FINAL REPORT

Aquatic Animal Health Subprogram: technical guidelines for the translocation of live aquatic animals – with reference to barramundi nodavirus

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LIST OF ABBREVIATIONS

AAHC	Aquatic Animal Health Committee
AAHL	Australian Animal Health Laboratory
ANZSDP	Australian and New Zealand standard diagnostic procedure
CVO	Chief Veterinary Officer
EHNV	Epizootic haematopoetic necrosis virus
ELISA	Enzyme linked immunosorbent assay
FRDC	Fisheries Research and Development Corporation
IFAT	Indirect immunofluorescent antibody test
IHCT	Immunohistochemistry test
IUCN	International Union for the Conservation of Nature and Natural Resources
NAAH-TWG	National Aquatic Animal Health Technical Working Group
NSW	New South Wales
NT	Northern Territory
OIE	World Organisation for Animal Health (in French: Office International des Épizooties)
PCR	Polymerase chain reaction (A method of copying DNA)
QLD	Queensland
RT-PCR	Reverse Transcriptase PCR
SA	South Australia
TAS	Tasmania
VER	Viral encephalopathy and retinopathy
VIC	Victoria
WA	Western Australia

2005/640 Aquatic Animal Health Subprogram: technical guidelines for the translocation of live aquatic animals – with reference to barramundi nodavirus

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Objectives

- *To progress a common approach to the translocation of live finfish across Australia for aquaculture and restocking purposes.*

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

A workshop was conducted involving input from most jurisdictions. Technical issues including hazard identification and risk management associated with the translocation of live finfish were documented.

A series of recommendations, which resulted from the workshop process, will be progressed through the National Aquatic Animal Health Technical Working group and the Aquatic Animal Health Committee.

The policy basis for translocation of aquatic organisms within Australia is set out in the 1999 '*National Policy for the Translocation of Live Aquatic Organisms – Issues, Principles and Guidelines for Implementation*' published by the Ministerial Council of Forestry, Fisheries and Aquaculture (Anon. 1999). This policy establishes that translocation of aquatic animals should be assessed on the basis of a risk assessment. Industry concern about the lack of information on practical processes for achieving translocation assessment and approval led to the funding of the FRDC project 2004/080[1]. In addition, recent concern among jurisdictions over outbreaks of viral encephalopathy and retinopathy (VER) in hatcheries rearing barramundi and other fish species has emphasised the need to develop technical guidelines to underpin testing and other management measures for finfish.

In cooperation with the National Aquatic Animal Health Technical Working Group (NAAH-TWG) a one day workshop was held in Melbourne on 5th May 2005 to coordinate a national approach to translocation including the use of diagnostic tools for surveillance and translocation protocols for VER. While the workshop concentrated on VER, the outcomes apply to most of the diseases of concern facing finfish in Australia.

1 FRDC Project 2004/080. Aquatic Animal Health Subprogram: development of a national translocation policy using abalone and prawns as templates for other aquatic species.

Validation and standardisation of diagnostic tests for diseases of national concern are required and few available tests (even commercially available kits) meet the criteria set down by the World Organisation for Animal Health (OIE). While there are diagnostic tests for VER, more work is required to validate the tests and to compare the performance of all tests (including commercial kits). It was recognised by participants in the workshop that there are considerable resources involved in validating a test, therefore - where available - validated tests should be used in Australia in preference to unvalidated tests. It was also recognised that because false positive results can have an economic impact at the farm level and on exports, it is recommended that multiple sample positive results be obtained by one test method (preferably at two separate laboratories) or a single positive sample be confirmed by at least two different test methods when reporting on the presence or absence of a causative agent in significant cases (for example, in emergency disease outbreaks, or where the agent is thought to have occurred in a new host or new geographic location).

There needs to be more research work done on sampling methodology to detect sub-clinical infections at low levels of prevalence, not only for VER but for all aquatic animal pathogens. For example, random sampling of 150 fish may not be sufficient to detect low prevalence of infection - but merely increasing sample sizes can be prohibitively expensive. Targeted sampling may be more appropriate but the methodology needs to be verified if it is to have credibility for health certification purposes.

With modern molecular methods, pooling of samples from several animals is common. There needs to be further work on the protocols used when pooling samples, for example, what tissues should be used for pooling, whether the relative proportions of tissue making up the pool are critical and how pooling affects the sensitivity[2] of the test.

Australia, as a continental landmass, has a unique aquatic fauna, including a unique viral fauna. Tests developed overseas may not be appropriate for detecting local strains of virus, and there may well be multiple geographically isolated strains within Australia. Therefore, there will be a need to update and regularly review the performance of tests used for surveillance and monitoring in Australia. Likewise, there is very little information on the epidemiology of most of the aquatic disease agents of national concern. This has a direct impact on sampling methodology, estimation of prevalence of infection in populations and on screening of fish for presence of agents.

There also needs to be more work on defining the concept of a ‘biosecure facility’ and protocols for the movement and quarantining of stock. We have very little scientific information on the environmental requirements (i.e. temperature and pH requirements) or disinfection protocols for Australian pathogens.

The use of scientifically based hazard identification, risk analysis and risk management is fundamental to managing unwanted effects. However, the lack of information on Australian aquatic animal diseases makes it extremely difficult for managers to apply risk management measures to mitigate the risk associated with translocation or restocking, not just for VER but any of the aquatic diseases of concern.

Note that this document only addresses those technical issues associated with pathogen transfer and associated disease. Other translocation issues such as genetic or environmental concerns are not addressed.

KEYWORDS: Finfish, aquaculture, translocation, restocking, diagnostics, surveillance, monitoring.

2 Sensitivity: the proportion of true positives that are detected by the method.

ACKNOWLEDGEMENTS

As part of this project, a workshop was conducted in Melbourne on 5th May 2005. Subsequently members assisted by providing detailed and helpful comments on various drafts of this final report. Thanks are particularly due to Jill Birrell and Mehdi Douroudi for organising the venue for the workshop.

The workshop attendees were:

Brian Jones	Principal Investigator. Department of Fisheries, Western Australia
Ian Anderson	Queensland Department of Primary Industries and Fisheries
Jill Birrell	Department of Fisheries, Western Australia
Roger Chong	Queensland Department of Primary Industries and Fisheries
John Creeper	Department of Fisheries, Western Australia
Kevin Ellard	Department of Primary Industries Water and Environment, Tasmania
Ingo Ernst	Australian Government Department of Agriculture Fisheries and Forestry
Judith Handlinger	Department of Primary Industries Water and Environment, Tasmania
Alistair Herfort	Australian Government Department of Agriculture Fisheries and Forestry
Neil Hickman	Department of Primary Industries, Victoria
Matthew Landos	NSW Department of Primary Industries
Ramesh Perera	Biosecurity Australia
Richard Whittington	University of Sydney
Belinda Wright	Australian Government Department of Agriculture Fisheries and Forestry

BACKGROUND

There is an existing FRDC funded project (FRDC 2004/080)[3], which develops a risk assessment for the translocation of prawns (as a model for crustaceans) and abalone (as a model for molluscs). However, there is, yet, no national translocation guideline for fish. Concern about the lack of technical translocation guidelines was raised at national level following an outbreak of viral encephalopathy and retinopathy (VER) in barramundi and Australian bass. The FRDC Aquatic Animal Health Subprogram Strategic R&D Plan for 2002-2007 lists as a key priority project: *To facilitate inter-jurisdictional harmonisation of domestic and international approaches (common tests, common protocols [e.g. translocation], common certification*). Likewise, *AQUAPLAN 2005-2010* lists as an objective under Strategy 2: Harmonisation of approaches to aquatic animal health in Australia, "*to implement a common / national approach for managing pathogens associated with the translocation of live aquatic animals across Australia*.

The international protocols and guidelines that underpin the Australian quarantine provisions require that the measures used to control movements of aquatic animals within Australia should, in cases where the risk is similar, be consistent with the requirements that Australia applies to imports of aquatic animals. While the federal government regulates the movement of aquatic animals into and out of Australia, the state and territorial governments share the legislative responsibility to control interstate movements of aquatic animals. A consistent approach to assessing and managing risk associated with interstate movements within Australia is therefore needed to support import controls and to avoid adoption of state policies that might undermine national import controls.

NEED

The international movement of fish for aquaculture and re-stocking purposes has been carried out for hundreds of years and has been particularly marked since the 19th century. The spread of pathogens with this international movement of aquatic animals has been well documented (Bauer 1991; Gaughan 2002; Murray & Peeler 2005) and in some cases has led to serious impacts on local environments.

The economic activity generated by movements of aquatic animals may be substantial, for example, the Tasmanian salmonid industry, based on a species imported into Australia, was worth A$115 million in the financial year July 2003 to June 2004 (ABARE 2005). About 34% of the gross value of production of the Australian fishing industry is generated by Aquaculture (ABARE 2005) and it has been estimated that in excess of 60 species of aquatic animal are being cultivated or are under consideration for cultivation (NAC 2004). To continue to grow, the industry requires access to broodstock and selectively bred juvenile stock. This demand has led various jurisdictions within Australia to develop protocols to allow industry growth while protecting the environment.

The policy basis for translocation of aquatic organisms within Australia is set out in the 1999 '*National Policy for the Translocation of Live Aquatic Organisms – Issues, Principles and Guidelines for Implementation*' published by the Ministerial Council of Forestry, Fisheries and Aquaculture (Anon. 1999). This policy establishes that translocation of aquatic animals should be assessed on the basis of a risk assessment. Industry concern about the lack of information on practical processes for achieving translocation assessment and approval led to the funding of the FRDC project 2004/080. In addition, recent concern among jurisdictions over outbreaks of viral encephalopathy and retinopathy in hatcheries producing fingerlings of barramundi and other fish species has emphasised the need to develop technical guidelines to underpin testing and other management measures for finfish.

3 FRDC Project 2004/080. Aquatic Animal Health Subprogram: development of a national translocation policy using abalone and prawns as templates for other species.

OBJECTIVES

This project was administered through the FRDC Aquatic Animal Health Sub-program with the objective:

- *To progress a common approach to the translocation of live finfish across Australia for aquaculture and restocking purposes.*

METHODS

With the current interest in nodavirus in finfish hatcheries, VER in barramundi was chosen as a suitable case study. It is a disease for which there is some Australian research information available. VER, or viral nervous necrosis as it is usually called, can be a devastating disease of larval and juvenile fish (Munday et al. 1992). It is a known translocation risk since VER has been translocated into South Australia with barramundi (DAFF 2004). The virus and the disease it causes have been documented in a disease strategy manual (DAFF 2004). The impact of the virus on a range of freshwater fish was examined in FRDC project 1999/205[4] while FRDC 2001/626[5] developed diagnostic methods for the virus.

In cooperation with the National Aquatic Animal Health Technical Working Group (NAAH-TWG) a one day workshop was held in Melbourne on 5th May 2005 to coordinate a national approach to translocation including the use of diagnostic tools for surveillance and translocation protocols for VER. The workshop included a group exercise based on 'failure mode and effect analysis' which looked at two scenarios: The first scenario was "*20 day old barramundi in Farm A are found infected with VER following mortalities*" while the second scenario was "*A routine sample is found to be positive for VER by PCR. The farm was thought to be free of VER and there are no mortalities*". Outputs from these group sessions have been included in the body of this report.

Translocation may be defined (Anon. 1999) as**:** the movement of live aquatic material (including all stages of the organism's life cycle and any derived viable genetic material): -beyond its accepted distribution; to areas which contain genetically distinct populations; or to areas with superior parasite or disease status. The Union for the Conservation of Nature and Natural Resources (IUCN) goes further and distinguishes three different classes of translocation:

- *Introduction of an organism: is the intentional or accidental dispersal by human agency of a living organism outside its historically known native range.*

- *Reintroduction of an organism: is the intentional movement of an organism into part of its native range from which it has disappeared or become extirpated in historic times as a result of human activities or natural catastrophe.*

- *Restocking: is the movement of numbers of plants or animals of a species with the intention of building up the number of individuals of that species in that habitat (IUCN 1987).*

4 FRDC Project 1999/205. The effect of barramundi nodavirus on important freshwater fishes.
5 FRDC Project 2001/626. Aquatic Animal Health Subprogram: development of diagnostic tests for the detection of nodavirus.

FRDC project 2004/080 has the objective to develop a single consistent translocation policy document for live temperate abalone and for prawns as a template for other translocation issues. Therefore, the technical guidelines workshop assumed that a translocation risk assessment had been carried out and that risk management was required to reduce the risk to the level of the jurisdictions' acceptable level of risk. For the purposes of the workshop, these management measures were divided into diagnostic capability, surveillance and translocation measures. Finally, research priorities were identified. The issue of certification was also discussed at a workshop associated with FRDC 2004/080 but does not form part of that report. The information has instead been included here.

BACKGROUND DOCUMENTS FOR WORKSHOP

The following documents were recommended to workshop participants:

OIE Aquatic Animal Health Code http://www.oie.int/eng/normes/fcode/en_sommaire.htm

OIE Manual of Diagnostic Tests for Aquatic Animals, Chapter 1.1.4: *Requirements For Surveillance For International Recognition of Freedom From Infection*: http://www.oie.int/eng/normes/fmanual/A_00013.htm

AQUAVETPLAN *Viral encephalopathy and retinopathy – disease strategy manual.* http://www.affa.gov.au/content/publications.cfm?Category=Animal%20fixand%20Plant%20Health&ObjectID=603F059F-025E-46DB-A65C498476FE27E6

Ministerial Council on Forestry Fisheries and Aquaculture. 1999. *National Policy for the translocation of Live Aquatic Organisms – Issues, principles and guidelines for implementation*. http://affashop.gov.au/product.asp?prodid=12105

RESULTS AND DISCUSSION

A. DIAGNOSTIC CAPABILITY

Knowledge of the health status of aquatic animal populations or stocks is an essential prerequisite for risk assessment and management of pathogen transfer. This knowledge of the health status of a population of fish requires that some form of surveillance has occurred and that there is an adequate diagnostic capability. Diagnostic capability rests on three factors:

- Availability of trained personnel;
- Adequate laboratory facilities and infrastructure;
- Diagnostic tests that are sensitive, validated and cost-effective.

How good are our diagnostic capabilities?

Unlike the terrestrial animal industries the aquaculture industry is based on a wide diversity of animal species, most of which are endemic to Australia and have a considerable number of poorly understood potential diseases. For this reason, overseas experience and literature are not always applicable to the Australian situation and "the task facing diagnosticians in aquaculture and plants is of a significant magnitude greater than that being undertaken by diagnosticians working with animal disease" (Anon. 2004). Given the magnitude of the task, there is a recognised shortage of trained personnel in Australia. This shortage is being addressed, in part, through other projects within *AQUAPLAN 2005-2010*. Though they are relatively few, a high proportion of the Australian personnel who are involved in diagnostic work on aquatic animal pathogens are internationally recognised for their expertise.

The laboratory facilities available in Australia consist of state and territory laboratories, university facilities, private laboratories and the national Australian Animal Health Laboratory (AAHL) in Geelong, Victoria. At present, diagnosis for both endemic and some exotic diseases is carried out in these institutions using a variety of procedures that are in no effective way harmonised or regulated (Anon 2004).

There are a limited number of aquatic Australian and New Zealand Standard Diagnostic Procedures (ANZSDPs) available, as well as the diagnostic procedures published in the OIE *Manual of Diagnostic Tests for Aquatic Animals*, and there are also a number of unpublished or unvalidated methods in use. The National Aquatic Animal Health Technical Working Group (NAAH-TWG) is coordinating efforts to update the existing aquatic ANZSDPs. While laboratories do attempt to standardise and validate tests used, the time and expense required are seldom justified for all but commonly needed procedures. Even when the ANZSDPs are available and are used, they require strict adherence to the protocols including the use of specified reagents. This point is not always appreciated. Molecular techniques, in particular, are very susceptible to variations in protocol including such issues as the batch of reagent used or, for example, whether *taq* polymerase or thermostable polymerases are used (Wiedbrauk et al. 1995; Moody 2004). Changes in equipment can also cause unwanted effects, for example, variations in the annealing temperature of a PCR thermocycler across the block may have significant effects on the outcome of the test (Claydon et al. 2004). Therefore, any change to a protocol, such as using a different commercial kit during preparation, requires validation steps to be performed.

Current diagnostic procedures for VER

Histology

In Australia, diagnosis of VER has been mainly based on histopathology of the brain, spinal chord and retina of juvenile fish. This is a procedure of low sensitivity and, although still a valuable test, it is not capable of detecting all nodavirus infected fish in a sample.

Routine testing by histology of juvenile fish (at 21 days old) for certification purposes is performed at laboratories in SA, WA, NT and QLD. Currently (November 2005), sample size for histology is 150 fish in all four states. In SA where histological interpretation is uncertain, samples are sent to QLD for PCR, in NT 60 fish are also tested by PCR.

PCR

PCR based detection techniques are proxy measurements, that is; they are indirect indicators of pathogen presence. The use of proxy measurements is associated with increases in the potential for misinterpretation of results and validation is essential (Hiney & Smith 1998). Laboratories in Queensland and the Northern Territory routinely perform testing by PCR. Oonoonba Veterinary Laboratory (Queensland) modified the OIE RT-PCR and the Thiery et al. (1999) nested PCR methods, retaining the prescribed primers for nodavirus as part of an FRDC project that finished in 2003 (Moody 2004). This is the PCR that is currently used in QLD, NSW, VIC and NT.

Tissue culture

The use of susceptible cell-lines in which the virus can be grown is the 'gold standard' test but cell lines that are susceptible to VER are not routinely available in Australia. One cell line which had been developed in Australia in the past was initially successful but transformed on passage 35 and is no longer useful (Moody 2004). Cell lines are available overseas, and at AAHL, but the OIE listed cell lines SSN-1 and E-11 are contaminated with a C-type retrovirus designated SnRV. Lee et al. (2002) provide evidence that snakehead cells infected with retrovirus support the growth of nodavirus. There is an urgent need to investigate the potential of such contaminated cell lines under Australian conditions and, by extension, the impact that multiple viral infections in host fish might be having on expression of the disease.

Other methods

A number of other laboratory procedures of varying sensitivity can be used to detect nodavirus in either clinically infected or apparently uninfected fish. An immunohistochemistry test (IHCT) and an indirect immunofluorescent antibody test (IFAT) were also developed as part of the FRDC project 2001/626. There was 100% agreement between the presence of nodavirus damage as seen by histology and positive immunodiagnostic test analysis of sections from infected tissue samples, but there was greater sensitivity in detecting unapparent carrier fish using the immunodiagnostic procedures. The immunodiagnostic procedures also detected exotic nodavirus isolates in fixed material from France, Israel, Norway, Japan and the Philippines (Moody 2004).

Issues with the methodology

Sample collection

There is a need to develop sampling protocols. For example, sampling from the top of cages (i.e. the healthy population) is of little use; the target samples should be taken from mortalities at the bottom of the cage. When sampling is carried out using this technique the estimated prevalence

goes up. A similar sampling strategy is used to detect low prevalence of Epizootic Haematopoetic Necrosis virus (EHNV) in trout (Whittington et al. 1999).

While there are methods to determine the theoretical prevalence of VER in a population of fish from the number of positive pooled-samples tested (see pooled prevalence calculator at http://www.ausvet.com.au/pprev/), there is as yet no PCR sensitivity information to input into such calculators. This is a particular problem with larger fish where, for example, the choice of blood or spawning fluids can give different results with PCR (Moody 2004).

There is also no information on what might be an appropriate sample size for surveillance and monitoring purposes given that background levels of infection in wild populations have not been established in different fish species.

Sample preparation

It is common to use fresh or thawed fish samples for testing by PCR and samples are often pooled to combine the tissue from a number of small fish (typically 5 heads). However, there are no Australian data on which organs are the most appropriate to use in pooled samples (in terms of viral titre) and how this in turn affects the sensitivity of the PCR.

Test methodology and standardisation

There are a growing number of published PCR's for nodaviruses appearing in the literature, few of which have undergone any validation (for technical guidelines on validation see the OIE *Manual of Diagnostic Tests for Aquatic Animals* http://www.oie.int/eng/normes/fmanual/A_00012.htm).

The Oonoonba modification of the OIE /Thiery et al. PCR is the most commonly used PCR in Australia for VER, and has been documented both in the FRDC Project report 2001/626 and as a draft ANZSDP. This PCR, when used by AAHL, did not pick up the Australian bass strain of VER and this led AAHL to develop a new set of primers to detect the virus (McColl et al. 2005)[6]. As a result, there was national concern that the Oonoonba method may not be the best test for use in translocation testing. However, the Oonoonba PCR, as routinely used by the Queensland laboratory, does pick up both strains of VER and does differentiate the two (Ian Anderson, pers. com., Moody 2005). A comparison needs to be made between the Oonoonba test, the AAHL test and also the commercial kits for nodavirus that are available overseas to determine their sensitivity and specificity for detecting variants of VER.

It should be noted that a key element in the validation of any PCR-based technique for field application is to establish the degree of certainty that the positive signal generated by the technique is a consequence of the presence of the target pathogen alone. This is a two-step process. (Hiney & Smith 1998). PCR must be shown to detect the correct target sequence and then it must be shown that the sequence is unique to the pathogen. For example, the OIE PCR method for WSSV does not detect the correct sequence, but may cross-react with host tissue (Claydon 2004). Ludert et al. (2004) provide an example of a widely used primer sequence for human caliciviruses that

6 Discussion at the July 2005 FRDC Subprogram scientific conference highlighted two problems with the Australian PCR methods for VER. The first was that, compared to barramundi VER, the Australian bass VER has two mismatches in the three terminal bases at the 3' end of the nested reverse primer set. This mismatch is probably the reason that the OIE primer set does not work under the protocols used at AAHL. Though a newly designed primer at AAHL does amplify the target sequence the gel photos presented to the meeting indicated that the PCR produces multiple products and needs further optimisation. The second issue was that AAHL do not, as a matter of policy, use published standard methods but use only the recommended primer sets and thermocycler settings together with their own in-house methods. The Oonoonba PCR is known to be sensitive to the polymerase used (Moody 2004) which is presumably why the miss-matched nested reverse primer works at Oonoonba but not at AAHL.

unexpectedly cross-reacts with rotaviruses. PCR methods are also very susceptible to inhibitory substances (Wilson 1997), excess host or target nucleic acids (Høie et al. 1997) and contamination (Borst et al. 2004). For these reasons, it is unwise to rely on a positive result from a single sample. Therefore it is recommended that reporting on the presence of a pathogen in a new host or area should be based on results from multiple positive samples by one test or from a single positive sample using at least two different test methods.

As part of a proposed national laboratory proficiency 'ring-test' AAHL have applied for funding to arrange a PCR ring test using barramundi nodavirus.

Strain variation

Moody et al. (2005) showed that all Australian sequence variations fall within the one RGNNV (red spot grouper nervous necrosis virus) genogroup that has been described by researchers (Nishizawa et al. 1997; Dalla Valle et al. 2001; Skliris et al. 2001). Sequence data analysis has shown that nodavirus from NSW and SA isolations are different from previous isolations suggesting that the outbreaks of VER from NSW and SA are independent of any translocation activity and represent local strains.

Development of new technology

The point was well made at the workshop that one could always devise a 'better' diagnostic method. The real question is whether the existing methodology is fit for the intended purpose. There are currently four uses for diagnostic tests in Australia:

- Screening of juveniles prior to movement from a hatchery;
- Screening of potential broodstock;
- Testing for nodaviruses as part of epidemiological research studies; and
- Outbreak investigation – confirmation of diagnosis.

Screening of juveniles prior to movement from a hatchery

The most cost effective test is still histology. Not only is histology able to detect lesions consistent with nodavirus; it can also detect a wide range of other potential problems. Suspicious lesions may be confirmed using IFAT. The existing Oonoonba PCR has proved reliable for detecting both Australian strains of VER. The issue of the effect of pooling and sample size on test sensitivity also influences the value of PCR testing of juveniles at this stage.

Screening of potential broodstock

This is currently problematical. Experience has shown that in broodstock testing, gonad fluids may be PCR positive but blood samples from the same fish may return negative results. Age is also a factor since farm-reared males may be tested before maturity and the reproductive tract fluid found to be negative for virus yet years later the same males can test positive. Current research in FRDC Project 2002/043[7] has also found that an individual mature male or female can test negative several times over a year and then subsequently test positive.

Therefore, at present, a single PCR test is not sufficient for determining the VER status of broodstock.

7 FRDC Project 2002/043 Aquatic animal subprogram: the production of nodavirus-free fish fry and the nodaviruses natural distribution.

Testing for nodaviruses as part of epidemiological research studies

This is also problematical at present. Both PCR and histology appear to be suitable for detecting virus in diseased juvenile fish but results from adult fish are not reliable. The most suitable test would probably be the use of tissue culture, but cell lines are not readily available.

"*Due to insufficient knowledge of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine screening method for assessing the viral status of fish populations*". (OIE Aquatic Manual, http://www.oie.int/eng/normes/fmanual/A_00024.htm).

Outbreak investigation – confirmation of diagnosis

This is relatively straightforward. For hatchery fingerling fish, a presumptive diagnosis can be made on the basis of vacuoles in the brain, spinal chord and retina. IFAT can be used in difficult cases. Characteristic viral particles can be seen using electron microscopy, and the virus can be grown on cell lines (that are available at AAHL).

B. SURVEILLANCE AND MONITORING

There are two conflicting issues associated with applications to translocate live fish, whether to farms or for restocking. One is the desire of industry to make a profit without hinderance from the state but without disease either. The other view was eloquently put subsequent to the workshop by one of the participants "*Our aim is not to keep industry free of nodavirus, it is to prevent entry of nodavirus to waters where it is exotic and to prevent the occurrence of outbreaks caused in endemic areas because of the influence of hatcheries/aquaculture*"

Both positions rely heavily on surveillance and monitoring both to detect disease agents in stock about to be translocated, and to determine the ongoing disease status of the waters put at risk by the translocation activities.

Lack of knowledge of epidemiology of the disease

Although VER has a world-wide distribution, is internationally reportable, is a common disease in barramundi aquaculture in Australia and, based on overseas experience, is probably common in the marine environment in Australia there is very little known about its epidemiology.

Experimental studies in Australia have shown that it is difficult to transmit infection except in very young fish; infection experiments using bath exposure with any fish over 21 days have failed. Research is hampered by lack of resources and the difficulty in obtaining young fish for experiments. Northern Territory and Queensland fish pathologists with help from Sydney University have been trying to understand farm outbreaks that occur despite strict biosecurity measures being in place.

Australia, as a continental landmass, has a unique fauna. Pathogens found in Australia include new strains of pathogens previously known from the Indo-Pacific. Tests developed overseas may therefore not be appropriate for detecting local strains of virus, and there may well be multiple geographically isolated endemic strains within Australia. Murray & Peeler (2005) point out that virulence may well increase as pathogens adapt to aquaculture. There will, therefore, be a need to update and regularly review the performance of tests used for surveillance and monitoring in Australia as new information on strain variation becomes available.

Need for surveillance?

The point was made at the workshop that the existing PCR is not being used for surveillance due to lack of time and resources. There will also always be the problem that testing is expensive and farmers will not test due to the cost. It was also suggested that testing of most marine finfish would detect the virus since snapper and various other species have been found to be positive for nodavirus overseas (Castri et al. 2001). Nodavirus has also been detected in freshwater guppies in the Singapore aquarium trade (Hegde et al. 2003).

As an alternative to intensively testing for specific disease (active surveillance), passive surveillance can provide disease information over time and, together with historical data on disease occurrence, might be a manageable process for jurisdictions. However, this strategy takes no account of the risk of spreading VER strains to new areas via translocations. If surveillance is to be carried out (and paid for) a commercially viable industry is needed to justify the expense. There is at present insufficient information on the epidemiology of the virus to carry out risk assessments associated with translocation applications. More disease information is required and surveillance would provide this.

C. TRANSLOCATION

A model risk assessment for translocation of live aquatic organisms is provided in FRDC 2004/080 for abalone and prawns. In addition, South Australia has issued a risk analysis for the translocation of live larvae and juveniles of barramundi (PIRSA 2005); Queensland has issued an aquaculture policy paper on management arrangements for potentially high-risk activities in the context of ecologically sustainable development for aquaculture facilities. This includes barramundi (DPIF 2004); Western Australia has issued a translocation management paper on barramundi translocation (Thorne 2002). Guidelines for Assessing Translocations of Live Aquatic Organisms in Victoria are on the following website: www.dpi.vic.gov.au .

Each jurisdiction has its own acceptable level of risk based on local conditions and more work needs to be done to rationalise this across jurisdictions. Disease risk, just like genetic risk, is strongly related to distance over which the translocation occurs as well as environmental factors such as catchment area. The regular presence of veterinary or aquaculture field officers at farms helps establish rapport with the farmers and provides added independent assurance of disease status. At present disease risk with respect to VER is based on a poor understanding of the distribution and epidemiology of the disease in Australia. This is particularly important for restocking: the VER status of the receiving population is often unknown; if VER is present, the local strain of VER may be unknown; the survival of subclinically infected stocked fish may also be uncertain.

It was identified that among risk management measures which are, or could be applied there is a need for certification standards for each species and a need to harmonise testing regimes i.e. methodology for determining “freedom-from-infection”. It is also possible to accredit hatcheries through surveillance and monitoring – i.e. by maintaining a 2-3 year record of freedom of infection through batch testing and with no new introduction of fish from the wild, it should be possible to provide “specific pathogen free” status (but see concerns about epidemiology and sampling above). The risk of undetected disease may be reduced to very low levels by surveillance and monitoring, but for an endemic disease the risk can never be reduced to zero.

The cost of testing is an important issue for industry i.e. the larger the size of fish to be tested the more expensive the testing becomes. Also, the use of PCR testing or tissue culture isolation can be very expensive when large sample sizes are involved.

Biosecurity and Disinfection protocols

There should be more documentation of existing aquatic animal zones in Australia (for example those in use in Tasmania) as an encouragement to use zoning as a management tool.

There needs to be more work on the concept of a 'biosecure hatchery or farm facility' and protocols for the movement and quarantining of stock. Biosecurity includes any practices, policies or procedures used on a farm to reduce the risk that pathogens will spread through the facility (Delabbio et al. 2004; Pruder 2004). Few finfish aquaculture farms in Australia see any need to protect their water supply, and even fewer would filter and treat their effluent for disease agents (as opposed to effluent treatment required for environmental reasons), yet the risk is real. Eide (1992) found that a fish farm with clinical infectious salmon anaemia could infect a farm 5-6 km away after a period of 6-12 months. Jarp et al. (1993) showed that hatcheries with two or more fish farms within 10 km radius and infected with *Aeromonas salmonicida* were at two times higher risk of having the disease compared to hatcheries that had fewer than two farms within 10 km. Studies in British Columbia have associated an increased risk of *A. salmonicida* in sea cages that are within 10 km of infected cages (Needham 1995). The impact of salmon farms in inadvertently breeding up large numbers of salmon lice that subsequently affect and kill wild fish has also been established after considerable debate (Glover et al. 2004; Krkosek et al. 2005). Farmers need to be educated about the risks associated with disease in aquaculture and the steps that they need to take to mitigate undesirable environmental impacts. Monitoring for environmental impacts may become a cost of doing business for aquaculture.

If there is a VER outbreak in a farm, simply removing infected fish is not sufficient to eradicate the disease. Newly stocked fish will be re-infected by the virus unless the farm or hatchery is disinfected thoroughly. There is very little information on the environmental requirements of, or disinfection protocols, for Australian aquatic animal pathogens.

Protocols to ensure that stocking programs use stock that are free from diseases of concern are lacking. Since it is difficult to ensure that stock are completely free of disease (zero prevalence) they need to come from a biosecure facility of proven disease status or there needs to be agreement within jurisdictions on the level of risk that will be tolerated for restocking. One-off certification of batches for 'freedom' from VER before restocking does not necessarily mean that the fish are free from the disease agent – just that it was not detected.

Health certification

Health certification and quarantine measures are integral parts of the overall health management process used by jurisdictions. This process should be practical, cost-effective and easy to implement.

The international trend is to move away from testing prior to translocation and to move towards using historical information and knowledge of the past and current biosecurity status of the establishment. In NSW the recently introduced Hatchery Quality Assurance Program is one such benchmark that could be used to achieve a minimum standard of surveillance and confidence in disease freedom within a compliant facility. The minimum requirements for declaring historical disease status are set out in the OIE website.

Disclosure of information prior to and post-translocation is sometimes required. The onus is usually on the importer to report significant mortalities after translocation. Vendor statutory declarations as to health status of aquatic animal stock are the exception rather than the norm and there are legal issues associated with enforcing them. Cross-jurisdictional certification would be required in most cases.

Where testing is required, there are sometimes different requirements for the confidence levels for testing – usually 95% is required, depending on the prevalence of the disease, sensitivity and specificity of the test and the number of animals to be translocated. However, the uncertainties around the sampling protocols and the sensitivity of the test are issues that are unresolved (as previously discussed).

Where the origin and destination of the stock are of similar known disease risk it is unlikely that disease testing will be required. An exception is where the receiving jurisdiction has a formal surveillance program in place testing local stock for disease, in which case translocated stock may need to be tested to the same level.

Issues associated with certification include:

- *What details should appear on the Certification? (i.e. a simple form such as the OIE model certificates, or a full pathology report?)*

  In TAS the terrestrial model is based on a declaration of the farmer countersigned by the Competent Authority. If testing is required, the laboratory report must be sighted by Competent Authority, but the report is not required to accompany the consignment. In SA, pathology reports are not required to accompany consignment, but must be sighted by the Competent Authority. Other details are dependent on the translocation requirements. In VIC and WA, for barramundi, laboratory testing is required and a translocation certificate is also required. In QLD a health certificate and a declaration are required. In NSW, a pathology report from the Competent Authority is to accompany shipments and records are to be maintained by the importer. Other details are dependent on translocation requirements.

- *Who is the "Competent Authority" who can sign health certificates in each jurisdiction?*

  In all states the Competent Authority is usually the Chief Veterinary Officer (CVO) or delegate, except in the case of pearl oysters in WA and NT.

- *What timeframe should the Certification be current for? (The OIE model certificates have a 3-day period).*

It was agreed by TAS, SA, VIC, NSW and WA that a timeframe of 2 weeks would be appropriate.

D. RESEARCH NEEDS

The workshop process identified a series of research needs with respect to VER. These include:

- The need to assess the risk to other native species.
- The need to assess sensitivity and specificity of tests.
- The need for new locally developed nodavirus susceptible cell culture isolation systems.
- To develop new tests- including:

  a. An antibody detection test (the Japanese and the French already use this technique), this will require specific antibodies.

  b. To develop an antigen capture ELISA as is now used for EHNV, this is a cheap test to detect viral antigen, and is 60% as sensitive as cell culture.

  c. To develop methodology based on mass spectrometry to enable the detection of fragments of viral coat protein so that we can detect very early infections.

- To fund more research work on sampling methodology to detect sub-clinical infections at low levels of prevalence, not only for VER but for all aquatic viruses.
- Research to determine the actual pathogenesis of a nodavirus infection in Australian euryhaline and marine finfish.
- To do more work on defining the concept of a ‘biosecure facility’ and for the movement and quarantining of stock.
- To research the epidemiology of infection.

CONCLUSIONS

While the workshop concentrated on VER, the principles outlined apply to most of the diseases of concern facing finfish in Australia. Validation and standardisation of diagnostic tests for diseases of national concern are required and few available tests (even commercially available kits) meet the criteria set down by the OIE. While there is a diagnostic test for VER, more work is required to validate the test and to compare its performance with commercial kits.

Where time and scarce resources have been committed to validating a test, it should be used in preference to unvalidated tests and commercial kits. As false positive results may have economic impact at the farm level and on exports, it is recommended that to confirm a positive result in significant cases (for example, in emergency disease outbreaks, or where the agent is thought to have occurred in a new host or new geographic location).

The information on which sampling protocols to use is scant. There needs to be more research work on sampling methodology to detect sub-clinical infections at low levels of prevalence, not only for VER but for all aquatic viruses. For example, random sampling of 150 fish may not be sufficient to detect low prevalence of infection. Perhaps targeted sampling would be more appropriate but the methodology needs to be verified if it is to have credibility for certification purposes.

With modern molecular methods, pooling of samples from several animals is common. There needs to be further work on the protocol for pooling samples, for example, on which tissues should be used for pooling, whether the relative proportions of tissue making up the pool are critical and how pooling affects the sensitivity of the test and on pooling rate (e.g. 5 or 50).

Tests developed overseas may not be appropriate for detecting local strains of virus, and there may well be multiple geographically isolated strains within Australia. There will be a need to update and regularly review the performance of tests used for surveillance and monitoring in Australia.

There is very little information on the epidemiology of most of the aquatic animal disease agents of national concern. There is very little information on the pathogenesis of many viral disease agents. This has a direct impact on sampling methodology, estimation of prevalence of infection in populations and on screening of fish for presence of agents. There is evidence that certain cell lines require a contaminant virus to support growth of barramundi nodavirus, which poses the question "What role do other disease agents play in expression of disease in barramundi?"

There needs to be more work on defining the concept of a 'biosecure facility' and for the movement and quarantining of stock. (For example, moving stock by road tanker may involve water exchanges en-route). We have very little information on the environmental requirements (i.e. temperature and pH requirements) or disinfection protocols for Australian pathogens.

All of these factors make it extremely difficult for managers to assess the risks associated with the translocation or restocking of live finfish, not just for VER but any of the diseases of concern. The uncertainty in information available, in the absence of any funding for research to address the situation, will tempt managers to err on the side of caution and limit aquaculture and restocking activity.

KEY RECOMENDATIONS

The following key recommendations, which resulted from the workshop process, will be progressed through the National Aquatic Animal Health Technical Working Group. Funding for recommendations will be sought through the Aquatic Animal Health Committee process, which has oversight of *AQUAPLAN 2005-2010*, and through the FRDC subprogram on Aquatic Animal Health.

1. Validation and standardisation of diagnostic tests for aquatic animal diseases of national concern are required. While there is an ANZSDP diagnostic test for VER, more work is required to compare its performance with commercial kits.

2. Where time and scarce resources have been committed to validating ANZSDPs they should be used in preference to unvalidated tests and commercial kits.

3. Since false positive results can have economic impact at the farm level and on exports, it is recommended that multiple sample positive results be obtained by one test method (preferably at two separate laboratories) or a single positive sample be confirmed by at least two different test methods when reporting on the presence or absence of a causative agent in significant cases (for example, in emergency disease outbreaks, or where the agent is thought to have occurred in a new host or new geographic location).

4. The information on which sampling protocols can be developed is scant. There needs to be more research work on sampling methodology to detect subclinical infections at low levels of prevalence, not only for VER but for all aquatic viruses. For example, random sampling of 150 fish may not be sufficient to detect low prevalence of infection. Targeted sampling may be more appropriate but the methodology needs to be verified if it is to have credibility for certification purposes.

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