

# **Submission to the senate - Biosecurity risks associated with the importation of seafood and seafood products (including uncooked prawns and uncooked prawn meat) into Australia**

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## **Terms of Reference**

1. The biosecurity risks associated with the importation of seafood and seafood products (including uncooked prawns and uncooked prawn meat) into Australia, with specific reference to:
2. Management of the emergency response and associated measures implemented to control the outbreak of White Spot Syndrome Virus;
3. The effectiveness of biosecurity controls imposed on the importation of seafood and seafood products, including, but not limited to, uncooked prawns and prawn meat into Australia, including the import risk analysis process concluded in 2009 that led to these conditions being established;
4. The adequacy of Commonwealth resourcing of biosecurity measures including Import Risk Assessments;
5. The effectiveness of post-entry surveillance measures and "end use" import conditions for seafood products including, but not limited to, uncooked prawns and uncooked prawn meat into Australia, since the import conditions implemented in 2010 were put into place;
6. The impact of the outbreak on Australia's wild and farm prawn sectors;
7. The economic impact on Australian wholesalers and retailers;
8. Domestic and foreign trade implications for Australian industries resulting from the suspension of importation of seafood and seafood products, including, but not limited to, uncooked prawns and uncooked prawn meat in Australia;
9. Matters to be satisfied in the management of biosecurity risk before imports of seafood and seafood products, including, but not limited to, uncooked prawns and uncooked prawn meat into Australia could recommence;
10. Any related matters.

## Background

**White spot syndrome virus** is the lone virus (and type species) of the genus *Whispovirus* (**white spot**), which is the only genus in the family *Nimaviridae*. It is responsible for causing white spot syndrome in a wide range of crustacean hosts.<sup>[1]</sup> **White spot syndrome** (WSS) is a viral infection of penaeid shrimp (prawns). The disease is highly lethal and contagious, killing shrimps quickly. Outbreaks of this disease have wiped out within a few days the entire **populations** of many shrimp farms throughout the world. ( Wikapaedia).

The virus itself has no effect on humans and other than in countries like Australia where it is screened for, so that a large percentage of the world's population has actually no idea that the shrimp they eat may be affected by or contain WSSV itself.

WSSV is present in the prawns in most of the oceans/seas especially in Asia where it may be carried by all types of crustaceans including and other marine species such as polychaete worms and small crustaceans such as crabs and copepods. These may be infected at low levels and act as carriers of WSSV without manifesting any symptoms.

Disease problems are normally manifest when WSSV gets into a prawn farm but may remain at low infectious levels for long periods and only manifest when the prawn population in a pond becomes overcrowded or has a number of other environmental factors change. Typically changes to the situation of the pond itself (water, salinity, temperature etc). Generally WSSV affects highly inbred prawns and may not cause disease in the wild.

## Routes of infection

WSSV infections in prawn ponds and in the wild populations have been extensively studied by scientists particularly in Asia where the problems first occurred since the early nineties and has spread to other continents from Asia and now occurs in nearly every country where prawns are farmed. As a result of this, the countries which have had their prawn production most affected have developed very sophisticated prawn farms with significant biosecurity measures to prevent re-infection or infection with other bacterial and viral diseases which also can occur or are present in Asia or elsewhere and cause significant losses and lost production. These state of the art prawn raising facilities both in hatcheries and farms now occur in most Asian countries as well as South America and Saudi Arabia where the disease has occurred and put in place to regain the lost production. Biosecurity in prawn farming/ hatcheries in Australia does not come close to international standards

WSSV can be transmitted both vertically and horizontally. This means that prawns in a pond can become infected by several potential methods in reality. The most common route of infection in Asia and shown over and over in the literature is through contaminated broodstock. This route is also the most difficult to control unless stringent biosecurity measures are adopted. Old practices such as harvesting prawns from the wild stock from the ocean have been shown to be the most likely to cause problems as wild stock can harbour very low levels of virus which are not manifest and only through extensive and meticulous testing for WSSV at all levels throughout the hatcheries and in each generation including the larval stages (PL) used to stock a pond ultimately for the final growth phase. Most countries are moving to the use of pathogen free raised broodstock to ensure WSSV is not transmitted vertically within a facility. No pathogen free facility is currently available in Australia. No wild caught prawns are adequately tested to ensure they are not potential carriers of WSSV or other diseases.

Other methods have been shown to also be responsible for infection and these include feed both manufactured commercially and in house with seafood or trash type materials or worms especially used to promote larval growth in hatcheries. Again screening and biosecurity control is vital to prevent these types of infections being transmitted via feed. Commercial feeds have specific protocols to ensure the constituent ingredients are cooked sufficiently to kill the virus. Locally made feeds using marine organisms should be thoroughly checked to ensure this cannot be a route of infection although this checking practice has been very poorly observed in Australian hatcheries and farms. Also in recent times locally made commercial feeds included the use of such material as trash marine waste from sources such as the Sydney fish markets. Was this waste ever checked for potentially contaminating viruses and was the processing in Australia adequate to ensure it wasn't contaminated albeit at low levels? Where are the results of this testing? Use of any contaminated feed within hatchery facilities without extensive testing both of the feed and raw ingredients and before and after processing could result in the virus being present at low levels in broodstock or larvae only to then manifest at the pond level when environmental changes occurred there at a later time.

The use of contaminated tools, equipment and etc are also significant routes of transmission from pond to pond, hatchery to pond and also includes the movement of people in and out of facilities. Modern prawn facilities in the world have very rigid biosecurity protocols to prevent any uncontrolled people movement and especially at hatcheries with full biosecurity including shower in shower out etc. The types of biosecurity already are used in other Australian industries like chickens and pigs where very rigid biosecurity measure are in place to prevent all types of disease transmission in these industries, but appear to be poorly demonstrated at Australian prawn farms and hatcheries.

Water also is a major source of transmission as the WSSV can live in water for some considerable period. This includes water in the ponds in the ocean, rivers and at subterranean pond levels and water which occurs in ships ballasts and boats hulls etc. Bilge or waste processing water of any type from an infected area can transmit the virus to other carriers such as small crustaceans worms etc, not just prawns. Prawns either escaped from ponds or other crustaceans can also be a source of transmission, particularly as the virus can be carried at subclinical levels and not affect the carrier. These carriers can easily persist for years and act as a continual source of re-infection.

Eradication of WSSV carriers is practically impossible in carriers as they may be in the mud at the bottom of oceans and rivers residing in crabs, copepods or worms or other marine creatures harbouring the WSSV. Transmission from an infected wild marine sources has been shown to travel quite rapidly thousands of kilometers in a very few years in the ocean (shown by South American and other Asian studies).

Other methods of transmission might include the introduction of an infected source of material directly to the pond since prawns are scavengers and will eat other marine material including prawns which may be infected. The exclusion of deliberate or inadvertent contaminated material being introduced directly into ponds is difficult to monitor. Again in Asia where this has been shown to be an issue only rigid biosecurity control, stopping of vehicles and people and high fencing or covered ponds and CCTV etc has been shown to prevent this from happening.

Other alternative sources of infection may include the use of contaminated material such as infected prawns used in recreational fishing or tipped off boats in by product of larger catches elsewhere or from general wastage of marine seafood waste in fish markets and restaurants etc . In order to establish this as a route of infection however it requires that the material be sufficiently infective, eaten by crustaceans rather than fish and the spread through those means to a pond via a wild crustacean, or worm infestation delivered by bird life or through water. WSSV can even be

transmitted pond to pond by air in infected particles. In properly biosecure facilities the use of concrete or plastic lined ponds, the use of crab fences, proper water filtration or recycled water systems, moving ponds inland with recycled water and covering of ponds are used to exclude other marine creatures or WSSV material from entering ponds from the wild. In general it has been shown that the this whole potential infection process may take several years to take place as the wild stock have to become infected first, potentially breed and establish a significant presence to represent an substantial infection source.

It is also quite possible to conceive that a prawn farm infected by one of the other routes could likewise then transmit the disease to the wild through escapees and or small crustaceans or though inadequately treated water sources. Recent findings of very large non native prawns ( tigers) caught in Moreton bay could easily attest to WSSV being present for some time and having escaped from prawn ponds in the vicinity into the rivers and then the ocean at much earlier times.

No infection route has thus far been established for the QLD outbreak and may never be known.

### **Monitoring WSSV**

WSSV is a virus that cannot be cultured in any cell culture system. The presence of WSSV in carriers or prawns at sub clinical manifestation cannot be recognised easily. When WSSV is at sufficient clinical pathological level to cause morbidity and mortality it may then be noticed as actual white spots present on the carapace and be at very high infectious levels.

To effectively monitor the presence of WSSV at low levels and in carriers and hatchery or larval stock to stock ponds the only useful test is based on DNA technology to measure the actual WSSV genome ( DNA sequence) rather than the virus itself. This is currently done on a world wide basis using what are called DNA/PCR techniques. RNA viruses such as yellow head virus ( YHV) is also tested for in a similar way. PCR techniques rely on the known DNA sequence of the WSSV and a short segment on the DNA is very specifically amplified and multiplied by the use of the PCR ( polymerase chain reaction).

Detection of WSSV using DNA methods and PCR techniques is described in scientific literature dating well back into the nineties to current day. PCR/DNA techniques have evolved over the years since the early nineties as the whole PCR methodology has developed. The entire reaction mechanisms are well understood and it is not a “black box” technology. Methods and instrumentation to get reliable, reproducible, robust and quantitative methods have been developed for WSSV and are published in the scientific literature where they can be peer reviewed and subsequently relied upon for usage in diagnosis. The current best PCR techniques are (quantifiable) PCR methods. qPCR methods are used today for all types of medical, animal, plant, bacterial, and viral monitoring of the agents causing diseases of all types.

When an outbreak occurs in a country in the world the local authorities will try and deal with the disease. The diagnosis of an outbreak requires the use of some sort of veterinary or aquatic scientifically based facility able to diagnose disease.

On a world wide basis this diagnosis is covered specifically in the aquatic diseases manual of the OIE ( World organisation for Animal Health) with WSSV diagnosis covered in the chapter 9.7 of the OIE AQUATIC ANIMAL HEALTH CODE

[http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre\\_wsd.htm](http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_wsd.htm)

This chapter outlines specifically the OIE code to deal with infections by WSSV the code of any country dealing with outbreaks of WSSV, notifications to the OIE and conditions for considering a country WSSV free or otherwise. It outlines the OIE regulations regarding this disease.

In addition the OIE AQUATIC Manual of Diagnostic Tests for Aquatic Animals has a chapter specifically on WSSV Chapter 2.2.7 see

[http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre\\_wsd.htm](http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_wsd.htm)

Which outlines the disease its diagnosis the methods of doing so and also includes as part of this diagnosis are details of the PCR methods that could be used for detecting WSSV DNA sequences of the WSSV genome. The section 4.3.1.2.4. Molecular techniques and 4.3.1.2.4.1. Polymerase chain reaction (PCR) are included.

The PCR method for WSSV detection is according to the latest manual described as “The PCR protocol described here is from [Lo et al., 1996a](#) and [Lo et al., 1996b](#), and uses sampling methods from [Lo et al., 1997](#). It is recommended for all situations where WSSV diagnosis is required. A positive result in the first step of this standard protocol implies a serious WSSV infection, whereas, when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated. Alternative PCR assays have also been developed (e.g. [Nunan & Lightner, 2011](#)), but before use they should first be compared with the protocol described here” So that the method described is in fact based on original methods for 1996 and 1997 and are in molecular science terms considered very old by modern standards. The method of Nunan and Lightner, 2011 comes from the WSSV laboratory which is considered the most up to date advisory and testing facility at the University Of Arizona USA. ( Prof Lightner was an original contributor to the setting up of the 2009 IRA by the Department of Agriculture). Currently the laboratory is headed by Dr Kathy Tang Nelson and considered and used by most laboratories as the best international back up reference laboratory for the testing of WSSV. She is a reference adviser for WSSV for the OIE. ( it is not CSIRO AAHL which is reference lab for Yellow Head Virus, YHV).

Diagnosis of an out break of WSSV has been dealt with by CSIRO AAHL Aquatic diseases unit and the QLD dept of Agriculture in the current outbreak. The methods have included veterinary diagnostic methods as they have facilities for pathological examination of specimens and are best placed for these techniques. This does not however mean necessarily that that these laboratories have the staff equipment or best diagnostic methodology or routine and ongoing expertise to diagnose and quantify WSSV in prawns by the use of qPCR methodology for routinely imported prawns.

Three other diagnostic laboratories Agrigen Pty Ltd, Advanced Analytical Australia Pty Ltd and The Elizabeth Macarthur Agricultural Institute a( EMAI) as part of NSW DPI have been specifically approved by DAWR to perform routine testing of imported prawns using PCR diagnostic methods according to OIE or equivalent methods. Since 2009 these three laboratories have tested hundreds of thousands of prawns for the presence of these viruses and would have to be considered the experts in the routine testing of commercially imported prawns and prawn meat for the viruses WSSV and YHV. CSIRO AAHL did not and has not since 2009 conducted routine commercial testing of WSSV or YHV in imported prawns.

CSIRO qPCR testing is not in any way “enhanced” as recently claimed by DAWR and any proper analysis of their methods or reports to importers will reveal this. The use of this “enhanced” method outside the OIE protocols on prawns tested negatively by other labs has lead to significant mistrust and confusion by importers generally. The results are not believed to be other than false positives on many of these reports.

## The IRA

As part of the 2009 IRA ( Import Risk assessment for imported prawns) devised over two or more years and available from the DAWR website and is attached. This over three hundred page document outlines in much greater detail the scientific reasoning and implementation of a protocol for the importation of uncooked green prawns or prawn derivatives or products to minimise the disease risk for three viruses. These included WSSV, YHV and IHNV. This is by no means an exhaustive list of potential nasty pathogens that could be imported into Australia in prawns. The requirement for IHNV testing was subsequently dropped from the requirements when found to be not exotic leaving only WSSV and YHV for screening of every and any container arriving into Australia containing wild caught and/ or farmed raw green prawns prepared in Asia or elsewhere.

Any IRA is required by the OIE under an ALOP ( Appropriate level of Protection) and to fulfill the protection requirements of the OIE to devise a protocol for the sampling and testing of imported prawns in order to reduce the risk of WSSV transferring infection to Australian prawns to a low risk. It specifically under the OIE guidelines and protocol for the protocols to ensure LOW RISK not NO RISK. The scientific basis for the 2009 IRA was carefully and extensively debated by an expert panel with significant contributions from expert scientists overseas as well as local and public input. There was an expert scientific review panel to review the submissions from all stakeholders including the importers as well as the local prawn farmers and anyone or any body who had to do with the importation and testing. While not a perfect document or seemingly accepted by some Australian prawn farmers after two years work it seemed to have most if not all the requirements to fulfill the requirements under the OIE and the acceptance of ALOP especially to have a **low level of risk** for any imported prawns.

### Two parts of the IRA

The IRA consisted of two major elements fulfilled by two parties. One was the sampling of prawns from the containers and second to test them for the presence of viruses. The basis of the sampling was on the concept of a container representing a “ batch” of prawns from a “pond” in Asia ( or other country) which had been harvested and sent for processing in another country. When the container was opened here in Australia a statistical sample of  $13 \times 5 = 65$  prawns was to be taken AT RANDOM from the inside of the container and then the 13 bags of prawns sent to an accredited testing lab of choice of the importer for testing at a choice of laboratory using a NATA ( National Australian Testing Authorities) accredited method. The results were then sent to the department of Agriculture, currently called DAWR, and the importer for the release of those prawns to the market. The importer paid the cost of sampling attendance by DAWR officers, for the testing and transport of the prawns. The costs are in the thousands of dollars all together.

If either of the two elements ie the sampling or the testing was to not be carried out correctly then the entire process would fail. The laboratories could only test on an “as received” basis, so if the prawns were not a random sample of the contents of the container and a statistical sample representing the “pond” being taken then the results would be biased and incorrect not in value but in misleading anyone into the percentage of potentially positive prawns and the level of risk to meet the ALOP.

The sampling required that the officers representing DAWR at the border would be fully understanding of their role in the process and that they understood sufficiently the need for statistical independent correct sampling of the container to ensure a properly representative result. This statistical sampling regime was unique and quite unlike other food sampling processes for the testing of eg chemicals or other biological pathogens in a container.

This in turn required a "sampling protocol" a document which appears to be an internal document of the department and only referred to once in the subsequent investigation by the Inspector General of Biosecurity in a review conducted when a container of prawns was accidentally released and found subsequently to contain WSSV infected product. Without an adequately informed and trained front line force of sampling officers understanding their role in the whole process then the whole regime was likely to fail.

Initial training sessions in 2009 called a "road show" was done by two departmental members Dr Mike Nunn and Dr Geoff Grossel of the department shortly after the process outlined in the IRA was instigated. This was rolled out to the departmental officers in each port of entry in each State at the time but over the intervening years as staff left or were replaced at the front line it would appear that none of these officers were trained or had any proper instruction or had any use of the "sampling protocol" which could explain their role in the overall sampling and testing of containers.

Eventually some years ago also both Dr Mike Nunn and Dr Geoff Grossel also left the Department to take other roles elsewhere and as a consequence of that the whole basis and scientific reasoning and understanding of the IRA and its two major elements sampling and testing were also lost and not replaced by senior staff understanding the whole picture with regard to the WSSV story and how to get the right representative results from the sampling and testing, the two elements required.

It was obvious at the testing laboratories that the sampling process was being poorly handled. Prawns would arrive unrefrigerated, sent on the wrong day, not properly bagged in 13 bags containing 5 prawns and may many other problems related to inadequate or improper sampling or transportation to the labs in Sydney. At Advanced Analytical while I was CEO and one of the major testing labs, there was an officer almost full time on the phone trying to educate the various State officers supposedly sampling prawns according to a "sampling protocol" clearly not doing so and despite trying to do this over many years there appeared no one at the department who would either listen or understand the importance of proper sampling or the testing would fail. There even appeared to be no understanding of the IRA and its scientific content and meaning.

Anecdotal information was supplied to the laboratories especially from brokers and importers of inadequate and improper sampling of containers not being accessible for inspection at the time, stacked atop each other and so on or of officers just simply requesting that someone else from the importer or their agent bring them 65 prawns of such and such a batch. This in turn clearly lead to the potential of exploitation of inadequate or substituted prawns. But the lack of understanding or training of the officers role was also significantly the cause of subsequent inadequate or false non statistical sampling.

Likewise despite the significant paperwork and the requirements of that paperwork, often and mostly handled by brokers of importers there was wrong information, lack of complete information so that no one least of all anyone in the department knowing what results meant. It appeared also that no one at the department was monitoring what the results were and what they meant scientifically. The reality is that the testing laboratories had the most information. It was they who interacted with importing clients, their brokers as it was the importer who paid the labs' bills. But there was no forum or mechanism to share results between labs or with the department in fact any communications were actively discouraged. There was no contact between the two main labs testing at that time Advanced Analytical (testing actually subcontracted internally to Agrigen scientists) and EMAI or CSIRO AAHL either. This has lead to significant breakdown on a scientific basis of the testing methodology which has evolved over the years as well as highlighting the problems with the sampling and the issue there also. The labs simply went on testing prawns

sending data to the department for years with no feedback or review of the results being obtained or what they meant. Despite my own repeated calls to the department from 2009 onwards nothing was ever organised amongst those who knew the most of what was happening in the sampling and testing of prawns for these viruses.

Australia has a unique problem. No one else in the world is interested in sampling every container at the border and testing it for these viruses. There is no major incentive to sample and test except to try and prevent major economic losses of production in other countries. The countries are varied in their responses to control the diseases which are mostly ubiquitously present in the seas around them as well. Some countries are more successful than others in having proper biosecure facilities in their countries and rigidly enforcing testing. For importers in Australia it is enormously difficult to find exporters or processing facilities or even farmers who can reliably provide prawns negative for these diseases including WSSV. There is no real incentive for exporters who can sell much greater volumes of product to other destinations such as Japan or Europe or America who have no testing or need for it in their countries. So importers try to secure suppliers of negative prawns but this is a constantly changing scene with problems of inadequate testing, cross border movement of broodstock, lack of biosecurity and so on meaning that a prawn pond may be negative this season and positive the next. Finding a reliable source of totally negative prawns for the importer is an almost impossible task. Such sources when found are guarded severely from the any other importer.

And on testing for WSSV or any other viruses there is no overseas protocols to pick up as there might be for many other pathogens, to have a uniform testing protocol which would be specifically applicable to routine commercial testing of imported material. This is not a problem for chemical testing where international bodies such as CODEX have international agreements on testing levels etc. So Australia does use this mechanism adopting testing for other agents especially in other human or other veterinary pathogens.

The OIE protocols are designed to help counties who have an outbreak of disease. They are NOT designed for routine diagnostic border testing. The only way to have done this was to set up mechanisms and scientific discussion to bring about standardised protocols and inter-laboratory proficiency testing overseen by independent providers of proficiency testing services. Yes, under accreditation requirements of ISO/IEC 17025:2005 the international requirements accredited by NATA there is some proficiency testing conducted. The labs in Australia have participated if and when this has been possible, but no discussion or evaluation of this process is done as there is no mechanism to do it.

With other viruses of international interest such as say Norovirus in food including particularly oysters and recently found in raspberries etc, there are international labs across the world and especially in Europe to bring about standardised international protocols/ methods, many laboratories contribute to that process and then an international committee oversees the implementation of the testing. In addition particularly where qPCR methods are used for virus detection, reports are standardised to an extent to allow international interpretation of any result.

For viruses in particular the qPCR DNA results have shifted specifically to equate them with viral genome copy number. The purpose is to try and decide whether a scientific qPCR DNA (Ct, Cq) value is actually representative of a number of virus particles which may be infective. qPCR DNA techniques do not measure live infective particles. They measure fragments of specific pieces of DNA. They are very accurate and sensitive at doing that, but without infectivity studies in parallel cannot be used to tell anything about a prawn containing viruses. The virus maybe alive or dead. You are only measuring DNA. But studies have been done with many viruses eg Norovirus to interpret how many viral particles are needed for that virus to be considered infective in that oyster,



raspberry etc. This for Norovirus has been considered to be in the vicinity of 600-800 virus particles. And Norovirus is a very infectious agent. Studies at low infective numbers for WSSV have not been done or remain unpublished if they have been.

In WSSV in recent times laboratories such as the University of Arizona reference lab has done studies and provides reports in viral genome equivalents. The theoretical limit and Level of Detection (LOD) is single viral genome equivalent for the qPCR test but that is theoretical and the research and methodology of this reference lab has indicated that it is only possible to report to 10 viral genome equivalents with values below 10 as being unreliable and not reproducible or repeatable. So they give out reports on that basis and are accepted world wide on that basis. It still does not tell you specifically if a value of 10 viral genome equivalents would produce an infection were that prawn be eaten by another prawn. This could be done but only by using well characterised infective material to test in pathogen free prawns if that is infective. Where this has been done it appears that thousands of viral genome equivalents are needed to obtain infection.

### **qPCR testing in Australia**

In Australia the qPCR testing has been expressed on reports as Ct or Cq values which pertains to the sigmoidal curve graphic plot obtained in the amplification of the DNA extracted from the start material. Its value which is used internationally by laboratories using qPCR for all types of diagnostic methods to indicate 1 viral genome equivalent in the extract. The acceptable level of this Ct Cq value is around a maximum value of 35 cycles. Anything greater than 35 cycles represents on a logarithmic scale hundreds or thousands of less likelihood of a single viral DNA equivalent being found and is not reproducible or reliable. In reality, this represents an extremely low level of potential infectivity of such material. In effect it represents absolute zero tolerance or no virus. In effect for any importer this level is impossible to achieve and as stated the actual level of reliable value to place on a report to be equivalent to 10 viral genome copies would be a value of 32-34 cycles or Ct Cq cycle values. Even specific pathogen free prawns have shown unreliable results of cycle value of 36-37 cycles which is why these are discarded by international reference laboratories. Its less than 1 viral genome equivalent. Any value of > 40 cycles is entirely meaningless. In fact the OIE reference manual refers to a maximum of 40 cycles being the used, and that is for diagnosis in an outbreak not for routine testing of imports to satisfy the ALOP and OIE.

DAWR has collected a huge amount of Ct Cq data from the testing of the laboratories. All laboratories have been obliged to collect and pass on all the data in reports to DAWR for more than a year. What precisely has been done with this data.? What collation or collection of this data and interpretation of this data and scientific discussion has taken place on this with scientists or experts in qPCR? None to my knowledge outside DAWR. There simply is no adequate scientific qPCR DNA interpretation expertise inside DAWR currently.

### **Monitoring the success of sampling and testing to achieve low risk**

To separately monitor the success or otherwise of the sampling and testing regime also required that independent surveys be conducted to measure the effectiveness at the retail level in Australia. This is done currently by DAWR for the purposes of their IFIS program to monitor the level of chemicals in products as well as microbial contamination. The process is well established and is done every few years to ensure compliance. Clearly no equivalent process was put in place to monitor the sampling and testing of WSSV and YHV until last year when it has emerged from the Senate enquiry so far that an operation was conducted in secret to test retail outlets and other sources such as fishing bait for WSSV in such materials. Given the lack of proper sampling for years it is not surprising that the results of this testing did not reflect the testing by conducted by the appointed labs. As

stated above the test becomes meaningless when the samples provided are not reflective of a statistical sample of the “pond”. Why aren’t independent end sampling done and tested previously to 2016/2017?

### **Testing our own waters/oceans**

Given also that Australia has seemingly ignored the potential problems of WSSV having spread to all manner of oceans/seas around Asia and South America alone in the last decade, why did Australian industry go on relying on a survey conducted by CSIRO (East et al, 2004) many years ago that there was no WSSV or other virus present in the oceans/seas to the north of Australia especially in the EEZ (Economic zone) in the north when we practically share waters and almost touch on at Torres Strait with Indonesia where these prawn diseases exist in the wild in their seas. Proper surveys of these waters could have been done and yet there is only one very limited survey in recent times done by CSIRO as part of an FRDC project published on their website <http://frdc.com.au/research/final-reports/Pages/2013-036-DLD.aspx> primarily for the testing of YHV and other agents. This work supported by the FRDC was limited in scope and only had WSSV added partly at the request of APFA and yet curiously although some Australian hatcheries provided samples of broodstock for testing for other pathogenic agents the testing of this material from hatcheries was specifically excluded from WSSV testing. Why? No other routine testing of Australia’s prawns around the costs have been tested either, so how is it known whether WSSV is not already present albeit at a low level?

### **Surveys of Northern waters**

Likewise survey testing of the prawns from Northern Australian waters could have been done by simply collecting random but statistical samples from the fishing vessels fishing in these northern waters and bringing back their catches to ports including Brisbane. With the cooperation of these prawn harvesters a representative survey using qPCR techniques using the testing labs could have been done but wasn’t. Why not? Catching and using these prawns as broodstock remains a very significant biosecurity risk for many emerging marine diseases including prawns. Wild caught prawns have often very low levels of WSSV present and is non pathogenic to wild caught prawns. Its difficult to detect but can be done with proper qPCR and statistical and well designed surveys.

WSSV disease only becomes a problem when wild collected, bred in hatcheries and the larvae introduced to the pond. In this regard also it appears that the protocols put in place to monitor and test for these diseases in hatcheries and larvae are also inadequate. Queensland only introduced such a protocol in late 2015. Is this protocol observed? Where are the results? The protocols by international standards are completely inadequate with the testing of 20 pleopods from a selection of prawns. Much more rigid and extensive testing and monitoring of broodstock and hatcheries and on any live imported prawn by all states will protect against not only WSSV but also the other emerging prawn diseases. There are not even protocols it seems from other states to monitor and test this major potential source of outbreak potential. And yet wild caught live prawns have been captured for broodstock from wild catches. How are these monitored?

### **Other DNA testing**

There has obviously been an attempt to try and connect an imported prawn with the recent outbreak in QLD on the Gold Coast. It is possible to do scientific studies to develop DNA protocols to test similarities between the DNA prawns of one area with another. This type of analysis is done by SNPs (Single Nucleotide Polymorphisms) and this technique is being used in other Australian industries like beef cattle and pig industry to trace point of origin. But the surveys and measurement

of the prawns in Asia and in from other all other locations has to be done first. It requires the extensive cooperation of the Asian and other exporters, importers and of prawn farmers every where to get the background to be then able to pinpoint the origin by DNA methods . It cannot be done by simply doing DNA sequencing of a particular virus from a particular location. So you can't simply do a DNA sequence of WSSV from a prawn from a retail outlet or bait and compare that to a WSSV sample from an outbreak pond. It doesn't work as the variability of sequences of DNA which are constantly altering or mutating would not allow comparison unless it happened to be absolutely identical. This is highly improbable.

The upshot of all this is there is no way to link any WSSV virus found from any import to the outbreak on the Gold Coast. No scientific way, currently. The corollary to this is that no WSSV outbreak whether now or in future could be linked to any specific action or event. So the source of the recent outbreak remains of unknown origin. Unless more evidence emerges to pinpoint the original source. It remains entirely speculative so far.

Equally however there is no reason to have imposed a ban on all imports. The IRA of 2009 was adequate to maintain a low level of risk based on strong scientific and risk principles but within the OIE requirements and for ALOP and for international trade and for a trade agreements of which Australia is signatory. It needed to be implemented and carried out properly. There is no proof of a scientific link between the out break and the imports, whether or not they had higher than anticipated viral levels.

Probably the 2009 IRA needed some changes.

### **Sampling**

These should include sampling to be conducted by trained officers who understand what they are doing and why they are doing it the way it needs to be done. It needs a transparent, accurate and proper sampling protocol to be followed. The inspections need to be done of locked and sealed containers only opened in presence of front line Biosecurity officer. It needs proper random sampling 13X5 samples from all over the container regardless of any labeling types of boxes etc. Simply randomly pull thirteen boxes and sample five prawns.

### **Testing**

The three labs two commercial and one Government ( EMAI) are more than adequate to handle the volume of testing required. This has already been shown over many years. A proper testing procedure based on the best internationally informed science of qPCR testing backed up by ISO 17025 accreditation delivered by NATA in a uniform type report which is internationally acceptable and backed up by international proficiency testing conducted regularly and preferably internationally also checked and ideally published in peer review literature and accepted by the OIE as a reliable adjunct for qPCR testing for WSSV. That method should also be accepted internationally. Such a process could be rapidly done given that the existing methods of the three labs are very similar and could be tweaked.

CSIRO AAHL should not provide routine testing and should stick to its proper role of disease diagnosis at outbreaks of any viral disease affecting any animals. It should also do research and publish the results of such research for all manner of diseases but not simply get involved in routine testing when there are other adequate labs to do this.

Testing routinely and allowing importers a choice in their lab was a fundamental principle of the need for competition in all testing services for food. This principle also seems poorly understood as a fundamental reason for having many testing services which was envisaged in the setting up of the IRA.

Testing and sampling should all be provided to allow the most rapid and efficient services. Containers of prawns cost hundreds of thousand of dollars and importers need to get their goods to market as rapidly as possible. Current wait times for DAWR to sample and release prawns are completely inadequate. By comparison routine testing labs can turn around tests within hours but importers wait weeks for sampling and getting releases. In other countries, the process of testing and disease control is entirely handled by commercial concerns with no government involvement. This has improved efficiencies, turn around times and production from prawn facilities.

WSSV disease is only one of the potential diseases of prawns which may cause an outbreak tomorrow. Does DAWR or any State government have the infrastructure and manpower to take on this challenge? It has already been shown that with a single outbreak the resources of both federal and state were stretched beyond breaking point and response times have been agonisingly slow. Can the emergency responses cope with the potential outbreaks of several aquatic or terrestrial crises at the same time. Which disease would take priority and what would happen to the international trade or the ability for others to trade were the services of a government lab the only one to do testing?