FRDC
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White Spot Disease R&D Needs Workshop

Workshop Report

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November 2017

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Executive Summary

Introduction

Following the white spot disease outbreak in SE Queensland a workshop was convened to provide the opportunity for stakeholders to articulate current and future research, prioritise research needs, coordinate collaboration between responsible agencies and identify potential funding sources. Thus key representatives from industry sectors and governments gathered for a 1-day workshop in October 2017 to identify future R&D priorities.

Background

FRDC has supported immediate response projects to provide information to guide industry and government agencies involved in the response to the WSSV in southern Queensland. It is evident that FRDC's Aquatic Animal Health & Biosecurity Subprogram and other funding providers will need to continue to support R&D to close key knowledge gaps. Consequently, it was proposed to convene a stakeholder workshop to identify and prioritise research needs and to coordinate collaboration between research providers. The workshop outputs should be a list of priorities for the next 12-18 months to invest on-farm, post-harvest, supply chain and out-of-water (and identify what has already been covered e.g. by FRDC response fund).

Aims/objectives

To Identify and assess the WSD R&D needs of governments and industry to support surveillance, biosecurity and production.

Methodology

The workshop was planned and convened by FRDC and was facilitated by Dr Len Stephens. Participants were invited from industry sectors and governments with the expectation they should be prepared to present on bio-security risks and previous R&D research. In addition, government and industry representatives were requested to prepare a presentation on:

- their sector/jurisdictional priorities
- identify R&D needs and gaps
- timeframes for research
- who can lead the research so PI's can be targeted in a call for EoI
- where will funding for research come from?

Results/key findings

Potential research projects identified during the workshop included:

- Improved diagnostics
- SPF centre and nuclear breeding centre
- Epidemiology
- Chemical use permits
- Industry training
- Gamma-irradiation of bait prawns
- FRDC Project proposal 2017-078
- Bait & burley use and disposal
- Diseases in non-prawn crustaceans
- Viability of disease agents in cooked prawns

- On-farm biosecurity standards
- Assessment of decontamination and disposal methods

Implications for relevant stakeholders

The workshop provided a forum for major stakeholders to discuss R&D needs following the white spot disease outbreak in SE Queensland. There was strong agreement between stakeholders on the key R&D priorities and in addition to development of project concepts, a further outcome of the workshop was a better understanding of industry/regulator needs by the workshop participants.

Recommendations

It is recommended that the project concepts developed during the workshop should be forwarded to key research providers in a call for expressions of interest (full proposals) for evaluation by FRDC.

Keywords

White spot syndrome virus; white spot disease; diagnostics; SPF centre; nuclear breeding centre; epidemiology; chemical use permits; training; bait prawns; bait & burley; diseases in non-prawn crustaceans; virus inactivation; on-farm biosecurity; decontamination and disposal methods

Introduction

Following the white spot disease (WSD) outbreak in prawn farms located on the Logan River, SE Queensland, that occurred late 2016 and into 2017, FRDC supported immediate response projects to provide information to guide industry and government agencies involved in the emergency disease response.

A summary of FRDC funded and completed WSD research can be found at: http://www.frdc.com.au/Environment/Aquatic-Animal-Health-and-Biosecurity/White-spot-syndrome.

At the time of the workshop all infected farms had been decontaminated and all water removed. The farms have agreed to not fill their ponds until after May 2018. Sampling for WSD in the wild has continued with the most recent report in October being negative to WSD for all sampling locations (see Queensland Biosecurity Update reports). The Commonwealth Government has announced a review of the *Generic Import Risk Analysis (IRA) Report for Prawns and Prawn Products*. The wild catch and farming sectors have significantly progressed the implementation of an Emergency Aquatic Disease Response Agreement (EADRA). Both wild catch and the farming sector are undertaking projects funded by Department of Agriculture and Water Resources (DAWR) to consult and communicate with their sectors on establishing biosecurity manuals and protocols to ensure the best protection possible either on the water or on farm. All these responses to the WSD have thrown up new challenges and unknowns for which targeted research responses will be required. It was therefore appropriate to gather industry, regulators and researchers to hear and determine what are the priorities for further research to ensure WSD does not impact the natural or farmed environments. With limited resources available those who attended focused on both the immediate and the future needs and identified how best to collaborate and partner across stakeholders both in the immediate effected zone and also outside. Finally, the research areas identified are capable of informing how future disease incursion can better managed and minimise this happening again.

It is evident that FRDC Aquatic Animal Health & Biosecurity Subprogram (AAHBS) and other funding providers will need to continue to support research and development (R&D) to close key knowledge gaps. Consequently, at the July meeting of the FRDC AAHBS, it was proposed that a workshop be convened to provide the opportunity for stakeholders to articulate current and future research, prioritise research needs, coordinate collaboration between responsible agencies and identify potential funding sources.

Objectives

Identify and assess the WSD RD&E needs of governments and industry to support surveillance, biosecurity and production.

Method

Workshop planning

Following the July meeting of the AAHBS when the workshop was proposed, FRDC issued a preliminary notice to State Governments and industry bodies with the intention to convene a stakeholder workshop. A workshop organising committee comprising Jo-Anne Ruscoe (Projects Manager – Research, FRDC), Wayne Hutchison (Projects Manager – Research, FRDC), Mark Crane (FRDC AAHBS Leader) and Joanne Slater (FRDC AAHBS Coordinator), met by teleconference (20 September, 2017) to discuss the purpose and format of the proposed workshop, and the potential participants. The 1-day workshop was planned to take place on 18 October, 2017, back-to-back with the October meeting of the AAHBS meeting.

It was agreed that Len Stephens should be invited to facilitate the workshop the outputs of which should be a list of priorities for the next 12-18 months to invest on farm, post-harvest, supply chain and out of water (and identify what has already been covered e.g. by FRDC response fund).

In addition, State CVO's or their delegates should be prepared to present on risks and previous RD&E research. Government and industry representatives need to come to workshop prepared to:

- Present on their sector/jurisdictional priorities
- identify R&D needs and gaps
- timeframes for research
- who can lead the research so PI's can be targeted in a call for EoI
- where will funding for research come from?

An invitation and draft agenda (Appendix 1) was prepared and forwarded to key stakeholders.

All logistics (accommodation/meeting room and facilities/catering) were coordinated through the AAHB Subprogram.

Workshop format

The workshop facilitator, Len Stephens, outlined the format of the workshop, including the expected outputs. All stakeholders present had the opportunity to outline their R&D needs and priorities. Following the sector/jurisdictional presentations, the workshop participants broke into stakeholder groups to develop the outlines (need, outputs, time-frame, potential Principal Investigator and Co-investigators, cost, funding sources) for potential research projects identified during the presentations and subsequent discussions:

- Improved diagnostics
- SPF broodstock production and nuclear breeding centre
- Epidemiology
- Chemical use permits
- Industry training
- Gamma-irradiation of bait prawns
- FRDC Project proposal 2017-078
- Bait & burley use and disposal
- Diseases in non-prawn crustaceans
- Viability of disease agents in cooked prawns
- On-farm biosecurity standards
- Assessment of decontamination and disposal methods

Results

There was a total of 20 participants (Appendix 2) in the workshop representing industry (QSIA/ACPF/APFA), federal (DAWR) and state/territory (Qld/WA/NSW/NT governments, FRDC and CSIRO-AAHL. Presentations were made by representatives of QDAF, DAWR, CSIRO-AAHL, ACPF, QSIA and APFA (Appendix 3). Several of the priorities identified during this session overlapped across jurisdictions and industry sectors and could be grouped into 12 project concepts. Subsequent sessions were devoted to development of project outlines, including the need, outputs, time-frame, potential principal investigator(s) and co-investigator(s), cost estimate and funding source(s), as detailed below. It was noted that some of the projects required the use of a QC3 facility to undertake bio-secure infectivity trials (bio-assays) to achieve the project outputs and it may be possible to achieve some efficiencies to undertake these projects together in such a facility.

The 12 project concepts are nominally listed only and the numbering does not represent R&D priority.

1. **Improved diagnostics**

Need: White spot disease, caused by white spot syndrome virus, and other infectious diseases of farmed prawns can result in 100% mortality within days of the initial infection. Thus, it is imperative that farms have the ability to identify presence of pathogens within hours of the initial detection of disease signs. To minimise potential losses, the prawn farming industry requires alternative technology to allow cheap, high-throughput testing for pathogens that provides a capability to detect the presence of pathogens rapidly (within hours), ideally on-farm, thus eliminating the time required for transport of samples from farms to laboratories and subsequent testing. This project would be concerned with evaluation of (1) commercially available test kits suitable for on-farm application and (2) multiplexed screening platforms.

Outputs:

- Determination of the diagnostic performance characteristics (DSe, DSp) of commercially available test kits for use on-farm
- Appraisal of multiplexed screening platforms that provide sensitive, specific, rapid and affordable pathogen detection and identification

Time-frame: 12 months

Principal Investigator(s): Nick Moody, Mark Crane

Co-investigators: Alistair Dick (APFA), Stephen Wesche (BSL, Qld), Matt Landos, Mike Snow

Cost: $165k

Funding sources: APFA IPA/CSIRO-AAHL/FRDC

2. **SPF Broodstock Production and Nuclear Breeding Centre (APFA)**

Need: The prawn farming industry remains reliant on wild broodstock which is a ‘major risk’ category for industry biosecurity. In other countries wild broodstock are generally considered the predominant disease incursion pathway for WSSV (and other pathogens). Establishment of an industry-owned and operated source of specific pathogen-free broodstock for all farms is the single most important action that can be taken to reduce the risk of WSSV recurring. The facility would need to initially house wild broodstock, screen them repeatedly for known diseases and breed them through several generations to achieve domestication. These domesticated broodstock could then form the basis of a nucleus breeding program to enable selection for WSSV resistance and other traits. This project would be concerned with developing a scope of work and engaging a suitable consultant to develop a blueprint for a 5-year plan for the SPF Broodstock Production and Nuclear Breeding Centre.

Outputs: Blueprint for a 5-year plan for the SPF centre and nuclear breeding centre

Time-frame: 3 months

Principal Investigator(s): Potential PIs to be considered include Len Stephens, Craig Foster, Ewan Colquhoun, Nick Robinson

Co-investigators: Tony Charles, APFA R&D Committee, Kim Hooper

Cost: $50k

Funding sources: APFA IPA/FRDC

3. Epidemiology

Need: The epidemiologic study is needed to identify potential sources of infection and transmission pathways factors that determine the spatial distribution of the WSSV during the outbreak. The results will be used for planning of future risk mitigation and surveillance strategies. This project would be concerned with undertaking an epidemiological study of the Logan River outbreak of WSD and would include collection of ALL existing data; investigation of all potential pathways (prawns, feed, bait etc.); spatial distribution (infection/prevalence); future risk mitigation/surveillance strategies.

Outputs:

- A peer-reviewed report on the epidemiology of the white spot disease outbreak on the Logan River, SE Queensland
- Communication package for regulators and industry to ensure uptake of the recommendations from the study

Time-frame: 12 months

Principal Investigator(s): Stephen Wesche, QDAF

Co-investigators: DAWR (Ingo Ernst), CSIRO-AAHL Fish Diseases Laboratory (Mark Crane et al.), NSW DPI EMAI (Peter Kirkland)

Cost: $100k

Funding sources: QDAF/FRDC

4. Chemical use permits

Need: Chemical use permits for a variety of chemicals are required to facilitate their use during an emergency disease outbreak. Robust information on the dosage, efficacy etc. of chemicals for use in management of disease outbreaks on prawn farms in Australia is required to support APVMA permit applications. This project would review the literature on compounds that could potentially be used during the stock destruction, disinfection and disposal phases of the response to an emergency disease outbreak on Australian prawns farms.

Outputs: All data to support permit applications for Trichlorfon, chlorine, hydrogen peroxide, benzalkonium chloride (BKC), copper sulphate ($CuSO_4$)

Time-frame: 12 months

Principal Investigator(s): Matt Landos

Co-investigators: Stephen Wesche (QDAF)

Cost: $300k

Funding sources: QDAF/FRDC

5. **Building Biosecurity Capability across the Wild Harvest Fisheries**

Need: A White Spot Disease R&D Needs Workshop held in Brisbane on Wednesday 18 October 2017 provided a forum to unpack research issues with respect to the detection of WSSV and the response to the outbreak. The government and industry response demonstrated gaps in capacity, unexpected operational problems and communication. The purpose of this project application is to address the information and education gaps identified amongst the commercial fishing sector.

Outputs:

- Development of biosecurity material that can be accessed online via videos or written material drafted by communications experts, government and industry.
- Delivery of "train the trainer" type workshops across Queensland.
- A better informed Queensland wild catch and post-harvest fishery with a higher appreciation of aquatic biosecurity.

Time-frame: 6 months

Principal Investigator(s): Eric Perez

Co-investigators: Biosecurity Queensland, Dr Matt Landos and Dr Ben Diggles

Cost: $100k

Funding sources: FRDC

6. **Industry training**

Need: The white spot disease outbreak on farms located on the Logan River, SE Queensland, has highlighted the need for training of industry sectors in disease preparedness and response. This project would be concerned with delivery of biosecurity training to the wild-catch and farming sectors, including a simulation exercise to assess level of preparedness and response capability for a prawn disease event and identify and explore opportunities for enhancement of preparedness and response capabilities.

Outputs:

- Commercial wild catch fishers and prawn farmers have the capability to respond to a disease outbreak.
- Existing AQUAVETPLAN manuals are updated with information for wild catch and farms.

Time-frame: 12 months

Principal Investigator(s): Matt Landos

Co-investigators: QDAF, DAWR, CSIRO-AAHL

Cost: $50k

Funding sources: DAWR/FRDC

7. Gamma-irradiation of bait prawns

Need: The white spot disease outbreak on farms located on the Logan River, SE Queensland, is the greatest threat to the Moreton Bay Fishery. Uncooked product, including bait prawns, cannot be sold outside of the containment area until April 2019, threatening the sustainability of this fishery that supplies 70% of bait prawns used by recreational fishers around Australia. The use of gamma-irradiation to inactivate WSSV in prawn tissue is a potential method to treat bait prawns and allow treated prawns to be sold outside the containment area potentially allowing fishers to maintain their businesses. Thus, there is a need to determine the appropriate dose of gamma-irradiation for treating prawns in such a way as to inactivate the virus while not destroying the marketability of the product. This project would be concerned with assessment of gamma-irradiation as a feasible method for treating wild-catch prawns from Moreton Bay for supplying the bait market.

Outputs:

- Technical/logistical assessment of gamma-irradiation (or other) to allow path to market (Go/No go point).
- Determine efficacious dose for a) maintenance of product quality and b) virus inactivation

Time-frame: 12 months

Principal Investigator(s): Stephen Wesche, QDAF

Co-investigators: CSIRO-AAHL, QSIA

Cost: $50k

Funding sources: Bait industry (e.g. Tweed), Steritech, QDAF, CSIRO, ACPF, FRDC

8. FRDC Project proposal 2017-078

Need: Following the recent incursion of white spot syndrome virus (WSSV) into SE Queensland, there is an urgent need to determine if WSSV has become established in Australian waters, and to determine the level of risk this virus poses to a range of commercially important penaeid species. Extensive environmental sampling of a variety of crustacean species, including large numbers of penaeid prawns has demonstrated a low prevalence of WSSV in localised areas of Moreton Bay and associated waterways, but it is not known if this is a transient detection or if the virus has become established. It is also necessary to assess the susceptibility of penaeid species of interest and to ensure that virus detection methods have high sensitivity to reliably determine the status of WSSV in Australian waters. Sensitive methods of detection are also required for screening of broodstock and early developmental stages to minimise risk in hatcheries and for the movement of stock for grow out. Critical information on infection dynamics of WSSV in penaeid prawns including the minimum dose required to induce infection, as detected by sensitive laboratory assays, is currently lacking. Determining the minimum infectious dose will underpin our understanding of situations likely to lead to infection of susceptible species in the wild, and provide a basis for assessing the sensitivity of surveillance methods where infection is subclinical. This project is concerned with developing highly sensitive methods for the detection of sub-clinical infections in crustaceans and determining the minimum infectious dose for virus transmission.

Outputs:

- Improved (efficiency) method for detection of WSSV
- Determination of minimum infective dose

Time-frame: 3 years

Principal Investigator(s): Cheryl Jenkins, NSW DPI

Co-investigators: CSIRO-AAHL, QDAF

Cost: $300k

Funding sources: FRDC/NSW/QLD/AAHL/APFA

9. Bait & burley use and disposal

Need: Previous *Bait and burley use surveys* (2002 and 2007) provided significant inputs into the *Generic Import Risk Analysis Report for Prawns and Prawn Products* (Prawn IRA, 2009). Since that time there are likely to have been changes in bait use behaviours. Up to date information is required to understand current bait use behaviours of recreational fishers (including other seafood e.g. cooked prawns, non-prawn seafood). Data will inform the exposure assessment component of the risk analysis for prawns and prawn products. Following the recent incursion of white spot syndrome virus (WSSV) into SE Queensland, it is clear that current data on bait and burley use and disposal patterns need to be reviewed. This project is concerned with gathering data on current bait & burley use and disposal patterns.

Outputs:

Current data on bait & burley use (and disposal) patterns:
- Imports, domestic translocation
- State-by-state (methodology: data in a form that meets RA process)

Time-frame: mid 2018

Principal Investigator(s): Specialist (put out to tender)

Co-investigators: end-users (to input design)

Cost: $200k

Funding sources: DAWR/FRDC

10. Diseases in non-prawn crustaceans

Need: Under current policy, ALOP (appropriate level of protection) for non-prawn crustaceans is met by:

- lower prevalence of diseases of biosecurity concern to Australia as product is predominantly wild-caught, AND
- lower risk of diversion due to high product value.

If the bait survey (see project 8 above) identifies that non-prawn seafood products are diverted for use as bait, information about prevalence of prawn diseases of biosecurity concern to Australia in these products would be useful to inform whether a policy review is required and ensure import conditions for non-prawn seafood meet ALOP. This information will be needed to inform the review of the *Generic Import Risk Analysis Report for Prawns and Prawn Products.*

Outputs: Determine prevalence of diseases of biosecurity concern in non-prawn crustacean products

Time-frame: mid 2018

Principal Investigator(s): Specialist (needs sound diagnostic capability)

Co-investigators: TBA

Cost: To be determined

Funding sources: DAWR/FRDC

11. Viability of disease agents in cooked prawns

Need: The Prawn IRA 2009 recognised that commercially cooking imported prawns achieves Australia's ALOP because:

- cooking may partially inactivate viruses, AND
- cooking reduces the likelihood of the prawns being diverted for use as bait, crustacean broodstock feed, or being further processed.

If the bait survey (see Project 8 above) indicates that cooked prawns are being diverted for use as bait, we need to confirm whether cooked prawns can transmit prawn diseases (of biosecurity concern to Australia) to live prawns, and under what cooking treatments.

This information will be used to inform the review of the IRA and ensure import conditions meet ALOP.

Outputs: Information on heat temperature required to inactivate pathogens in host tissues

Time-frame: 12-18 months

Principal Investigator(s): Specialist (needs bio-secure bio-assay capability)

Co-investigators: DAWR/end-users

Cost: $200k (tied into other projects requiring bio-assay for efficiency)

Funding sources: DAWR/FRDC

12. On-farm biosecurity standards

Need: In response to the WSD outbreak, APFA and QDAF have developed minimum biosecurity standards for prawn farms to minimise disease risk. The proposed enhanced biosecurity measures include technologies such as the use of mechanical filtration (drum filters) and/or disinfection (e.g. ozone treatment) of input and/or output water on prawn farms. However, the effectiveness of these treatments have not been validated for Australian prawn farms. This project would be concerned with determining the biosecurity benefits of filtration and ozone treatment on-farm, understanding that both adverse and beneficial factors may be affected (replacement of beneficial factors may be required).

Outputs: Determine the effects of filtration (drum filters)/disinfection (ozone) of input/output water on disease biosecurity and prawn pond management.

Time-frame: 12-18 months

Principal Investigator(s): David Mann (Bribie)

Co-investigators: Industry/suppliers

Cost: To be determined

Funding sources: Prawn IPA/FRDC

13. Assessment of decontamination and disposal methods

Need: During the emergency response to the white spot disease outbreak in SE Queensland liquid chlorine was used for decontamination and disposal. Selection of this chemical was based on scientific literature, recommendations in AQUAVETPLAN and the OIE Manual. Alternative chemicals are available and confirmation of the efficacy of these treatments is required.

Outputs: Determine the efficacy of disinfection protocols for inactivation of WSSV in pond/river water and prawn tissues.

Time-frame: 12-18 months

Principal Investigator(s): Stephen Wesche, QDAF

Co-investigators: Nick Moody (AAHL) - Bio-secure bio-assay capability required

Cost: $200k

Funding sources: QDAF/FRDC

Discussion and Conclusion

At least 12 projects were identified as high priority by stakeholders participating in the workshop. Some projects were relatively short-term (<6 months) with a relatively low estimated cost (<$100k), and identified as a high priority by industry groups, e.g., SPF Centre and nuclear breeding facility and y-irradiation of bait prawns. Medium-term (12-18 month; $100k-$200k) projects included evaluation of diagnostic tests, training, epidemiology, bait & burley survey, non-prawn diseases of concern and WSSV inactivation. The remaining projects were longer term (>18 months to 3 years; >$200k) and included detection of sub-clinical infections and input/output water treatments. The total cost (excluding in-kind contributions) for all projects is likely to be around $2 million.

Implications

The workshop provided a forum for major stakeholders to discuss R&D needs following the white spot disease outbreak in SE Queensland. There was strong agreement between stakeholders on the key R&D priorities and in addition to development of project concepts, a further outcome of the workshop was a better understanding of industry/regulator needs by the workshop participants.

Recommendations

It is recommended that the project concepts developed during the workshop should be forwarded to key research providers in a call for expressions of interest (full proposals) for evaluation by FRDC.

Appendices

Appendix 1: Draft Workshop Agenda

FRDC White Spot Disease R&D Planning Workshop

Brisbane, 18 October 2017

Draft Agenda

08.00	Coffee on arrival	
08.30	Introduction and purpose of the workshop	Len
08.45	Biosecurity QLD up-date and future requirements	Kerrod/Steve
09.15	DAWR up-date and future requirements	Ingo/TBA
09.45	FRDC up-date and future requirements	Patrick
10.15	Coffee break	
10.45	FRDC AAHBS/AAHL up-date	Mark/Nick
11.15	APFA priorities	Matt/Tony
11.45	ACPF priorities	Annie
12.15	QSIA priorities	Eric
12.45	Lunch	
13.30	Re-cap of morning presentations – R&D priorities	Len
14.00	Introduction to workshop exercise	Len
14.15	Group discussion re: R&D priorities	Gov/Ind groups
15.00	Tea	
15.30	Prioritisation exercise: “Dots on the wall”	All
15.45	Top priorities re-visited and confirmed	Len
16.00	Project titles/potential research providers/funding	All
16.30	Next steps/wrap-up	Len/Mark/Patrick
17.00	Workshop close	

Appendix 2: Workshop participants

Name	Position	Email address
Kerrod Beattie	A/Director, QDAF	kerrod.beattie@daf.qld.gov.au
Tony Charles	Chair, APFA R&D Committee	tony@australianprawnfarms.com.au
Mark Crane	Leader, FRDC Aquatic Animal Health & Biosecurity Subprogram	mark.crane@csiro.au
Alistair Dick	Member, APFA R&D Committee	
Ben Diggles	Principal, DigsFish Services Pty Ltd	ben@digsfish.com
Kitman Dyrting	Veterinary Pathologist, DPIR, NT	kitman.dyrting@nt.gov.au
Ingo Ernst	Director, Aquatic Animal Health, DAWR	ingo.ernst@ agriculture.gov.au
Jeffrey Go	Veterinary Officer, DPI, NSW	jeffrey.go@dpi.nsw.gov.au
Kim Hooper	Executive Officer, APFA	kim.hooper@apfa.com.au
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Annie Jarret	Chair, ACPF	annie.jarrett@bigpond.com
Matt Landos	Director, Future Fisheries Veterinary Service Pty Ltd.	matty.landos@gmail.com
Nick Moody	Team Leader, CSIRO-AAHL Fish Diseases Laboratory	nick.moody@csiro.au
Eric Perez	Chief Executive Officer, QSIA	eo@qsia.com.au
Joanne Slater	Coordinator, FRDC Aquatic Animal Health & Biosecurity Subprogram	joanne.slater@csiro.au
Mike Snow	Supervising Research Scientist, DPIRD, WA	mike.snow@dpird.wa.gov.au
Len Stephens	Consultant	lrstephens@bigpond.com
Stephen Wesche	Policy and Planning Manager, QDAF	stephen.wesche@daf.qld.gov.au
Matt West	President, APFA	matt@australianprawnfarms.com.au

Appendix 3: Presentations

Queensland's WSSV Program

White Spot Disease R and D Planning Workshop

18 October 2017

WSD Program
Biosecurity Queensland

Overview

- Current status
- Chronology
- Structured Surveillance
- Stakeholder engagement
- Critical Program Needs

Current Status

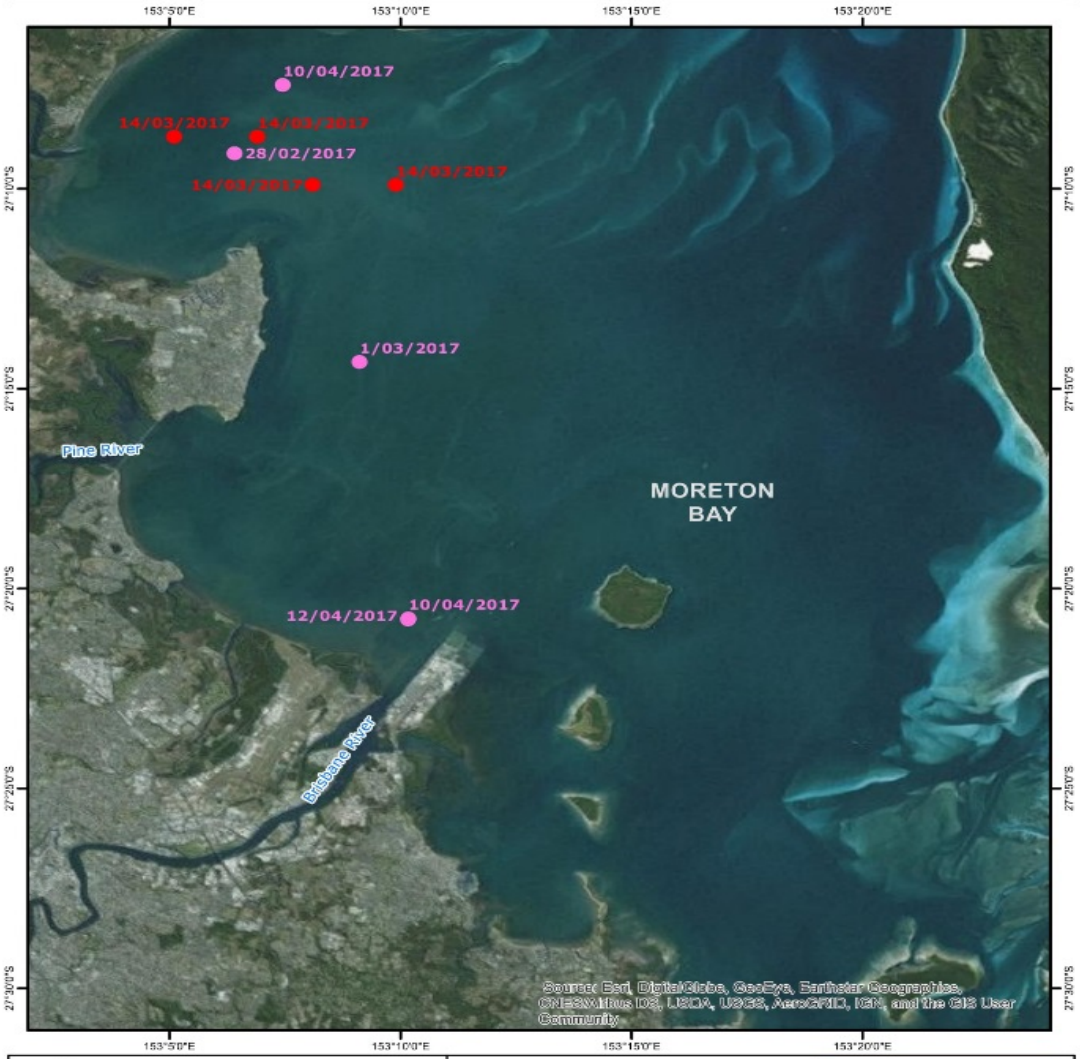
- Logan farms-all dry and will remain till 31 May 2018
- BQ undertaking auditing inspections
- Retail surveillance testing-as needs basis
- National Surveillance Plan-Qlds intent.
- Recent surveillance sampling results
- Industry/ Stakeholder Impacts
- Assessment of D and D phase

Chronology

- First confirmed in Farm 1 December 2016 then Logan River 7 December 2016
  - Additional detections in January and February 2017
- Confirmed detection in Moreton Bay early March 2017 in samples collected from commercial fishers
  - +VE detection again from samples collected during structured survey 14th March
  - And commercial samples collected second week of April

- Ist Round Official Structured Sampling-ALL NEGATIVE
- Next Round Feb 2018

WSD Response - Northern Moreton Bay Positive Detections



Author: Department of Agriculture & Fisheries

Date: 28/04/2017

Co-ord Sys: GCS GDA 1994
Datum: GDA 1994
Units: Degree

Queensland Government

N

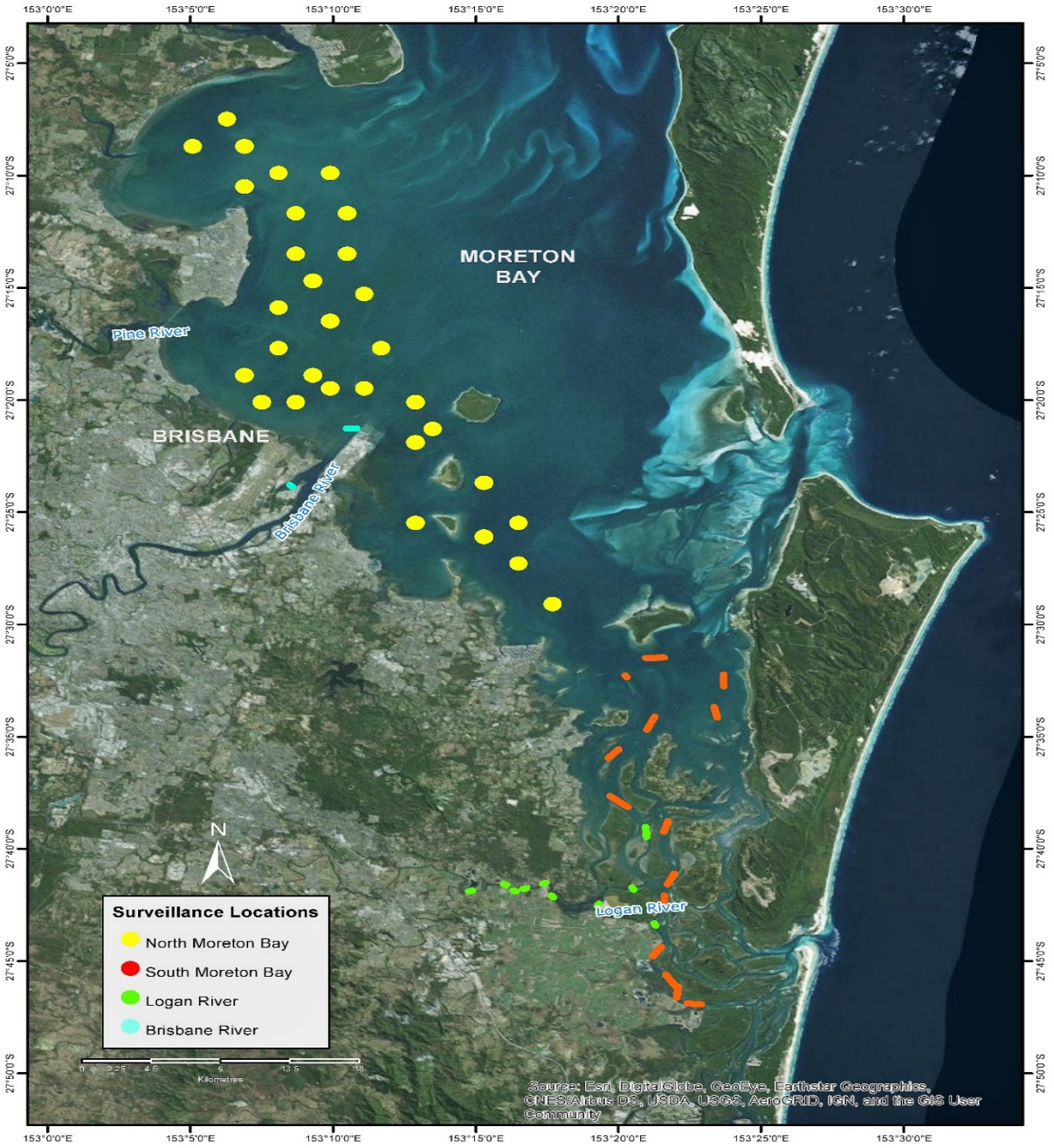
0 1.5 3 6 9 12
Kilometres

- Positive Detection (Structured Surveillance)
- Positive Detection (Commercial)

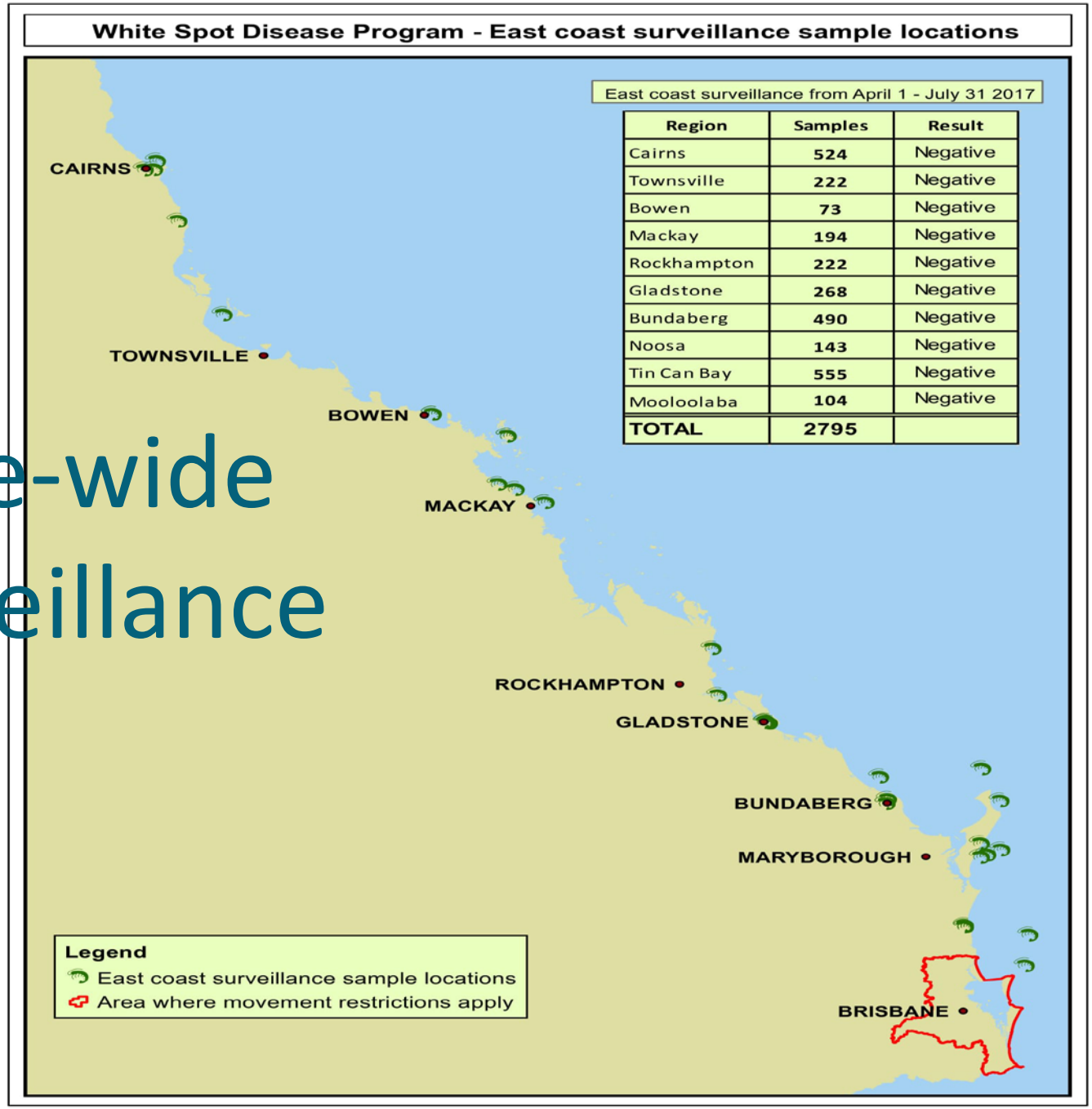
Structured Surveillance

- Wild - Samples collected by DAF or commercial fishers
  - Moreton Bay -45 sites
  - Brisbane River- 2 sites
  - Logan River-9 sites
  - East Coast-opportunistic (major commercial and or recreational activity)

Surveillance areas within the movement restriction area



State-wide surveillance



Region	Samples	Result
Cairns	524	Negative
Townsville	222	Negative
Bowen	73	Negative
Mackay	194	Negative
Rockhampton	222	Negative
Gladstone	268	Negative
Bundaberg	490	Negative
Noosa	143	Negative
Tin Can Bay	555	Negative
Mooloolaba	104	Negative
TOTAL	2795	

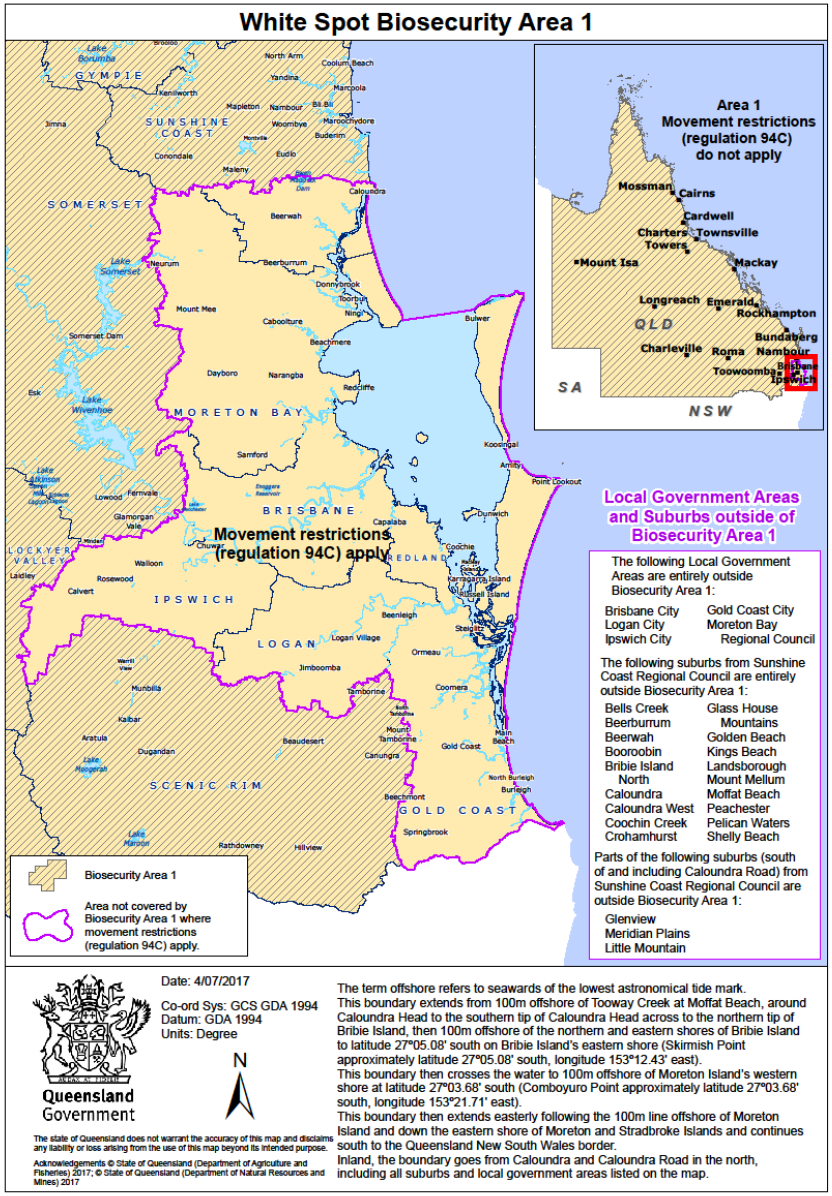
Statistics

- 160 Departmental staff involved in the response
  - More than 50,000 samples tested during surveillance
  - 25,000 samples from prawn farms
  - 25,000 samples from the wild
- 6.8 million litres of chlorine used
- Up to $17.6 million dollars committed by Queensland Government to fund the response in 2016/17.
- 174 days to complete destruction and decontamination work

Movement restrictions

- Cannot remove (uncooked)
  - prawns
  - yabbies
  - worms

- Fishing
  - ✓ allowed
  - ✓ fish caught can be moved
  - ✓ clean equipment before leaving the area
  - ✓ Decontamination Guide



Media-vehicle to get messages out.


"BE A MATE
CHECK YOUR BAIT"
If using prawns as bait, make sure
you use Australian wild-caught.
For more info, visit www.qld.gov.au/checkyourbait
Queensland
Government

Help stop the spread of **white spot disease**

White spot disease could have a devastating impact on our natural waterways and fish stocks if it spreads.

www.daf.qld.gov.au/wsd or call 13 25 23

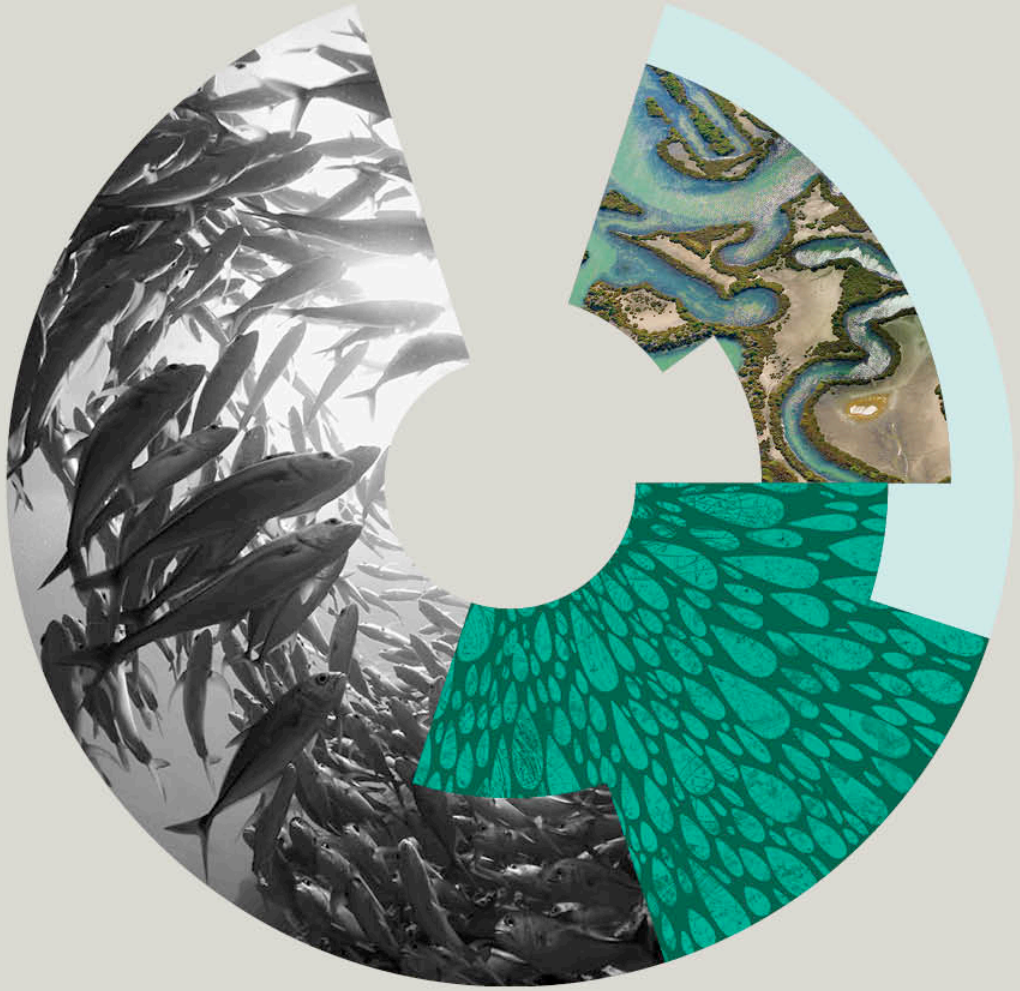
Queensland Government

Program Needs

- Assessment of D and D phase on farms
- Epidemiology Review/Report?
- Proof of Freedom Surveillance
- Working with Prawn farms to implement minimum biosecurity standards
- Finding ways forward for other Commercial Industries affected by movement restrictions

Questions?

DAWR update and R&D requirements



Ingo Ernst
Aquatic Pest and Health Policy
Animal Division
18 October 2017

Key activities / issues

- Coordination of surveillance (proof of freedom)
- Lab diagnostics
- Contingency planning (AQUAVETPLAN)
- Aquatic deed
- Review of prawn import biosecurity risks

Review of prawn import conditions

- A review of the biosecurity risks and import conditions for prawns and prawn products for human consumption was announced on 16 May 2017
- The review will consider all biosecurity risks, not just those associated with white spot syndrome virus
- A draft report is being prepared which will outline the identified biosecurity risks and propose risk management measures to achieve Australia's appropriate level of protection (ALOP).
  - It is anticipated the draft report will be available in 2019.
  - The draft report will be released for a 60-day stakeholder comment period.

Information requirements:

1. Current Bait and Burley Use Survey

- Previous *Bait and burley use surveys* (2002 and 2007) provided significant inputs into the *Generic import risk analysis report for prawns and prawn products* (Prawn IRA 2009).
- Since that time there are likely to have been changes in bait use behaviours
- Up to date information is required to understand current bait use behaviours of recreational fishers (including other seafood e.g. cooked prawns, non-prawn seafood).
- Data will inform the exposure assessment component of the risk analysis for prawns and prawn products.
- Outcomes will also help inform the necessity for two additional studies:
  1. Cooked prawn bioassays
  2. Prevalence of diseases of concern in imported non-prawn seafood products

2. Cooked prawn bioassays

- The Prawn IRA 2009 recognised that commercially cooking imported prawns achieves Australia's ALOP because:
  - cooking may partially inactivate viruses, AND
  - cooking reduces the likelihood of the prawns being diverted for use as bait, crustacean broodstock feed, or being further processed.
- If the bait survey indicates that cooked prawns are being diverted for use as bait, we need to confirm whether cooked prawns can transmit prawn diseases (of biosecurity concern to Australia) to live prawns, and under what cooking treatments.
- This information will be used to inform the review and ensure import conditions meet ALOP.

3. Prevalence of diseases of concern in imported non-prawn crustacean products

- Under current policy, ALOP for non-prawn crustaceans is met by:
  - lower prevalence of diseases of biosecurity concern to Australia as product is predominantly wild-caught, AND
  - lower risk of diversion due to high product value.
- If the bait survey identifies that non-prawn seafood products are diverted for use as bait, information about prevalence of prawn diseases of biosecurity concern to Australia in these products would be useful to inform whether a policy review is required and ensure import conditions for non-prawn seafood meet ALOP.

Role of CSIRO Australian Animal Health Laboratory in the emergency response to the white spot disease outbreak in farmed prawns in Queensland, 2016-17

AUSTRALIAN ANIMAL HEALTH LABORATORY (AAHL)
www.csiro.au

Moody NJG, Mohr PG, Hoad J, Williams LM, Cummins DM, Slater J, Crane MStJ and Eagles D

4th FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity

AFDL's role in aquatic disease responses

Diagnostic submissions from State authorities:

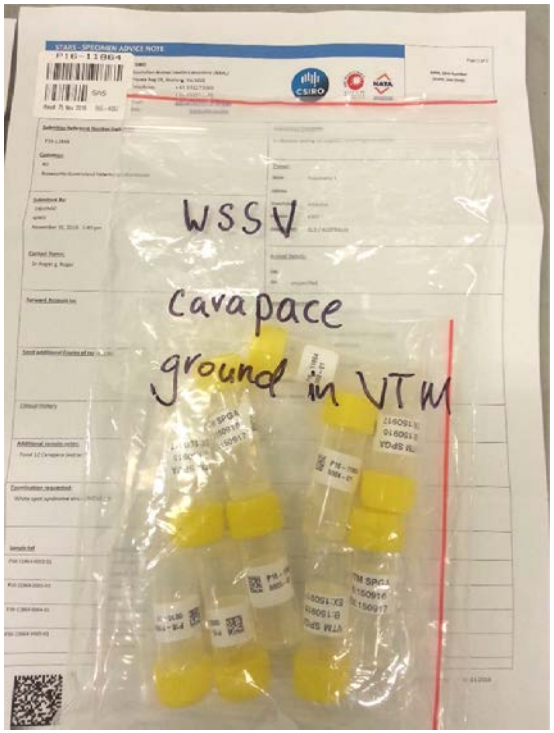
Category 1: Routine samples (e.g. health surveillance, no disease suspected, fee-for-service)

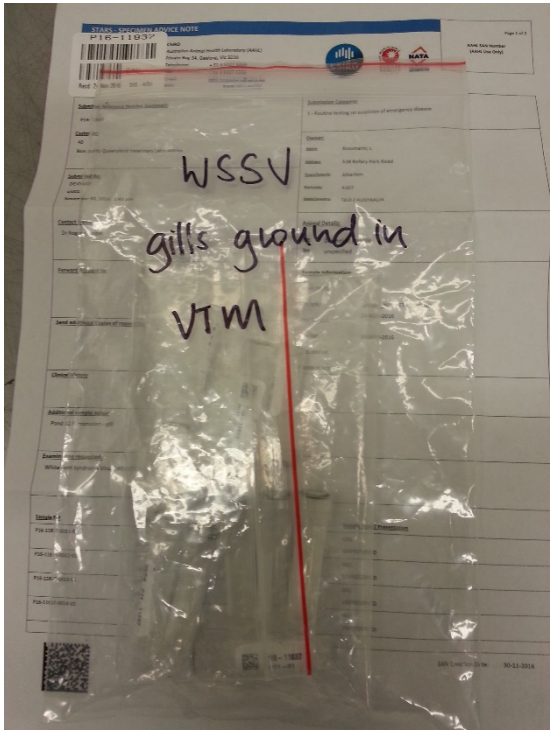
Category 2: Exotic disease exclusion (low likelihood) – test results required within 72 hours

Category 3: Exotic disease exclusion/confirmation (high likelihood) – test results required within 24 hours. Diagnostic test report issued to submitting laboratory, CVO of the submitting state, Australian CVO and Director of AAHL.

Prawns submitted for WSSV confirmation

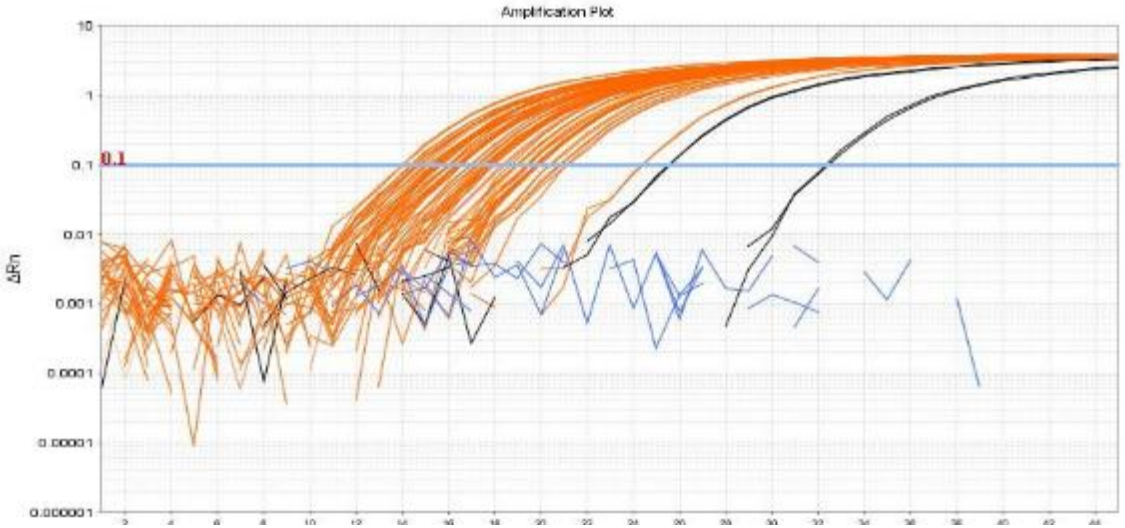
November 30, 2016: Samples received from Queensland laboratory at 8:30pm



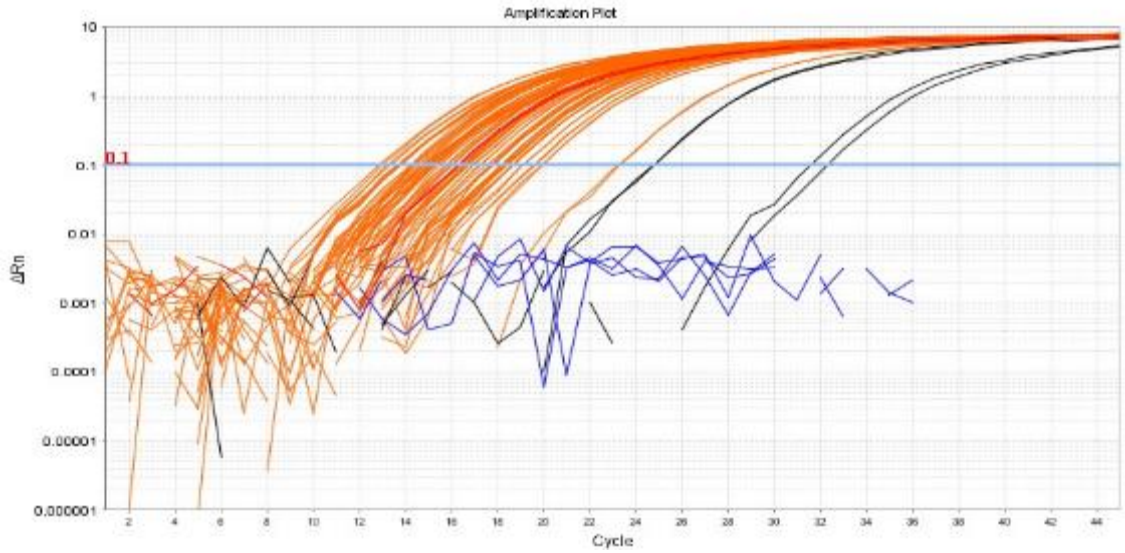


December 1, 2016: WSSV qPCR POSITIVE result at ~1:00am, WSSV PCR POSITIVE and sequence reported at 9:30am, aqCCEAD convened, OIE notified

Index pond was very positive



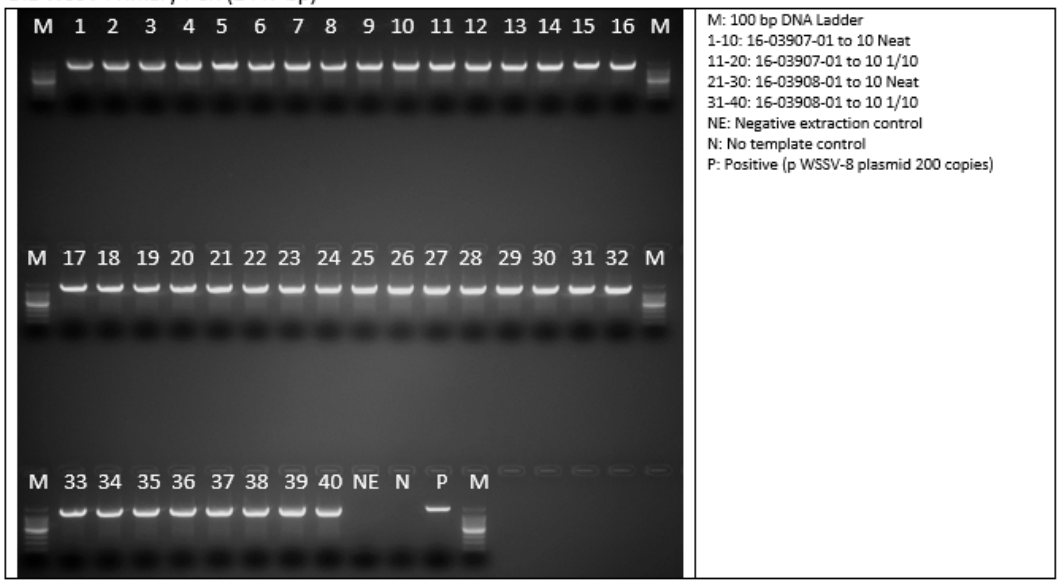
OIE WSSV qPCR amplification curves for samples (orange) and positive (black) and negative (blue)controls.



CSIRO WSSV qPCR amplification curves for samples (orange) and positive (black) and negative (blue) controls.

Confirmation by sequence analysis

OIE WSSV Primary PCR (1447 bp)



Amplicons from Lanes 1-4 (16-03907-01 to 04) and Lanes 21-24 (16-03908-01 to 04) were excised, purified and submitted for sequencing.

Shares 100% nucleotide identity with WSSV in the NCBI database, including WSSV [redacted]

TTCTCTCTTGATAGCTAGAGCAAAGGCATTTTGGTTAGTATTTGGTGGACAGTTAGTATAGTCAGAAGAAATATTACGTTCGATATCGTTGAGGATATCCTTGAATTCAGCATCAGTTACTTGCTTAGAGATGGCTTCGAAAACTCGAGCCAAATGCTTACGAAGAGTAGTGTTAGAAGAGGAAGAATAGGTAGAGGATACGGCAGCTGCTGCACCTTGTTCGGCGTTCTTTTCTTCGAATGTA
TCTCCCTTTGACAGAAGGAAATCAAAGGCGAGAGGGCCGCTGGCGCATGAGGCGAATGGTACATTTTCCGGGCGAGCTGCCTTGCCGGAAATTAGTGTGTGATAGACGGCATTCTTCATGGCTTCTGAGATGAGGACGGGACCATTGTTGGATGTTGTTTCGCTATCTTCATAATCCATTGATTCTACTCCTCCATTATTATTAGATTTGAAGAGGTCGTCAGAGCCTAGTCTATCAATCATTTC
CTTGGTGTTTTCATATACGCTTTCTGGTACAGATTCAGCGATGGTGCCGGCCAGTTCGTTCACGATAACCTTTCCAAGATCGGCGATAAGTTGTTCCAAAACATTAGCAAACACGACAGTTTGAGCGTTCATTGTGCTCTTTTGGATGTATCTGTTCCTTGGCAGAGCATTCTGGATCAGTTCAGATTCGTTACCGTTTCCATCGGTTGCCTGGAAGACGGCTGGCATGGCTGAAGCGACAGTTCC
CTTGGCGTCGGCGGTGCACACGTGCTGGCGATGACATTCCAATATTCCTAGTACACTTCTTGCATTATTTGGTTTGACAATAACGTCGAGGTTGACTCCTGCCGTTGTCATGCCAGAGTTGGAGAGGGCGAGTCTTGATGGTTCAAATGTTCCATTAAAGTTGGAGTTCATTCTCATTTCAGTCCATTCGCTAACCTTCTTAAGGAGGGAAGATCCTGTTACTCTAGAATCGATCTTGGACATGTT
CATCACGTAATGTTTGTCACTAATAAGCTTGTTGGCCTGATAGGGTGTGGCTCTAATTTCTTCCATGGAGACTTCTGGGCGGGCGAATGCGAAAGAGGATACCAGATGCTCGTTATCTGAGGGAGTAAAAGTGTGGTGGAGATGGAAGGGGCAGTTACAATTTTGCTTGTGGGACATGGCGTATTCGTCCCCATATCTTCCCAAGAATTTCTCAAATCGTTCAATGTTCTTGAAGAAGCATTGA
GTATCAGTCATGTGCAATTTGATGTTGTCCATGAAAGATGACTTTGTATGGGAATCCATGGAAGAAAGCATGAGGTGAGAAGCGCAGGTGAGGGCGGCAGGGAAGGCTGGGTCAA

Whole genome sequencing at AAHL of samples from 1IP, 5IP and northern Moreton Bay indicate it was not a multiple source incursion

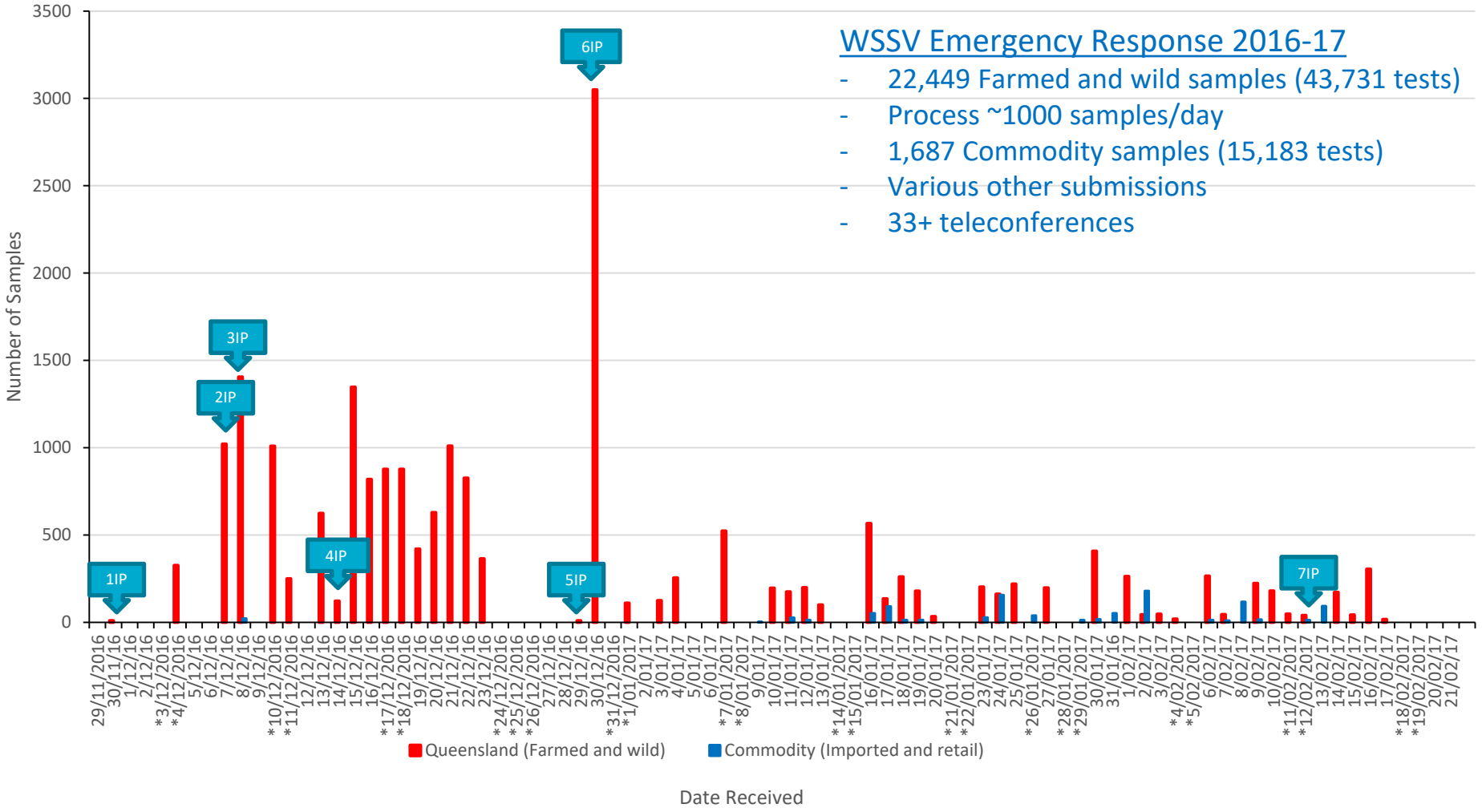
Enacted the AAHL EDRP – 9 December, 2016

- AAHL Emergency Disease Response Plan
  - This plan has been drawn up to describe the range of resources that must be provided within AAHL in the event of responding to an outbreak of an emergency disease and to outline the organisational structure required to meet the demand for technical excellence, quality performance and efficient laboratory output.
  - The activation of the plan is the responsibility of AAHL's Director (or delegate) who will also appoint the Laboratory Response Coordinator. It is the Laboratory Response Coordinator's responsibility to implement the plan, together with the assistance of the Scientific Services, Veterinary Services and Resources Coordinators.
  - Role descriptions and responsibilities are set out in job cards. To ensure that the handover of a role is carried out with minimal loss of function, debriefing will take place.

- Laboratory Response Co-ordinator: Dr Debbie Eagles
- Veterinary Services Co-ordinator: Dr Mark Crane
- Scientific Services Co-ordinator: Dr Peter Mohr
- Resource Co-ordinator: Dr Nick Moody

- Everyone in AAHL available for the response

Largest disease outbreak in Australia



WSSV Emergency Response 2016-17

- 22,449 Farmed and wild samples (43,731 tests)
- Process ~1000 samples/day
- 1,687 Commodity samples (15,183 tests)
- Various other submissions
- 33+ teleconferences

A logistical challenge

- Specimen receipt (4-8 staff)
  - Unpacking, specimen registration, tube labelling
  - Sorting to 96-well format
- Sample preparation (8-10 staff)
  - 8 staff
  - PBSA to bead beating tubes, samples to bead tubes, bead beating (5 bead-beaters)
  - Limited robotics
- Nucleic acid extraction (3-4 staff)
  - Sample clarification, buffer preparation
  - 2 x MME-96 systems
  - Very important robotics
- Real-time PCR (3-4 staff)
  - Loading
  - Data retrieval and analysis
  - 6 x 7500 FAST Thermal Cyclers

A logistical challenge

- 203 x 5ml TaqMan Fast Universal Master Mix

- 44 x 5x MagMax-96 Viral 1 Kit

- 29,000 x 2mL Lysis Matrix M tubes

- Purchase Order with Thermo Fisher (Life Technologies)
- Other companies happy for me to pay when I got the invoice

A logistical challenge

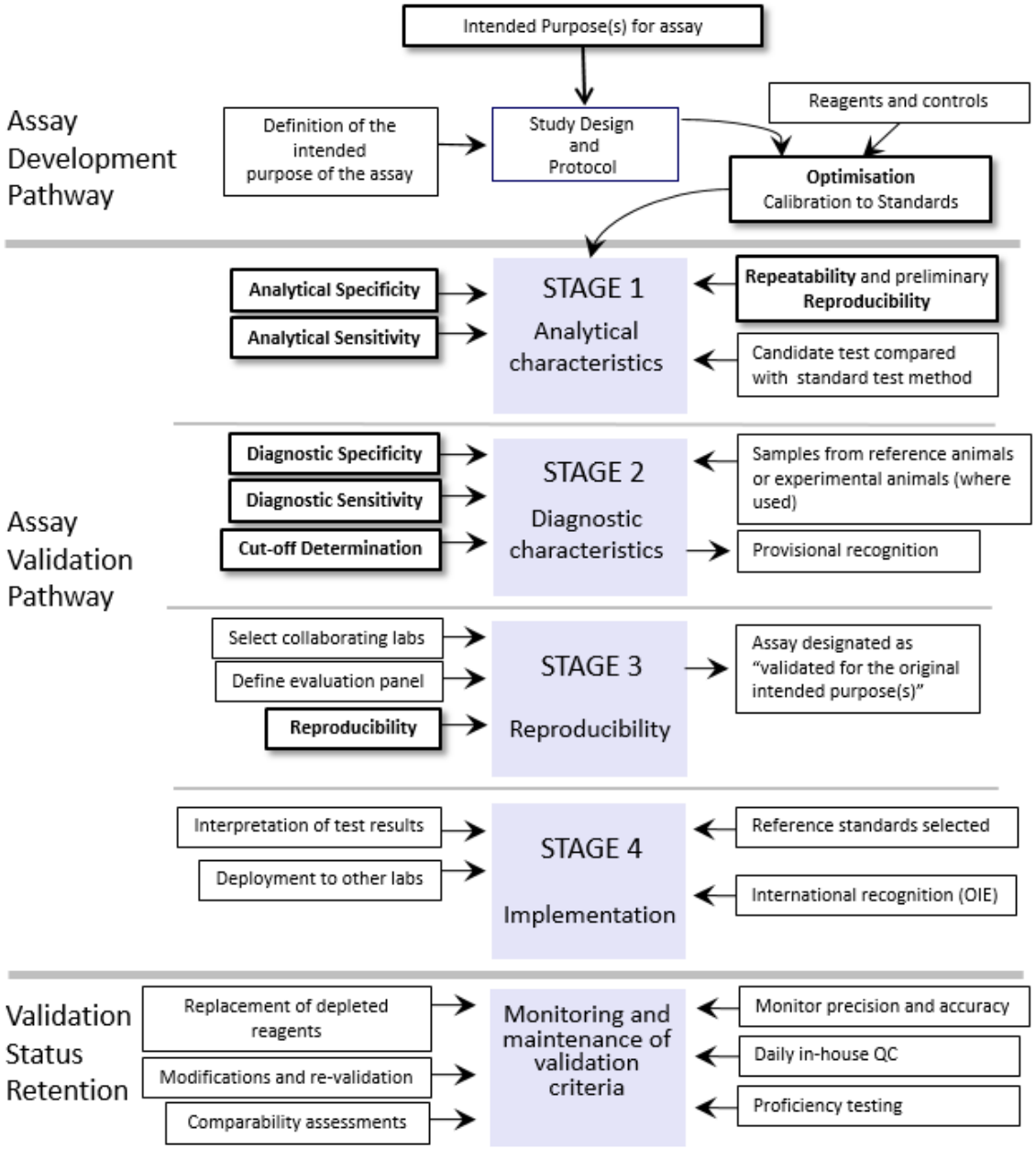
Assay validation?

- Validation is the process that determines the fitness of an assay, which has been properly developed, optimised and standardised, for a specific, defined diagnostic purpose (OIE)

- Assay validation is a core requirement of a laboratory quality management system based on ISO 17025
  - Section 5.4.5.1: “Provision of objective evidence…..”

- The process to attain assay validation is not a simple task

- Worthwhile doing:
  - Gives you confidence in your results
  - Gives stakeholders confidence in your results
  - Greater ability to withstand legal scrutiny



(A) Assay Development Pathway:

- definition of intended purpose(s),
- optimisation,
- standardisation

(B) Assay Validation Pathway:

- repeatability,
- analytical sensitivity (ASe),
- analytical specificity (ASp),
- thresholds (cut-offs),
- diagnostic sensitivity (DSe),
- diagnostic specificity (DSp),
- reproducibility,
- fitness for intended purpose(s)

Not always completed in order (1, 2, 3, 4)

(C) Validation Status Retention

- ongoing monitoring through the Quality System

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.01.05_VALIDATION.pdf

(A) Definition of the intended purpose

The most common purposes, based on terrestrial pathogens, are to:

1. Contribute to the demonstration of freedom from infection in a defined population:
    a) 'Free' with and/or without vaccination,
    b) Re-establishment of freedom after outbreaks
2. Certify freedom from infection or presence of the agent in individual animals or products for trade/movement purposes.
3. Contribute to the eradication of disease or elimination of infection from defined populations.
4. Confirm diagnosis of suspect or clinical cases (includes confirmation of positive screening test).
5. Estimate prevalence of infection or exposure to facilitate risk analysis (surveys, herd health status, disease control measures).
6. Determine immune status of individual animals or populations (post-vaccination)

Simplified for aquatic pathogen detection:

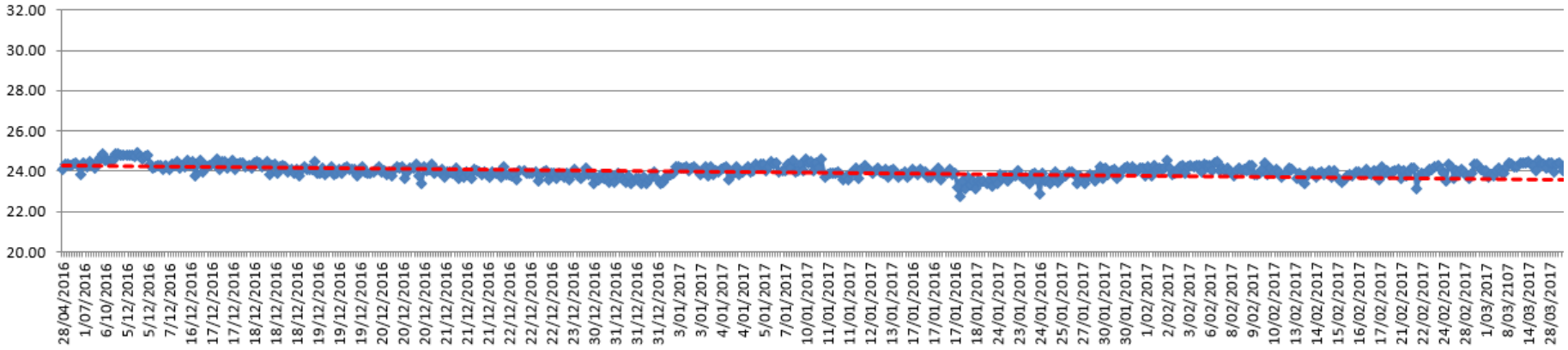
1. Detection of sublinical infections (surveillance) $\rightarrow$ **apparently healthy animals**
2. Confirmation/exclusion of clinical disease (disease investigation) $\rightarrow$ **clinically-diseased animals**

(B) Repeatability

Repeatability is the level of agreement between results of replicates of a sample both within and between runs of the same test method in a given laboratory.

Between-run variation is determined by using the same samples in multiple runs involving two or more operators, done on multiple days.

It is not acceptable to prepare a final working dilution of a sample in a single tube from which diluted aliquots are pipetted into reaction vessels, or to create replicates from one extraction of nucleic acid rather than to extract each replicate before dilution into the reaction vessels (depends on what you are doing and what is practical)



CSIRO WSSV qPCR Positive control – multiple operators

(B) Analytical sensivity (ASe)

Limit of detection (LOD)

The LOD is the estimated amount of analyte in a specified matrix that would produce a positive result at least a specified percent of the time. Typically, estimated LOD will be based on spiking of the analyte into the target matrix.

Plasmid diluted in water

Plasmid Copies	AbHV ORF49	AbHV ORF66	AbHV ORF77	WSSV CSIRO	WSSV OIE	OsHV-1 Martenot	OsHV-1 EMAI	Megalo CSIRO	AHPND OIE	VHSV Jonstrup	VHSV Garver	TSV OIE	TSRV CSIRO
200,000,000	11.85	11.04	11.42	5.68	10.48	9.19	10.75	10.33	11.35	NA	6.33	9.89	8.79
20,000,000	15.64	14.9	15.64	7.55	13.49	14.47	14.99	14.87	14.71	NA	12.83	14.05	13.98
2,000,000	19.01	18.21	18.84	12.21	17.11	17.21	18.54	18.81	18.39	8.99	17.01	17.51	17.43
200,000	22.48	21.5	22.58	15.78	20.66	20.47	22.02	21.98	21.39	14.58	18.24	21	20.69
20,000	25.44	25.3	26.04	19.49	24.31	24.68	24.94	25.96	24.78	18.03	22.06	24.67	24.9
2,000	29.52	28.73	30.21	23.15	27.45	27.82	28.84	29.28	29.48	1.41	26.24	28.17	28.49
200	34.39	32.99	34.83	27.3	31.97	31.44	34.6	33.62	33.11	25.16	28.76	31.91	31.2
20	Und	37.08	38.61	30.82	35.6	33.95	36.11	37.11	36.60	28.64	30.71	34.86	36.72
2	Und	Und	Und	34.51	39.02	36.8	Und	Und	Und	31.64	34.27	Und	Und
0.2	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	36.27	Und	Und

Plasmid diluted in host DNA

Plasmid Copies	AbHV ORF49	AbHV ORF66	AbHV ORF77	WSSV CSIRO	WSSV OIE	OsHV-1 Martenot	OsHV-1 EMAI	Megalo CSIRO	AHPND OIE	VHSV Jonstrup	VHSV Garver	TSV OIE	TSRV CSIRO
200,000,000	10.39	10.18	11.07	6.94	10.85	8.62	10.76	10.07	8.54	NA	8.20	10.27	8.37
20,000,000	14.73	13.57	13.57	11.11	14.28	13.56	14.42	14.06	13.10	NA	12.78	13.73	12.83
2,000,000	18.2	17.14	18.26	15.84	17.47	16.82	17.91	17.61	16.38	12.60	15.87	17.58	16.53
200,000	21.71	20.36	22.19	18.3	21.09	20.3	20.52	20.72	19.56	15.18	19.19	20.49	19.78
20,000	24.96	24.16	26.54	22.91	24.74	23.6	24.9	24.38	22.87	18.32	22.42	24.32	23.89
2,000	28.39	28.49	29.6	26.86	28.18	26.94	27.27	27.81	26.23	23.26	25.46	27.03	27.31
200	32.54	31.45	32.81	30.31	31.61	30.76	30.96	31.66	29.53	26.53	27.85	29.95	29.98
20	34.35	36.54	38.18	34.13	35.87	33.79	35.78	34.73	32.93	29.75	30.34	34.81	33.91
2	Und	38.01	39.31	Und	37.96	37.13	Und	38.13	35.92	32.67	33.79	37.54	36.09
0.2	Und	Und	Und	Und	Und	Und	Und	Und	Und	41.57	Und	Und	Und

(B) Analytical specificity (ASp)

Inclusivity is the capacity of an assay to detect several strains or serovars of a species, several species of a genus, or a similar grouping of closely related organisms

Both the CSIRO and OIE WSSV qPCRs detect WSSV in samples from eight countries including [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] and [redacted]

(B) Analytical specificity (ASp)

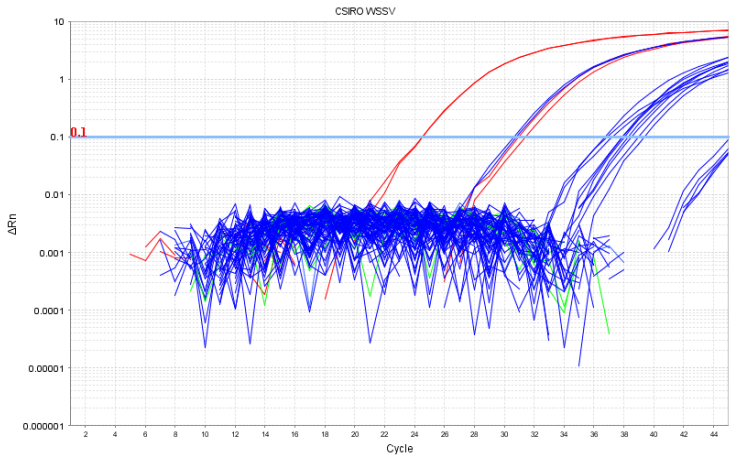
Exclusivity is the capacity of the assay to detect an analyte or genomic sequence that is unique to a targeted organism, and excludes all other known organisms that are potentially cross-reactive.

Agent	CSIRO WSSV qPCR	OIE WSSV qPCR
Acute hepatopancreatic necrosis disease (AHPND)	Negative	Negative
Infectious hypodermal and haematopoietic necrosis virus (IHHNV- C)	28.41*	30.09*
Infectious hypodermal and haematopoietic necrosis virus (IHHNV- H)	Negative	Negative
Infectious myonecrosis virus (IMNV)	Negative	Negative
Taura syndrome virus (TSV)	Negative	Negative
Monodon baculovirus (MBV)	Negative	Negative
Mourilyan virus (MoV	Negative	Negative
Yellow head virus genotype 1 (YHV1)	Negative	Negative
Yellow head virus genotype 2 (YHV2)	Negative	Negative
Yellow head virus genotype 7 (YHV7)	Negative	Negative
Yellow head virus genotype 9 (YHV9)	Negative	Negative
Yellow head virus genotype 9b (YHV9b)	Negative	Negative
Yellow head virus genotype 10 (YHV10)	Negative	Negative
P. monodon	Negative	Negative
P. merguiensis	Negative	Negative
L. vannamei	Negative	Negative

*WSSV co-infection in tissue supplied as positive control material for IHHNV

(B) Cut-off determination (thresholds)

To obtain DSe and DSp estimates of the candidate assay, which is measured on a continuous scale, the test results first must be reduced to two (positive or negative) or three (positive, intermediate [doubtful] or negative) categories of test results. This is accomplished by insertion of one or two cut-off points (threshold or decision limits) on the scale of test results.



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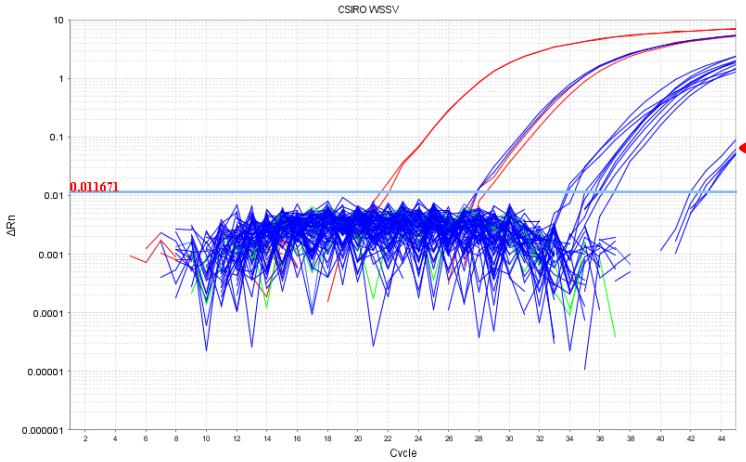

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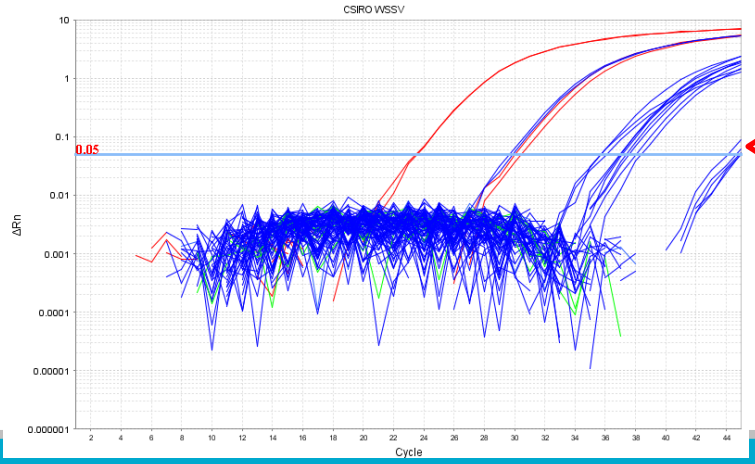

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What is the correct threshold?
What is the correct cut-off?
What is the correct answer?

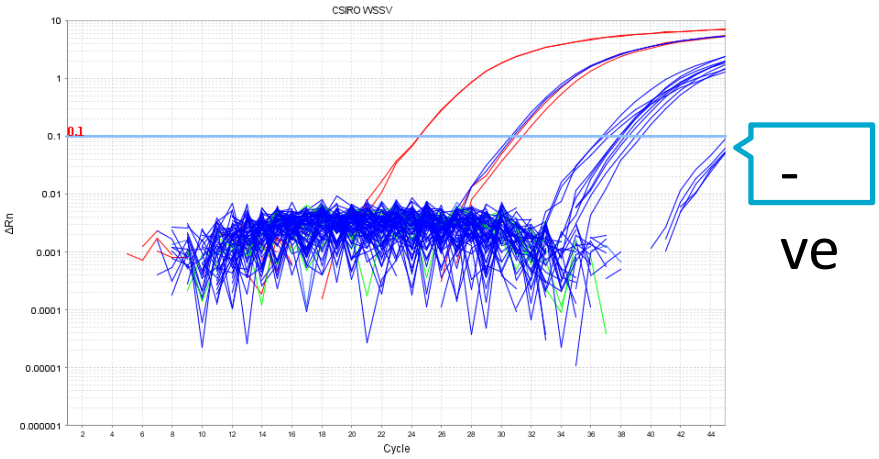
(B) Cut-off determination (thresholds)

What is the correct threshold?
What is the correct cut-off?
What is the correct answer?

Depends of the purpose of the laboratory as much as the purpose of the test

AFDL: threshold of 0.1 for monitoring positive control results and don't use cut-offs. Anything with a typical amplification curve is considered a presumptive positive and subject to additional testing.

"Additional verification by conventional PCR and sequencing is considered an acceptable alternative to using a C_T cut-off to determine positive or negative status of a test sample (Caraguel *et al*, 2011)."



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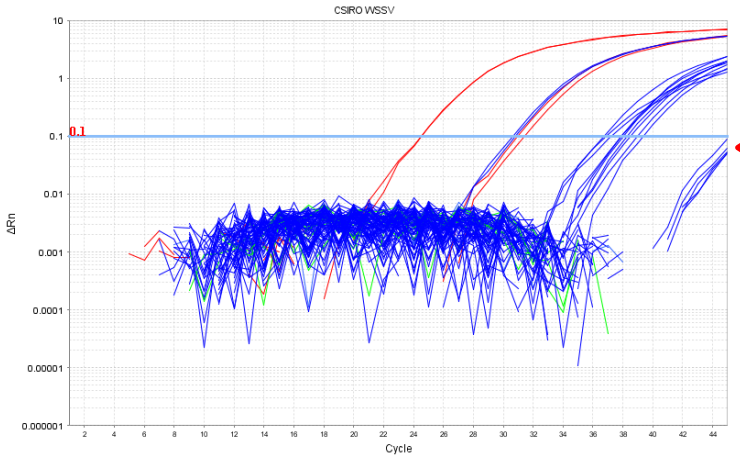

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Caraguel C.G.B., Stryn H., Gagné N., Dohoo I.R. & Hammell K.L. (2011). – Selection of a cutoff for real-time polymerase chain reaction results to fit a diagnostic purpose: analytical and epidemiologic approaches. *J. vet. diagn. Invest.*, 23, 2–15.

(B) Diagnostic sensitivity (DSe)

Diagnostic sensitivity (DSe) is the proportion of samples from known infected reference animals that test positive in an assay. Ideally, they are derived from testing a panel of samples from reference animals, of known history and infection status relative to the disease/infection in question and relevant to the country or region in which the test is to be used.

Theoretical number of samples from animals of known infection status required for establishing diagnostic sensitivity (DSe) and specificity (DSp) estimates depending on likely value of DSe or DSp and desired error margin and confidence

	2% error in estimate of DSe and DSp			5% error in estimate of DSe and DSp		
	Confidence			Confidence		
Estimated DSe and DSp	90%	95%	99%	90%	95%	99%
90%	610	864	1493	98	138	239
92%	466	707	1221	75	113	195
94%	382	542	935	61	87	150
95%	372	456	788	60	73	126
96%	260	369	637	42	59	102
97%	197	279	483	32	45	77
98%	133	188	325	21	30	52
99%	67	95	164	11	15	26

More difficult if you no not have known infected reference animals, to determine DSe for surveillance purposes

(B) Diagnostic sensitivity (DSe)

It is generally problematic to find sufficient numbers of true positive reference animals, as determined by isolation of the pathogen. It may be necessary to resort to samples from animals that have been identified by another test of sufficiently high accuracy, such as a validated nucleic acid detection assay.

Samples of animals of unknown status

When the so-called reference standard is imperfect, which is the rule with any diagnostic tests, estimates of DSe and DSp for the candidate assay based on this standard will be flawed. A way to overcome this problem is to perform a latent class analysis of the joint results of the two tests assuming neither test is perfect.

Because these statistical models are complex and require critical assumptions, statistical assistance should be sought to help guide the analysis and describe the sampling from the target population(s), the characteristics of other tests included in the analysis, the appropriate choice of model and the estimation methods based on peer-reviewed literature (see *Terrestrial Manual* Chapter 3.6.5 [footnote 14] for details).

→ Consult an epidemiologist

In aquatic pathogen testing, finding adequate numbers of known-positive subclinically-affected animals is a major hurdle especially when there may only be one assay available.

(B) DSe estimation for WSSV

- >2000 samples of unknown status, assumed to be clinically healthy:
  - Tested by 2 real-time assays (CSIRO and WSSV qPCRs), with nucleic acid undiluted and diluted 1/10
  - 2 populations/3 populations
  - Data subjected to Latent Class analysis (complicated): Markov Chain Monte Carlo algorithm

- **DSe for CSIRO WSSV qPCR = 90-93%**

- **DSe for OIE WSSV qPCR = 91-92%**

- Variables include:
  - Testing in duplicate or singlicate?
  - If testing in duplicate do both replicates have to be positive or not?
  - Effect of pooling and sample type for pooling

(B) Diagnostic specificity (DSp)

Diagnostic specificity (DSp) is the proportion of samples from known uninfected reference animals that test negative in an assay. It is often possible to obtain these samples from countries or zones that have eradicated or have never had the disease in question. Such samples may be useful as long as the targeted population for the assay is sufficiently similar to the sample-source population.

From AFDL point of view, DSp is relatively easy (most assays are for exotic pathogens)

General guideline is 300 known uninfected animals

For the CSIRO and OIE WSSV qPCR assay validation- DSe component:

- Positive interpretation affects DSp
  - Decrease from 99% to 95%

(B) Reproducibility

Reproducibility is the ability of a test method to provide consistent results, as determined by estimates of precision, when applied to aliquots of the same samples tested in different laboratories, preferably located in distinct or different regions or countries using the identical assay (protocol, reagents and controls).

Minimum of 20 samples using a standardised assay.

Not always possible so harmonisation as an alternative to standardisation.

Difficult to achieve for molecular detection aquatic pathogens (different platforms, reagents, chemistries, analysis).

Proficiency Testing (PT) used as an alternative.

- Australian National Quality Assurance Program (ANQAP)
  - Panels for WSSV in 2010, 2011, 2012, 2013, 2015 and 2017

Validation

Validation is very important

- Gives you confidence in your results
- Gives stakeholders confidence in your results
- Greater ability to withstand legal scrutiny

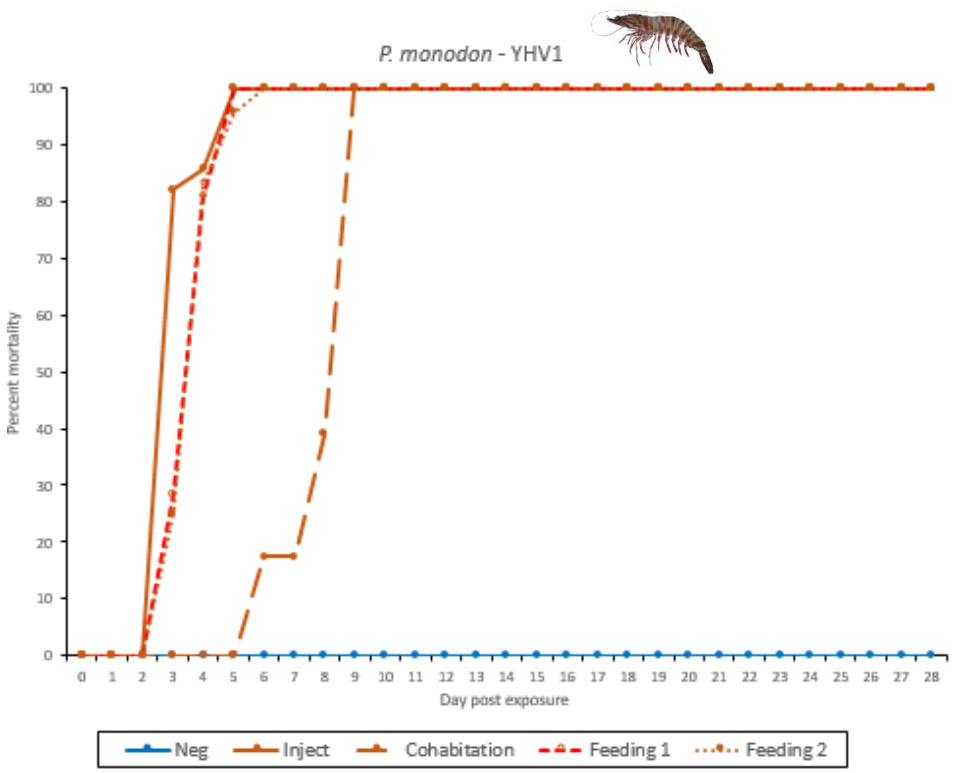
However, validation is also an onerous, complicated, time-consuming and costly exercise, particularly with regard to molecular assays targeting pathogens of aquatic animals.

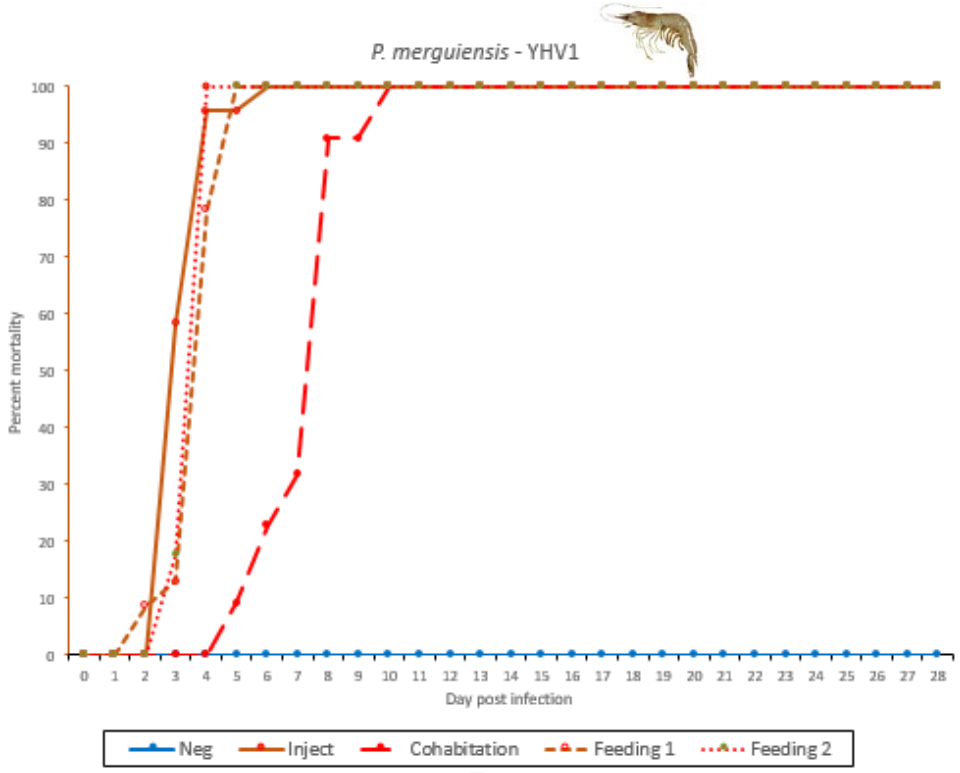
- Absence of alternative, secondary, confirmatory assays (serology, virus isolation)
- Reliance on herd testing and lethal sampling (no option to resample an individual)
- Difficulties obtaining adequate numbers of known-positive animals
- Difficulties obtaining adequate numbers of known-negative animals

Moody and Crane (2015) Validation of diagnostic tests in the Aquatic Manual. 3rd OIE Global Conference on Aquatic Animal Health "Riding the wave to the future". Ho Chi Minh City, Vietnam, 20–22 January 2015

CSIRO and OIE WSSV qPCR assay validation is ongoing and is expected to be completed by September

YHV Complex - Infectivity Trial 2: November, 2016 @ 30°C





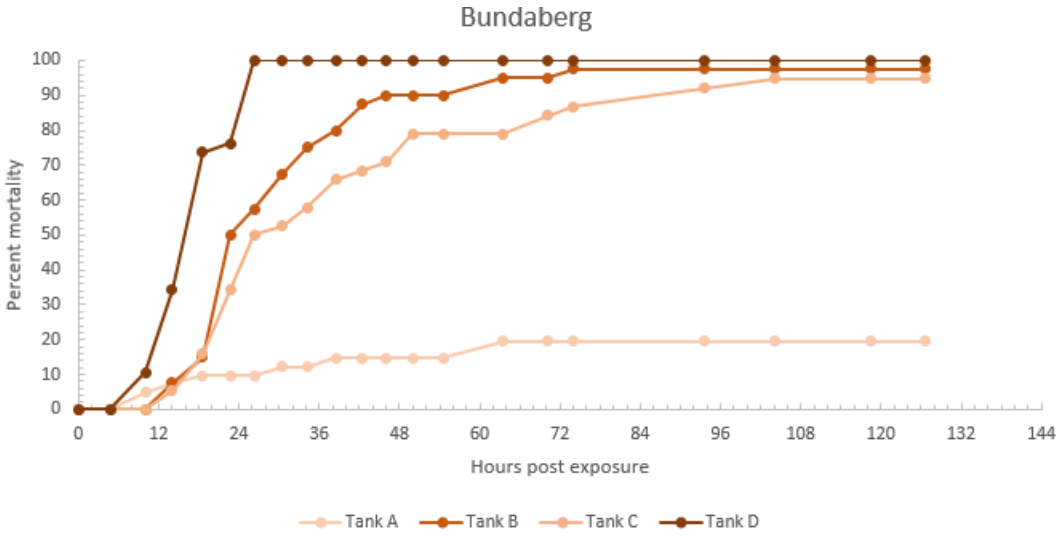
YHV1 is pathogenic to *P. monodon* and *P. merguiensis* by co-habitation and feeding

Confirms OIE criteria for susceptible species (pending molecular testing and histology)

PMMS/AHPND – Trial 1

Bundaberg

- Mortalities



AHPND Broth qPCR Summary

	Tank A	Tank B	Tank C	Tank D
n	16	26	29	25
n (POS)	68.80%	100%	100%	100%
n (POS)	11	26	29	25
min	18.50	17.40	17.70	17.00
max	33.50	24.80	22.90	20.30
ave	28.04	19.86	19.22	18.34
sd	6.72	1.67	1.11	0.89
Mortality Rank	4	2	3	1

- Toxin gene in the bacterial isolate used
- Greater exposure seems to lead to greater mortality

Conclusions

- Involved in confirmation and subsequent national emergency response
- Validation of CSIRO and OIE WSSV real-time assays to OIE standard
- Expertise with bioassays for pathogens of prawns
- Application for OIE Reference Laboratory for infection with white spot disease virus

1. Interested in comparative evaluation of on-farm test kits

Acknowledgements

- Australian Government Department of Agriculture and Water
- AAHL Fish Diseases Laboratory

Thank you

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Senior Research Scientist

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APFA – WSSV Research Priorities

AUSTRALIAN Prawn Farmers ASSOCIATION

APFA represents our members - farm and hatcheries

Our priorities

- minimise risk of major disease event happening again
- Improve practices that will help mitigate the damage caused by future potential disease events

APFA – WSSV Research Priorities

Research requiring PC3 high containment laboratory	
Enhance surveillance by investigating species susceptibility and the effect of temperature stress on viral load. Testing sensitivity of WSSV in PLs of crustaceans prior to PCR to improve detection of white spot syndrome virus.	**High**
Other research	
APFA Quarantine Facility - Establishment on Specific Pathogen Free (SPF) supply of prawn broodstock	**Very High**
Breeding Program – Establishment on Specific Pathogen Free (SPF) supply of prawn broodstock. Development of SPR lines.	**Very High**
What (if anything) is living in sediment from treated ponds and how infectious is it?	**High**
Alternative technology to allow cheap, high throughput testing for white spot disease	**High**
Validation of desktop commercial diagnostic tests for WSD and other diseases by farmers	**High**
Broodstock testing / Complete wild broodstock survey	**High**
Nursery Systems	**High**
Prawn translocation protocol	**High**
Assessment of different sterilisation and filtration systems for destroying WSD	**M/high**
Assessment of different sterilisation and filtration systems for destroying WSD	**M/high**
Evaluation of ability to hold PL at high temperature to eliminate WSSV	**M/High**
Plankton testing	**Med**
Use of alternative technologies to prevent disease	**Med**
Aeration strategies	**Med**

APFA – WSSV Research Priorities

AUSTRALIAN Prawn Farmers ASSOCIATION

Nuclear Breeding Centre

- **Establishment of Specific Pathogen Free (SPF) supply of prawn broodstock**

Viral Screening Capability

- Alternative technology to allow cheap / high throughput testing for viruses
- Validation of desktop commercial diagnostic tests for WSD and other diseases
  - Farmers need to be able to rapidly (ie hours!) test for present of virus to minimise potential losses and spread.

APFA – Nuclear Breeding Centre

Term

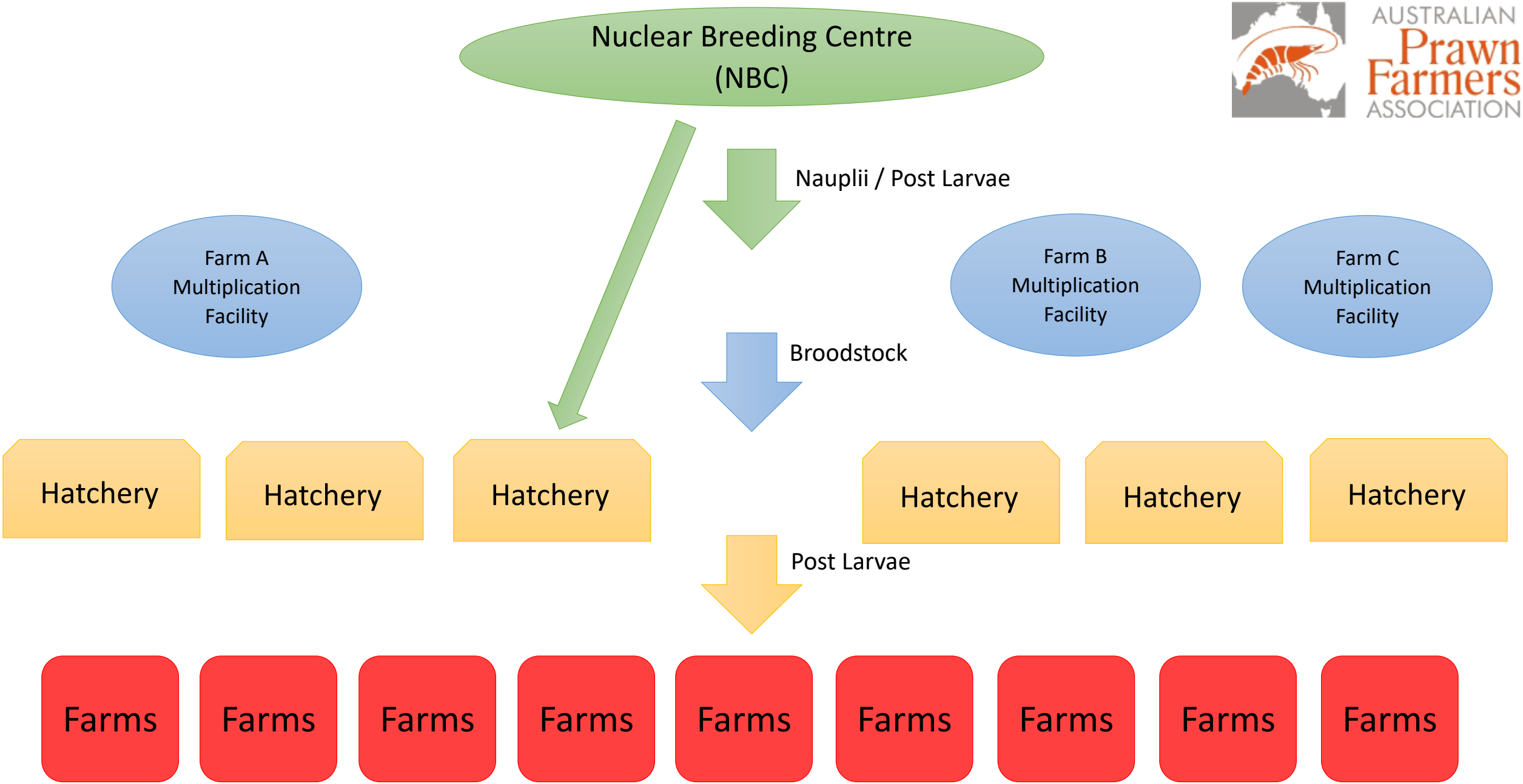
- 5 year project
- (Depending on stock may need 7-10 year)

Objective

- Reduce the reliance of wild sourced broodstock for Australian prawn farms by 50% (100%?)

Need

- Industry still reliant on wild broodstock
- Wild broodstock 'major risk' category for industry biosecurity
- Wild broodstock - predominant disease incursion pathway for WSSV (and other pathogens)
- Establishment of an industry NBC is the best long term strategy to reduce the risk of another outbreak.


Nuclear Breeding Centre (NBC)
Nauplii / Post Larvae
Farm A Multiplication Facility
Farm B Multiplication Facility
Farm C Multiplication Facility
Broodstock
Hatchery
Hatchery
Hatchery
Hatchery
Hatchery
Hatchery
Post Larvae
Farms
Farms
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Farms

APFA – Nuclear Breeding Centre

Stock

- Australian SPF wild?
- Overseas SPF domesticated?
  - as once off founder stock to fast track establishment of a breeding population
  - Save 8-10 years
  - Robins Macintosh (Senior Vice President CP) - '8 years of hell' just to establish a domesticated population of *P.monodon*, *before* starting selective breeding
  - Only 2 worldwide *P.monodon* domestication programs still going (out of many)
  - Significant greater chance of establishing a successful breeding population

Funding

- APFA
- FRDC
- Individual Farms – 'shareholders' (more $, greater % of NBC production)
- NBC operators (e.g CSIRO? / JCU? / CP? Etc)
- ARC Grant? CRC for Developing Northern Australia? Innovations Connections ?

APFA – Nuclear Breeding Centre

Funding Example

$	Year 1	Year 2	Year 3	Year 4	Year 5	
APFA	$150,000	$150,000	$150,000	$150,000	$150,000	**$750,000**
FRDC	$150,000	$150,000	$150,000	$150,000	$150,000	**$750,000**
Farm 1	$100,000	$100,000	$100,000	$100,000	$100,000	**$500,000**
Farm 2	$75,000	$75,000	$75,000	$75,000	$75,000	**$375,000**
Farm 3	$150,000	$150,000	$150,000	$150,000	$150,000	**$750,000**
Farm 4	$100,000	$100,000	$100,000	$100,000	$100,000	**$500,000**
NBC Operator (in kind contrib?)	$150,000	$150,000	$150,000	$150,000	$150,000	**$750,000**
	$875,000	**$875,000**	**$875,000**	**$875,000**	**$875,000**	**$4,375,000**

APFA – Nuclear Breeding Centre

AUSTRALIAN Prawn Farmers ASSOCIATION

Challenges

- Facility – lease existing ?/ build?
- Longer term business model
- Stock – is importing live SPF broodstock a realistic option
- Finding a service provider / NBC operator – put out to tender?
- $$$

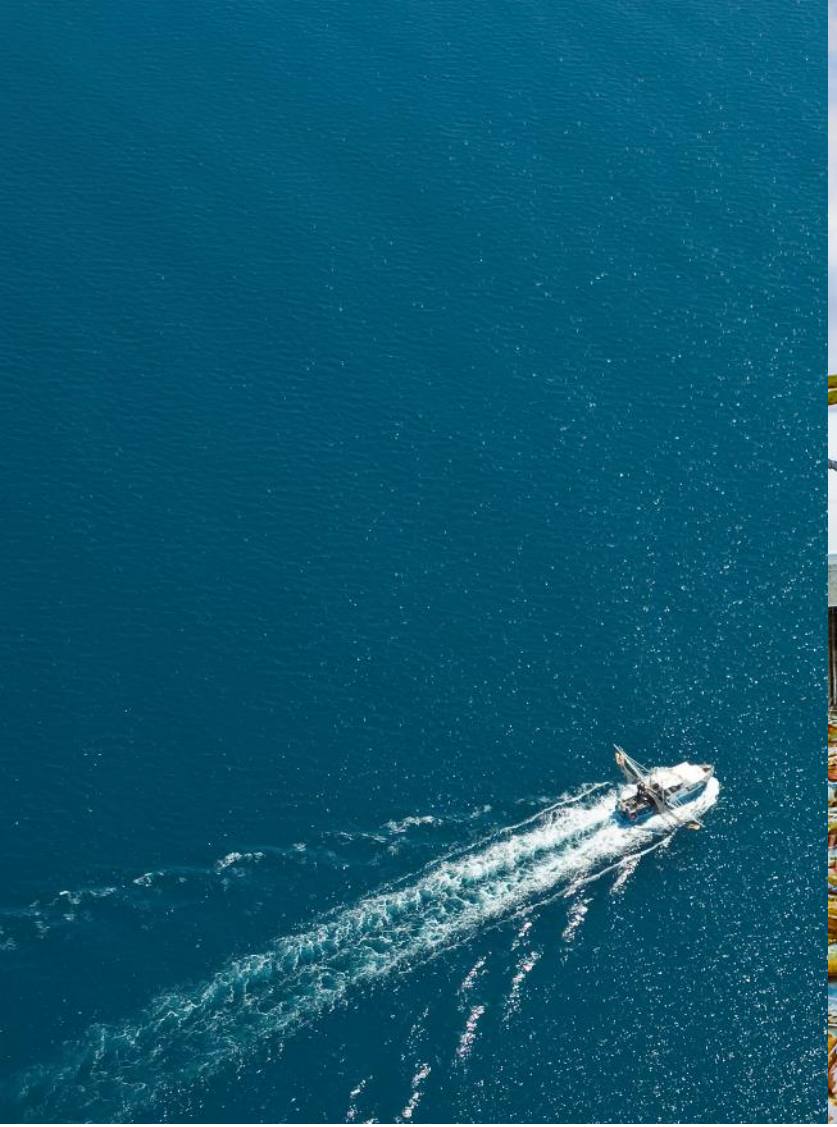
APFA – Nuclear Breeding Centre

Recommendation

- Run a scoping project
    - Appoint a steering committee / board
    - Investigate the challenges – what's feasible / what's not
    - Develop a NBC model that works for industry and stakeholders
    - Develop a longer term business model

Output

- 5 year Nuclear Breeding Centre blue print that can be put out to tender



WSSV R&D priorities

Australian Council of Prawn Fisheries

WSSV in context

Literature suggests that WSSV poses a low mortality risk for wild catch prawns. ACPF's research priorities are therefore driven by the need to reduce risk of quarantine and/or restricted market access *and* inform the IRA review eg:

1. Reducing biosecurity risk from farms to wild
2. Reducing biosecurity risks via bait pathway
3. Managing around established disease (in a zone and if endemic)
4. Identify knowledge gaps to inform the IRA review

Reducing biosecurity risks

For ACPF - Border biosecurity is more a policy, process and enforcement issue – less of a research issue.

- Most research has already been undertaken (and analysed by Landos) on viability of **known** virus(es) in green prawns but need **to identify knowledge gaps to inform the IRA**
- Closing the bait pathway for uncooked prawns is imperative to Australia's biosecurity:
  - Increased education to the community/rec fishers to reduce use of imported prawns as bait (QSIA project?)
  - Market research to demonstrate effectiveness (or otherwise) of education campaigns about the use of uncooked imported prawns for bait

Managing around established disease

What level of introduced WSSV dose is required before WSSV can be detected in the wild (EMAI proposal?)

- Risk to wild catch posed by infected animals via border biosecurity failure (reputational/market)
- Risk posed by trade of stock from inside a Managed Zone to other areas ie can it be demonstrated that wild stock (eg bait) from very low WSSV incident areas can be sold uncooked out of the area at insignificant risk to the wild?

Reducing biosecurity risk from farms to wild

Intensive production can be a catalyst for disease expression and is a risk for adjacent wild catch sector

- Any risks to farm biosecurity pose a threat to wild catch (reputational/market)
- Any measures to reduce the risk of disease (water intake systems, disease resistance breeding, early detection, etc) are a bonus to wild catch to minimise risk but are essentially the remit of the farmed sector

ACPF/ APFA R&D priorities

- Research needs to inform IRA review – what are they?
- What level of introduced WSSV dose is required before WSSV can be detected in the wild (EMAI proposal?)
- Education to reduce risk through bait pathway
- Market research to determine effectiveness of education program

- Identified APFA R&D needs are all currently *low* priority for wild catch in terms of wild-catch specific priorities *but* ACPF supports APFA's listed high priorities to decrease risk of disease/improve on-farm bio-security
- ACPF does NOT support the introduction of exotic species broodstock (eg live vannemei) into Australia

Queensland Seafood Industry Association

Promoting profitable & sustainable seafood

White Spot Disease R&D Planning Workshop

Eric Perez
CEO
Queensland Seafood Industry Association

QRAC Research Priorities

Priority	Future Proofing Wild Harvest Fisheries – Education / Communications
Need	The outbreak of White Spot demonstrated just how under prepared government and industry were in terms of managing its response across the key stakeholder groups: • Commercial fishers; and • Recreational fishers. A more integrated protocol is needed when responding at the eradication stage: • Social media, print and television media, emails etc.

QRAC Research Priorities

Planned Outcomes	Development of a government-industry and government-recreational fisher response protocol and education/information material: • What could this look like? • What is world's best practice?

QRAC Research Priorities

Priority	Industry recovery from White Spot

Need	The FRDC has undertaken economic impact studies across both the aquaculture and wild harvest fisheries in Moreton Bay. The overall market impacts for all categories of crustaceans (particularly prawns and crabs) is unclear.
	• Wild harvest fishers have had their markets changed overnight.
	• There is no definitive understanding of medium and long-term market impacts during and post-White Spot outbreak.
	• There is a need to understand the impacts at the following levels:
	  ▪ Wild harvest fishers;
	  ▪ Retail seafood businesses; and
	  ▪ Wholesale seafood businesses.
	• What recovery options are available to industry in Moreton Bay?

QRAC Research Priorities

Planned Outcomes	FRDC has already invested funds into understanding the economic impact at the business level. However, the incursion of White Spot and the extent of the market impacts in the Moreton Bay region are not fully understood.

Building Industry Capacity

- White spot demonstrated the lack of understanding and language around biosecurity within the commercial seafood industry in Queensland (nationally).

- Biosecurity Roundtable meetings demonstrated a lack of awareness of wild capture fisheries within the land-based agriculture sector.

Research Priorities

Project 1: Methods (e.g. gamma-irradiation) for virus inactivation

Project 2: Response protocol – managing the industry response.

Project 3: Medium to long-term impacts of White Spot in Moreton Bay – understanding of impacts at the following levels:

- Wild harvest
- Retail
- Wholesale

Understand market impacts by sectors:

- Crab
- Prawns
- Bloodworm / Beachworm

Project 4: Building industry capacity

Biosecurity and Industry Liaison Officer

- Industry information materials developed in collaboration with industry, the Department of Agriculture and Fisheries and Biosecurity Queensland.
- Draft biosecurity plan being developed with a meeting to discuss current draft with DAF and BQ set for Monday 23 October.
- Information sheets also being drafted.
- A detailed response plan that industry can follow will also be developed amongst government and industry.