World Organisation for Animal Health

# Launch of the Regional AquaticAnimal Health Laboratory Network for Africa (RAAHLN-AF)

## 5 – 7 December 2023 Pretoria, South Africa

ARC • LNR

UNION AFRICAINE
BUREAU INTERAFRICAIN DES RESSOURCES ANIMALES

FishGov 2 Project

World Organisation for Animal Health

# ACDP Fish Diseases Laboratory

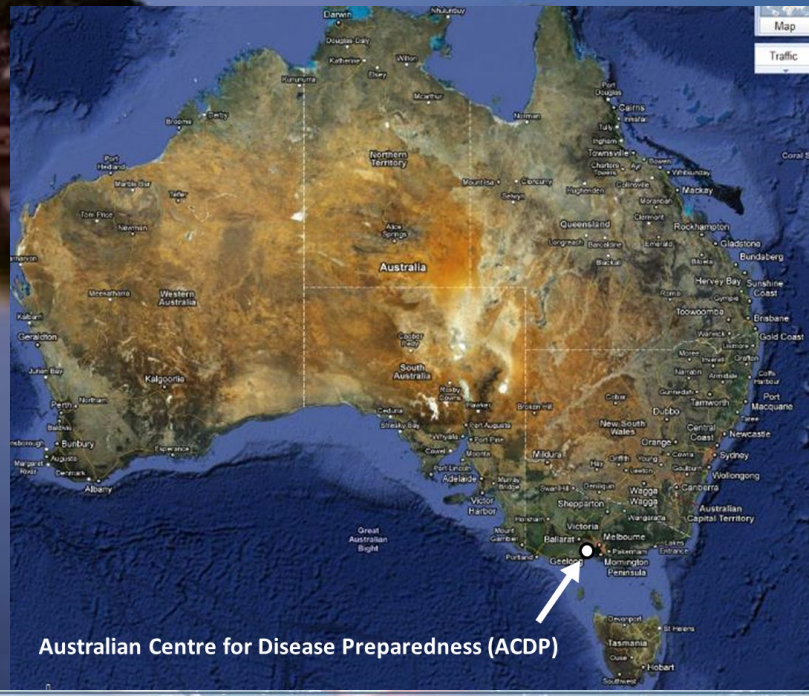
(Facilities, services and challenges)

Dr Nick Moody

CSIRO ACDP Fish Diseases Laboratory

Australia

World Organisation for Animal Health

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INTERAFRICAN BUREAU FOR ANIMAL RESOURCES

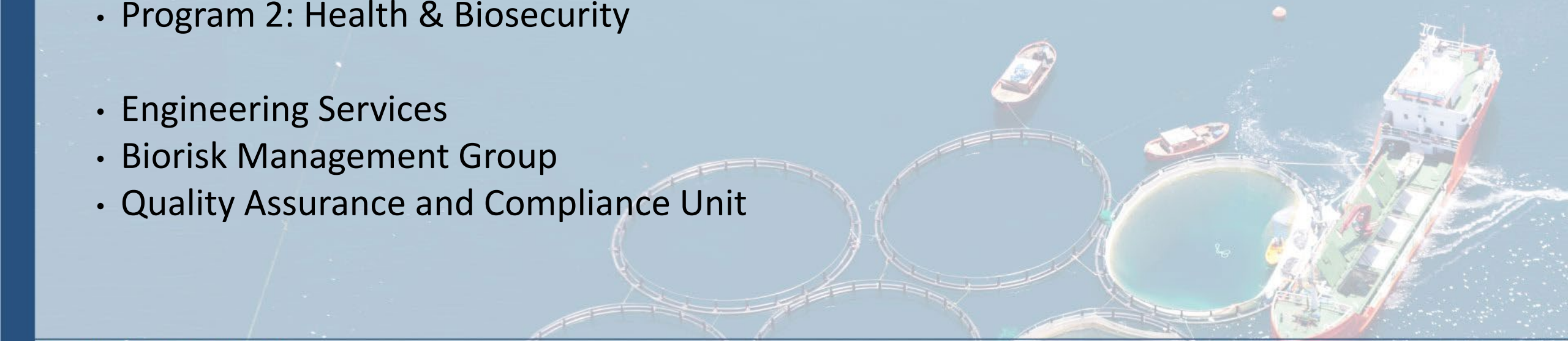
FishGov 2 Project

- CSIRO Australian Centre for Disease Preparedness
  - Australia's National Reference Laboratory
  - Terrestrial and aquatic animal diseases
  - PC2, PC3 and PC4 (*in vitro* and *in vivo*)

- Program 1: Diagnosis, Surveillance and Response (DSR)
  - Open 24/7, 365 days of the year

- Program 2: Health & Biosecurity

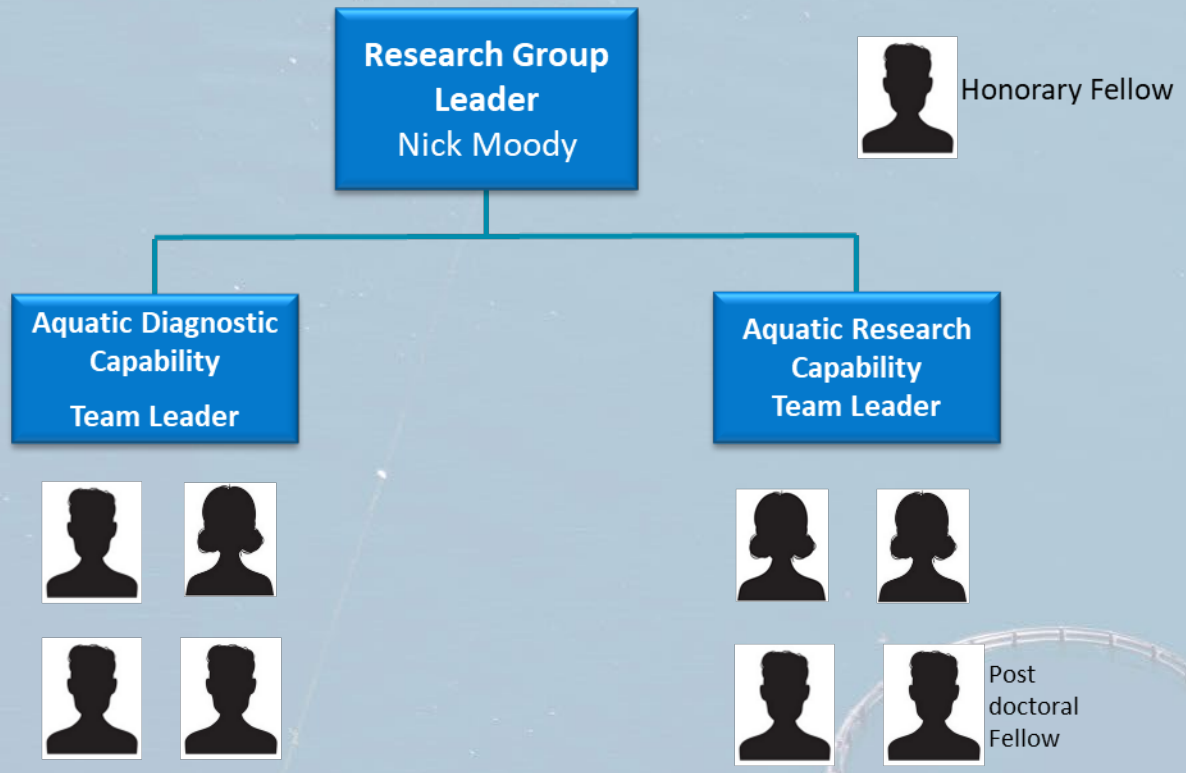
- Engineering Services
- Biorisk Management Group
- Quality Assurance and Compliance Unit



# Diagnosis, Surveillance and Response (DSR)

- ACDP Fish Diseases Laboratory (AFDL)

# ACDP Fish Diseases Laboratory




- Samples referred through the states (no direct submissions)
- We focus on emerging and exotic infectious pathogens (reduce competition with the States)
- Quality Assurance (ISO 17025)

## AFDL Roles and Responsibilities

- Diagnosis of exotic and emerging diseases of aquatic animals (mainly finfish/molluscs/crustaceans)
- Provision of technical advice on exotic and emerging diseases
- Proficiency testing (National, Asia-Pacific PT Program)
- Research (generally results from diagnostic testing)

- Funding from the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF)
  - 24/7 service, 365 days per year

- Funding from Fisheries Research and Development Corporation
  - Industry/government funding
  - In the national interest

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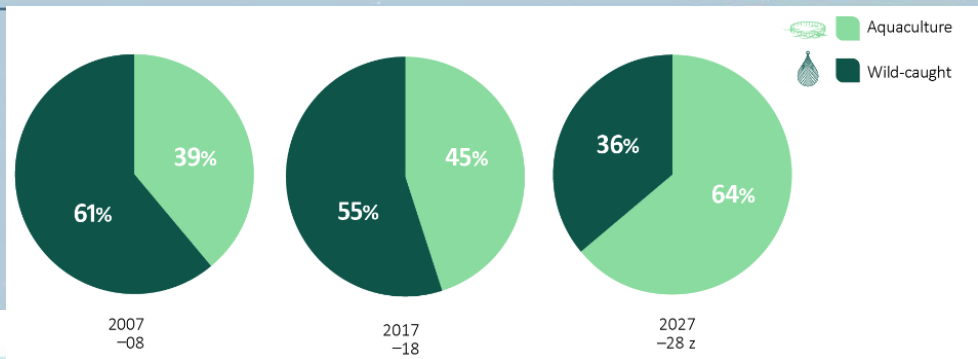
# Australian aquaculture industries





## Australian fisheries and aquaculture production

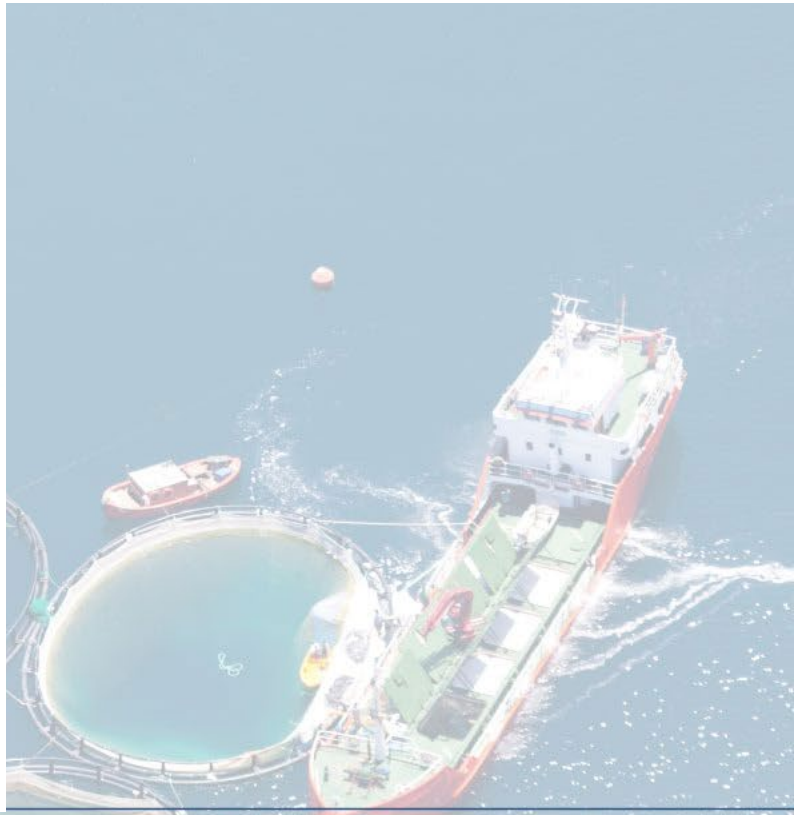
**Aquaculture GVP increased by 9%** to **$1.73 billion**, accounting for 56% of total GVP

**Wild-catch GVP decreased by 12%** to **$1.39 billion**, accounting for 44% of total GVP

**Aquaculture volume increased by 24%** to **131,578 tonnes**, accounting for 43% of total volume

**Wild-catch volume increased by 0.3%** to **172,657 tonnes**, accounting for 57% of total volume

Note: 2020–21 figures are preliminary. Wild-catch and aquaculture figures may not sum to total GVP and volume presented due to rounding and adjustment for Southern Bluefin Tuna caught in the Commonwealth Southern Bluefin Tuna Fishery as an input to farms in South Australia.
Source: ABARES



AFDL Submission Categories

## Diagnostic submissions from State authorities:

Category 1: Routine samples (surveillance/health certification) 

Category 2: Exotic/emergency disease exclusion (low likelihood) – NO CHARGE

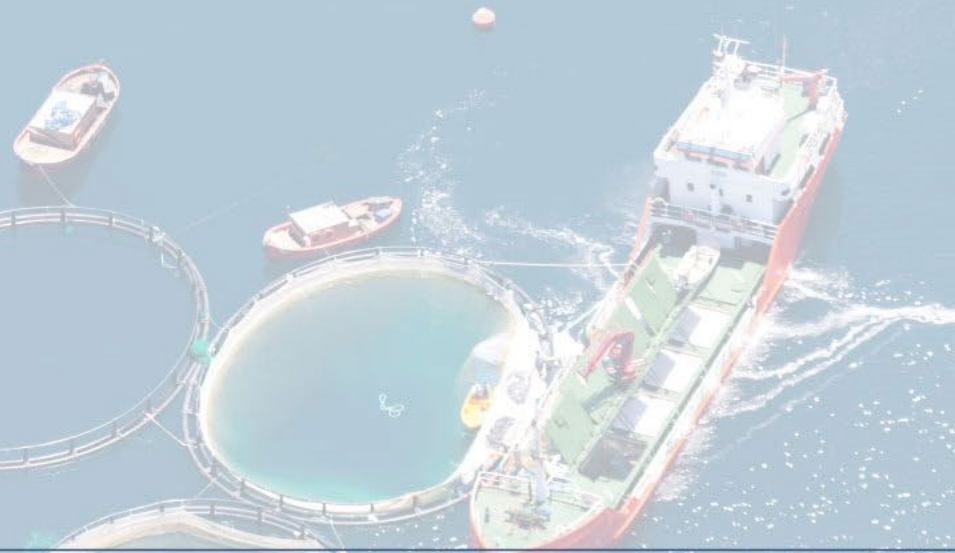
- Test results required within 48 hours (depends on submission)

Category 3: Exotic disease exclusion/confirmation (high likelihood) – NO CHARGE

- Test results required within 24 hours (work overnight)
- Director of ACDP notified. Diagnostic test report issued to submitting laboratory, CVO of the submitting state, Australian CVO
- AqCCEAD (National emergency response committee)

## AFDL Capability

- Molecular testing (real-time and conventional PCR)
- Sanger and high-throughput sequencing
- Cell culture and Virology
- Immunohistochemistry
- Electron microscopy
- *In vivo* aquatic facility (PC3)
- All AFDL staff are competent in ISO 17025 testing
- All AFDL staff participate in research projects



How do we identify pathogens of concern?

- WOAH Listed pathogens
- Australia's National List of Reportable Diseases of Aquatic Animals
  - 24 finfish pathogens (19 exotic)
  - 11 molluscan pathogens (6 exotic)
  - 13 crustacean pathogens (9 exotic)
  - 3 amphibian pathogens (1 exotic)
- Network of Aquaculture Centres in Asia-Pacific (NACA)
- *ad hoc* Steering Committee of the Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific (OIE Japan)
  - Regional Collaboration to respond to Emerging Diseases
- Scientific literature, media, conferences etc.
- Diagnostic submissions

# Responses since 2008

**2008:** Mortalities (*Streptococcus* sp.) in (wild) grouper (Qld), **abalone viral ganglioneuritis (TAS),** white tail disease - *Macrobrachium rosenbergii* nodavirus (Qld), **new strain (previously exotic) of IHHNV (QLD)**

**2010:** *Edwardsiella ictaluri* in native catfish (NT), **Ostreid herpesvirus in Pacific oysters (NSW),** *Aquabirnavirus* in trout (VIC)

**2011:** **AVG in farmed abalone (TAS)**

**2012:** *Megalocytivirus* in ornamental fish farm (Qld); Orthomyxo-like virus in salmonids (SE. Tas), YHV genotype 7 in farmed prawns (QLD), issues with specificity of OIE YHV assays

**2013:** **YHV genotype 8 and 10 in imported commodity prawns**, issues with specificity of OIE YHV assays

2014: *Edwardsiella ictaluri* in wild catfish (Qld)

**2015:** Turtle mortalities (NSW), Hepatopancreatitis (AHPND-like) in prawns (Qld), ***Bonamia exitiosa*, *Perkinsus olseni*,** *P. beihaiensis* **in native flat oysters (VIC)**

**2016:** **Hepatopancreatitis (AHPND-like) in prawns (QLD); Ostreid herpesvirus in Pacific oysters (TAS)**
**WSSV in farmed prawns (QLD)**

**2017:** **WSSV in farmed and wild prawns (QLD)**

**2018:** **Ostreid herpesvirus in wild Pacific oysters (SA), WSSV in wild prawns (QLD)**

2019:

**2020:** **SARS-CoV-2, WSSV in wild and farmed prawns (QLD)**

**2021:** **SARS- CoV-2, AbHV in wild abalone (VIC)**

**2022:** **WSSV in farmed prawns (NSW)**

**2023:** **WSSV in farmed and wild prawns (NSW)**

**RED** = AqCCEAD (National teleconference)

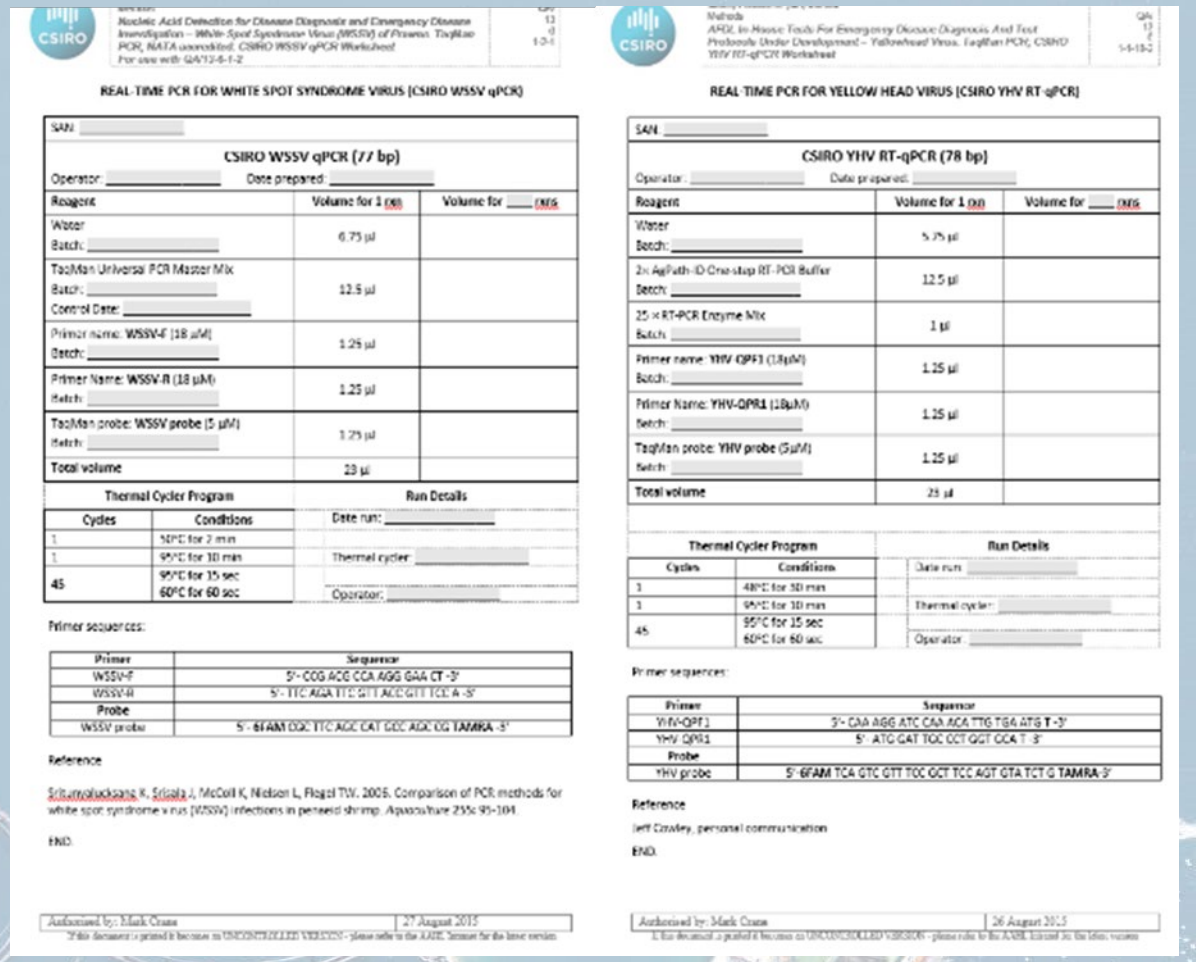
Screen by real time PCR (88 assays) and confirm by conventional PCR (110 assays) and sequence analysis.
Also use housekeeping gene assays (18 assays)

# AFDL molecular tests: diagnostics & research

| | |
|---|---|
| 2009 | FRDC 2009-032: Tactical Research Fund - Validation of tests for **Abalone herpesvirus** |
| 2010 | FRDC 2010-032: Characterisation of **Tasmanian Aquabirnavirus** |
| 2011 | FRDC 2011-048: Tactical Research Fund - Aquatic Animal Health Subprogram: determining the susceptibility of Australian species of prawns to **infectious myonecrosis virus** |
| 2011 | FRDC 2011-004: Development of Improved Molecular Diagnostic Tests for ***Perkinsus olseni*** in Australian molluscs |
| 2011 | FRDC 2011-224: Characterisation of **TSRV** |
| 2013 | FRDC 2013-001: **AbHV** Pathogenicity |
| 2013 | FRDC 2013-033: Characterisation and identification of **Salmon Orthomyxo-like virus** (SOMV) and associated pathology in Atlantic Salmon |
| 2014 | FRDC 2014/002: Development of stable **positive control material** and development of internal controls for molecular tests for important endemic and exotic pathogens. |
| 2015 | FRDC 2015-005: Determining the susceptibility of Australian *Penaeus monodon* and *P. merguiensis* to newly identified enzootic (**YHV7**) and exotic (**YHV9** and **YHV10**) Yellow head virus (YHV) genotypes. |
| 2015 | FRDC 2015-001: Whole genome sequencing of **Bonamia** |
| 2016 | FRDC 2016-013: Aquatic Animal Health Subprogram: Comparative pathogenicity of exotic **AHPND** and the presumptive bacterial **hepatopancreatitis** detected in farmed Penaeus monodon in Queensland. |
| 2017 | FRDC 2017-190: Assessment of gamma irradiation as a feasible method for treating prawns to inactivate **White Spot Syndrome Virus**. |
| 2018 | FRDC 2018-147: Diagnostic detection of aquatic pathogens using real-time next generation sequencing |
| 2018 | DAWE Determine pool level sensitivity for **WSSV**, **YHV-1** and **megalocytivirus** testing |
| 2019 | FRDC 2019-089: Evaluation of point of care (POC) tests for **White Spot Syndrome Virus** |

# AFDL molecular tests: diagnostics & research

- Same protocols for both diagnostics and research
- Evaluate published methods, can also develop our own tests
- Real-time PCRs are all TaqMan probe based
- Don't use $C_T$ cut-offs
- All staff continually demonstrate competency
- Continual review of procedures
- We document everything



REAL-TIME PCR FOR WHITE SPOT SYNDROME VIRUS (CSIRO WSSV qPCR)

CSIRO WSSV qPCR (77 bp)

Operator: Date prepared:

| Reagent | Volume for 1 rxn | Volume for ___ rxns |
|---|---|---|
| Water<br>Batch: | 6.75 µl | |
| TaqMan Universal PCR Master Mix<br>Batch:<br>Control Date: | 12.5 µl | |
| Primer name: WSSV-F (18 µM)<br>Batch: | 1.25 µl | |
| Primer Name: WSSV-R (18 µM)<br>Batch: | 1.25 µl | |
| TaqMan probe: WSSV probe (5 µM)<br>Batch: | 1.25 µl | |
| Total volume | 23 µl | |

| Thermal Cycler Program | |
|---|---|
| Cycles | Conditions |
| 1 | 50°C for 2 min |
| 1 | 95°C for 10 min |
| 45 | 95°C for 15 sec<br>60°C for 60 sec |

Run Details: Date run: Thermal cycler: Operator:

Primer sequences:

| Primer | Sequence |
|---|---|
| WSSV-F | 5'- COG ACG CCA AGG GAA CT -3' |
| WSSV-R | 5'- TTC AGA TTC GTT ACC GTT TCC A -3' |
| Probe | |
| WSSV probe | 5'- 6FAM CGC TTC AGC CAT GCC AGC CG TAMRA -3' |

Reference

Sritunyalucksana K, Srisala J, McColl K, Nielsen L, Flegel TW. 2006. Comparison of PCR methods for white spot syndrome v rus (WSSV) infections in penaeid shrimp. Aquaculture 255c 95-104.

END.

Authorised by: Mark Crane | 27 August 2015

REAL-TIME PCR FOR YELLOW HEAD VIRUS (CSIRO YHV RT-qPCR)

CSIRO YHV RT-qPCR (78 bp)

Operator: Date prepared:

| Reagent | Volume for 1 rxn | Volume for ___ rxns |
|---|---|---|
| Water<br>Batch: | 5.75 µl | |
| 2x AgPath-ID One-step RT-PCR Buffer<br>Batch: | 12.5 µl | |
| 25 x RT-PCR Enzyme Mix<br>Batch: | 1 µl | |
| Primer name: YHV QPR1 (18µM)<br>Batch: | 1.25 µl | |
| Primer Name: YHV-QPR1 (18µM)<br>Batch: | 1.25 µl | |
| TaqMan probe: YHV probe (5µM)<br>Batch: | 1.25 µl | |
| Total volume | 25 µl | |

| Thermal Cycler Program | |
|---|---|
| Cycles | Conditions |
| 1 | 48°C for 30 min |
| 1 | 95°C for 10 min |
| 45 | 95°C for 15 sec<br>60°C for 60 sec |

Run Details: Date run: Thermal cycler: Operator:

Primer sequences:

| Primer | Sequence |
|---|---|
| YHV-QPF1 | 5'- CAA AGG ATC CAA ACA TTG TGA ATG T -3' |
| YHV QPR1 | 5'- ATC GAT TGC CCT GGT CCA T -3' |
| Probe | |
| YHV probe | 5'-6FAM TCA GTC GTT TGC GCT TCC AGT GTA TCT G TAMRA-3' |

Reference

Jeff Cowley, personal communication

END.

Authorised by: Mark Crane | 26 August 2015

# AFDL Proficiency Testing (with ACDP PTRM team)

- National Aquatic PT Program (since 2013)
  - Crustacean panel: WSSV, YHV1
  - Finfish panel: NNV, RSIV
  - Molluscan panel: AbHV, OsHV-1, *Bonamia* spp., *Perkinsus* spp.
  - Participants: 14 laboratories

Australian Government
Department of Agriculture, Fisheries and Forestry

- Asia-Pacific PT Program (since 2017)
  - Crustacean panel: AHPND, IHHNV, IMNV, TSV, WSSV, YHV1
  - Finfish panel: KHV, Megalocytivirus, NNV, SVCV
  - Participants: up to 55 laboratories in 15 countries

Australian Government
Department of Agriculture, Fisheries and Forestry

- AFDL participates in:
  - National Aquatic PT Program
  - Interlaboratory Ring Test – Finfish
  - Interlaboratory Ring Test – Crustacean
  - Other (*ad hoc*)

DTU
EU Reference Laboratory for Fish and Crustacean Diseases
Technical University of Denmark

## Provision of positive control material and protocols

- Plasmid positive controls
- Genomic positive controls
- Gamma-irradiated material
- Pre-publication molecular test worksheets
- All provided under an MTA and generally no charge for national capability development
- Assistance with test implementation and any troubleshooting

## Challenges encountered in assisting other countries

- **Confidentiality**: important for submitters to know results will be handled in a confidential manner and any further use of the samples will be with prior approval. WOAH notification obligations for WOAH-listed pathogens.

- **Communication**: the submitting country Delegate must be aware the of the submission, the tests that will be undertaken and that the results will be reported to them. AFDL will notify the Australian Chief Veterinary Officer of overseas submissions and results (no surprises, for positive feedback).

- **Delivery**: as requested in the time agreed (for information, test turn-around times). Need to make sure you can undertake the work.

- **Advice**: may be other organisations/individuals providing advice and this needs to be handled sensitively.

## Benefits encountered in assisting other countries

- Challenges are reduced through open and transparent communication, which develops trust.
- Accurate results obtained in a timely manner
- More rapid access to robust diagnostic tests, leading to quicker outcomes for stakeholders (no need to reinvent the wheel)
- Generally, ACDP do not charge for submissions associated with disease
- Always potential for further collaborations (surveillance, other activities)
- Develops networks with upskilling and sharing knowledge

Thank you very much for the opportunity to participate in this meeting