trade issues. The first component of this project involved the development and validation of methods to confirm the presence and pathogenicity of *V. cholerae, V. parahaemolyticus* and *V. vulnificus* in prawns. We envisage that these methods will be utilised by industry to confirm positive samples (in routine regulatory testing) as virulent or non-virulent and to support future risk assessment and adoption of policies that are commensurate with risk.

Component 2 - **Cadmium**:
There have been trade failures in China and the EU related to the detection of cadmium in Australian prawns through port of entry testing. This relates to the implementation of a 0.5 mg/kg cadmium Maximum Level (ML) in prawns. In 2007 the Australian Government made a submission to the European Commission (EC) to review the limit for cadmium in crustaceans. The submission included a large data set on levels of cadmium in key commercial prawn species from a range of Australian fisheries. The submission was unsuccessful in persuading the EC to review their cadmium level for crustaceans, and several jurisdictions in China have now adopted the EC level. Given the foregoing, a systematic survey of Australian wild caught prawns followed by a risk assessment constructed in line with current Food and Agriculture

Organization / World Health Organization (FAO/WHO) guidelines was undertaken to inform future risk management measures in Australia and overseas.

The survey is based on a systematic sample of Australian wild-caught prawns, with a sampling strategy directed by sample size estimates derived from the historical database of cadmium analysis in Australian prawns, and a sampling frame constructed from intra-fishery spatially-defined production data. Chemical analyses were conducted on de-veined prawn muscle tissue, according to standards published by European reference laboratories. This latter feature addresses a source of variability introduced into previous analyses of cadmium in Australian prawns, insofar as measurement of cadmium in whole prawns and measurement in de-veined muscle can be expected to vary because of heterogeneous distribution of cadmium in various prawn tissues. Statistical analysis of survey results investigated the proportion of variability in prawn muscle cadmium attributable to prawn species, fishery location, season and average prawn size (weight).

Analysis of selenium concentrations in prawn tissues complements the survey. Selenium is an essential trace element, with important antioxidant functions. While selenium supplementation is somewhat controversial because of a narrow window between sufficiency and toxicity, selenium-rich foods are also beneficial in the context of a comprehensive dietary intake of trace minerals and vitamins. Seafood is generally presented as a good source of dietary selenium.

A risk assessment for cadmium exposure through prawn consumption was informed by the results of the survey, and considerations from published dietary surveys on consumption patterns for crustaceans.

The original project brief proposed a risk-benefit assessment incorporating cadmium (Cd), iron (Fe), zinc (Zn), selenium (Se) and calcium (Ca) concentrations in prawns. Consideration of Zn, Fe and Ca was made on the basis of discussion in the scientific literature regarding enhanced bioavailability of cadmium from foods in the context of marginal deficiency states for Zn, Fe and Ca in experimental animals, and of increased cadmium absorption in humans with iron-deficiency status. An expert opinion report by a European panel noted that:

> "In general, cadmium absorption from food…and its bioavailability varies according to a number of factors [including] the presence in food of divalent or trivalent cations such as zinc, iron and calcium that compete with cadmium for absorption (Reeves and Chaney, 2008)."
>
> *Alexander J et al (2009) Scientific opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food EFSA J 980; 1-139.*

However, on further investigation the statement that the presence in food of "zinc, iron and calcium that compete with cadmium for absorption" is arguably not supported by the review of Reeves and Chaney (2008). More precisely, any possible interpretation of the above statement to the effect that an individual food item containing beneficial levels of zinc, iron and calcium might result in decreased bioavailability of the cadmium *also contained in the same food item* would undoubtedly be incorrect. The published literature refers to cadmium bioavailability in marginal Zn, Ca and Fe deficiency status *in vivo*. Extrapolation of those findings to the broader population in developed countries, where such dietary deficiency states can be expected to be uncommon, would be inappropriate. Of the three elements discussed here in terms of marginal deficiency status (Zn, Fe, Ca), a relative deficiency in iron may be expected to be seen most often in developed countries. But prawns contain only moderate levels of Fe: 16 ppm in one paper, about the same as in white rice, and much less than in red meat, chickpeas or peanuts, (Hercberg, S., *Iron deficiency*, in *Medical practice of preventive*

nutrition, M.L. Wahlqvist and J.S. Vobecky, Editors. 1993, Smith-Gordon: London). Therefore prawns are unlikely to be able to be marketed as a good source of dietary iron for groups such as premenopausal women that may be at higher risk of having lower body iron stores. Likewise prawns are not particularly rich sources of either calcium or zinc, therefore could not be "sold" as a priority source of nutrition to that small proportion of the population in Western countries that might suffer relative or marginal deficiency status.

This component of the project aims to provide a risk assessment of cadmium in Australian wild caught prawns. Selenium was considered by the Australian Council of Prawn Fisheries (ACPF) to be a desirable micronutrient to include in the survey for potential future marketing benefits.

Component 3 – Risk Ranking

The key food safety risks for prawns include both microbiological and chemical hazards. Of concern, a recent survey undertaken on the retail quality of Australian prawns in the Australian Capital Territory revealed that 43% of the samples taken exceeded the maximum allowable limits for standard plate counts (SPC). The retailers were investigated and found to be compliant with sanitary requirements, indicating that the prawns may have already had unacceptable levels prior to receipt by the retailers. A supply chain study undertaken previously by SARDI (FRDC Project 2002/425) also indicated elevated SPC counts. SPC are a general indicator of hygienic quality of foods and these results indicate the potential for food safety issues.

There was a need to identify, assess and compare the risks posed by various contaminants in prawns (food safety and trade risk) and prioritise opportunities for reducing these risks through targeted initiatives. The process of comparing risks and ordering them in some manner is known as Risk Ranking. The Risk Ranking will assist industry to direct scarce resources into high priority areas to support sustainable business development.

1.1 Need

There have been numerous trade failures regarding exportation of Australian prawns into Asia and Europe (e.g. see http://ec.europa.eu/food/food/rapidalert/rasff_portal_database_en.htm). These mainly relate to cadmium and pathogenic *Vibrio* species. Approximately 4800 tonnes of prawns were exported in 2008/2009 compared to the wild-capture total of 20,000 tonnes.

- Due to ongoing trade issues ACPF have ranked pathogenic *Vibrio* species and cadmium as 'high priorities'.
- Pathogenic *Vibrio* species have been responsible for illness outbreaks in Australia. Due to the role of vibrios in illness outbreaks, the Codex Alimentarius Commission is progressing standards on the control of these pathogens, and domestic requirements for testing are increasing (e.g. Woolworths requires testing for *V. cholerae*).
- Standard laboratory methods are time-consuming and do not differentiate disease-causing and non-disease-causing strains, and therefore can return a high rate of false positive results. The lack of capability in this field hampers efforts to challenge trade detentions related to the presence of *Vibrio* species.
- This project aims to address this need by developing methods that distinguish pathogenic and non pathogenic *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* to underpin domestic and international trade requirements.
- Cadmium trade failures are related to the low maximum level (0.5 mg/kg) set by the EC and some Chinese jurisdictions. By contrast, Australian and Japanese food safety regulators do not consider the cadmium content of prawns to be of sufficient concern

to warrant the establishment of specific maximum levels. Attempts by the Australian Government have been unsuccessful in negotiating a higher limit.

- This project aims to provide a risk assessment of cadmium in prawns to underpin further multi-lateral trade and Codex negotiations. To support the assessment a systematic survey incorporating an unbiased sampling program was undertaken to gather data on cadmium levels in Australian wild-caught prawns.
- Other potential food safety risks to the prawn industry were assessed and key areas of concern prioritised to assist in directing resources to issues of high business risk.

1.2 Objectives

Component 1: Establish a recognised capacity to assist with potential public health and trade issues related to *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in prawns.

Component 2: Conduct a representative survey of Australian wild-caught prawns to measure cadmium and selenium concentrations, and develop a scientific risk assessment on cadmium in prawns to underpin future trade negotiations.

Component 3: Assess and rank the risks (food safety and trade risks) to the prawn industry and prioritise key areas of concern to target risk reduction initiatives in the future.

2. Methods

Component 1 (Pathogenic *Vibrio* species):

Refer to attached report *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns* for full methodological approach used.

Component 2 (Cadmium):

See attached reports *2102-13 survey of Australian wild-caught prawns for analysis of cadmium and selenium*, and *Risk assessment of cadmium in Australian wild-caught prawn muscle tissue.*

Component 3 (Risk Ranking):

Refer to published ASCRC report *Food safety risks associated with prawns consumed in Australia* by John Sumner, September 2011.

3. Results

Component 1: ***Vibrios***

Refer to attached report: "*Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*"

A literature review identified four methods as potentially useful for the detection and enumeration of pathogenic *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in prawns. These methods were: (i) colony picks from TCBS agar followed by DNA hybridisation, (ii) MPN-LAMP, (iii) MPN-conventional PCR and (iv) MPN-Real-Time PCR. Initial screening to assess the robustness and accuracy of each method, as well as considerations on time, safety and appropriate positive and negative controls, determined that MPN-Real-Time PCR was the most effective method.

The MPN-Real-Time PCR method was subsequently found to be highly accurate using a *Vibrio* strain library containing both target and non-target *Vibrio* species, developed as part of the project. Each gene target was detected from overnight cultures containing each of the target organisms along with six other non-target *Vibrio* species (*V. cholerae* was detected by mixing previously prepared DNA lysates with DNA lysates from overnight suspensions due to restrictions on the propagation of *V. cholerae*). Highly linear results were also obtained from a prawn inoculation trial using the target species (except *V. cholerae* due to restrictions on its propagation).

Lastly, a small survey of raw prawns was undertaken. A total of 29 samples collected from around Australia were assessed for the presence of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae.* This work identified a very low prevalence of these pathogens in the prawns assessed. All samples were below the level of detection (0.3 MPN/g) except one sample that was positive for *V. parahaemolyticus* (present at the detection limit). No virulence genes were present in this sample indicating that the strain present was unlikely to cause human illness, particularly at this very low concentration.

Component 2: **Cadmium and Selenium**

Refer to attached documents: Appendix 4: "Survey of Australian wild-caught prawns for analysis of cadmium and selenium" and Appendix 5: "Risk assessment of cadmium in Australian wild-caught prawn muscle tissue."

This component of the project involved undertaking a survey of cadmium and selenium levels in prawns across the principal wild-capture fisheries. The data generated from the survey were used to underpin a quantitative risk assessment of cadmium in wild-caught Australian prawns.

The survey analysed 140 prawn samples collected from the Commonwealth-managed Northern Prawn Fishery, the Queensland east coast prawn fishery, Spencer Gulf in South Australia, and Shark Bay and Exmouth Gulf in Western Australia. While the majority (95.7%) of prawns surveyed showed low cadmium levels, six of the 140 samples (i.e. 4.3%) were found to have cadmium levels in de-veined muscle tissue that exceeded the EC regulatory limit of 0.5 mg/kg. Two of these were found in the two samples of *Metapenaeopsis crassissima* collected from Western Australia; *M. crassissima* is the main component species marketed as coral prawns, which is a small by-catch fishery. Some of this product is exported to countries in Asia. *M. crassissima* from WA has been known since the 1990s as having an unusual capacity to bioaccumulate high cadmium levels. The other four samples were collected from waters in the Gulf of Carpentaria; three were endeavour prawns (*Metapenaeus endeavouri*) and one brown tiger prawns (*Penaeus esculentus*).

Statistical evaluation of cadmium in prawn muscle has revealed some important species-related variability in cadmium levels. Aside from the high cadmium concentrations found in *M. crassissima*, endeavour prawns were found to have higher levels than any other species. Banana prawns (*P. merguiensis*) and western king prawns (*P. latisulcatus*) had the lowest cadmium levels.

There were also significant differences found in the cadmium levels according to fishery location. Western king prawns from Spencer Gulf in South Australia had lower cadmium concentrations than those from Shark Bay and Exmouth in Western Australia; and brown tiger prawns (*P. esculentus*) from the Northern Prawn Fishery had higher cadmium concentrations than those from the Queensland East Coast Prawn Fishery.

Selenium levels in prawns were also found to be related to species and fishery, with some evidence of seasonal variability.

Cadmium levels in prawn hepatopancreas were seen to be higher than in muscle tissues. This finding supports the results of observational and experimental studies reported from elsewhere in the world; the decapod hepatopancreas is the organ that concentrates cadmium, other toxic metals and other natural and anthropogenic contaminants.

Experiments to test the contribution of cadmium in prawn alimentary tracts to prawn muscle cadmium showed that such contribution is insignificant, presumably due to a dilution effect.

Quantitative risk assessment modelling of Australian wild-caught prawns indicated that removing product with cadmium levels above 0.5 ppm from prawns exported to Europe may have a negligible impact on reducing exposure to cadmium. This implies that the current regulatory limit imposed by the European Commission is an ineffective risk management strategy.

There were two species found to have relatively high cadmium levels among Australian wild-caught prawn species, the coral prawn *Metapenaeopsis crassissima* and the blue endeavour prawn *Metapenaeus endeavouri*. Removal of these species from the risk assessment model had the same impact on reducing cadmium exposure from prawns as the removal of samples >0.5 mg/kg. Removal of these species would be a more reliable strategy for lowering cadmium exposure from prawns than application of a 0.5 mg/kg ML. The degree of benefit realised by a strategy of high-cadmium species removal has not been determined with sufficient certainty by this assessment, and would need to be considered in the context of costs to the prawn industry.

Component 3: **Risk Ranking**

The report scientifically evaluated the human health impact of chemical and microbial hazards associated with prawns. Risk ratings indicated a very low risk of human illness associated with the consumption of prawns produced domestically, imported prawns and exported prawns. The completed report has been published as: *Food safety risks associated with prawns consumed in Australia – Seafood CRC project 2009/787: Prawn market Access Defenders* by John Sumner, September 2011.

4. Discussion

Component 1: **Vibrios**

Refer to attached document: *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*.

The MPN-Real-Time PCR method was found to be effective at detecting and enumerating pathogenic *Vibrio* species in prawns. This method was developed for use by the US Food and Drug Administration (FDA). The FDA plays a primary role in leading international development of methods for the detection of pathogenic *Vibrio* species in seafood. The turn-around time for a single sample from receipt to obtaining results with the MPN-Real-Time PCR is approximately 48 hours. Utilisation of the developed methods by industry will provide support for future risk assessment and the subsequent development of policies that are commensurate with the established risk. Furthermore, if trade disputes occur with respect to *V. parahaemolyticus,* determination of likely pathogenesis can be ascertained using these methods. This is a beneficial capability for industry given that over 50% of border rejections for crustaceans into the EU market between 2008 and 2013 due to pathogenic *Vibrio* species were related to the presence of *V. parahaemolyticus*.

Initially, it was intended that the established method include full accreditation. However, it was found that the cost of such accreditation and on-going maintenance thereof was prohibitive, with unknown levels of future business. To counter this, a quality system for the laboratory, including quality assurance procedures and all appropriate documentation, has been developed and implemented by SARDI Food Safety and Innovation.

Component 2: **Cadmium**

This survey of Australian wild-caught prawns has clearly identified species and fishery location as the most significant causes of variability in cadmium levels. *Metapenaeopsis crassissima* was found to have the highest cadmium concentrations, although this species was only sampled in small numbers, consistent with its proportion of overall prawn production from Western Australia fisheries. Endeavour prawns (*Metapenaeus endeavouri*) were also significantly higher in cadmium than other prawn species. By contrast, the major commercial species (banana prawns and king prawns) had low levels of cadmium. Targeting prawn species is a potential strategy worthy of further investigation for future export market access initiatives, with the aim of minimising border control non-compliance notifications and therefore enhancing Australia's reputation as a source of high quality, low-risk seafood. An assessment of the benefit of such a strategy in the context of the cost to industry would need to be considered.

Component 3: **Risk Ranking**

Refer to the completed Australian Seafood CRC report: *Food safety risks associated with prawns consumed in Australia* by John Sumner, September 2011.

Risk ratings indicate a very low risk of human illness associated with the consumption of prawns produced domestically, imported prawns and exported prawns. This finding is consistent with the public health record which shows few reports of illness related to the consumption of prawns that have been handled appropriately. The scientific findings contained in the report may assist negotiations for improved trade access conditions into domestic and overseas markets, and risk-commensurate testing requirements for retail outlets.

5. Benefits and Adoption

Adoption

Component 1: **Vibrios**

Technology and capability has been developed to support industry access to timely diagnostic services for assessing levels of pathogenic *Vibrio* species in Australian prawns, underpinning quality and integrity claims. This will greatly assist in any future international trade issues and domestic detections related to these pathogens in Australian prawns.

International networks have been increased through visiting and networking with:

- Center for Southeast Asian Studies (Japan)
- USFDA (USA)
- Australian Institute of Marine Science (Australia)
- Centre for Environment, Fisheries and Aquaculture Science (UK)

As discussed within the attached technical document: *Assessment of methodologies for the enumeration of pathogenic* Vibrio *species in Australian prawns*, changing international and

domestic dynamics may create business risks for the Australian prawn industry through increased testing costs to comply with regulations and the potential for positive testing results for *Vibrio* species. Introduction of any new standards should be commensurate with the risk and as such should be underpinned by risk assessment. Adoption of the developed methods by industry will provide support for future risk assessment and the subsequent development of policies that are commensurate with the established risk.

Component 2: **Cadmium**

A contemporary risk assessment for dietary exposure to cadmium from Australian wild-caught prawns, informed by a systematic survey and analysis of cadmium and selenium in samples from the main prawn fisheries, can direct appropriate risk management decision-making and interventions. This work can be used by the Australian prawn industry to direct strategies to reduce the likelihood of rejection of exported prawns to the EU on the grounds of cadmium levels (e.g. by selectively targeting prawn species or fisheries for export). The work can also be presented to national and international regulatory agencies and seafood industry bodies to address information gaps and identify remaining ambiguities regarding cadmium in Australian prawns. The work may be used by industry to contend for revision of trade regulations on cadmium in prawns into the EU. The project will be a tangible measure of the Australian seafood industry's endorsement of a food safety risk assessment approach, and of its support for targeted research programs that seek to address this particular issue.

Component 3: **Risk Ranking**

Food safety hazards in prawns in international trade have been identified and ranked. Key areas of concern have been prioritised to assist in directing resources to issues of high business risk for Australian prawns.

The present project ratings are in line with public health data linking prawns with illness. Despite being a huge commodity in international trade, there are few reports of illness where handling standards are maintained according to those contained in the Food Standards Codes of importing countries.

6. Further Development

The following information details the specific deliverables and adoption pathways that could be implemented to ensure uptake of research outputs.

Component 1: Vibrios

- Protocols for the detection and enumeration of pathogenic *Vibrio* species, namely *V. cholerae*, *tdh*$^+$/*trh*$^+$ *V. parahaemolyticus* and *V. vulnificus*, by MPN-Real-Time PCR are available for adoption by commercial laboratories.
- The developed MPN-Real-Time PCR methods could be used to undertake a more comprehensive survey of Australian prawns to inform an appropriate risk analysis with the view of using the results to negotiate improved access conditions to key markets.

Component 2:

The ability to conduct this risk assessment for exposure to cadmium from prawns by Australian consumers was limited somewhat by information gaps on specific consumption patterns; the

most recent dietary survey conducted for the Australian Bureau of Statistics was published in 1995 and cannot discriminate below the category of consumption of crustaceans, and did not characterise variability in consumption patterns throughout the year. A survey of individuals in Melbourne indicated annual consumption patterns in that city, but did not collect information on the quantities of prawns consumed. A community-based survey of prawn consumption patterns would be a valuable addition to the knowledge base. Further, information on consumption habits with regard to the relative proportions of individuals that routinely eat prawn tomalley (aka "brown meat"), i.e. hepatopancreas tissues, compared to those that never eat these tissues, will greatly assist future food safety risk assessment considerations. For Australia, detailed information on the market distribution of the species with higher cadmium levels: coral prawns (*Metapenaeopsis crassissima*) and endeavour prawns (*Metapenaeus endeavouri*), would provide a better risk assessment of cadmium exposure for Australian consumers.

High levels of cadmium have been found in female prawns of *Metapenaeopsis crassissima* in Western Australia. *Metapenaeopsis* spp. are also caught in small quantities from other Australian prawn fisheries. An investigation of other *Metapenaeopsis* species would indicate whether similar levels occur elsewhere or whether this finding is associated with a genetically isolated group of prawns in the Western Australia Shark Bay and Exmouth Gulf fisheries.

Recommendations regarding standard approaches to future laboratory analysis of cadmium in prawn tissues have been presented. As prawns are not regulated for cadmium content by Australian food safety agencies, there is no specific direction from regulators regarding analysis of prawn tissues. Unless specified by clients, whether government or industry, laboratories may analyse either whole prawns or shelled muscle tissue. This lack of specificity may be a source of variability for reported cadmium levels in prawns.

Options for the industry to minimise risks for compliance failures in prawns exported to countries that monitor and regulate cadmium concentrations in prawns have been presented. The lowest-risk – and highest-risk – prawn species and fisheries have been identified in the accompanying report: *2012-13 survey of Australian wild-caught prawns for analysis of cadmium and selenium*.

The key conclusion of the risk assessment is that the currently regulatory limit imposed by the European Commission does not significantly reduce exposure to cadmium in typical consumers (*Risk assessment of cadmium in Australian wild-caught prawn muscle tissue*). The risk assessment is available to support future trade negotiations on appropriate regulatory limits for cadmium.

Release of data (aims, methods, results and conclusions) from both the *2012-13 survey of Australian wild-caught prawns for analysis of cadmium and selenium* and *Risk assessment of cadmium in Australian wild-caught prawn muscle tissue* to the study authors so they can prepare relevant publications in international, peer-reviewed, open-access scientific journal/s would facilitate broader dissemination of the work and could be acknowledged to show that Australia's prawn industry supports rigorous science.

Component 3:

The risk ranking component of the project identified prawns as a relatively low risk food type, with few outbreaks of human illness associated with hazards in Australian prawns. The risk ranking document (*Food safety risks associated with prawns consumed in Australia*) can be used by prawn companies and associations to support trade and marketing initiatives.

7. Planned Outcomes

1. Increased product compliance with domestic and international quality assurance requirements:
The key component for achieving this outcome is provision of a method for detection of pathogenic *Vibrio* species in prawns. Integration of this method into testing regimes will allow identification of pathogenic strains, and enable a more accurate assessment of the risks from *Vibrio* species in prawns. We envisage that this could lead to fewer trade detentions due to pathogenic *Vibrio* species of concern than is currently the case, as established methods do not discriminate virulent and non-virulent strains. The development of appropriate risk assessments for cadmium in wild-caught prawns will enable industry and regulators to negotiate and implement management policies that are appropriate in scale, both domestically and internationally.

2. Increased understanding of hazards that are likely to affect the Australian prawn industry in terms of food safety and/or trade access in the future:
The risk ranking developed in this project has provided a comprehensive understanding of the key hazards which are of importance to the prawn industry. Importantly, the risk ranking demonstrated that prawns are a relatively low risk food. The risk ranking document is being used by the prawn industry to objectively underpin safety claims and for marketing initiatives.

3. Improved market access for Australian prawns exported to Asia and Europe:
This project has generated data on the presence of cadmium and selenium in wild caught prawns. The data generated have been used to inform a risk assessment on cadmium that can underpin multilateral government trade negotiations on revised cadmium standards in line with current Codex recommendations. Data on selenium may support industry nutrition labelling claims and assist with marketing initiatives.

Private Benefit Outcomes

Component 1: **Vibrios**

Companies that utilise the described method will be able to confirm samples as positive for potentially pathogenic *Vibrio* species based on the presence of pathogen-associated genes (*ctx*$^+$ for *V. cholerae*, *tdh*$^+$/*trh*$^+$ for *V. parahaemolyticus*) or as environmental strains (isolates that do not possess these genes). This may mean that they can release previously detained products based on the absence of risk to consumers.

Component 2: **Cadmium**

Prawn export companies will be able to more accurately predict the risks to their industry attributable to cadmium. This work will enable exporters to factor in considerations such as lower and higher-risk species and fishery location into their business models.

Component 3: **Risk Ranking**

Companies can utilise the risk ranking document to support assertions that their products represent a relatively low food safety risk to consumers.

Linkages with CRC Milestone Outcomes

Milestone 2.2.6: Technology and capability developed to support industry access to timely diagnostic services underpinning quality and integrity claims.

Milestone 2.4.2: Two completed, internationally reviewed, integrated health benefit and risk assessments available for market access negotiations and for consumer risk advisories.

Milestone 2.4.3: Integrated health benefit and risk assessment methodology accepted internationally and available for use with standard-setting, market access negotiations and "clean and green" claims and for differentiating Australian product in premium price markets.

8. Conclusions

Component 1:

The work undertaken has developed the capability to assess prawns for the presence/absence of pathogenic *Vibrio* species. This provides Australia with a recognised capacity to assist with potential public health and trade issues related to *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in prawns.

Component 2:

This component of the project has determined that cadmium levels in Australian wild-caught prawns are generally low. A small proportion of samples (4.3% of the total survey) collected from the Gulf of Carpentaria and Shark Bay had cadmium concentrations in de-veined muscle tissue that exceeded regulatory levels for importation into the European Union.

Cadmium levels in hepatopancreas tissues were considerably higher than in muscle tissues, a finding that is consistent with previously published observations by other research workers internationally. Prawn abdominal tracts did not contribute to measureable cadmium concentrations in muscle tissue homogenates.

Coral prawns (*M. crassissima*) sampled from Shark Bay in Western Australia were found to have accumulated high concentrations of cadmium, a result consistent with previously published reports. This species is currently subject to ongoing investigations in order to determine the reasons for its unusual capacity to bioaccumulate this toxic metal.

Prawns from South Australia's Spencer Gulf Prawn Fishery had exceptionally low levels of cadmium detected in muscle tissue.

Prawn consumption is likely to represent only a minor contribution to dietary cadmium intake for the majority of the population. Only a minor proportion of Australian wild-caught prawns had a cadmium concentration in their muscle meat above 0.5 mg/kg, and it appeared likely that this low frequency of prawns with higher cadmium levels occurs in prawns from other sources that are imported into European countries. The risk assessment found that the 0.5 mg/kg ML, the current regulatory limit in Europe, is an ineffective risk minimisation strategy for exposure from cadmium in prawns as it does not significantly reduce exposure to cadmium for most consumers, and could result in inappropriate rejection of prawn consignments. The risk assessment is available to support future trade negotiations on appropriate regulatory limits for cadmium.

The ability to conduct food safety risk assessments for prawns is limited somewhat by information gaps on specific consumption patterns; the most recent dietary survey conducted

for the Australian Bureau of Statistics cannot discriminate below the category of consumption of crustaceans. Community-based surveys of prawn consumption patterns, including information on consumption of "brown meat" (i.e. hepatopancreas tissues) would be a valuable addition to the knowledge base.

Measured selenium levels place Australian prawns as a good source of this trace nutrient, in common with seafood products overall. Formal criteria for labelling and marketing Australian wild-caught prawns as a "good source" of selenium have been met.

Component 3:

Please refer to ASCRC report:
Food Safety Risks Associated with Prawns Consumed in Australia
John Sumner
September 2011

Appendix 1: Intellectual Property

There is no intellectual property generated from this work. All methods developed were closely based on those developed by other researchers or accredited standard methods.

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Appendix 3: Risk Ranking component: steering group and external reviewers

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