



**Assessing compliance and efficacy of import conditions for uncooked prawn in relation to White Spot Syndrome Virus (WSSV) through testing retail commodities and comparison of stringency of import measures with other imported commodities into Australia**

**FRDC Project 2016-066 report to:**

**Australian Prawn Farmers Association**

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2016-066**

2017

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## Acronyms

ALOP	-Appropriate Level of Protection
APFA	-Australian Prawn Farmers Association
DAWR	-Australian Government, Department of Agriculture and Water Resources
IRA	-Import Risk Analysis
IGB	-Inspector General of Biosecurity
IIGB	-Interim Inspector of Biosecurity
OCVO	-Office of the Chief Veterinary Officer
OIE	-Office International des Epizooties World Organisation for Animal Health
qPCR	-Quantitative Polymerase Chain Reaction (real-time PCR)
WTOSPS	-World Trade Organisation Sanitary and Phytosanitary Agreement
TSV	-Taura Syndrome Virus
WSSV	- White Spot Syndrome Virus
WSD	-White Spot Disease
WTO	-World Trade Organisation
YHV	-Yellowhead Virus

## Glossary

<i>Fenneropenaeus merguensis</i>	- Banana prawn
<i>Haliporoides sibogae</i>	Royal red prawn
<i>Litopenaeus vannamei</i>	Pacific White shrimp
<i>Melicertus latisulcatus</i>	King prawn
<i>Metapenaeus benettiae</i>	Greentail prawn
<i>Peneaus esculentus</i>	Brown tiger prawn

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## Executive Summary

Subsequently to the outbreak of WSD on Australian prawn farms in November-December 2016, this project was initiated to generate data on some of the likely entry pathways for WSSV to enter Australian waters via the retail purchase of uncooked prawns and crabs. The project also sought to compare the import procedures across different commodity types including chicken meat, pork, salmon and stock feeds to those which were being applied to prawns, prior to the WSD outbreak at the Logan River prawn farms.

The project's study design sought to identify the test status of retail commodities based on the assumption that the within-commodity prevalence of a positive commodity was >10% (Assumptions: test sensitivity 80%; test specificity 100%; confidence 95%)

The level of testing in this project is set at a less intensive level of examination than that which is detailed for Australian border testing of imported prawn commodities, where the target level of prevalence for identification is 5%, with 95% confidence in the result.

The project collected 173 commodities (168 prawn and 5 crab) from retail outlets between 4 December 2016 and 14 February 2017. The distribution of sampling is illustrated in the table below.

<b>Sampling region</b>	<b>Human grade commodity outlets</b>	<b>Bait grade commodity outlets</b>
Moreton Bay, QLD	27	8
Northern NSW	14	2
WA	1	0

Locations of sampling were targeted to include: the catchment adjacent the WSSV outbreak (Logan River/Moreton Bay); sites up to 250km south to Grafton NSW on the Clarence River catchment; and one location in Western Australia.

The prawn commodities included 131 products destined for human consumption and 37 bait commodities. The project also collected 5 samples of human consumption grade softshell crab for similar testing. From each commodity, up to 6 pools of 5 prawns (less if numbers of prawns within the commodity were <30) were prepared for screening qPCR analysis (40 cycles) for the detection of WSSV nucleic acid at Sydney University. WSSV is comprised of a chain of nucleic acids and the tests are designed to look for a specific unique sequence of these nucleic acids, which belongs only to WSSV. A total of 910 pools were screened. When a commodity generated a positive result, it was subsequently tested with the OIE nested PCR (which looks for a different piece of WSSV sequence) for confirmation of the result. Those samples which gave all negative results in screening qPCR were not tested with the OIE nested PCR.

The screening qPCR is semi-quantitative, so it can be used as a coarse indicator of the amount of the target nucleic acid present in the test sample. Results are reported as a  $C_t$  value for each homogenised pool tested. Broadly,  $C_t$  values are the inverse of the amount of nucleic acid that is in the sample, and correlate to the number of copies of the target nucleic acid in the sample. Lower  $C_t$  values indicate high amounts of target nucleic acid, while higher  $C_t$  values mean lower amounts of target nucleic acid (down to extremely low trace levels).

Positive tests were recorded for all types of prawn commodities including peeled, whole, cutlets, marinated products and bait.

The numbers of commodities from each country of origin is displayed in the table below. The  $C_t$  scores for positive commodities ranged from ~17.68-39.99. Some DAWR-approved import

testing laboratories set a test to operate at a C<sub>t</sub> of 35, which is more conservative. Results at both the 35 and 40 cycle are illustrated below.<sup>1</sup>

Country of Origin		Screening qPCR					OIE nested PCR Confirmatory (only performed on positive qPCR commodities)		
		Number of commodities	Number with at least one positive pool result	% positive	Number with a CT value <35	% with a CT value <35	Number of commodities tested	Number positive	% positive
1	Australia	12	2	16.67	0	0.00	2	1	50.00
2	Australian caught, Processed and packed overseas	5	3	60.00	1	20.00	3	1	33.33
3	China	36	28	77.78	18	50.00	28	20***	71.43
4	Malaysia	29	28	96.55	23	79.31	28	24***	85.71
5	Vietnam	20	20	100.00	19	95.00	19	19	100.00
6	Thailand*	5	3	60.00	1	20.00	3	1	33.33
7	Indonesia	2	2	100.00	2	100.00	2	2	100.00
8	Myanmar**	1	0	0.00	0	0.00	0	0	0.00
9	Labelled imported (country not labelled)	11	9	81.81	9	81.81	9	9	100.00
<b>TOTAL LABELLED IMPORTED FOOD GRADE (3-9)</b>		<b>104</b>	<b>90.00</b>	<b>86.53</b>	<b>72</b>	<b>69.23</b>	<b>89.00</b>	<b>75.00</b>	<b>84.27</b>
10	Unlabelled	15	13	86.67	12	80.00	13	12	92.31
11	Bait - no country labelled	37	5	13.51	0	0.00	5	0****	0.00

\*4/5 products were soft shell crab

\*\* 1/1 product was soft shell crab

\*\*\* Trace level in one commodity reported as negative (inconclusive)

\*\*\*\*Trace level in two commodities reported as negative (inconclusive)

<sup>1</sup> Notwithstanding that a formal quantitative standard was not used, a C<sub>t</sub>>35 is consistent with a very low viral load. However, when testing pooled samples this quantitative information is difficult to interpret as it might indicate a low prevalence of high viral load or a high prevalence of uniform low viral load

86.5% of the prawn commodities which were labelled as imported, returned a positive WSSV screening qPCR in one or more of the pools. Of these positives, 69.2% had  $C_t$  values below 35 and 84.3% were confirmed with the OIE nested PCR as positive.

By comparison only 16.7% of prawn commodities which were labelled as product of Australia, returned a positive WSSV screening qPCR in one or more of the pools. Of these positives, none had  $C_t$  values under 35 and only one of the two tested, was confirmed as positive by the OIE nested PCR.

Of the five products which were labelled product of Australia, with processing and packing in Thailand or Vietnam, three of five samples returned a positive WSSV screening qPCR in one or more of the pools. Of these positives, one had a  $C_t$  value under 35 and one was confirmed positive by the OIE nested PCR.

Collectively these results indicate a failure at the border to accurately detect WSSV positive imported prawn commodities and prevent their entry into Australian retail. Information from the Senate Inquiry from DAWR suggests there are two components to this failure. Firstly, illegal evasion of import conditions by some importers, and secondly a failure of the DAWR import inspection and testing to accurately identify WSSV positive batches and prevent their entry into Australia.

The results identified WSSV positive human grade commodities in every retail outlet tested. Many different brands of product returned WSSV positive results with product origins from a variety of countries including Malaysia, China, Vietnam, Thailand and Indonesia.

The identification of positive commodities in Queensland, NSW and WA retail is suggestive of a national issue with border control, rather than an individual port issue.

The quarantine measures such as testing at the border are intended to reduce the risk of disease entry into Australia to a level, which is commensurate with Australia's ALOP. The intention is that outbreaks and significant impacts on Australia's prawn farming and wild industries should be avoided.

The results from this project are strongly suggestive that the import measures which were in place, prior to the Logan River WSD outbreak, to control the entry of WSSV into Australia on uncooked prawn commodities were ineffective. The corollary of this import regulatory failure, is the escalation of risk above Australia's ALOP, which has permitted substantial quantities of WSSV a potential pathway and release into the Australian environment.

Other Australian industries including chicken meat, pork, salmon and stock feed, are afforded greater protection from imported disease risks, through establishing a system which tolerates a lower level of risk for disease entry.

A national response to this incursion is warranted and should include: ongoing containment and eradication processes; surveillance to determine proof of freedom in other areas outside the outbreak zone; a complete review of the Import Risk Analysis of uncooked prawn commodities; and a review of bait biosecurity risks encompassing the potential for diversion of product intended for human consumption.

## **Recommendations**

### **a) Review the IRA for prawns and crustacea**

Numerous risks have shifted within existing commodities and consumer behaviours since the 2009 IRA. Additionally, new prawn diseases have continued to emerge which warrant thorough assessment.

New technologies have also emerged that may assist in better mitigating illegal activities such as product substitution and mislabelling country of origin.

The extent of issues which have been identified over the last 12-18 months in the imported prawn trade together with results of this study seem sufficient grounds for DAWR to maintain current restrictions on uncooked prawn imports and to activate its provision to review the import protocols.

### **b) Enhanced surveillance and inspection rigor**

Reports from DAWR through the prawn liaison officer signalled that enhanced surveillance is being undertaken on imported containers of prawns, after the outbreak of WSSV on prawn farms on the Logan River. These measures appear to have substantially increased the detection rate of positive containers from <15% to >50%.

This likely signals that the previous level of surveillance was substantially underperforming its target of identifying WSSV at a prevalence of above 5% at the batch level.

The survey results from product purchased in January 2017, indicate that even with enhanced surveillance and product recalls, there was uncooked imported WSSV positive product available for retail purchase.

Future surveillance of the prawn trade could consider implementing the recommendations of the Interim Inspector-General's report into the pork trade in 2013 including:

- regular reviews and staff visits to confirm country claims of freedom from disease
- establishment of a random inspection regime;
- unannounced audits of importers facilities

### **c) Mandatory cooking/gamma-irradiation**

Heat through cooking and to a lesser extent, gamma-irradiation, have been documented to destroy the infectivity of prawn viruses including WSSV. Cooking is considered effective for pork and chicken viral pathogens, which underpins the trade arrangements for these products from disease positive countries, to only be allowed entry to retail if cooked.

Offshore cooking or irradiation (pending further confirmatory research) would be preferable to reduce handling risks of uncooked product within Australia, and minimise risk of illegal leakage of uncooked imported prawn into the Australian retail market.

Cooking/gamma irradiation in an approved premise within Australia, would be analogous to arrangements for imported pork products, such that no uncooked product is permitted entry to retail.

#### **d) Implement forensic trace element analysis**

Sample randomly through every container. Such testing could confirm the validity of claims about the number of batches per container, and provide some confidence around the country of origin claims from the paperwork. Testing costs should be fully cost recovered from importers.

#### **e) Unannounced independent retail testing for exotic pathogens**

Fully cost-recovered by levy on prawn importers, to ensure that risks in imported commodities are at levels anticipated by the current IRA

#### **f) Alter definition of highly processed prawns**

Presently the action of processing a prawn by breaching it, or marinating it, can facilitate illegal activity to bypass the intended biosecurity barrier. Both breaching and marinade can be washed off and product re-packaged. There is no scientific evidence that either breaching or marinating are sufficient sanitary measures to inactivate WSSV or other prawn pathogens. Nor is there evidence that such processing entirely prevents the use of these commodities as bait or burley by recreational fishers.

Definitions of permitted processed products should only include those product types which cannot be modified upon entry to Australia to return to an uncooked prawn meat product, which could in turn be diverted into the recreational bait release pathway.

#### **g) Develop National WSSV strategy in response to incursion**

The widespread detection of WSSV retail outlets and the apparent cross-contamination onto Australian origin prawns at retail suggest a national response is necessary, in addition to the review of the IRA. This will include: ongoing eradication and containment of the outbreak; surveillance for freedom from disease in zones outside of the infected area; IRA review; review bait biosecurity including potential for diversion of product intended for human consumption into the bait or berley use pathway; and development of strategies to control risks associated with mixing of imported and domestic commodities at the point of sale.

## **Conclusion**

It is the view of the author that taking into consideration all of the available controls, the most cost effective measure to reduce risk to an acceptable level, in line with other imported commodities ALOP, is to require mandatory cooking or sufficient irradiation to achieve disinfection of uncooked imported prawn commodities. This measure leaves the least room for further illegal activity to divert high risk WSSV carrying uncooked prawns into Australian retail outlets, and thereby removes the risk of such potentially infectious product being released into Australian waterways.

Concurrently, there is clearly a case for a complete review of the 2009 IRA to take into account the serious weaknesses identified.

## Background

Australia imports a range of uncooked prawn commodities. This trade was the subject of the Generic Import Risk Analysis Report for Prawns and Prawn Products (Biosecurity Australia 2009). The risks associated with the import of these uncooked commodities were subject to a risk mitigation protocol (Annex 5) to reduce risk of importation of White Spot Syndrome Virus (WSSV), which causes the internationally OIE reportable White Spot Disease (WSD), Taura Syndrome Virus (TSV) and Yellowhead Virus (YHV) to an acceptable level.

Up until November 2016 Australia had been declared free from WSSV. There had been one historic outbreak of WSD reported in Darwin Aquaculture Centre which was traced to feeding of bait prawns, which were an imported product from Indonesia. This outbreak was successfully eradicated and a national survey utilising PCR testing supported a freedom from WSSV claim across Australia (East et al 2004).

In late November 2016, an outbreak of clinical WSD occurred in a prawn farm on the Logan River, in South East Queensland. Over the ensuing few months all farms in the area became infected. The Queensland Government undertook an eradication campaign on all infected farms employing chlorine to destroy stock and sanitise water.

The preliminary epidemiological assessment does not suggest an entry via broodstock and PL stocking. Rather it suggests a potential wild source had been pumped into farms.

Several positive detections have occurred in multiple species of crustacea in the adjacent wild populations to the farming area. Recreational anglers have been detected using imported uncooked prawns, purchased from retail outlets which were intended for human consumption only. Testing results from some of these prawns by the Commonwealth Government revealed positive WSSV detection.

Two historical surveys (Kewagawa Research 2002, 2007) illustrated a release pathway existed for imported green prawns, through purchase and use as bait by recreational anglers in waterways, whereby viral transmission to wild stock could occur. It then becomes possible for prawn farms to pump in the disease agent or be exposed by wild carriers to the virus and suffer consequent epizootics.

The prawn industry sought data on the risks posed by retail sales of uncooked prawns. This project commenced prior to the Minister for Agriculture and Water Resources announcement in early January 2017 of a temporary import ban on uncooked prawns to Australia. The project was also commenced prior to details being made public of the compliance actions against some prawn importers associated with illegal activity uncovered by DAWR's Operation Cattai.

The third objective of this study was only able to be completed in part, due to the ongoing compliance actions of DAWR, which limited the information which this department was able to provide the Principal Investigator.

## **Project Objectives**

1. Sample uncooked prawn commodities at retail outlets in Logan and Clarence River Catchments
2. Test commodities for presence of WSSV DNA using qPCR at NATA accredited laboratory
3. Review import testing and processes associated with import of prawns into Australia and compare to other commodities. Make recommendations which could reduce risks associated with prawn importation into Australia.

## **Methods**

### **Objective 1: Sampling**

Sampling locations included retail outlets (supermarkets, bait and tackle shops) of uncooked prawn commodities in the affected Logan River catchment and other reference catchments (Richmond River, Clarence River, Brisbane River, Western Australia). Sampling commenced on 4 December 2017.

Opportunistic sampling locations of available uncooked prawn commodities included:

- a) Supermarkets and specialty seafood outlets n=27 stores (85 commodities sampled) and bait outlets n=8 (34 commodities sampled) with catchments draining into Moreton Bay (Postcodes: 4207; 4507; 4216; 4212; 4209; 4210; 4216; 4217; 4007; 4221; 4220; 4228; 4208; 4215; 4127).
- b) Supermarkets n=14 (46 commodities); and bait outlets n=2 (3 commodities) located in catchments to the south of the index WSSV area in Northern NSW (Postcodes: 2478; 2460; 2464; 2487; 2485).
- c) Specialty seafood outlet n=1 (6 commodities) located in Perth, Western Australia (Postcode: 6154).

Sampling commenced at retail locations closest to WSSV positive index prawn farms on the Logan River, Queensland, and radiated around the catchments entering Moreton Bay.

Up to six pools, each containing up to five prawns were sampled from each commodity purchased. This provided a total of 992 pools for testing. Suitable uncooked commodity types to included pre-packaged and deli items: frozen peeled prawn meat, frozen peeled tail-on prawn cutlets, whole frozen prawn, frozen soft shell crab, frozen marinara mix, marinated frozen prawn, chilled marinated prawn, chilled peeled prawn meat, chilled whole prawn.

Frozen commodities were split into pools for testing and kept frozen. Chilled product was frozen, split into pools and kept frozen. Storage was maintained below -20°C until processing for testing by qPCR could be undertaken at the NATA accredited Sydney University laboratory.

## Design of study

The study examined the presence/absence of WSSV DNA in uncooked prawns. It should be noted that the detection of WSSV nucleic acid by these PCR test methods, does not necessarily mean the sample contains infectious “live” virus.

The following assumptions apply to the design, from which the sample numbers are derived.

All prawns within each purchased commodity have an equal chance of being sampled.

### Assumptions

- Prawn level prevalence of WSSV DNA >10% (in a commodity)
- Diagnostic sensitivity = 0.80
- Diagnostic specificity = 1.00
- Prawn population = 1 to 200

If a random sample of 30 units is taken from a population of 200 and 0 or fewer reactors are found, the probability that the population is diseased at a prevalence of 0.1 is 0.0478.<sup>2</sup>

The sampling locations of retail outlets are designed to provide a representative coverage of the outlets for the range of uncooked commodity types of interest.

The targeting of locations around the outbreak site and reference sites allow comparison of the WSSV status of commodities in each catchment area which are available for retail sale.

Commodities were purchased and moved from retail outlets in iceboxes to storage in -20°C freezers. Each commodity was allocated a sample ID and details of purchase and commodity were recorded onto the project keylist (Table 2). Frozen commodities were -re-packed into pools of 5 prawns per pool with each pool going into a separate zip-lock bag. Up to a total of 6 pools of 5 prawns were sampled from each commodity. Where less than 30 prawns were available for testing, fewer pools were collected. Disinfection was completed between each commodity. The commodity and pool ID was attached to each bag.

## Objective 2: Laboratory Methods

### Transport and storage of specimens

Specimens were shipped to Sydney University frozen, packaged with freezer bricks in foam boxes by overnight courier. Signed packing tape confirmed the chain of custody. The samples

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<sup>2</sup> Calculation from Ausvet FreeCalc

<http://epitools.ausvet.com.au/content.php?page=FreeCalc2&Option=0&N=200&Sens=.90&Spec=1&prevtype=1&prev=0.10&type1=0.05&type2=0.05&analysis=0&popthreshold=10000&maxSS=3200&Prec=4&SampleSize=1&Pos=1>

were transferred directly for storage at -80°C and removed in small batches onto ice at the time of processing to minimize the time at room temperature and number of freeze-thaw cycles.

A total of 910 samples were received by Sydney University in four shipments. The majority of samples were pools of 5 prawns in various states of preparation for human consumption. Other samples included whole prawns and individual soft shell crab. Animals within a pool were presented in a single plastic snap-lock bag with identification labels attached.

## **Dissection**

The most appropriate target specimen available for detection of WSSV depended on the state of preparation of the product for consumption. Tissue types were ranked preferentially according to the expected distribution of WSSV (OIE, 2016). Each animal contributed equally to the pool for tissue homogenization by dissection of the chosen specimen:

- *whole prawns*: all pleopods, not exceeding 0.1g.
- *peeled prawns*: 0.1 g of abdominal muscle excised as a wedge to maximize cuticular surface area (Figure 1).
- *peeled prawns with tail on*: a similar wedge of muscle with corresponding portion of shell excised and included in the sample.
- *soft shell crab, whole*: 0.25 g of tissue per individual (no pooling of animals). This was comprised of a small portion of gill (~0.08 g from each side) and half (split longitudinally) of the last and second last segment of each 5th pereopod (~0.1g).

The state of processing of the product and the tissues dissected to produce each pooled tissue homogenate is indicated in Table 2. In cases where marinade was present, it was removed from the region where the specimen was excised by wiping with sterile absorbent paper moistened with sterile purified water (Milli-Q). After dissection the remaining tissues were replaced in the original labeled bags and stored at -20°C. Tubes containing dissected tissue specimens were incubated on ice.

Weighed tissue samples from each individual from a pool were placed into a 2 ml snap-lock laboratory tube for a total mass of 0.5 g per pool unless indicated otherwise. The weight of tissue was adjusted to < 10% deviation from the target weight at the time of dissection. Each tube was prepared with 2 stainless steel beads, 3 mm diameter (Aussie Sapphires) and 0.1 g of sterile sand prior to addition of tissues. The dissected tissues pooled in tubes were returned to -80°C prior to further processing.

The workflow and procedures were conducted to prevent cross contamination between pools sampled from the same commodity ID. This entailed working in a class 2 biosafety cabinet and using sterile scalpel blade and autoclaved tooth pick to dissect and manipulate

specimens. Between each pool the work surfaces were disinfected using 1% Virkon, gloves were changed and a new sterile absorbent bench mat was applied.

Positive and negative control samples were prepared at the time of dissection and followed each batch of specimens through the laboratory workflow. A batch was defined as the set of tissue homogenate tubes prepared by an operator on a day. The negative control samples were homogenizing medium only, one negative control was prepared at the beginning and completion of each batch of samples. Positive control samples were prepared from a homogenate of WSSV positive shrimp tissue that was diluted in homogenizing medium to a concentration expected to produce a Ct value between 30 and 35. The positive control was prepared from the same shrimp that were tested as commodity ID M45. Reported to be *Litopenaeus vannamei* from Malaysia. It was used at a dilution of  $10^{-4}$  and  $10^{-5}$ . It was confirmed positive based on the OIE nested PCR and 2 qPCR assays with different amplification targets. A synthetic DNA positive control for the OIE nested PCR (Durrand 2002) assay was used initially to identify this sample, M45, as a positive control after the initial round of assay optimization. Results are reported for the (East 2004) qPCR assay in which this was the control for all PCR plates.

### **Tissue homogenization**

The samples were homogenized by bead beating with 1 ml of RLT buffer (Qiagen) and 5  $\mu$ l Dx reagent (Qiagen) to provide antifoaming properties. A Tissue Lyser II (Qiagen) was used at maximum oscillation frequency of 30 Hz for 60 sec, and then the insert was rotated 180° before another cycle at 30 Hz for 60 sec. The tubes were centrifuged at 12 000 *g* for 2 min. The external surfaces of tubes were disinfected with 1% virkon between addition of tissue and reagents and opening after completion of homogenisation. An aliquot of the supernatant was removed directly for nucleic acid purification. An additional aliquot of the supernatant in a sterile nuclease free PCR strip tube and the remainder of the bead-beating tube and content were archived at -80°C. Tissue homogenates represented 0.5 g of tissue in 1 ml (500 mg/ml).

### **Nucleic acid purification**

Nucleic acids were purified from an aliquot that represented 50 mg of pooled tissue using the All-for-One Vet Biosprint Kit (Qiagen). This entailed adding 100  $\mu$ l of the supernatant from the tissue homogenate to a reaction with a total volume of 400  $\mu$ l RLT buffer. The procedure was automated using a Biosprint magnetic particle workstation (Qiagen) according to the directions of the manufacturer.

The positive and negative WSSV homogenization control samples were also the control samples for the nucleic acid purification procedure. The Xeno positive control system (Life Technologies) was used to assess potential inhibition in a proportion of samples (n=247); 2  $\mu$ l of Xeno DNA (20 000 copies) was added to these nucleic acid purification reactions.

## Screening qPCR assay

Based on evaluation in the laboratory (data not shown) the oligonucleotide primer and probes for the qPCR assay described initially by East et al., (2004) was selected as preferable to those described by Durand and Lightner (2002). Oligonucleotides were supplied by Sigma Aldrich with HPLC purified probes labeled with a 5' FAM reporter and BHQ1 at the 3' end (Table 2). Pools were tested in duplicate 25  $\mu$ l reactions prepared using the Path ID qPCR mastermix (Life Technologies) with 300 nM of each primer and 100 nM probe, 5  $\mu$ l of nucleic acid template and molecular biology grade water. Thermocycling was conducted using an Mx300P qPCR system (Stratagene) with thermocycling: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 sec. The FAM fluorescence signal acquired at the completion of each cycle was normalized to the ROX passive reference dye and a corrected to a baseline according to the Stratagene software. Threshold fluorescence was calculated according to the amplification of positive controls by Mx3000 software. A negative result was assigned to samples in which the FAM signal did not exceed the threshold. When the FAM signal exceeded the threshold in either replicate a  $C_t$  value was assigned for samples according to the fractional cycle number at which this occurred. All raw fluorescence plots were examined visually before assigning a final call of positive and negative. Results were reported dichotomously as positive or negative. The  $C_t$  value can provide some indication of the relative quantity of WSSV DNA sequence in a sample. Typically for a qPCR assay a decrease in  $C_t$  value by 3.3 is consistent with a 10-fold increase in the amount of WSSV DNA. However, quantitative information is influenced by the efficiency of the reaction and the nature of the sample matrix for each pool (Bustin, et al., 2009). In the absence of a formal quantitative standard, the  $C_t$  values should be interpreted cautiously as an indication of the load of WSSV.

Assignment of a  $C_t$  value was interpreted as a positive result for the screening qPCR assay and is consistent with the presence of WSSV specific nucleic acid sequence at the currently unspecified sensitivity of the assay. Further verification of positive results obtained in the qPCR assay was obtained by testing the samples with a confirmatory assay. A negative result indicates that the sample is free from WSSV nucleic acids or it was present and was not detected by virtue of being below the limit of detection of the assay.

Results from each qPCR experiment were accepted when expected results were obtained for the following control reactions: (i) PCR positive control (purified nucleic acids prepared from a shrimp (muscle tissue from *Litopenaeus vannamei* from Malaysia) that tested positive for WSSV by qPCR and confirmed by conventional nested PCR; (ii) the relevant positive control sample from tissue homogenization and nucleic acid purification for each PCR run; No template control (water only qPCR reaction). Note, synthetic DNA containing sequence corresponding to the region of the target amplicon was applied during experiments to compare assay conditions.

The Vet Max Xeno-VIC internal positive control assay was used according to the instructions of the manufacturer (Life Technologies) to evaluate potential inhibition in a selection of samples.

### **Confirmatory conventional nested PCR**

Confirmatory testing was undertaken at the commodity level. For each commodity in which there was a positive test in the screening qPCR assay, the sample (pool) which produced the lowest  $C_t$  value was selected for further testing. The nested qPCR assay described in the Manual of Diagnostic Tests for Aquatic Animals (OIE, 2016) for detection of WSSV DNA is derived from the method described by Lo et al. 1996. It was used according to the following protocol:

**Primary PCR.** Reactions were prepared in a 50  $\mu$ l volume containing 5  $\mu$ l of appropriate purified nucleic acid template from the appropriate sample with: 5  $\mu$ l of 10X PCR buffer (66.6 mM Tris-HCl, 16.6 mM  $(\text{NH}_4)_2\text{SO}_4$ , 2.5 mM  $\text{MgCl}_2$ , 1.65 mg/mL bovine serum albumin), 10  $\mu$ l of 1 mM dNTPs, 500 nM each forward and reverse primer (Table 2), 4U Taq polymerase (Roche) and 27.1  $\mu$ l nuclease free water. A 96-place thermocycling machine (Biorad) was used according to the following protocol: 94°C for 4 min, 55°C for 1 min, 72°C for 2 min for 1 cycle; then 34 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 2 min and a final extension at 72°C for 5 min.

**Nested (secondary) PCR.** The template for the second round amplification was 5  $\mu$ l of a 1/100 dilution of the primary PCR reaction mixture in nuclease free water. Each sample was tested using the same composition of the 50  $\mu$ l reaction volumes with the nested PCR primers (Table 2) at a final concentration of 500 nM each. And thermocycling at 94°C for 4 min, 55°C for 1 min, 72°C for 2 min for 1 cycle and then 29 cycles at 94°C for 30s, 62°C for 30s, 72°C for 1 min.

Nested PCR products were visualized by loading after electrophoresis on a 1% agarose gel stained with Redsafe (iNtRON). 10  $\mu$ l of each reaction was loaded and products were viewed under uv transillumination and imaged using a Geldoc system (Biorad). A positive result was indicated by a visible DNA band with a size of 941 base pairs. The specificity of this test has not been determined in all of the species of crustacea tested. Determining the sequence of the amplicon is recommended to increase confidence that WSSV DNA has been detected. (Claydon, et al., 2004)

### **Sample retention**

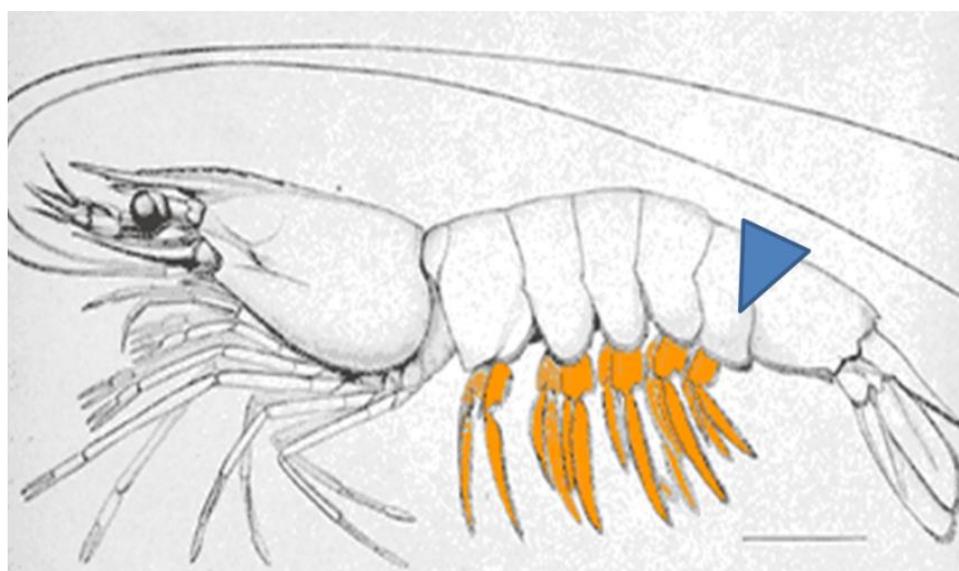
The laboratory has retained extracts from all positive samples and all original source pools should further testing be required. This could feasibly include screening for other viruses such as YHV or Taura Syndrome, or testing by another laboratory for WSSV.



Table 1 Primers and probes used in this study were supplied in the freeze dried form from Sigma-Aldrich Australia.

Assay		Primer name	5'→3' sequence	Reference
Purpose	Type			
Screening assay	Real-time, qPCR	WSSV-QPF1	CCGACGCCAAGGGA ACT	(East, et al., 2004)
		WSSV-QPR1	TTCAGATTCGTTACCGTTTCCA	
		WSSV-Pr1	FAM-CGCTTCAGCCATGCCAGCCG-BHQ1	
Secondary test	Real-time, qPCR	WSS1011F	TGGTCCCGTCCTCATCTCAG	(Durand, Lightner, 2002)
		WSS1079R	GCTGCCTTGCCGAAATTA	
		WSS-Probe	FAM-AGCCATGAAGAATGCCGTCTATCACACA-BHQ1	
Confirmatory test	Conventional nested PCR (primary)	146F1	ACTACTAACTTCAGCCTATCTAG	(OIE, 2016)
		146R1	TAATGCGGGTGTAATGTTCTTACGA	
	Conventional nested PCR (secondary)	146F2	GTA ACTGCCCTTCCATCTCCA	
		146R2	TACGGCAGCTGCTGCACCTTGT	

Figure 1: Location of tissue sample used for prawns that had been processed for human consumption is indicated by the blue triangle. From each of 5 shrimp in a pool a 0.1 g wedge of abdominal muscle was excised (not shown to scale). When there was shell present in this region the corresponding portion of shell and sub-cuticular tissues were included in the sample.



### **Objective 3: Review of import procedures**

Data on the number and timing of tests being conducted on containers was sought from DAWR and OCVO. A request for all “Approved Arrangements” which were in place for prawn importers was placed with DAWR.

It was intended that the project would undertake a review of the management of imported containers through making requests to DAWR for information on test results, location of containers and security of containers whilst awaiting test results.

It was also intended that information on additional inspection measures will be requested from DAWR in relation to how many containers of cooked prawns are inspected for illegal undeclared uncooked prawn commodities. A list of questions was formulated to cover these areas (Annex 6 Questions posed to DAWR in relation to import processes for prawns).

However, the extent of information available from DAWR has not allowed for external verification of these issues at the time of publication of this report.

In the absence of these responses the project team reviewed the application of Australia’s Appropriate Level of Protection across several commodities which each contained comparable pathogen transfer risks including chicken meat, pork, salmon and stock feed.

## **Results**

### **Laboratory QC report**

Survey samples were tested using 13 plates for nucleic acid purification for which the negative controls were all negative and the positive control was always positive with a Ct of  $30.28 \pm 1.86$ . There were 23 qPCR plates on which NTC samples were all negative and the Ct value for the positive control was  $25.80 \pm 1.50$  or  $30.33 \pm 1.99$ , for a  $10^{-4}$  and  $10^{-5}$  dilution, respectively (mean  $\pm$  standard deviation). The internal positive control was positive for all samples in which it was used, with a Ct value of  $31.46 \pm 1.61$  (n=247). This is indicated that inhibition of the PCR was not occurring and creating the potential for false negative results across the range of samples types being tested that included marinated retail product. Expected results were obtained for positive and negative samples in the conventional nested PCR based on the presence or absence of a visible DNA amplicon if appropriate size.

### **Survey samples**

The qPCR results for all pools for all commodities tested are documented in Table 3 below. Where positive results were reported in one or more pools from an individual commodities,

the nucleic acid sample purified for the pool that produced the lowest  $C_t$  value was selected to evaluate the status of the commodity according to the OIE conventional nested PCR (Table 3, Table 4, Table 5, Figure 2)

*Table 2 Codes for retail seafood specimens tested for WSSV DNA.*

<b>Product code</b>		<b>Retailer code</b>	
1	Peeled	1	Supermarket
2	Whole-human food	2	Specialist seafood retail outlet
3	Whole- bait grade	3	Bait outlet
4	Marinated		
5	Marinara mix		
6	Soft shell crab		

Table 3: Pool PCR sample ID and test results

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35 <sup>3</sup>	OIE nested PCR
A1	4/12/2016	Beenleigh QLD 4207	1	1	Peeled deli	n/a	n/a	1	31.95						Pos	Pos	Pos
A2	7/12/2016	Beenleigh QLD 4207	1	1	Cutlet peeled deli	n/a	Malaysia	1	27.5						Pos	Pos	Pos
A3	7/12/2016	Beenleigh QLD 4207	1	1	Peeled deli	n/a	China	1	23.9						Pos	Pos	Pos
M3	27/12/2016	Biggera Waters QLD 4216	1	1	Cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	23.84	23.69	23.83	20.19	24.43	23.66	Pos	Pos	Pos
M4	27/12/2016	Biggera Waters QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	No Ct	38.1	No Ct	No Ct	No Ct	No Ct	Pos	Neg	Pos
M6	27/12/2016	Helensvale QLD 4212	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	39.2	No Ct	No Ct	Pos	Neg	Neg
M8	27/12/2016	Helensvale QLD 4212	1	1	Cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	18.92	23.23	23.75	24.82	23.17	23.68	Pos	Pos	Pos

<sup>3</sup> Notwithstanding that a formal quantitative standard was not used, a Ct>35 is consistent with a very low viral load. However, when testing pooled samples this quantitative information is difficult to interpret as it might indicate a low prevalence of high viral load or a high prevalence of uniform low viral load

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M9	27/12/2016	Helensvale QLD 4212	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	No Ct	No Ct	No Ct	No Ct	39.5	<b>39.1</b>	Pos	Neg	Neg
M16	27/12/2016	Coomera QLD 4209	1	1	Cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	<b>33.7</b>	34.5	34.2	36.1	35.3	34.7	Pos	Pos	Pos
M17	27/12/2016	Coomera QLD 4209	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	No Ct	<b>35.6</b>	No Ct	No Ct	No Ct	No Ct	Pos	Neg	Pos
M18	27/12/2016	Coomera QLD 4209	1	1	Cutlet Deli	<i>Litopenaeus vannamei</i>	Malaysia	6	23.78	34.93	19.8	26.83	29.58	24.39	Pos	Pos	Pos
M19	27/12/2016	Coomera QLD 4209	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	28.9	28.66	27.7	28.7	27.48	30.62	Pos	Pos	Pos
M20	27/12/2016	Upper Coomera QLD 4210	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	39.17	37.32	38.78	36.82	38.84	39.4	Pos	Neg	Neg
M23	27/12/2016	Oxenford QLD 4210	1	1	Peeled Chilled vacupak	<i>Litopenaeus vannamei</i>	n/a	6	30.8	31.04	31.76	30.64	31.33	29.54	Pos	Pos	Pos
M24	27/12/2016	Oxenford QLD 4210	1	1	Peeled Chilled vacupak	<i>Litopenaeus vannamei</i>	n/a	6	24.63	24.02	23.44	24.25	<b>24.01</b>	29.5	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M26	27/12/2016	Oxenford QLD 4210	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	30.54	29.91	31.4	32.72	33.04	32.95	Pos	Pos	Pos
M27	27/12/2016	Oxenford QLD 4210	1	1	Cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	<b>34.3</b>	36.6	35.2	35.9	34.4	34.6	Pos	Pos	Pos
M31	30/12/2016	Yamba NSW 2464	1	1	Peeled Deli	n/a	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M32	30/12/2016	South Grafton NSW 2460	1	1	Cutlet FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	35.59	35.86	36.45	35.14	Pos	Neg	Neg
M33	30/12/2016	South Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	37.53	Pos	Neg	Neg
M34	30/12/2016	South Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	32.54	32.64	31.39	32.81	31.15	32.1	Pos	Pos	Pos
M42	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	28.01	29.4	27.53	28.18	29.11	30.92	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M43	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Melicertus latisulcatus</i>	Australia raw prawn, meat packed in Thailand	6	No Ct	36.94	Pos	Neg	Neg				
M44	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	Royal Red ( <i>Haliporooides sibogae</i> )	Australia caught, peeled and packed in Thailand	6	No Ct	32.22	37.52	35.43	34.55	34.88	Pos	Pos	Pos
M45	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	25.75	17.68	17.81	25.09	25.42	25.39	Pos	Pos	Pos
M46	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	35.71	39.4	36.66	No Ct	33.52	34.93	Pos	Pos	Pos
M49	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	33.47	32.48	32.2	34.22	34.04	32.75	Pos	Pos	Pos
M50	30/12/2016	Grafton NSW 2460	1	1	Peeled Cutlet	<i>Litopenaeus vannamei</i>	China	6	36.31	37.75	35.93	36.67	35.08	39.12	Pos	Neg	Neg
M51	30/12/2016	Grafton NSW 2460	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	36.85	No Ct	No Ct	36.81	37.71	Pos	Neg	Neg

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M53	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	28.3	29.1	28.8	<b>26.9</b>	27.1	28.9	Pos	Pos	Pos
M54	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled Cutlet	<i>Litopenaeus vannamei</i>	China	6	No Ct	37.3	34.6	<b>33.2</b>	No Ct	No Ct	Pos	Pos	Pos
M55	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M56	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M59	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	<b>28.6</b>	30.67	28.96	28.6	27.7	27.1	Pos	Pos	TRACE
M60	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	25.02	<b>24.5</b>	24.7	25.4	24.9	24.9	Pos	Pos	Pos
M61	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	34.6	35.3	35.7	36.95	<b>34.5</b>	35.8	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M62	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	28.3	38.2	20.4	24.04	<b>16.3</b>	24	Pos	Pos	Pos
M63	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	39.03	<b>36.95</b>	No Ct	No Ct	No Ct	Pos	Neg	Pos
M66	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	25.9	25.7	25.6	25.4	26.2	<b>25.3</b>	Pos	Pos	Pos
M67	11/01/2017	Beenleigh QLD 4207	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	No Ct	No Ct	No Ct	No Ct	No Ct	<b>38.9</b>	Pos	Neg	Neg
M68	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	25.1	25.1	<b>24.9</b>	25.5	25.3	25.4	Pos	Pos	Pos
M69	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	36.4	<b>35.8</b>	No Ct	37.9	36.5	36.96	Pos	Neg	Neg
M70	11/01/2017	Runaway Bay QLD 4216	1	1	Peeled cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	<b>34.5</b>	37.5	37.5	36.4	35.6	36.6	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M71	11/01/2017	Runaway Bay QLD 4216	1	2	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M73	18/01/2017	Ballina NSW 2478	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	34.2	34.1	34.4	36.7	<b>33.3</b>	36.5	Pos	Pos	Pos
M74	18/01/2017	Ballina NSW 2478	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	n/a	6	30.7	31.6	31.1	30.2	32.1	<b>29.9</b>	Pos	Pos	Pos
M75	27/01/2017	Springwood QLD 4127	1	1	Peeled Deli	n/a	Malaysia	6	32.4	30.96	32.7	32.01	<b>29.9</b>	32.2	Pos	Pos	Pos
M76	27/01/2017	Springwood QLD 4127	1	1	Peeled Deli	n/a	China	6	28.2	28.5	27.6	<b>26.9</b>	32.04	27.8	Pos	Pos	Pos
M78	27/01/2017	Springwood QLD 4127	1	1	Peeled cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	26.5	<b>20.9</b>	23.3	27.5	25.7	27.9	Pos	Pos	Pos
M79	27/01/2017	Springwood QLD 4128	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	28.4	28.6	<b>28.3</b>	29.7	28.9	28.8	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M80	21/01/2017	Ballina NSW 2478	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	36.8	<b>35.7</b>	No Ct	No Ct	No Ct	No Ct	Pos	Neg	trace
N3	5/01/2017	Hamilton QLD 4007	1	2	Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	China	6	28.7	29.8	29.6	28.9	<b>28.4</b>	29.1	Pos	Pos	Pos
N4	5/01/2017	Hamilton QLD 4007	1	2	Peeled Deli	<i>Fenneropenaeus merguensis</i>	Australia (Cairns, wildcaught)	6	No Ct	No Ct	No Ct	No Ct			Neg	Neg	not performed
N7	5/01/2017	Hamilton QLD 4007	1	2	Peeled FZN	<i>Fenneropenaeus merguensis</i>	Australian Prawns processed in Vietnam	6	No Ct	39.3	No Ct	<b>38.5</b>	39.6	39.3	Pos	Neg	Neg
N8	23/12/2016	Hamilton QLD 4007	1	2	Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	China	6	36.8	35.6	36.1	36.2	<b>33.6</b>	34.8	Pos	Pos	Pos
N9	23/12/2016	Hamilton QLD 4007	1	2	Peeled skewer Deli	n/a	n/a (Aus skewers)	1	No Ct						Neg	Neg	not performed
N10	23/12/2016	Hamilton QLD 4007	1	2	Peeled skewer Deli	n/a	n/a (Aus skewers)	1	No Ct						Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N11	23/12/2016	Hamilton QLD 4007	1	2	Peeled skewer Deli	n/a	n/a (Aus skewers)	1	No Ct						Neg	Neg	not performed
N15	23/12/2016	Hamilton QLD 4007	1	2	Peeled Deli	<i>Fenneropenaeus merguensis</i>	Australia, Cairns (wild caught)	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N22	29/12/2016	Casuarina NSW 2487	1	1	Peeled Deli	n/a	n/a	1	No Ct	No Ct					Neg	Neg	not performed
N26	28/12/2016	Elanora QLD 4221	1	1	Peeled Cutlet Deli	n/a	China	3	27.3	<b>26.8</b>	28.4				Pos	Pos	Pos
N27	28/12/2016	Elanora QLD 4221	1	1	Peeled Deli	n/a	China	6	21.9	19.8	21.7	<b>18.95</b>	23.1	23.7	Pos	Pos	Pos
N31	28/12/2016	Elanora QLD 4221	1	1	Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	China	6	23.7	24.4	<b>18.6</b>	24.1	24.7	23.2	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N32	28/12/2016	Elanora QLD 4221	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	30.8	30.5	29.5	26.3	<b>26.1</b>	27.01	Pos	Pos	Pos
N35	28/12/2016	Elanora QLD 4221	1	1	Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	25.7	26	<b>24.7</b>	25.1	25.4	25.8	Pos	Pos	Pos
N36	28/12/2016	Elanora QLD 4221	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	n/a	6	32.9	32.2	23.3	<b>18</b>	28.7	28.3	Pos	Pos	Pos
N38	5/01/2017	Burleigh Waters QLD 4220	1	1	Peeled Deli	n/a	n/a	4	27.4	<b>26.6</b>	27.9	27.99			Pos	Pos	Pos
N39	5/01/2017	Burleigh Waters QLD 4220	1	1	Peeled FZN	<i>Halipororides sibogae</i>	Australian caught, packed in Thailand	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N40	5/01/2017	Burleigh Waters QLD 4220	1	1	Peeled FZN	<i>Melicertus latisulcatus</i>	Australian caught, peeled and packed in Thailand	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N41	5/01/2017	Burleigh Waters QLD 4220	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N50	19/12/2016	Ascot QLD 4007	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	37.5	39.3	36.97	<b>34.4</b>	34.8	Pos	Pos	Neg
N51	19/12/2016	Ascot QLD 4007	1	1	Cooked Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	Thailand	6	30.6	31.2	30.6	30.4	<b>30.4</b>	30.6	Pos	Pos	Pos
N54	19/12/2016	Ascot QLD 4007	1	1	Peeled Deli	n/a	Imported	6	26.4	<b>22.6</b>	26.8	26.1	27.7		Pos	Pos	Pos
N55	19/12/2016	Ascot QLD 4007	1	1	Peeled Cutlet Deli	n/a	n/a	3	33.7	34.3	<b>32.7</b>				Pos	Pos	Pos
N56	19/12/2016	Ascot QLD 4007	1	1	Peeled FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N61	3/01/2017	Myaree WA 6154	1	2	Peeled Cutlets FZN	Tiger	Indonesia	6	<b>32.8</b>	33.5	34.6	34.7	37.8	38.2	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N62	3/01/2017	Myaree WA 6154	1	2	Peeled FZN	<i>Litopenaeus vannamei</i>	Indonesia	6	27.3	27.6	26.9	<b>26.1</b>	28.01	26.3	Pos	Pos	Pos
V1	10/01/2017	Robina QLD 4228	1	2	Peeled Cutlet FZN	<i>Litopenaeus vannamei</i>	Vietnam	6	32.7	<b>31.2</b>	33.1	33.1	32.6	33.5	Pos	Pos	Pos
V2	10/01/2017	Robina QLD 4228	1	2	Peeled FZN	<i>Litopenaeus vannamei</i>	Malaysia	6	30.9	30.7	28.7	<b>28.5</b>	32.02	30.1	Pos	Pos	Pos
V3	10/01/2017	Robina QLD 4228	1	2	Peeled FZN	n/a	Vietnam	6	<b>32.7</b>	33.2	34.3	33.6	33.6	34.6	Pos	Pos	Pos
M7	27/12/2016	Helensvale QLD 4212	2	1	Whole Deli	<i>Fenneropenaeus merguensis</i>	MSC	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
N2	5/01/2017	Hamilton QLD 4007	2	2	Whole Deli	Tiger prawn	Imported	6	No Ct	No Ct					Neg	Neg	not performed
N5	5/01/2017	Hamilton QLD 4007	2	2	Whole Deli	<i>Metapenaeus bennettiae</i>	Australia (Morteon Bay)	6	No Ct	No Ct	No Ct	<b>39.99</b>	No Ct	No Ct	Pos	Neg	Neg

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N12	23/12/2016	Hamilton QLD 4007	2	2	Whole Deli	Tiger prawn	n/a	6	<b>34.97</b>	36.2	36.04	35.2	35.4	37.3	Pos	Pos	Pos
N16	23/12/2016	Hamilton QLD 4007	2	2	Whole Deli	Endeavour prawn ( <i>Metapenaeus</i> )	Australia Harvey Bay, QLD	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N17	23/12/2016	Hamilton QLD 4007	2	2	Whole Deli	Tiger prawn ( <i>Penaeus esculentus?</i> )	Australia ( Rainbow beach QLD)	6	No Ct	No Ct	No Ct	No Ct			Neg	Neg	not performed
N57	3/01/2017	Myaree WA 6154	2	2	Whole FZN	Coral	Australia	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N58	3/01/2017	Myaree WA 6154	2	2	Whole FZN	Banana ( <i>Fenneropenaeus merguensis?</i> )	Australia	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N59	3/01/2017	Myaree WA 6154	2	2	Whole FZN	King	Australia	6	no Ct	No Ct	No Ct	No Ct	38.9	<b>38.7</b>	<b>Pos</b>	Neg	Pos
N60	3/01/2017	Myaree WA 6154	2	2	Whole FZN	Tiger	Australia	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
B1	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B2	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B3	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B4	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B5	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B6	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	39.7	No Ct	<b>36.6</b>	No Ct	No Ct	37.9	Pos	Neg	TRACE

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
B7	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	<b>No Ct</b>	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
B8	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	39.7	No Ct	No Ct	39.7	<b>37.9</b>	39.5	Neg	Neg	Neg
B9	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	<b>36.7</b>	No Ct	No Ct	No Ct	No Ct	Pos	Neg	Neg
B10	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B11	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	<b>No Ct</b>	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
B12	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
B13	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	<b>No Ct</b>	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
B14	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	38.98	<b>38.5</b>	No Ct	No Ct	No Ct	No Ct	Pos	Neg	Neg
B15	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B16	10/12/2016	Bribie Island QLD 4507	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	<b>No Ct</b>	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
B17	12/12/2016	Steiglitz QLD 4207	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	<b>37.2</b>	38.5	37.3	No Ct	Pos	Neg	TRACE
B18	12/12/2016	Steiglitz QLD 4207	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
B19	13/12/2016	Steiglitz QLD 4207	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
B20	14/12/2016	Jacobs Well QLD 4208	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B21	15/12/2016	Jacobs Well QLD 4208	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B22	16/12/2016	Southport QLD 4215	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B23	17/12/2016	Southport QLD 4215	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B24	18/12/2016	Southport QLD 4215	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B25	19/12/2016	Southport QLD 4215	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	6	No Ct	No Ct	Neg	Neg	not performed				
B26	20/12/2016	Southport QLD 4215	3	3	Whole FZN	<i>Metapenaeus ?</i>	n/a	4	No Ct	No Ct	No Ct	No Ct			Neg	Neg	not performed

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M1	27/12/2016	Biggera Waters QLD 4216	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
M2	27/12/2016	Labrador QLD 4215	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M11	27/12/2016	Stapylton QLD 4207	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	
M12	27/12/2016	Stapylton QLD 4207	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
M14	27/12/2016	Jacobs Well QLD 4208	3	3	Whole FZN	n/a	n/a (suspect Aus)	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	Neg
M15	27/12/2016	Jacobs Well QLD 4208	3	3	Whole FZN	n/a	Australia (Brisbane River??)	4	No Ct	No Ct	No Ct	No Ct			Neg	Neg	Neg
M29	30/12/2016	Yamba NSW 2464	3	3	Whole FZN	n/a	n/a	6	No Ct	39.5	No Ct	<b>36.8</b>	No Ct	No Ct	Pos	Neg	Neg

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N45	30/12/2016	Burleigh Heads QLD 4220	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N46	30/12/2016	Burleigh Heads QLD 4220	3	3	Whole FZN	n/a	n/a	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N47	27/12/2016	Burleigh Heads QLD 4220	3	3	Whole FZN	n/a	n/a (suspect Aus)	3	27.4	27.2	<b>26.9</b>				Pos	Pos	Pos
N48	4/01/2017	Tweed Heads NSW 2485	3	3	Whole FZN	n/a (Yamba River Prawn)	Australia (Yamba River?)	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
N49	4/01/2017	Tweed Heads NSW 2485	3	3	Whole FZN	n/a (Yamba River Prawn)	Australia (Yamba River?)	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed
M10	27/12/2016	Helensvale QLD 4212	4	1	Peeled Marinated Deli	n/a	Malaysia	6	31.9	31.7	34.5	32.1	<b>29.8</b>	31.7	Pos	Pos	Pos
M21	27/12/2016	Upper Coomera QLD 4210	4	1	Peeled Marinated Deli	n/a	Vietnam	6	32.7	34.4	<b>32.6</b>	34.4	33.3	34.4	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M22	27/12/2016	Upper Coomera QLD 4210	4	1	Peeled Marinated Deli	n/a	China	6	<b>30.3</b>	39.1	39.6	37.5	38.7	38.1	Pos	Pos	Pos
M25	27/12/2016	Oxenford QLD 4210	4	1	Peeled Marinated Vacupak chilled	<i>Litopenaeus vannamei</i>	n/a	6	29.9	29.5	<b>29.1</b>	No Ct	33.7	31.2	Pos	Pos	Pos
M28	27/12/2016	Hope Island QLD 4212	4	1	Peeled Marinated Deli	n/a	Vietnam	6	<b>28.4</b>	34.5	33.8	34.99	33.1	32.7	Pos	Pos	Pos
M30	30/12/2016	Yamba NSW 2464	4	1	Peeled Marinated Deli	n/a	Vietnam	6	29.5	28.9	31.7	29.7	30.1	<b>28.5</b>	Pos	Pos	Pos
M35	30/12/2016	South Grafton NSW 2460	4	1	Peeled Marinated Deli	<i>Litopenaeus vannamei</i>	Vietnam	6	No Ct	38.8	<b>37.2</b>	40	39.97	39.51	Pos	Neg	Pos
M36	30/12/2016	South Grafton NSW 2460	4	1	Peeled skewer marinated Deli	n/a	Vietnam	5	35.57	34.65	36.02	34.87	35.07		Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M37	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated FZN	<i>Litopenaeus vannamei</i>	China	6	29.99	30.8	<b>25.9</b>	29.7	31.1	30.8	Pos	Pos	Pos
M38	30/12/2016	Grafton NSW 2460	4	1	Peeled Cutlet marinated FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	Np Ct	No Ct	No ct	No Ct	No Ct	Neg	Neg	not performed
M39	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated FZN	<i>Litopenaeus vannamei</i>	China	6	No Ct	Np Ct	No Ct	No ct	No Ct	No Ct	Neg	Neg	not performed
M40	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated Deli	n/a	Vietnam	5	26.4	26.9	27.3	28.6	<b>26.3</b>		Pos	Pos	Pos
M41	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated Deli	n/a	China	5	38.7	36.99	38.9	<b>36.4</b>	37.6		Pos	Neg	Pos
M47	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated Deli	n/a	Vietnam	6	29.52	28.11	28.85	27.41	28.23	28.39	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M48	30/12/2016	Grafton NSW 2460	4	1	Peeled Marinated FZN	<i>Litopenaeus vannamei</i>	China	5	No Ct	No Ct	No Ct	No Ct	No Ct		Neg	Neg	not performed
M57	11/01/2017	Runaway Bay QLD 4216	4	1	Peeled Marinated Deli	n/a	China	5	<b>35.3</b>	37.2	No Ct	36.3	36.5		Pos	Neg	Pos
M58	11/01/2017	Runaway Bay QLD 4216	4	1	Peeled Marinated Deli	n/a	Vietnam	5	32.6	32.9	<b>32.3</b>	36.8	36.6		Pos	Pos	Pos
M64	11/01/2017	Beenleigh QLD 4207	4	1	Peeled Marinated Skewer Deli	n/a	Vietnam	6	38.3	30.9	30.5	<b>26.1</b>	29.1	30.5	Pos	Pos	Pos
M65	11/01/2017	Beenleigh QLD 4207	4	1	Peeled Marinated Skewer Deli	n/a	China	6	<b>33.8</b>	34.6	35.6	34.95	34.8	34.3	Pos	Pos	Pos
M72	11/01/2017	Labrador QLD 4215	4	2	Peeled Cutlet marinated FZN	<i>Litopenaeus vannamei</i>	Vietnam	6	31.7	31.2	32.2	30.8	<b>30.04</b>	30.5	Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
M77	27/01/2017	Springwood QLD 4127	4	1	Peeled Marinated Deli	n/a	Vietnam	6	No Ct	No Ct	No Ct	35.8	<b>32.3</b>	33.9	Pos	Pos	Pos
M81	14/02/2017	Ballina NSW 2478	4	1	Peeled Marinated Vacupak chilled	<i>Litopenaeus vannamei</i>	Imported (packed in Aus)	6	26.4	<b>25.5</b>	27.6	25.7	28.6		Pos	Pos	Pos
N18	29/12/2016	Casuarina NSW 2487	4	1	Peeled Marinated Skewer Deli	n/a	n/a	2	<b>35.7</b>	35.95					Pos	Neg	Pos
N19	29/12/2016	Casuarina NSW 2487	4	1	Peeled Marinated Skewer Deli	n/a	n/a	2	<b>26.3</b>						Pos	Pos	Pos
N21	29/12/2016	Casuarina NSW 2487	4	1	Peeled Marinated Deli	n/a	n/a	6	37.2	<b>32.8</b>					Pos	Pos	Neg
N23	29/12/2016	Casuarina NSW 2487	4	1	Peeled Marinated FZN	n/a	n/a	4	<b>33.4</b>	35.6	35.6	38.4			Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N24	28/12/2016	Elanora QLD 4221	4	1	Peeled Marinated Deli	n/a	Vietnam	4	<b>33.1</b>	32.8	33.1	34.7			Pos	Pos	not performed
N25	28/12/2016	Elanora QLD 4221	4	1	Peeled Marinated Deli	n/a	Vietnam	3	<b>29.3</b>	32.9	31.9				Pos	Pos	Pos
N29	28/12/2016	Elanora QLD 4221	4	1	Peeled marinated Deli	n/a	Vietnam	4	25.3	<b>24.8</b>	25.7	25.3			Pos	Pos	Pos
N30	28/12/2016	Elanora QLD 4221	4	1	Peeled Marinated Skewer Deli	n/a	Vietnam	3	34.2	<b>30.2</b>	31.5				Pos	Pos	Pos
N34	28/12/2016	Elanora QLD 4221	4	1	Peeled Marinated FZN	<i>Litopenaeus vannamei</i>	Local and Imported Ingredients	5	No Ct	No Ct	No Ct	No Ct	No Ct		Neg	Neg	not performed
N43	5/01/2017	Burleigh Waters QLD 4220	4	1	Peeled Marinated Deli	n/a	n/a	2	<b>31.6</b>	31.9	31.9	31.9			Pos	Pos	Pos
N52	19/12/2016	Ascot QLD 4007	4	1	Peeled Marinated Skewer Deli	n/a	Imported	5	30.8	31.1	30.8	<b>29.5</b>	30.1		Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N53	19/12/2016	Ascot QLD 4007	4	1	Deli	n/a	n/a	3	<b>34.6</b>	36.98	No Ct				Pos	Pos	Pos
M5	27/12/2016	Helensvale QLD 4212	5	1	Marinara FZN	<i>Litopenaeus vannamei</i>	Vietnam	3	36.9	32.7	<b>22.7</b>				Pos	Pos	Pos
N20	29/12/2016	Casuarina NSW 2487	5	1	Marinara FZN	n/a	n/a	2	25.1	<b>23.97</b>					Pos	Pos	Pos
N28	28/12/2016	Elanora QLD 4221	5	1	Marinara Deli	n/a	Imported and Local Ingredients	1	<b>24.9</b>						Pos	Pos	Pos
N33	28/12/2016	Elanora QLD 4221	5	1	Marinara FZN	<i>Litopenaeus vannamei</i>	Vietnam	2	30.5	30.3	17.9				Pos	Pos	Pos
N37	5/01/2017	Burleigh Waters QLD 4220	5	1	Marinara Deli	n/a	n/a	2	24.2	<b>22.8</b>					Pos	Pos	Pos
N42	5/01/2017	Burleigh Waters QLD 4220	5	1	Marinara FZN	<i>Litopenaeus vannamei</i>	Vietnam	3	29.9	29.3	29.2				Pos	Pos	Pos

SAMPLE ID	Date collected	Purchase Location	Product Code	Retailer Code	Product ID	Species	Country Of Origin	No. of Pools	Pool 1 Ct	Pool 2 Ct	Pool 3 Ct	Pool 4 Ct	Pool 5 Ct	Pool 6Ct	qPCR Ct <40	qPCR Ct <35	OIE nested PCR
N1	5/01/2017	Hamilton QLD 4007	6	2	Soft shell crab	n/a	Thailand	1	No Ct	No Ct					Neg	Neg	not performed
N6	5/01/2017	Hamilton QLD 4007	6	2	Soft shell crab	n/a	Thailand	1	No Ct	<b>37.4</b>	No Ct	No Ct			Pos	Neg	Neg
N13	23/12/2016	Hamilton QLD 4007	6	2	Soft shell crab	n/a	Thailand	1	No Ct	No Ct	No Ct	<b>37.3</b>			Pos	Neg	Neg
N14	23/12/2016	Hamilton QLD 4007	6	2	Soft shell crab	n/a	Thailand	1	No Ct	No Ct	No Ct	No Ct			Neg	Neg	not performed
V4	10/01/2017	Robina QLD 4228	6	2	Soft shell crab	n/a	Myanmar	6	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Neg	Neg	not performed

Table 4 Summary of results by commodity type

Code	Descriptor (Imported. Australian and unknown combined)	Total No.	Screening qPCR					OIE nested PCR Confirmatory (only performed on positive screening qPCR commodities)		
			No. +ve	% +ve	Average % of +ve pools within a positive commodity	No. with CT values <35	% Ct <35	No. commodities test	No. +ve	% +ve commodities of those tested by nested PCR
1	Peeled prawn	80	66	82.5	85.6	66	82.5	66	52*	78.8
2	Whole (unpeeled) prawn - food grade	11	4	36.4	62.5	2	18.1	4	3	75.0
3	Whole prawn - bait	37	5	13.5	36.7	0	0.0	5	0*	0.0
4	Marinated prawn	34	30	88.2	94.4	22	64.7	29	28	96.6
5	Marinara mix	6	6	100.0	100.0	6	100.0	6	6	100.0
6	Soft shell crab	5	2	40.0	25.0	0	0.0	2	0	0.0
Total		173	113.0	65.3		96.00	55.4	112	89	79.5

Table 5 Summary of test results by retail outlet type

Code	Retail outlet	Total No. commodities	Screening qPCR				OIE nested PCR Confirmatory (only performed on positive screening qPCR commodities)		
			No. positive	% positive	No. with CT values <35	% CT <35	Total No. of tested commodities	No. positive	% positive
1	Supermarket	106	93	87.7	75	70.8	93	78	83.9*
2	Seafood specialty outlet	30	15	50.0	10	33.3	15	11	73.33
3	Bait outlet	37	5	13.5	0	0.0	5	0	0.00*
Total		173	118.00	67.82	89.00	51.15	118.0	92.00	

\*2 commodities in each category returned a trace result and were not recorded as positive test results

Table 6 Summary of results by country of origin

Country of Origin		Screening qPCR					OIE nested PCR Confirmatory (only performed on positive qPCR commodities)		
		Number of commodities	Number with at least one positive pool result	% positive	Number with a CT value <35	% with a CT value <35	Number of commodities tested	Number positive	% positive
1	Australia	12	2	16.67	0	0.00	2	1	50.00
2	Australian caught, Processed and packed overseas	5	3	60.00	1	20.00	3	1	33.33
3	China	36	28	77.78	18	50.00	28	20***	71.43
4	Malaysia	29	28	96.55	23	79.31	28	24***	85.71
5	Vietnam	20	20	100.00	19	95.00	19	19	100.00
6	Thailand*	5	3	60.00	1	20.00	3	1	33.33
7	Indonesia	2	2	100.00	2	100.00	2	2	100.00
8	Myanmar**	1	0	0.00	0	0.00	0	0	0.00
9	Labelled imported (country not labelled)	11	9	81.81	9	81.81	9	9	100.00
<b>TOTAL LABELLED IMPORTED FOOD GRADE (3-9)</b>		<b>104</b>	<b>90.00</b>	<b>86.53</b>	<b>72</b>	<b>69.23</b>	<b>89.00</b>	<b>75.00</b>	<b>84.27</b>
10	Unlabelled	15	13	86.67	12	80.00	13	12	92.31
11	Bait - no country labelled	37	5	13.51	0	0.00	5	0****	0.00

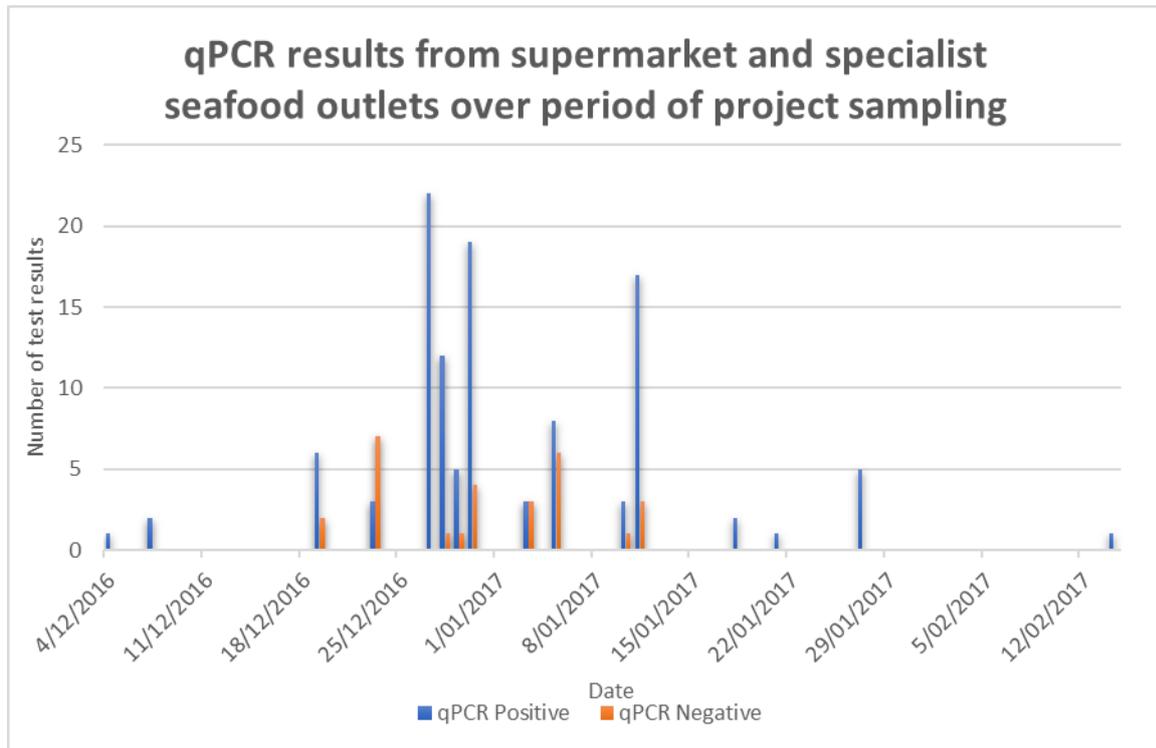
\*4/5 products were soft shell crab

\*\* 1/1 product was soft shell crab

\*\*\* Trace level in one commodity reported as negative (inconclusive)

\*\*\*\*Trace level in two commodities reported as negative (inconclusive)

Figure 2 Graph documenting the number of positive and negative test results for commodities collected over the date range of the project



From a total of 173 commodities (human and bait grade) 65.3% were detected as positive for WSSV using the screening qPCR, based on a detection of the target nucleic acid occurring at any point during the 40 cycles of the test (Table 3).

Of the samples from these commodities that were selected for confirmatory testing, 79.5% were positive based on the results of the conventional OIE nested PCR. However, where these results are from an unknown species of shrimp they should be considered inconclusive until sequence analysis of the amplicon is completed. A false positive result with these primers is possible for some host species (Claydon, et al., 2004). Four results are noted in the results as trace indicating that a band of appropriate size was present, but was very faint which is consistent with either a very low load of WSSV or possibly a non-specific amplification product.

The data were further analysed to consider the proportion of commodities which would be considered positive if a Ct value of 35 was utilized (Table 3).

Positive WSSV human grade commodities were detected at every retail outlets across regions sampled during the project.

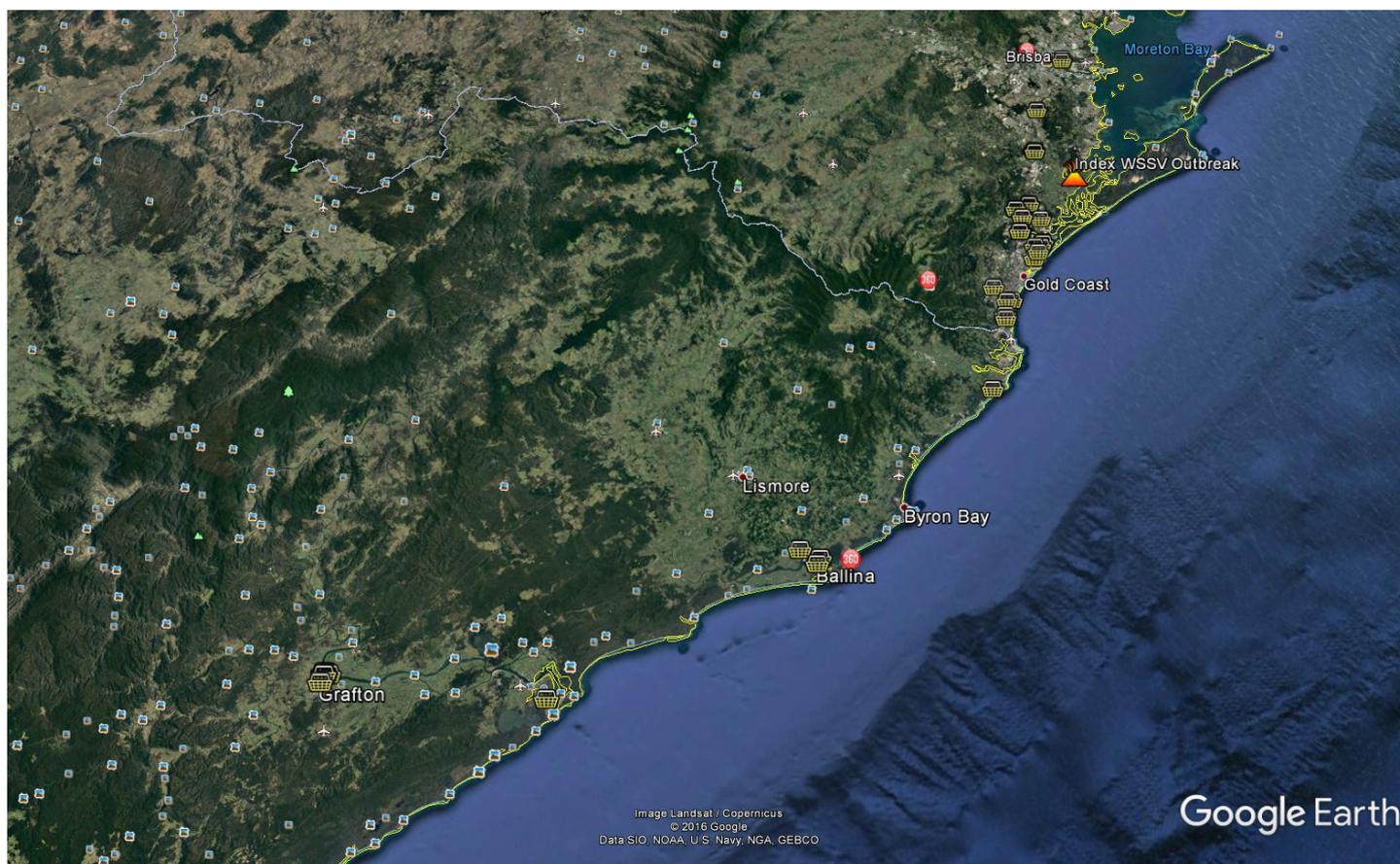
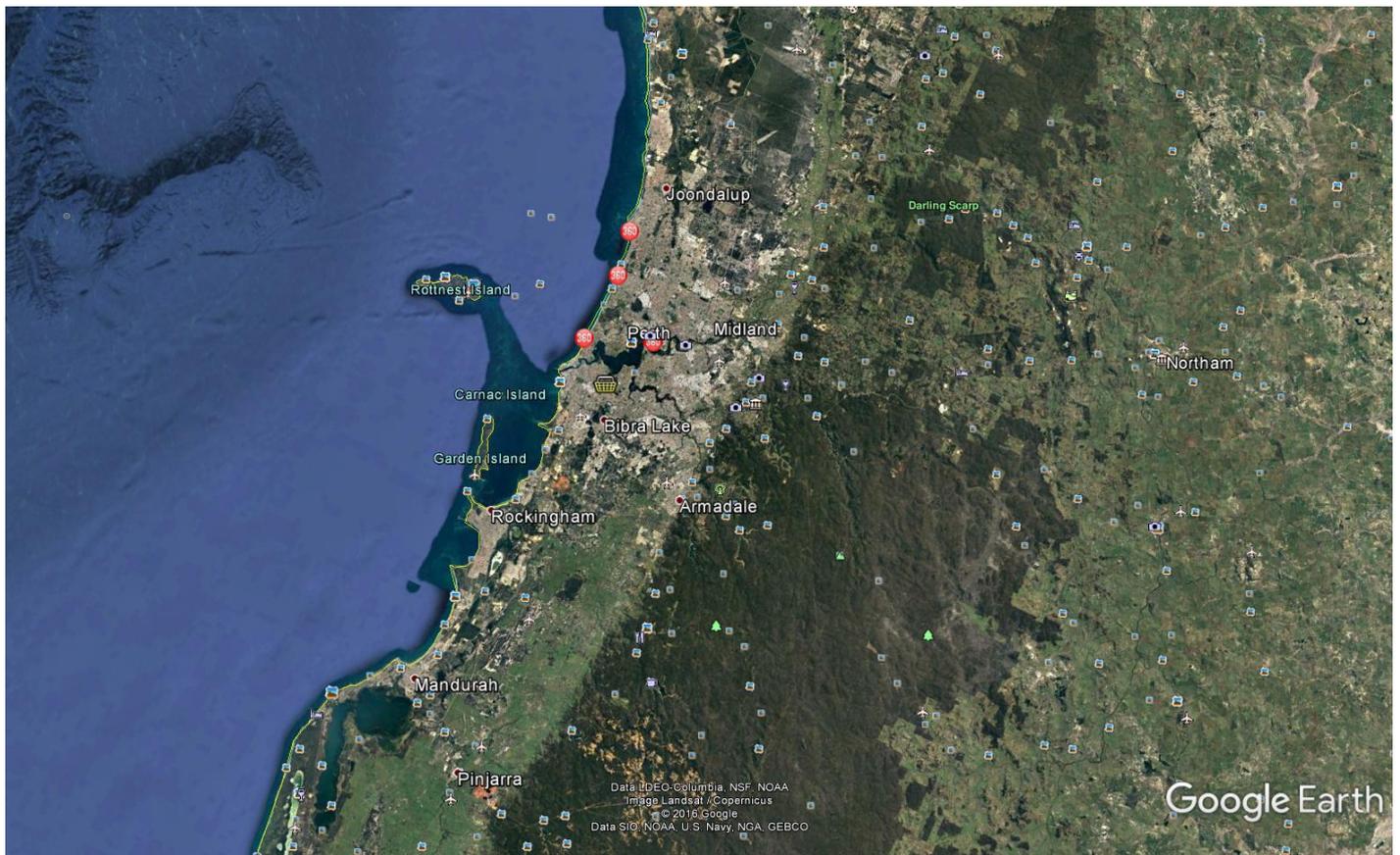


Figure 3 Yellow basket icons indicate sample locations on East Coast Australia



*Figure 4 Yellow basket icon indicates sampling location in Western Australia*

Positive commodities were recorded throughout the time course of the sampling, indicating the continued presence of WSSV test-positive product for retail sale from the timing of first collected product through until the last collected product on 14/02/2017 (Figure 1).

## **Discussion**

The project identified that a wide range of prawn commodities intended for human consumption (peeled, cutlets, skewers, marinated, marinara mix) from retail outlets, in all areas of sampling which tested positive to the presence of nucleic acid from WSSV by qPCR. The data suggests that the location of WSSV test positive commodities in retail was not limited to the Logan River area, where the index case of WSD was observed in late November 2016.

This type of testing is not designed to demonstrate the infectivity risk of such test-positive frozen commodities. Other authors have demonstrated that WSD can be transmitted to naive prawns from WSSV from test-positive frozen commodity prawns (Oidtmann and Stentiford 2011, Bateman et al. 2012), flagging the risk of commodity trade for the extension of the distribution of WSD.

The testing protocol on imported prawns destined for human consumption at the border was designed to detect positive shipments where the prevalence of test-positive prawns was greater than 5%. The level of positive imported commodities detected at retail was ~86.5%

by qPCR. Even taking a conservative  $C_t$  of 35, ~69.2% were still test-positive. This is suggestive of a failure to accurately detect positive commodities at the border and avoid their entry into the Australian marketplace, or illegal actions circumventing the border controls.

The results indicate that positive commodities were found in many different supermarkets and specialty seafood outlets. Many different brands of product returned positive results with product origins from a variety of countries including Malaysia, China, Vietnam, Thailand and Indonesia. The identification of positive commodities in WA retail is suggestive of a national issue with border control, rather than an individual port issue. Testimony given in the recent senate estimates committee hearing concerning DAWR's Project Cattai, also suggested that multiple ports were involved.

This project examined two potential retail pathways for WSSV to entry to waterways:

- 1) Via uncooked bait prawns
- 2) Via use/disposal of uncooked prawns/crabs intended for human consumption as bait or burley

The methodology of qPCR is exquisitely sensitive at detecting the presence of any of the target nucleic acid, which generates risks of surface cross-contamination leading to positive detections, where the individual prawn may not have actually been infected with the virus. The lower the  $C_t$  value reported, the less likely that a result could be due to such surface cross-contamination. Similarly, where all pools are affected it is less suggestive of trace surface cross-contamination.

There are numerous pre and post-border opportunities for food grade prawns to become cross-contaminated in the supply chains from nations which have endemic WSD status. These opportunities include at the time of sampling for import approval at the Australian border. Imported prawns may be re-packed in Australia. Should the re-packing facilities have inadequate sanitation in between prawn batches, a single WSSV positive batch could result in cross contamination of subsequent batches.

Commodities that were purchased from open delicatessen displays are at an elevated risk of cross-contamination, relative to pre-packaged product. Both staff handling, liquid and aerosol movement between commodities in the delicatessen windows is likely. Hence should a WSSV positive commodity be present in the delicatessen display, it will increase risk that all commodities in the display will test positive. Positive commodities collected from this location may not necessarily have been infected at the point of entry to Australia. They do however still pose a potential risk for virus release, if used as recreational fishing bait and burley and disposed of into a waterway. The size of this risk requires further quantification, however these results suggest a more cautious approach is warranted, given the recent emergence in Australia of WSSV in the Logan River in association with use of imported prawns as bait at this location.

Some positive bait commodities were detected by the screening qPCR test which would be unexpected if all bait was domestically sourced. Unfortunately, this could not be verified as none of the bait products were labelled with their country of origin. Bait prawns are not allowed to be imported into Australia unless they are irradiated. It is notable that many of the bait prawn commodities where a positive pool was detected, also had negative pools from the same commodity. And that generally the  $C_t$  values were high, suggesting the quantity of nucleic acid detected was low. Upon re-testing with the OIE nested PCR these commodities all returned either a negative or a trace result (which is different from a positive result and generally considered inconclusive). A similar pattern was found with soft-shell crab commodities. This situation differed however for imported prawns intended for human consumption where ~84.27% of positive commodities detected by qPCR also returned positive results using the OIE nested PCR.

The possibility cannot be excluded that some of these high  $C_t$ , positive pools are due to surface cross-contamination at any stage from packaging at manufacturer, through to the preparation of pools of 5 frozen prawns in the field sampling for this project, as opposed to the positive detections representing truly infected prawns. During field sampling complete control of aerosol risks could not be achieved due to the absence of a laminar flow cabinet. Every effort was taken through sanitary procedures to minimise this risk. The test results data does not indicate a widespread contamination issue as many commodities tested negative in all 6 pools (Table 4).

The level of positive commodities detected in this project is similar to the 50-70% detection frequency reported by DAWR during enhanced screening of imported prawns after the suspension of uncooked prawn imports. The data from DAWR testing has not been made available to determine whether the types and sources of commodities tested are similar to this project. Such data would assist in improving the understanding of the size of the risks posed by the positive retail products.

Five commodities of Australian caught prawns which had been processed in Thailand or Vietnam and re-imported to Australia were tested. Given at the time of capture of these prawns it is likely Australia was still considered free from WSSV, it was expected that these commodities would return negative test results. There are however some positive results that require follow-up to clarify whether there may have been cross contamination of these samples either in Asian processing plants, or during field pool preparation for this project. Two of these commodities tested negative in all 6 pools tested. One commodity tested positive in 4 of 6 pools, another in 6 of 6 pools but both then tested negative on OIE nested PCR, suggesting levels of WSSV nucleic acid were low. One of these commodities tested positive on 6/6 pools and positive on the OIE nested PCR. The results have been reported as positives, until further clarification is made (where residual sample material permits further examination).

The role of testing at the border is to reduce the risk of virus entry into Australia to a level, which is commensurate with Australia's ALOP. The intention is that outbreaks and significant impacts on Australia's prawn farming and wild industries should be avoided.

The results indicated there was a substantial quantity of imported uncooked prawn commodities carrying WSSV, in retail outlets around the area of the outbreak of WSSV on the Logan River, as well as elsewhere in Australian retail outlets. Only imported product was identified to have high loads based on the low  $C_t$  values reported.

### **Importation issues that may have contributed to high rates of WSSV test-positive prawns in retail samples.**

From speaking with industry participants and reviewing senate estimates Hansard from 28 February 2017, the project team were able to identify the following issues that may have been associated with high rates of WSSV carrying prawns reaching Australian retail outlets prior to and immediately after the WSSV outbreak in Logan River prawn farms:

- 1) Containers leaving port and biosecurity control prior to results being received.
- 2) Containers being opened prior to Border Officials arriving to collect samples for testing
- 3) Samples for testing not being collected first hand by Government officers
- 4) Samples being sent from overseas, prior to packing container
- 5) Empty boxes included in container, to fill with "WSSV" negative samples on arrival
- 6) Illegal packing of uncooked product within containers of cooked product
- 7) Illegal branded of product as processed, when it is in fact unprocessed
- 8) Washing off marinade /breeding and re-packaging
- 9) Different coloured straps on cartons

The project team also considered that it was likely that there had been an increased rate of recreational use of imported "human consumption only" prawns as bait based on reports of use of these commodities in the Logan River, after the closure to such bait use had been ordered. Results of recent angler surveys were not available at the time of publication of this project, but will be of utility to determine any shifts in angler use of imported bait.

### **Australia's Appropriate Level of Protection (ALOP) and Viral Diseases**

There are several significant issues relating to conditions that meet Australia's ALOP as it relates to prawn imports.

- The conditions in place for uncooked imported prawns prior to 6 January 2017 lacked consistency with other imported products. The conditions on uncooked prawns were weaker than those placed upon other animal and plant products with lesser or comparable viral or fungal risks associated with the commodities.
- The 2009 risk analysis did not place sufficient weight on the capacity for non-scientific or trade related practices such as product substitution or product integrity to be managed.
- The 2009 risk analysis failed to take into account that the purchasing behaviour of recreational anglers could change, thereby altering the volumes of “human consumption” product being diverted into bait use.
- <http://www.agriculture.gov.au/biosecurity/risk-analysis/animal/prawns>
- Submissions from APFA and QSIA prior to the conclusion of the 2009 IRA argued that the risk of disease entering was high, rather than low as the risk assessment had concluded.
- The volume of farmed prawn trade also increased over time, amplifying the risk of incursion of disease agents associated with this trade (<http://frdc.com.au/trade/Pages/Crustacean-Full.aspx>)

## **Background - The WTO SPS Agreement**

- The risk analysis process generally, as it is required to do under the WTO Sanitary and Phytosanitary (SPS) Agreement, focuses on the scientific justification in the context of least trade restrictive risk. The following two extracts from the text of the WTO SPS Agreement are relevant.
  - Article 5 Para 2 In the assessment of risks, Members shall take into account available scientific evidence; relevant processes and production methods; relevant inspection, sampling and testing methods; prevalence of specific diseases or pests; existence of pest — or disease — free areas; relevant ecological and environmental conditions; and quarantine or other treatment.
  - Article 5 Para 6 Without prejudice to paragraph 2 of Article 3, when establishing or maintaining sanitary or phytosanitary measures to achieve the appropriate level of sanitary or phytosanitary protection, Members shall ensure that such measures are not more trade-restrictive than required to achieve their appropriate level of sanitary or phytosanitary protection, taking into account technical and economic feasibility.

Notwithstanding the above, the reality of trade and commerce practice is such that Article 5 (2) above does not provide sufficient security in the context of Australia's ALOP as it relates to prawns.

## **The Risk Analysis Process**

The clear focus of the biosecurity risk analysis process over many years has been on the assessment of exotic infectious disease agents and their ability to spread if introduced into Australia and negatively impact on Australian production industries and the environment. The underlying assumption is that commodity entry will be through an approved/controlled channel. Very few past incursions of exotic pest and disease agents have occurred through controlled channel Importations.

A lesser focus has been on the entry of exotic disease agents through international trade practices, including clearly illegal practices. One area of real concern is country of origin labelling where the transshipment of product can easily lead to a "new passport" being issued for product. The level of security over the identity of fresh / frozen product at many ports in the world are definitely problematic as the commodities have both an international production base and an international market promoting movement. Many ports are of such a size and with high volumes of product movement that effective monitoring of the integrity of product is extraordinarily difficult. Adding to this difficulty is the tendency for product to spend minimal time in port dues to significant port charges.

For example, in the case of New Caledonia, it is questionable whether there is the level of security at the port, sufficient to provide the necessary assurances for product from that country to comply with Australian import conditions. It is doubtful also that with other countries that the level of product identity security is at a level to provide the necessary level of protection required by Australia ALOP.

Evidence presented at the Senate Additional Estimates hearings on 28 February 2017 demonstrates that even within Australia's own border control activities there has been an inability to maintain integrity in inspection processes. This has contributed to the entry of significant volumes of non-compliant WSSV test positive commodities into Australia. Australian border inspection services have allowed the development of "approved arrangements" with some individual importers which permitted importers to present the imported containers with security seals already broken. Such measures rely on the integrity of importers to not exploit these arrangements through partaking in illegal behaviour such as product substitution. The evidence provided at the Additional Estimates hearings detailed that such "trust" measures had been breached by many importers.

Presently compliance testing measures which could help control prawn species substitution, such as microsatellites or forensic trace element analysis, are not in place to determine if the labelling of prawn species and country of origin are correctly stated on traded prawn

commodities. Use of such technologies in conjunction with “unannounced” periodic compliance audits could serve as a regulatory tool to check the validity of importer product claims such as how many batches are in a consignment, or the country of origin of prawns sent for WSSV testing.

## **Conditions related to the import of fresh uncooked / partially cooked prawns to Australia- prior to 6 January 2017 (Detail in Annex 5)**

Whole uncooked or partially cooked prawns can only be imported from countries or zones that are free of all three of the following disease agents:

WSSV, YHV and TSV

Prior to the Biosecurity suspension notice of 6 January 2017 the only country assessed as meeting the above conditions without testing at the border was New Caledonia. Uncooked prawn imports from other countries were subject to testing at the border (13 pools of 5 prawns each per batch within the shipment). Nevertheless, it appears that uncooked prawns that for example tested positive for WSSV could still be sold on the Australian market after further processing (see details in Annex 5 below).

## **Comparison of ALOP for prawns with similar products**

It is apparent when considering the ALOP for uncooked prawns that several other commodities (both animal and plant) have more stringent biosecurity measures in place having regard to a possible incursion by an exotic infectious disease agent. A summary of the differences is shown in the Table 7 below:

*Table 7 Comparison of conditions relating to fresh prawns (Prior to 9 January 2017) and chicken meat, pork, salmon and stockfeed*

<b>Commodity</b>	<b>Pre entry differences</b>	<b>Post entry differences</b>
Chicken meat	The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from Newcastle disease.	Secure transport and handling requirements for post entry cooking at approved processing establishments. If cooking establishment is regionally based, enhanced security requirements.  No imported uncooked product (outside of disease free country (eg New Zealand)) available at retail market level.

Pork (Canada)	A statement that the establishment where the pigs from which the meat was derived were slaughtered, the establishment where the meat was prepared and the establishment where it was stored, all have current approval from the Veterinary Authority of Canada (and therefore Department of Agriculture approval) for their facilities and hygienic operation.	Mandatory cooking on arrival at approved processing establishments.  There is no scope for any uncooked product to move to retail without prior cooking, unless illegal activity occurs.
Salmon (Canada and Denmark)	Have been derived from a population for which there is a documented program of disease surveillance and monitoring administered by the relevant Canadian and Danish agencies.  Product must be washed, gilled, eviscerated and in head-off form.	Post entry control arrangements including, weight and premises approval plus record keeping.  Product prohibited from entering Tasmania and hence geographically separating the main production area for Atlantic Salmon through and additional layer of biosecurity protection enacted by the Tasmanian Government.
Stockfeed	Stock feed import permit applications are subject to a desk audit. Detailed information regarding the stock feed manufacturing process, provision of quality assurance manuals and operating procedures are required to perform the desk audit.  A checklist and details of Department of Agriculture and Water Resources requirements for the desk audit will be forwarded to the applicant by the Plant Import Operations upon receipt of an import permit application. Following a successful desk audit, the manufacturing facility and export pathway may be subject to a Department of Agriculture and Water Resources site inspection.	Typically, stringent post entry requirements have existed with past imports including sanitising by heat or irradiation.

	The importer is responsible for all costs associated with any site inspection (including business class airfares, accommodation, meals and fee for service).	
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All commodities highlighted in the table above have secure post entry requirements designed to increase the level of safety in line with Australia’s conservative ALOP. None rely on sampling to be undertaken by border officers/importers at Australia’s border. None rely on test results at the Australian border in order to gain release, in contrast to uncooked prawns.

The current processes associated with prawn importation do not sit well with the widely held biosecurity principle that safety is enhanced when disease risks are managed before commodities enter Australia.

Current conditions for pig meat, chicken meat, salmon, stockfeed and uncooked prawns are attached as Annexes 1, 2, 3, 4, 5.

## **Importation levels and processes associated with selected imported commodities**

### **Chicken meat**

Effectively due to the stringency of biosecurity conditions relating to chicken meat there are minimal imports. In the ten years to 2010 around 60 tonnes of mainly shelf stable heat treated product was imported (From New Zealand), with around 7,000,000 tonnes produced in Australia during that period. (Source: Australian Chicken Meat Federation (2011)).

### **Pig meat**

During 2016 approximately 160,000 tonnes of pig meat was imported into Australia from a range of countries. The major quantities imported are from Denmark, Canada, USA and Netherlands. (Source: Market News for the Australian Pork Industry). This entire volume was either imported as cooked product or cooked on arrival and the meat may only be sent to processing establishment(s) that have entered into an approved arrangement. If the processing facility is located outside the metropolitan area, transport of pig meat from the wharf or from an approved cold store must be by shipping container or other sealable, secure transport (e.g. Pantech). Heat treatment for pig meat is mandatory and not dependent on any results from a testing regime.

The Interim Inspector-General reviewed the conditions for pork in 2013 (DAFF 2013) and made several observations that are relevant to the present issue with WSSV entry in uncooked prawns. Selected excerpts of the report are inserted below, with the relevant findings highlighted. It appears that the recommendations which were largely generic in nature, such as: regular reviews and staff visits to confirm country claims of freedom from disease were valid; establishment of a random inspection regime; and unannounced audits were not applied across to importation of uncooked prawns.

### ***“Key findings***

11. *DAFF relies heavily on the assurances provided by the competent authorities of exporting countries, importers, quarantine approved premises and facilities operating under compliance agreements that all import conditions have been met. Biosecurity risk management measures identified in the pig meat import risk analysis (DAFF 2004) include such things as country or zone freedom from specified diseases, testing of the carcass, cooking, freezing, curing, canning and removal of certain tissues or parts of the carcass (the head and neck, major peripheral lymph nodes and bones).*

12. *Imported uncooked, cooked and cured pig meats are products with different biosecurity risks and therefore different import requirements. On importation, uncooked pig meat is directed by DAFF to an approved facility for heat processing under specific conditions and biosecurity controls, whereas eligible cooked or cured pig meat can be released after DAFF’s verification of documentation completeness at the border (also dependent on the compliance history of the importer).*

13. *Import consignments of uncooked, cooked and cured pig meat are profiled and targeted by DAFF as part of arrival clearance procedures. This means that all consignments need to be cleared by a DAFF officer. The relevant regional office is responsible for clearing imported cooked and cured pig meat consignments in their region and DAFF’s entry management national coordination centre is responsible for centrally clearing all imported uncooked pig meat consignments.*

14. *The entry management national coordination centre has a well developed approach with good systems and procedures in place for clearing and monitoring the importation of uncooked pig meat. This is evident through the limited number of adverse findings reported during the fieldwork component of this audit.”*

### ***Approved countries***

15. *The IIGB found that DAFF undertakes an adequate, detailed assessment process prior to approving a country to export pig meat to Australia. DAFF monitors the performance of approved countries in reporting World Organisation for Animal Health (OIE) listed diseases, and notifying Australia of changes in disease status. DAFF also investigates any issues of non-compliance or discrepancies in health certification. All approved countries remain under*

general review and approval can be suspended on an emergency basis at any IIGB Audit Report – The effectiveness of controls for imported uncooked, cooked and cured pig meat

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“The IIGB noted DAFF’s advice that under current review arrangements, to date, none of the countries currently exporting pig meat to Australia has required further assessment relating to change in disease status or non-compliances. It is apparent that the system of review of approved country status does leave room for question regarding its robustness, including documentation of procedures.

16. In view of the biosecurity risk status of imported pig meat, the increased levels of imports and the essential reliance placed on approved countries to ensure imported pig meat meets Australia’s import requirements, the IIGB noted that DAFF should develop a formal, cyclical review process for approved countries. This should include some periodic visits to selected countries for inspection of control systems and a sample of approved export facilities. This process will enhance assurance in the DAFF control system for pig meat and more tangibly reinforce good governance commitment.

19. As DAFF services are focused on a risk return approach, a lack of complete, accurate and useable data will make it difficult to make informed decisions on risk. The risk return approach is based on and underpinned by quality data and information. Risks are evaluated using scientific and economic consequence evidence and projections as well as operational experience. Under the risk return model, resources, including staff, are allocated to areas that pose the highest biosecurity risk.

22. The IIGB noted that no cartons are opened and inspected by DAFF to ensure that they contain the correct product. In biosecurity risk management, tracking cartons, rather than the product places complete reliance on exporter-importer supply chain integrity and vigilance of export certification authorities to ensure the correct type of pig meat is contained within. The IIGB noted that the establishment of a random inspection regime undertaken by DAFF that involves a visual assessment of a sample of cartons would enhance biosecurity risk management.

#### **Audit requirements for processors operating under a compliance agreement**

23. DAFF’s audit policy for pig meat processors operating under a compliance agreement is detailed in the operational procedures statements for the processing of imported uncooked pig meat. DAFF officers are required to conduct a minimum of three formal audits annually to monitor the compliance. At least one of these formal audits will be unannounced. The IIGB found that the processors operating under compliance agreements in Adelaide did not have an unannounced audit for 2012. The last unannounced audits for these facilities were conducted in June 2011.”

## Salmon

The conditions for the import of fresh salmon include:

- A statement that the intended end use for salmon is one of the following:
  - Sale for commercial processing, or
  - For processing for direct retail/food service sale/use, or
  - For direct retail/food service sale/use.
- A statement that the fish and/or fish products are processed to at least a gilled, eviscerated and head-off form.
- A statement that the fish and/or fish products are packaged by one of the following methods:
  - Individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
  - Loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less, or
  - Loose packed in cartons weighing greater than 27.3 kg (60 lbs).

## Stockfeed and grain

Stockfeed and grain have stringent conditions related to their import into Australia reflecting viral and fungal disease risks as well as weed seed contamination risks. Post entry security is extremely high, particularly if product is to be moved to a rural area for processing. The Inspector General of Biosecurity reviewed the effectiveness of biosecurity controls for importing stockfeed of plant origin in 2014 (IIGB 2015) and recommended:

- The department should review and update its import risk analysis and/or policies for importing plant-based stockfeed into Australia.
- The department's Biosecurity Plant and Biosecurity Animal divisions should jointly undertake desk and verification audits of overseas stockfeed manufacturing establishments to facilitate more effective pre-border compliance and reduce the risk of both plant and animal disease incursions.
- The department should consider implementing a programme of strategically targeted audits of overseas manufacturing facilities and export pathways for plant-based stockfeed. These audits should focus attention and resources on identified areas of higher risk and ensure that compliance is further promoted.
- The department should (consistently across all ports) routinely record the import permit number, consignment description and inspection and testing outcomes (where applicable) of all bulk and bagged stockfeed consignments imported into Australia. These data should be available to relevant areas in the department to establish whether policy and regulation are effectively addressing biosecurity risks.
- The department should centrally process all entries for 'bulk in ships' holds' stockfeed imports at the Bulk Commodities National Coordination Centre, Newcastle.
- The department should maintain ongoing testing for animal biosecurity risk material at an appropriate level and record results in a central register that is accessible to the department's policy and operational staff.

- The department should finalise the job card and a staff training package for inspectors of imported stockfeed within the next six months. The training package should include a step-by-step procedure for recording (in the Department of Agriculture Import Management System, AIMS) details of each stockfeed consignment imported into Australia. These details should be available for analysis and policy development.

The Department agreed with all recommendations.

## **Uncooked prawns**

Fresh or frozen uncooked prawns are imported into Australia subject to country freedom from a range of serious viral diseases: White spot syndrome virus (WSSV); Yellowhead virus (YHV); and Taura syndrome virus (TSV); and in addition, Necrotising hepatopancreatitis bacterium (NHPB) provided the product is not frozen.

Product from countries which have not declared freedom from WSSV could be released for retail sale following the return of negative test results from sampling undertaken by border officers and sent to accredited laboratories.

The stated aim of the testing was to achieve detection with 95% confidence of batches of prawns which contained a prevalence >5% of WSSV. Thirteen batches of five prawns are sampled. The assumptions which appear to underlie this aim are that test sensitivity is 90% and test specificity is 100%<sup>4</sup>. And that sampling of prawns is random. That is, every prawn in every box within the container has an equal chance of being selected for testing. It is apparent from the Senate Additional Estimates hearing on 28<sup>th</sup> February 2017, that this is not how the sampling protocol was being operated prior to the outbreak. Details on the heightened surveillance methods were not made available by DAWR, so this sampling issue may have been corrected. The impact of the lack of randomisation of sample collection on the testing regime is likely to have reduced confidence that all >5% prevalence batches would be detected, prior to release to retail.

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<http://epitools.ausvet.com.au/content.php?page=FreeCalc2&Option=0&N=10000&Sens=0.90&Spec=1&prevty pe=1&prev=0.05&type1=0.05&type2=0.05&analysis=0&popthreshold=10000&maxSS=3200&Prec=4&SampleSi ze=1&Pos=1>

Minimal conditions exist post negative testing through to retail sale. Controls exist over positive tested product where the product must be treated in an approved establishment or exported back out of Australia.

WSSV positive uncooked prawns are allowed entry provided they are processed offshore into marinated or breaded products, which are deemed highly processed, which may then proceed into the Australian retail market. It was found through sampling in this project that some product such as breaded uncooked prawns are at times so lightly breaded as to appear no different to an uncooked, unbreaded product. This appears to be a labelling loophole that could be exploited to facilitate the entry of WSSV positive uncooked prawn into the unprocessed uncooked prawn retail market.

Non-compliance with the marinade requirements for a minimum 12% by weight of product to be marinade was detected in samples purchased for this project, illustrating the difficulty in maintaining such regulations.

Reliance on such measures to lower risk of an incursion, or reduce risk of product diversion into use as bait by anglers appears inappropriate. Similar risks were reported anecdotally, but unable to be confirmed, exist with marinated uncooked prawns, which can potentially have the marinade washed off after entry, prior to prawns being repacked and moved into Australian retail outlets as uncooked unprocessed prawn. An example of a “lightly” breaded product is illustrated below.



Figure 5: Product labelled breaded (upper image), but upon closer inspection appears to have little if any bread on the prawns (lower image)

## **Risk comparison across chicken meat, pork, salmon, stockfeeds / grain and uncooked prawns**

Knowledge of the presence of viable exotic viruses in prawn commodity imports to Australia is longstanding (McColl et al 2004) and was subject to a full risk analysis. Similar risks exist in chicken, pork, salmon and stockfeeds. The risks of uncooked imported prawn product being released into the environment is very high given the proven propensity for the product to be used as bait by recreational anglers (Kewagama Research 2002, 2007).

The significance of this risk pathway appears to have been understated during the 2009 IRA process and not fully reflected upon within the 2010 Interim Inspector-General of Biosecurity examination of what caused a consignment of imported raw peeled prawns that tested positive for White Spot Syndrome Virus (WSSV) to be mistakenly released into Australia by the Biosecurity Services group (BSG).

Survey results in the “National Survey of Bait and Berley use by Recreational Fishers in 2002” (Kewagama Research 2002) suggest using uncooked human consumption seafood grade prawns for bait represented only 6% anglers. Later research (Kewagama Research 2007) suggested the rate had risen to 7.9% associated with enhanced availability/convenience. The authors estimated that the volume of potentially imported human consumption seafood grade prawn being used as bait had risen by 225% by 2006.

These figures reflected survey results at the time, but with the relative increase in price of bait prawns it is unlikely to be reliable as a long-term indicator that imported uncooked prawns are a low probability of entering a high-risk release pathway- such as being diverted to be used as bait in a river adjacent a prawn farm. Prior to the trading halt in January 2017, imported uncooked prawns could be purchased at major supermarkets for significantly less than the price of pre-packed bait prawns from Tackle Shops and Service Station bait outlets. There are more supermarket outlets offering these uncooked products also and greater urban populations which continue to develop in the catchment of the Logan River, near to the Logan River prawn farms. There are multiple high volume boat ramps available for anglers to access the Logan River, with large car parking facilities. Collectively these factors have altered the risk since 2009.

The import conditions state that uncooked unprocessed prawn products must be labelled with 'for human consumption only' and 'not to be used as bait or feed for aquatic animals.'

Numerous uncooked and unprocessed prawn products were purchased from delicatessen windows which contained no such labelling, in addition to some pre-packed frozen commodities Figure 6: Retail purchased imported (Product of Indonesia) peeled prawn meat- with non-compliant labelling with respect to use as bait.

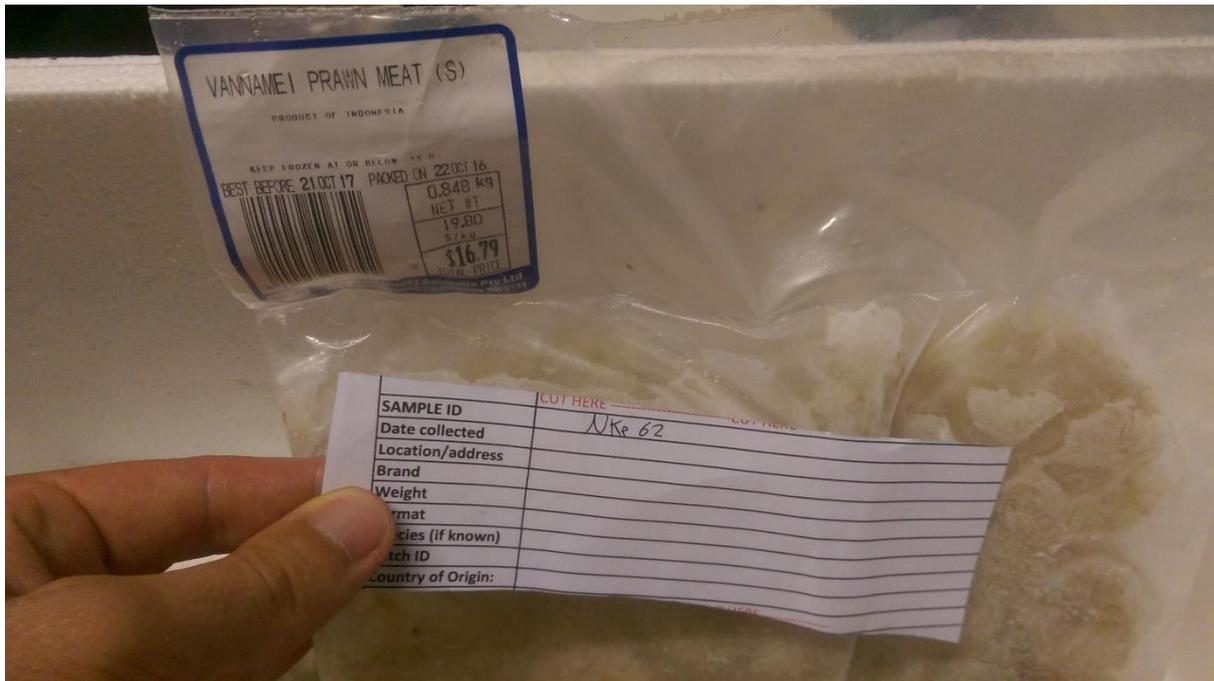


Figure 6: Retail purchased imported (Product of Indonesia) peeled prawn meat- with non-compliant labelling with respect to use as bait





Figure 7: Supermarket purchased uncooked imported prawns- with non-compliant labelling with respect to use as bait

Even if these measures were enforced, the responses of the Kewagama Research 2007 report suggest it would make little difference to angler's diversion of these products into use as bait. The survey recorded 85.6% of angling respondents had no awareness of this regulatory requirement.

In 2010 the Interim Inspector-General reviewed that the accidental release of a container of WSSV positive prawns into the Australian retail market DAFF (2010). His assessment was:

*“there is an **extremely low** likelihood of the raw peeled prawns entering high risk pathways and a **negligible** likelihood of them then causing WSSV to establish in Australia”*

It is unclear what evidence formed the basis to this conclusion. Should this conclusion have maintained currency, without deeper questioning, into the present DAWR investigations of entry of WSSV positive material into Australian retail as part of Project Cattai from March 2016, it may explain the absence of regulatory action to instigate product recall in response to the widespread retail detection of WSSV in June 2016.

The level of risk permitted in imported prawns is considered higher than for pork, chicken meat or salmon, as with those commodities reliance is more directed to product discarded and this will predominantly follow cooking. With pig meat and chicken meat Australia's production is largely sourced from biologically secure facilities. As such although feral animals are a genuine concern as far as potential spread is concerned, the risk compared to prawn diseases being introduced in waterways is significantly reduced. Salmon is unlikely to represent as significant a risk as prawns given the product is invariably cooked.

The likelihood of eradication of aquatic diseases from open wild water systems which contain many potential carrier species is remote, hence the consequences of an incursion are actually higher, and likely longer lasting than any land based incursion relating to chicken meat or pig meat.

The persistence of WSSV in wild populations is documented in several studies (Pena et al 2007, Powell et al 2015, Chapman et al 2004).

The 2013 Interim Inspector-General report on pork (DAFF 2013), noted that DAFF (now DAWR) had found overseas authorities were reporting appropriately on OIE listed pig diseases. An online review of recent OIE data on crustacean diseases for calendar year 2016 suggests that several countries may not be capturing their outbreaks and reporting them through the OIE system. A paucity of information makes it difficult to determine if there had been an elevation in risk from the exporting countries prior to Australia’s 2016 outbreak.

Table 8 below was assembled from searches on the OIE website for the period of Jan-Dec 2016 and illustrates all of the reports of White spot disease (WSD) outbreaks. Notably some countries where WSD is considered endemic apparently failed to report any outbreaks including Malaysia, Indonesia, Philippines and China during the last 12 months. It may be that no outbreak occurred in these countries for this time period. This contrasts strikingly with reports from Vietnam, which appear to represent a different level of internal reporting. Notably the retail test results from this project identified WSSV positive commodities from China, Malaysia, Indonesia and Vietnam.

*Table 8 Reporting of WSD outbreaks by OIE member countries in 2016*

<b>Country</b>	<b>Number of incidents</b>
Chinese Taipei	34
Iran	3
Japan	3
Singapore	2
Thailand	3
Vietnam	2711
Brazil	+ Mar-April 2016
Costa-Rica	+ Mar-Apr 2016
Honduras	5
Ecuador	0
Mexico	6
Panama	+ both halves of 2016
Australia	1
India	None reported

Indonesia	None reported
China	None reported
Malaysia	None reported
Philippines	None reported
Myanmar	None reported

Evidence has not been presented that uncooked salmon is utilised by recreational fishers as a bait source, unlike uncooked prawns. Measures such as the removal of the head and viscera of salmon are effective at reducing some of the pathogen loads for that species. Unfortunately, such processing measures for prawns do not make a considerable difference to the potential infectivity of prawns infected with WSSV, as there can be sufficient virus in the tail muscle meat of infected individuals to transmit the disease to other prawns (Oidtmann and Stentiford 2011). Positive PCR results for TSV and IHNV have been reported in imported tail meat in Australian retail (Ueda et al 2008)

The introduction of exotic diseases of salmon is further mitigated by Tasmanian controls which do not permit the importation of salmon. There are no additional State based biosecurity protections in place for the prawn farming industry (in NSW, Qld, NT, WA) to protect them from incursion of exotic disease via imported uncooked prawn commodities.

The list of serious and emerging prawn diseases has expanded since the 2009 IRA to include such diseases as Acute hepatopancreatic necrosis disease (AHPND, formerly known as EMS), Yellowhead Virus -1 (YHV1) and *Enterocytozoon hepatopenaei* (EHP). These pose significant threats to Australia's prawn farming sector. The risks associated with the trade of uncooked prawns has not been assessed and updated to reflect these new threats.

The risk of uncooked prawn imports is more comparable to stockfeed, where invariably the product is transported through rural areas. However, the level of sanitary measures in place prior to this movement are higher on stock feeds, compared to uncooked prawns.

## **Conclusion**

Overall the ALOP afforded to prawns (and salmon) seems to have been considered significantly lower by comparison with pork, chicken meat and stock feed. Such a disparity within Australian commodity imports is highly undesirable

## **Measures which could alter risk profile of the trade in imported uncooked prawns into Australia**

### **a) Review the IRA for prawns and crustacea**

Numerous risks have shifted within existing commodities and consumer behaviours since the 2009 IRA. Additionally, new prawn diseases have continued to emerge which warrant thorough assessment.

New technologies have also emerged that may assist in better mitigating illegal activities such as product substitution and mislabelling country of origin.

The extent of issues which have been identified over the last 12-18 months in the imported prawn trade seem sufficient grounds for DAWR to activate its provision to review the import protocols.

### **b) Enhanced surveillance**

Reports from DAWR through the prawn liaison officer signalled that enhanced surveillance would be undertaken on imported containers of prawns, after the outbreak of WSSV on the Logan River. These measures appear to have substantially increased the detection rate of positive containers from <15% to >50%.

This likely signals that the previous level of surveillance was substantially underperforming its target of identifying WSSV at a prevalence of above 5%.

The survey results from product purchased in January 2017, indicate that even with enhanced surveillance and product recalls, there was uncooked imported WSSV positive product available for retail purchase.

Future surveillance of the prawn trade could consider implementing the recommendations of the Interim Inspector-General's report into the pork trade in 2013 including:

- regular reviews and staff visits to confirm country claims of freedom from disease
- establishment of a random inspection regime;

-unannounced audits of importers facilities

### **c) Mandatory cooking/gamma-irradiation**

Heat through cooking and to a lesser extent, gamma-irradiation, have been documented to destroy the infectivity of prawn viruses including WSSV. Cooking is considered effective for pork and chicken viral pathogens, which underpins the trade arrangements for these products from disease positive countries, to only be allowed entry to retail if cooked.

Offshore cooking or irradiation (pending further confirmatory research) would be preferable to reduce handling risks of uncooked product within Australia, and minimise risk of illegal leakage of uncooked imported prawn into the Australian retail market.

Cooking/gamma irradiation in an approved premise within Australia, would be analogous to arrangements for imported pork products, such that no uncooked product is permitted entry to retail.

### **d) Implement forensic trace element analysis**

Sample randomly through every container. Such testing could confirm the validity of claims about the number of batches per container, and provide some confidence around the country of origin claims from the paperwork. Testing costs should be fully cost recovered from importers.

### **e) Unannounced independent retail testing for exotic pathogens**

Fully cost-recovered by levy on prawn importers, to ensure that risks in imported commodities are at levels anticipated by the current IRA

### **f) Alter definition of highly processed prawns**

Presently the action of processing a prawn by breaching it, or marinating it, can facilitate illegal activity to bypass the intended biosecurity barrier. Both breaching and marinade can be washed off and product re-packaged. There is no scientific evidence that either breaching or marinating are sufficient sanitary measures to inactivate WSSV or other prawn pathogens. Nor is there evidence that such processing entirely prevents the use of these commodities as bait or burley by recreational fishers.

Definitions of permitted processed products should only include those product types which cannot be modified upon entry to Australia to return to an uncooked prawn meat product, which could in turn be diverted into the recreational bait release pathway.

### **g) Develop National WSSV strategy in response to incursion**

The widespread detection of WSSV retail outlets and the apparent cross-contamination onto Australian origin prawns at retail suggest a national response is necessary, in addition to the review of the IRA. This will include: ongoing eradication and containment of the outbreak; surveillance for freedom from disease in zones outside of the infected area; IRA review; review bait biosecurity including potential for diversion of product intended for human consumption into the bait or berley use pathway; and development of strategies to control risks associated with mixing of imported and domestic commodities at the point of retail.

## **Recommendation**

It is the view of the author that taking into consideration all of the available controls, the most cost effective measure to reduce risk to an acceptable level, in line with other commodities ALOP, is to require mandatory cooking or sufficient irradiation to achieve disinfection of the commodity. This measure leaves the least room for further illegal activity to divert high risk WSSV carrying uncooked prawns into Australian retail outlets, and thereby removes the risk of such potentially infectious product being released into Australian waterways.

Concurrently, there is clearly a case for a complete review of the 2009 IRA to take into account the serious weaknesses identified.

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## **Annex 1 Chicken Meat Requirements from IRA (Newcastle Disease Specific)**

3.2.1 The chickens from which the meat was derived have been continuously resident in the source country since hatching and were slaughtered on ..... (dates).

In the case of Newcastle Disease

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from Newcastle disease.

**AND**

Any live vaccines used on chickens from which the meat was derived were produced from lentogenic strains of Newcastle disease virus.

**OR**

(ii) The chicken meat has been processed to ensure the destruction of the Newcastle disease virus and has been heated to a minimum core temperature of 70 °C for at least 8.2 minutes (or time/temperature equivalent).

Similar requirements apply for other significant exotic avian diseases and a further requirement is:

Where chicken meat is identified as requiring processing in Australia, as specified in 3.3, the following conditions apply. **(Note this condition does not appear in 2009 prawn conditions)**

4.1 The chicken meat and its derivatives must be securely transported from the port of entry to the approved storage establishment(s) thence to the processing establishment(s) and finally, with respect to inadequately processed surplus wastes and by-products, to the place(s) of disposal of quarantinable waste. The transport of imported chicken meat will require appropriate security arrangements to prevent spillage (e.g. refrigerated container) and be transported by the most appropriate route as determined by AQIS.

The meat must be processed in accordance with an approved AQIS arrangement. The approved AQIS arrangement also covers such things as disposal of packaging, wastewater and trimmings.

## Annex 2 Fresh Pork Canadian Import Requirements

Case: Uncanned or unretorted meat for human consumption Effective: 08 Feb 2017

Other — Pork — Other — Country of origin is Canada — Uncooked

### Import Conditions

The following Import Conditions are applicable to this Import scenario. The department will assess the suitability of your import against the import conditions.

- a. Prior to the importation of [goods](#) into Australia, a valid import permit issued by the Department of Agriculture and Water Resources is required.

An import permit may be obtained by submitting an [import permit application](#) to the department (if viewed online, use the 'Apply Now' button at the bottom of this page).

If the import conditions cannot be met then a permit application for a non-standard commodity will be required. Note: additional information (e.g. product names, approved arrangement sites) will not be considered during permit assessment or included in the standard permit conditions.

- b. These import conditions apply to the importation of pig meat that is uncooked but is to be directed for cooking in Australia at a processing establishment.
- c. **Conditions of Administration**
  1. Documents must be provided with each consignment which:

1.1. identify the consignment e.g. entry number

1.2. identify all goods being imported as part of this consignment e.g. invoice or waybill or importer's manifest

1.3. describe the goods being imported (where not clear). Example 1: Product X Rab = Purified protein derived from rabbits. Example 2: Product AX = Synthetic antibiotic. Example 3: Comte = Cheese.

2. For further information please contact:

Regional - Clearance assistance:  
<http://www.agriculture.gov.au/about/contactus/phone/regional>

Canberra - Administrative assistance or technical assistance: email [imports@agriculture.gov.au](mailto:imports@agriculture.gov.au) or phone 1800 900 090

- d. **No composite products**

These conditions do not allow for the importation of composite products.

For the purpose of these conditions, a composite product is a product that contains the commodity as an ingredient, mixed together with other ingredients.

- e. The meat must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Official Government Certificate](#):

1. The names and veterinary approval numbers of:
  - 1.1 the establishment at which the animals from which the meat was derived were slaughtered
  - 1.2 the cutting-up establishment at which the meat was prepared
  - 1.3 the establishment at which the meat was stored prior to export.
2. The names and addresses of the exporter and the consignee.
3. A statement that the pigs from which the meat was derived have been continuously resident in Canada and/or the United States (USA) since birth and were slaughtered on [*insert dates*].
4. A statement that the pigs from which the meat was derived passed ante-mortem and post-mortem veterinary inspection under official veterinary supervision; the meat was considered to be fit for human consumption.
5. A statement that Canada is free from:
  - 5.1 Foot and mouth disease (without vaccination)
  - 5.2 African swine fever
  - 5.3 Classical swine fever
  - 5.4 Swine vesicular disease
  - 5.5 Aujeszky's disease
  - 5.6 Nipah virus
  - 5.7 Vesicular exanthema.
6. A statement that the meat has not been derived from the head or neck (Note: This includes all head neck tissues cranial to the fourth cervical vertebrae but does not include shoulders).
7. A statement that the major peripheral lymph nodes have been removed (Note: This includes popliteal [if present], iliac, inguinal, axillary [if present], ventral, middle and dorsal superficial cervical).
8. A statement that the meat has been de-boned.
9. A statement that the establishment where the pigs from which the meat was derived were slaughtered, the establishment where the meat was prepared and the establishment where it was stored, all have current approval from the

Veterinary Authority of Canada (and therefore Department of Agriculture approval) for their facilities and hygienic operation.

10. A statement that Officials of the Veterinary Authority of the source country were present in plants at all times when pigs were being slaughtered for export to Australia.
11. A statement that the establishment where the meat was prepared did not prepare or process pig meat not eligible for export to Australia while pig meat was being prepared for export to Australia.
12. A statement that the meat has been prepared for export and packed on [*insert dates*], and the bags, wrappers or packaging containers were clean and new.
13. A statement that the identification number of the establishment where the meat was prepared is readily visible on the meat or, where the meat is wrapped or packed, was marked on the package or wrapping containing the meat, in such a way that the numbers cannot readily be removed without damaging the meat, package or wrapping.
14. A statement that the meat was not exposed to contamination prior to export.
15. A statement that the meat is being transported to Australia in a clean packing container sealed with a seal bearing the number or mark [*insert number or mark*]; and the container contains only meat eligible for entry into Australia.

**f. Post Entry Requirements**

1. Copies of the import permit and relevant quarantine directions must accompany each consignment of imported pig meat and its derivatives during transport to both storage and processing establishments, and until the meat has been adequately processed.
2. If the processing facility is located outside the metropolitan area, transport of pig meat from the wharf or from an approved cold store must be by shipping container or other sealable, secure transport (e.g. pantech).
3. Potentially suitable control systems may include leak-proof packing containers sealed with a numbered, tamper-proof seal at the point of origin for removal and retention at the point of destination. Alternatively, a system based on despatch and receipt weights may be used to accurately account for control of the product. The transport of imported pig meat outside urban areas associated with the Australian first point of entry will require appropriate security arrangements to prevent spillage (e.g. refrigerated container) and be transported by the most direct route.
4. The meat may only be sent to processing establishment(s) that have entered into an approved arrangement (class 2.8 or 2.52) under the *Biosecurity Act 2015*.
5. Meat must be processed by heating to a minimum core temperature of at least:

56 °C for 60 minutes, or

57 °C for 55 minutes, or

58 °C for 50 minutes, or

59 °C for 45 minutes, or  
60 °C for 40 minutes, or  
61 °C for 35 minutes, or  
62 °C for 30 minutes, or  
63 °C for 25 minutes, or  
64 °C for 22 minutes, or  
65 °C for 20 minutes, or  
66 °C for 17 minutes, or  
67 °C for 15 minutes, or  
68 °C for 13 minutes, or  
69 °C for 12 minutes, or  
70 °C for 11 minutes.

Note: The minimum core temperatures shown above must be maintained continuously for the minimum times stipulated.

6. Following its release from biosecurity control, the processed product can then be distributed solely for the end use of human consumption.
7. All solid waste material associated with imported pig meat must be treated as quarantine waste and disposed of by one of the following methods:
  - 7.1. high temperature incineration, or
  - 7.2. deep burial, or
  - 7.3. autoclaving (121 °C core temperature for 15 minutes) prior to disposal as general waste (landfill), or
  - 7.4. heat treatment on-site to a minimum core temperature of 100 °C for 30 minutes prior to disposal as general waste (landfill), or
  - 7.5. other method approved in writing by the Department of Agriculture.

Note: Solid waste materials must not be on-sold, recycled or dealt with in any other way or for any other purpose, unless specifically approved in writing by the Department of Agriculture.

8. Solid waste is any material not destined for human consumption. This includes (but is not restricted to) trimmings, spoiled product, contaminated cartons, contaminated wrapping, sludge and any processed product that cannot be distributed solely for the end use of human consumption.
9. Liquid waste must be disposed of by direction to the municipal sewerage system or other method approved in writing by the Department of Agriculture.
- g. The product may also be imported from various suppliers/exporters in the USA.
- h. These conditions prohibit the goods or any derivatives, to be distributed, sold or used for:
  1. veterinary or agricultural purposes including stock feed
  2. veterinary therapeutic or vaccine manufacture
  3. environmental use associated with livestock
  4. aquaculture/fisheries or for bait purposes.

## Annex 3 Fresh Salmon from Canada BICON Requirements

Case: Finfish for human consumption Effective: 28 Jan 2017

**Salmonidae — Not canned or retorted — Not for personal use — Sourced, processed and exported from Canada — Standard permit conditions**

### Import Conditions

The following Import Conditions are applicable to this Import scenario. The department will assess the suitability of your import against the import conditions.

- a. Prior to the importation of [goods](#) into Australia, a valid import permit issued by the Department of Agriculture and Water Resources is required.

An import permit may be obtained by submitting an [import permit application](#) to the department (if viewed online, use the 'Apply Now' button at the bottom of this page).

If the import conditions cannot be met then a permit application for a non-standard commodity will be required. Note: additional information (e.g. product names, approved arrangement sites) will not be considered during permit assessment or included in the standard permit conditions.

- b. For a list of scientific names for Department of Agriculture and Water Resources approved finfish in family *Salmonidae*, please refer to the [family Salmonidae list](#). Only the listed finfish may be imported using the import permit.

- c. **Conditions of Administration**

1. Documents must be provided with each consignment which:

- 1.1. identify the consignment e.g. entry number

- 1.2. identify all goods being imported as part of this consignment e.g. invoice or waybill or importer's manifest

- 1.3. describe the goods being imported (where not clear). Example 1: Product XRab = Purified protein derived from rabbits. Example 2: Product AX = Synthetic antibiotic. Example 3: Comte = Cheese.

2. For further information please contact:

Regional - Clearance assistance:  
<http://www.agriculture.gov.au/about/contactus/phone/regional>

Canberra - Administrative assistance or technical assistance: email [imports@agriculture.gov.au](mailto:imports@agriculture.gov.au) or phone 1800 900 090

- d. **No composite products**

These conditions do not allow for the importation of composite products.

For the purpose of these conditions, a composite product is a product that contains the commodity as an ingredient, mixed together with other ingredients.

- e. The fish must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Official Government Certificate](#):

Statements that the salmon and/or salmon products:

1. Have been processed in a processing plant that is operating a Quality Management Program audited by the Canadian Food Inspection Agency, in accordance with the Government of Canada's Fish Inspection Regulations.
2. Have been thoroughly washed, inspected and graded under the processing plant's Quality Management Program.
3. Are free from visible lesions associated with infectious disease and is fit for human consumption.
4. Are not juvenile salmonids or sexually mature adult salmonids.
5. Have not been derived from a population slaughtered as an official disease control measure.
6. Have been derived from a population for which there is a documented program of disease surveillance and monitoring administered by the Canadian Food Inspection Agency.
7. (for Atlantic salmon and rainbow trout only) did not come from a farm infected by or officially suspected of being infected by ISAV, or from waters within 10 kilometres or one tidal interchange (whichever is greater) of an infected farm.

Certification issued by the competent authority of the country of origin.

- f. The fish must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Official Government Certificate](#):

1. A statement that the fish and/or fish products are processed to at least a gilled, eviscerated and head-off form.
2. A statement that the fish and/or fish products are packaged by one of the following methods:
  - 2.1 individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
  - 2.2 loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less, or
  - 2.3 loose packed in cartons weighing greater than 27.3 kg (60 lbs).

Certification issued by the competent authority of the country of origin.

- g. The [competent authority](#) of the country of origin is recognised by the Department of Agriculture and Water Resources.
- h. The salmon product must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Importer declaration](#):

A statement that the intended end use for salmon is one of the following:

- 1. sale for commercial processing, or
  - 2. for processing for direct retail/food service sale/use, or
  - 3. for direct retail/food service sale/use.
- i. The conditions for salmon and/or salmon products end uses are as follows:
    - 1. Imported salmon intended for direct retail/food service sale/use can be sold to any retailer. These retailers are not required to be registered with the Department of Agriculture. Where an importer stores product that is intended for direct retail/food service sale/use, there is no requirement for the Department to be notified when that product is distributed.

Retail processors may only receive fish or fish pieces:

- 1.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
- 1.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less.

- 2. Imported salmon intended for retail processing (where the amount processed will be less than 300 kg per day) must only be sold to a premises that is registered with the department\*. The importer must nominate registered buyer(s) at the time of import. If the imported salmon has not been sold, the quarantine entry will remain open and the importer must advise the department of the registered buyer before or at the time that the product is sold. It is the importers responsibility to advise the department of the sale of all imported salmon in each consignment, which will allow the Department to finalise the quarantine entry.

Retail processors may only receive fish or fish pieces:

- 2.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
- 2.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less.

\*Premises that are processing imported product for retail sale must be registered with the department. Registration of premises does not require prior inspection and no fees will be charged for the registration. Registration

forms are available on the [Department of Agriculture and Water Resources Biosecurity website](#) or alternatively from the [Import Services Team](#).

3. Imported salmon intended for commercial processing or processing for direct retail/food service sale/use (where the amount processed will be more than 300 kg per day) must be sold to a processor that has entered into an approved arrangement with the department. Products that are intended for commercial processing may not be stored by the importer (this does not apply if the importer is the commercial processor).

These processors can receive fish or fish pieces:

- 3.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
  - 3.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less, or
  - 3.3. loose packed in cartons weighing greater than 27.3 kg (60 lbs).
- j. The importer must keep records (including the name and department registration number of purchaser (if relevant), date of sale and quantity (weight) of salmon sold) for all imported salmon. The department may audit these records at any time. If the end use of the imported salmon changes from that in the original declaration to the department, the importer must notify (within 24 hours) the department's [Import Services Team](#).
  - k. Once biosecurity requirements have been met, it is the importers responsibility to ensure that all [imported food](#) complies with the [Imported Food Control Act 1992](#) including the [Australia New Zealand Food Standards Code](#).
  - l. Non-commodity information requirements for imported cargo also apply, please refer to the BICON case [Non-Commodity Cargo Clearance](#).

#### **Case: Finfish for human consumption Effective: 28 Jan 2017**

#### **Salmonidae — Not canned or retorted — Not for personal use — Sourced, processed and exported from Denmark — Standard permit conditions**

#### **Import Conditions**

The following Import Conditions are applicable to this Import scenario. The department will assess the suitability of your import against the import conditions.

- a. Prior to the importation of [goods](#) into Australia, a valid import permit issued by the Department of Agriculture and Water Resources is required.

An import permit may be obtained by submitting an [import permit application](#) to the department (if viewed online, use the 'Apply Now' button at the bottom of this page).

If the import conditions cannot be met then a permit application for a non-standard commodity will be required. Note: additional information (e.g. product names, approved arrangement sites) will not be considered during permit assessment or included in the standard permit conditions.

- b. For a list of scientific names for Department of Agriculture and Water Resources approved finfish in family *Salmonidae*, please refer to the [family Salmonidae list](#). Only the listed finfish may be imported using the import permit.

c. **Conditions of Administration**

1. Documents must be provided with each consignment which:

1.1. identify the consignment e.g. entry number

1.2. identify all goods being imported as part of this consignment e.g. invoice or waybill or importer's manifest

1.3. describe the goods being imported (where not clear). Example 1: Product XRab = Purified protein derived from rabbits. Example 2: Product AX = Synthetic antibiotic. Example 3: Comte = Cheese.

2. For further information please contact:

Regional - Clearance assistance:  
<http://www.agriculture.gov.au/about/contactus/phone/regional>

Canberra - Administrative assistance or technical assistance: email  
[imports@agriculture.gov.au](mailto:imports@agriculture.gov.au) or phone 1800 900 090

d. **No composite products**

These conditions do not allow for the importation of composite products.

For the purpose of these conditions, a composite product is a product that contains the commodity as an ingredient, mixed together with other ingredients.

- e. The fish must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Official Government Certificate](#):

Statement that the salmon and/or salmon products:

1. Were processed in premises under the general supervision of the Danish Veterinary and Food Administration, in accordance with the health rules laid down by Regulations (EC) No. 852/2004, (EC) No. 853/2004 and (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004,
2. are headed, gilled, gutted and the external and internal surface thoroughly washed,

3. are individually inspected and graded, ensuring the product for export is free from visible lesions associated with infectious disease,
4. are fit for human consumption,
5. are sexually immature, or sexually maturing but not sexually mature (not fully developed roe),
6. are not juvenile salmonid fish,
7. were harvested from a population for which a documented health surveillance programme exists which is administered by the Danish Veterinary and Food Administration, (Note this is not a requirement for fresh farmed prawns)
8. Were not slaughtered as an official disease control measure as a result of an outbreak of disease.

Certification issued by the competent authority of the country of origin.

- f. The fish must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Official Government Certificate](#):

1. A statement that the fish and/or fish products are processed to at least a gilled, eviscerated and head-off form.
2. A statement that the fish and/or fish products are packaged by one of the following methods:
  - 2.1 individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or
  - 2.2 loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less, or
  - 2.3 loose packed in cartons weighing greater than 27.3 kg (60 lbs).

Certification issued by the competent authority of the country of origin.

- g. The salmon product must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an [Importer declaration](#):

A statement that the intended end use for salmon is one of the following:

1. sale for commercial processing, or
  2. for processing for direct retail/food service sale/use, or
  3. for direct retail/food service sale/use.
- h. The [competent authority](#) of the country of origin is recognised by the Department of Agriculture and Water Resources.
- i. The conditions for salmon and/or salmon products end uses are as follows:

1. Imported salmon intended for direct retail/food service sale/use can be sold to any retailer. These retailers are not required to be registered with the Department of Agriculture. Where an importer stores product that is intended for direct retail/food service sale/use, there is no requirement for the Department to be notified when that product is distributed.

Retail processors may only receive fish or fish pieces:  
1.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or  
1.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less.

2. Imported salmon intended for retail processing (where the amount processed will be less than 300 kg per day) must only be sold to a premises that is registered with the department\*. The importer must nominate registered buyer(s) at the time of import. If the imported salmon has not been sold, the quarantine entry will remain open and the importer must advise the department of the registered buyer before or at the time that the product is sold. It is the importers responsibility to advise the department of the sale of all imported salmon in each consignment, which will allow the Department to finalise the quarantine entry.

Retail processors may only receive fish or fish pieces:  
2.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or  
2.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less.

\*Premises that are processing imported product for retail sale must be registered with the department. Registration of premises does not require prior inspection and no fees will be charged for the registration. Registration forms are available on the [Department of Agriculture and Water Resources Biosecurity website](#) or alternatively from the [Import Services Team](#).

3. Imported salmon intended for commercial processing or processing for direct retail/food service sale/use (where the amount processed will be more than 300 kg per day) must be sold to a processor that has entered into an approved arrangement with the department. Products that are intended for commercial processing may not be stored by the importer (this does not apply if the importer is the commercial processor).

These processors can receive fish or fish pieces:  
3.1. individually packaged in plastic sleeves, pouches or other packaging, in cartons of any weight, or  
3.2. loose packed within a plastic bag in cartons weighing 27.3 kg (60 lbs) or less, or  
3.3. loose packed in cartons weighing greater than 27.3 kg (60 lbs).

- j. The importer must keep records (including the name and department registration number of purchaser (if relevant), date of sale and quantity (weight) of salmon sold) for all imported salmon. The department may audit these records at any time. If the end use of the imported salmon changes from that in the original declaration to the department, the importer must notify (within 24 hours) the department's [Import Services Team](#).
- k. Once biosecurity requirements have been met, it is the importers responsibility to ensure that all [imported food](#) complies with the [Imported Food Control Act 1992](#) including the [Australia New Zealand Food Standards Code](#).

All documentation presented to the department to assist in determining the level of biosecurity risk posed by transportation pathways and packaging must also meet the requirements of the non-commodity case.

## Annex 4 Stock Feed

Case: Stock feed, supplements and ingredients of plant origin Effective: 28 Jan 2017

Product does not contain ingredients of animal or microbial origin — For other than laboratory analysis — Stock feed

### Import Conditions

The following Import Conditions are applicable to this Import scenario. The department will assess the suitability of your import against the import conditions.

- a. Prior to the importation of [goods](#) into Australia, a valid import permit issued by the Department of Agriculture and Water Resources is required.

An import permit may be obtained by submitting an [import permit application](#) to the department (if viewed online, use the 'Apply Now' button at the bottom of this page).

You will need to provide a completed [production questionnaire](#) during the permit application, please ensure that this questionnaire is completed by the manufacturer of the product.

- b. Stock feed import permit applications are subject to a desk audit. Detailed information regarding the stock feed manufacturing process, provision of quality assurance manuals and operating procedures are required to perform the desk audit.

A checklist and details of Department of Agriculture and Water Resources requirements for the desk audit will be forwarded to the applicant by the Plant Import Operations upon receipt of an import permit application. Following a successful desk audit, the manufacturing facility and export pathway may be subject to a Department of Agriculture and Water Resources site inspection.

The importer is responsible for all costs associated with any site inspection (including business class airfares, accommodation, meals and fee for service).

For additional information regarding the importation of stock feed of plant origin, please see the Department of Agriculture and Water Resources [Plant based animal feed information](#) or contact the [Import Services Team](#).

- c. The importer is to ensure that the product complies with the relevant State/Territory regulatory requirements on labelling as per the Australian Ruminant Feed Ban (ARFB) National Uniform Rules.
- d. All consignments must be free of live insects, soil, disease symptoms, contaminant seed, other unprocessed plant material (e.g. leaf, stem material, fruit pulp, pod material, etc.), animal material (e.g. animal faeces, feathers, etc.) and any other extraneous contamination of biosecurity concern.
- e. If the conditions of import are met, each consignment will be subject to an inspection as specified in the permit conditions on arrival by a biosecurity officer to ensure that

the product is packaged in clean and new packaging, free of live insects, soil, contaminant seed, weed seeds, animal material and other biosecurity risk material.

- f. Testing for restricted animal material (RAM) will be required in any of the following instances:
1. The import permit specifies a requirement for testing.
  2. The product is transported in bulk and the cleanliness of containers or ships holds before export cannot be guaranteed to the satisfaction of officers from the Department of Agriculture and Water (e.g. through a pre-approved arrangement).
  3. The product is transported in bulk but, at inspection upon arrival, the cleanliness of containers/holds is not confirmed.
  4. The product is packaged but is packed in packages that are not clean and new.
  5. At inspection on arrival the integrity of packaging is found to be deficient.

Consignments must be held in quarantine at an appropriate AA site until such time as all batches/lots have been verified free from RAM and the consignment has been released from biosecurity control by the department's regional office.

- g. Under the [Biosecurity Charges Imposition \(General\) Regulation 2016](#) and Chapter 9, Part 2 of the [Biosecurity Regulation 2016](#), fees are payable to the Department of Agriculture and Water Resources for all services. Detail on how the department applies fees and levies may be found in the [charging guidelines](#).
- h. Non-commodity information requirements for imported cargo also apply, please refer to the BICON case [Non-Commodity Cargo Clearance](#).

## **Annex 5 Summary Fresh Prawn Import Requirements (Assumed to be in place up to the 9 January 2017 suspension)**

### **Quarantine measures for the importation of prawns and prawn products for human consumption**

The following quarantine requirements apply to the importation of prawns and prawn products for human consumption (other than shelf-stable prawn-based food products<sup>44</sup>), and are issued under the authority of *Quarantine Proclamation 1998*.

NOTE: Imported food, including prawns and prawn products must comply with the *Imported Food Control Act 1992* and the *Australia New Zealand Food Standards Code* (FSC) in its entirety. Under the *Imported Food Control Act 1992*, the Australian Quarantine and Inspection Service (AQIS) may inspect, or inspect and analyse imported prawns and prawn products to determine compliance with the FSC. These food safety and labelling requirements are separate from, and additional to, Australian quarantine requirements. Information on the FSC may be obtained from Food Standards Australia New Zealand.

### **1 Import Permit**

The importer must obtain a permit to import all *uncooked* prawns and prawn products into Australia for human consumption from AQIS, before the goods are imported.

The application to import must include:

- ☐☐ the name and address of the importer and exporter; and
- ☐☐ a description of the commodity to be imported.

The application will be assessed on the above information as well as any other criteria deemed relevant by the Australian Director of Animal and Plant Quarantine.

*Cooked* prawns and prawn products do not require an import permit but will be required to meet conditions that are specified in the *Quarantine Proclamation 1998* (see point 3).

### **2 Uncooked prawns**

**2.1** All imported *uncooked* prawns must:

☐☐ be sourced from a country or zone that is recognised by Australia to be free of ALL the following pathogenic agents:

- White spot syndrome virus (WSSV),
- Yellowhead virus (YHV), and
- Taura syndrome virus (TSV), and in addition
- Necrotising hepatopancreatitis bacterium (NHPB) if the product is not frozen (i.e. the product is chilled);

OR

☐☐ be highly processed<sup>46</sup>, that is with the head and shell removed (the last shell segment and tail fans permitted) and;

- coated for human consumption by being breaded (crumbed) or battered, or

- coated for human consumption by being marinated in a wet marinade (the marinade must be no less than 12% of the total weight of the product), or
- coated for human consumption by being marinated in a dry marinade (the marinade must be clearly seen to cover the product), or
- coated for human consumption by being marinated and placed on skewers (the marinade must be clearly seen to cover the product), or
- the raw prawn meat processed into dumpling, spring roll, samosa, roll, ball or dim sum-type product;

OR

They have had the head and shell removed (the last shell segment and tail fans permitted), be frozen and each batch tested on arrival in Australia and found to be free of WSSV and YHV. Batch testing for a pathogenic agent does not occur if sourced from a country or zone recognised by Australia to be free of that agent.

Testing is based on the polymerase chain reaction (PCR) tests in the current version of the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests for Aquatic Animals* or equivalent, and a sampling regimen that would provide 95% confidence of detecting the agent if present at 5% prevalence.

All consignments of prawns to be tested will be held under quarantine control in Australia where they will be sampled for testing. Prawns will remain under quarantine control until the results of the tests are available. Batches that return positive results must be re-exported, destroyed or further processed (i.e. cooked) in a facility approved by AQIS for that purpose. For the purpose of this testing, a batch is defined as a population from a different pond population or fishing period population. Documentation from the exporter, supplier or the Competent Authority verifying the number of batches in the consignment must be provided to AQIS. This documentation must clearly detail the labelling of each batch in the consignment.

If the number of batches cannot be determined from documentation, full unpacking and inspection may be required in order to determine the number of batches.

**2.2** For all *uncooked* prawns and prawn products (including those that are considered to be highly processed, as defined in Section 2.1), the Competent Authority in the exporting country must certify that the prawns or prawn products:

They are fit for human consumption, and

They have been processed, inspected and graded in premises approved by and under the control of the Competent Authority, and are free from visible signs of infectious disease.

In addition, for *uncooked* prawns that are not considered to be highly processed (as defined in Section 2.1), the Competent Authority must certify that:

Each package is marked with the words '*for human consumption only*' and '*not to be used as bait or feed for aquatic animals*'.

**2.3** *Uncooked* prawns and prawn products that are considered to be highly processed (as defined in Section 2.1) will be randomly inspected by AQIS to ensure the imported commodity complies with the product description on the import permit and health certificate.

### 3 Cooked prawns

For *cooked* prawns and prawn products, the Competent Authority in the exporting country must certify that the prawns or prawn products:

are fit for human consumption, and  
have been cooked in premises approved by and under the control of the Competent Authority to a minimum time and temperature standard where all the protein in the prawn is coagulated and no uncooked meat remains.

#### 4 Review

Conditions for importation may be reviewed if there are any changes in the source country's import policy or its animal disease status, or at any time at the discretion of the Australian Director of Animal and Plant Quarantine.

### **Annex 6 Questions posed to DAWR in relation to import processes for prawns**

- 1) What is the number/tonnage of shipments/containers of imported uncooked prawns that were received by Australia each month from October 2009 to January 2017 by port of origin?
- 2) How many of these shipments/containers were rejected from entry into Australia each month from October 2009 to January 2017, due to positive lab WSSV/YHV detections?
- 3) How many containers were rejected for other reasons? Please state what other circumstances resulted in rejection of containers (uncooked prawns were <15g).
- 4) How many of the uncooked prawn shipments that Australia received each month from October 2009 to January 2017 were tested for WSSV?
- 5) What quantity of prawn is exported from Australia for processing to a WSSV positive country, and subsequently imported to Australia after processing?
- 6) Are these commodities in question 5 subject to the same import conditions and testing?
- 7) Have any tests been conducted by the Commonwealth officers, on samples from the retail environment or external to Quarantine facilities. Please include data of the dates of the sample collection, WSSV PCR test results, source of the product (importer, distributor and retailer).
  - a. What responses, if any, followed these surveillance findings?
- 8) What sampling protocol/rate is used for WSSV surveillance in imported uncooked prawns? e.g. 1 test of 5 prawns per 300 boxes of imported uncooked prawns
- 9) What is the size of batch, or production/shipment unit that defines the number of samples to be collected (eg. 8 samples per "batch" – how big is the batch? (eg 1 hours production at factory, 1 farm pond (what tonnage per pond), 1 day, 1 container?)
  - a. Is the entire container unpacked on each occasion?

- b. How are pallets selected for testing from each container?
  - c. How many pallets are selected from the total number?
  - d. How are boxes selected for testing from each pallet?
  - e. How many prawns are selected from each box for testing?
  - f. Who undertakes the sampling (Govt officer or Importer)?
  - g. What is the required chain of custody of samples to the laboratory?
  - h. Who ships the prawns to the testing laboratory?
  - i. Are uncooked prawns of all sizes, style (peeled, whole) and marinated tested?
- 10) Are any whole uncooked small prawns (<15g) permitted for import? What conditions (if any) support such imports?
- 11) What is the diagnostic test and which laboratories are approved for WSSV testing of imported uncooked prawns?
- a. Are positive and negative controls used to each test at every laboratory?
  - b. Are testing laboratories required to undertake ring proficiency testing and accreditation?
  - c. What is the frequency of proficiency testing at all laboratories undertaking surveillance tests for WSSV?
- 12) How long does it take for each test to be performed?
- 13) Are containers held at the Port while waiting for the test result?
- 14) What oversight on the containers is undertaken whilst testing results are pending?
- 15) If any of the tests came back positive what was the response to each of the positive containers? E.g. repeat test/ re-export/destruction/ other? Where is this done, and what auditing and oversight?
- 16) How many instances have there been of WSSV positive consignments accidentally being diverted into Australia's food supply chain instead of being subjected to additional quarantine measures (eg cooking, re-export, destruction or other)?
- a. Were any import conditions altered subsequently to prevent repeat incidents of this kind
  - b. What were the specific changes made (if any)?
- 17) What requirements and testing must be met by countries exporting uncooked prawns to Australia in order to be approved for export? (ie pre-export requirements)
- 18) Has Australia audited /inspected the competence and reliability of the exporting countries measures? If so, what were the results of the visit?

- 19) What level of inspection (% of containers, level of container unpacking and frequency of examination) was undertaken on containers of cooked prawns from 2009 to January 2017 to ensure that uncooked prawn was not illegally hidden within those containers?
- 20) How many containers of cooked prawns were found to contain uncooked prawns or other non-compliant commodities (e.g. other uncooked crustacea) from 2009 to January 2017?
- 21) How often is the process of sampling imported uncooked prawns for WSSV testing audited?
- 22) How often is training for personnel in charge of sampling imported uncooked prawns for WSSV testing performed?
- 23) Please supply all changes to the import conditions associated with uncooked crustaceans since the original conditions approved in October 2009.