Aquatic Animal Health Subprogram Scientific Conference

Four Points Hotel, Sheraton
Eastern Beach, Geelong
8-10 October, 2003

Australian Government
Department of Agriculture, Fisheries and Forestry

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Aquatic Animal Health Subprogram Scientific Conference

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Aquafin CRC Health Program, *Barbara F Nowak*

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Development and Preliminary Characterisation of Pilchard (*Sardinops sagax neopilchardus*) Cell Lines Derived from Liver and Heart Tissues, *Lynette M Williams, Mark StJ Crane & Nicholas Gudkovs*

Aquatic Animal Health Subprogram
Strategic Research and Development Plan
2002 – 2007

(Update July 2003)

Aquatic Animal Health Subprogram
Strategic Research and Development Plan
2002 – 2007
(Update July 2003)

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1 Introduction

This strategic R&D plan ('the Plan') of the Fisheries Research and Development Corporation's Aquatic Animal Health Subprogram ('the Subprogram') will guide the Subprogram to fulfill its objectives to provide leadership, direction and focus for aquatic animal health research and development (R&D) and other related non R&D activities. The Plan will assist the Subprogram in assessing aquatic animal health project applications. A compilation of current R&D priorities is included.

This strategic R&D plan is a 'working document'. It has been developed for a five-year period (2002-2007) after which a full review will be conducted.

However, the Plan will also be reviewed annually and amended accordingly.

The Plan:

- Outlines the background to the establishment of the Subprogram;
- Describes the Subprogram including its role, objectives, structure and funding basis;
- Describes criteria used in defining a project under the Subprogram;
- Outlines the key research areas;
- Will be used by the Subprogram to assist in assessing animal health project applications;
- Lists current R&D priorities; and
- Will be reviewed annually with wide stakeholder consultation.

2 Background

Australia's fisheries and aquaculture are the fastest growing sectors of our primary industries in terms of both job creation and average growth in production, currently running at 13% growth p.a. Their capacity to contribute through export earnings and job creation especially in regional Australia is a vital part of our future prosperity. Australia is fortunate to have our aquatic animal sector free from many diseases that occur elsewhere in the world, and this provides us with a comparative advantage in both production and trade. Australia's capacity to produce 'clean green' seafood of superior quality allows ready access to overseas markets, enhances competitiveness and also provides value adding through the capacity to attract premium prices.

It is vital for Australia to maintain this relative disease free status. Industry and government have recognised the importance of an integrated and planned approach on aquatic animal health. This led to the cooperation between industry and government in developing AQUAPLAN, Australia's five year National Strategic Plan for Aquatic Animal Health. AQUAPLAN is a comprehensive document describing initiatives ranging from border controls and import certification through to enhanced veterinary education and improved capacity to manage incursions of exotic diseases. The eight programs described in this plan represent a world first in proactive management of aquatic animal health.

In addition, Australia has a unique and poorly understood endemic parasite flora and fauna which is becoming of increasing importance and concern to our export trade. Examples include the internationally reportable gill associated virus and spawner-isolated mortality virus in prawns, and QX disease (*Marteilia sydneyi*) in oysters. This concern over endemic diseases and the lack of surveillance and diagnostic services has already compromised

attempts to export live shellfish to the European Union. Such trade barriers, based on our lack of understanding of our own diseases, will continue to be imposed and provide an incentive to Australia to not only improve basic research knowledge on endemic disease agents but more critically to improve the quality control and thus international acceptance of our diagnostic and surveillance capacity.

3 Aquatic Animal Health Subprogram

The Subprogram was established by the Fisheries Research and Development Corporation (FRDC) in mid 2001 to provide a cohesive and national approach to aquatic animal health research and development in Australia, and in particular to address AQUAPLAN Program 6: Research and Development. The Subprogram has a national focus, consistent with international obligations.

Mission

"To provide leadership to aquatic animal health R&D and its adoption in Australia".

3.2 Objectives

The Subprogram's key objectives are to:

1. Provide leadership, coordination, management and planning for aquatic animal health R&D;
2. Set and review national priorities of aquatic animal health research; and
3. Oversee the communication, extension and adoption of results of aquatic animal health research projects.

3.3 Role

The role of the Subprogram is to:

- Implement the Subprogram strategic R&D plan;
- Set R&D priorities to maximise investment in aquatic animal health, avoid duplication and achieve the greatest potential return;
- Invite R&D applications to address those priorities;
- Maximise collaboration between researchers, and between researchers, fisheries managers and fishing industry interests;
- Attract other R&D funding and influence the way in which other funding entities apply their investments in that field;
- Standardise on the best scientific methods;
- Communicate regularly with potential beneficiaries; and
- Influence the adoption of R&D results.

3.4 Outcomes

The Subprogram's activities will contribute to:

1. Reduced risk of a major disease impact on Australia's fisheries resources;
2. Improved productivity and profitability of the fishing and aquaculture sectors;
3. Market access/biosecurity/meeting international obligations;
4. Improved standard/productivity of research and analysis;
5. Cost-effective research and analysis; and
6. Increased awareness of aquatic animal health issues.

3.5 Scope and links with other FRDC subprograms

The scope of the Subprogram is 'health' with a focus on infectious diseases. The Subprogram adopts a special responsibility for health-related project applications originating in industry sectors for which there is no specific subprogram. In particular the Subprogram would manage health-related projects on new or emerging aquaculture species ('orphan species').

In situations where a species-specific aquaculture subprogram exists[1], these subprograms would be responsible for the prioritisation and management of any health related projects involving those specific species. The Subprogram would provide advice on these health related projects where necessary.

The preferred process for submission and assessment of such applications is as follows:

1. The pre-proposal or full application should be submitted to the species-specific subprogram[1] who would assess its need and priority.
2. If supported by the species-specific subprogram, the pre-proposal or full application would be forwarded to the Subprogram for advice on technical feasibility and merit.
3. The full application should gain support from both subprograms before submission to the FRDC Board for final assessment.
4. If approved, the project would then be managed by the species-specific subprogram; the Subprogram would provide advice on milestone reports and the final report as required.

3.6 Scope and links with other bodies

The Subprogram consults on health R&D priorities and strategies with the Aquatic Animal Health Committee (AAHC) which was established in late 2002 as the primary industry/government interface for policy, communication and awareness related to aquatic animal health, thereby superseding the Fish Health Management Committee (FHMC). Consultation is primarily through AAHC's technical and scientific support body, the National Aquatic Animal Health – Technical Working Group (NAAH-TWG).

3.7 Steering Committee

The Steering Committee (STC) comprises both government and industry representatives. When established in 2001, its composition was deliberately identical to the then FHMC's AQUAPLAN Business Group (ABG) to ensure linkages to AQUAPLAN Program 6 (R&D)

[1] E.g. Atlantic Salmon Aquaculture, Southern Bluefin Tuna Aquaculture, Abalone Aquaculture, Rock Lobster Enhancement and Aquaculture

as well as to the FRDC and greatly reduced administrative overheads. FHMC was disbanded in 2002 and superseded by the AAHC (see above).

Amongst the key tasks of the STC are:

- To develop a strategic R&D plan with key performance measures and timeframes. This should be regularly reviewed.
- To ensure that research objectives are commercially focused and outcome driven.
- To coordinate industry and research provider involvement to maximise usage of available resources.
- To facilitate industry extension and technology transfer.

<u>STC members</u>

- *Industry members:* Simon Bennison[2] (joint chair)
  Russ Neal[3] (joint chair)
  Pheroze Jungalwalla[4]
  Brian Jeffriess[5]
- *Government Members:* Jim Gillespie[6]
  Eva-Maria Bernoth[7]
- *FRDC member:* Patrick Hone[8]

3.8 Scientific Advisory Committee

The Scientific Advisory Committee (SAC) consists of a small core group that may co-opt additional scientists as needed. The SAC members were chosen so that a Commonwealth laboratory as well as a State laboratory are represented and a formal linkage to the Health Program in the 'CRC for Sustainable Aquaculture of Finfish' (AQUAFIN CRC) is guaranteed.

Amongst the key tasks of the SAC are:

- To scientifically assess new research proposals, *inter alia* to ensure that the research proposed is scientifically feasible, and to advise the STC on new funding applications.
- To advise on scientific problems with project progress as well as identify remedial action, to ensure scientific objectives and milestones are met.
- To foster and develop collaboration amongst researchers.
- To facilitate research extension and technology transfer.

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[2] National Aquaculture Council
[3] Australian Seafood Industry Council
[4] Tassal Limited
[5] Tuna Boat Owners Association
[6] Queensland Fisheries Service
[7] Agriculture, Fisheries and Forestry - Australia
[8] Programs manager, Fisheries Research and Development Corporation

SAC members

- Mark Crane[9]
- Brian Jones[10]
- Barbara Nowak[11]

4 Stakeholders

The key stakeholders in the Subprogram, i.e. those beneficiaries that have the greatest stake in the success of the Subprogram and with whom the Subprogram consults to identify aquatic animal health R&D needs, are (alphabetical order):

- Australian Seafood Industry Council
- Commonwealth Department of Agriculture, Fisheries and Forestry – Australia
- Aquatic Animal Health Committee
- FRDC
- Major aquaculture industries (salmon, tuna, edible oysters, pearls, prawns)
- National Aquaculture Council
- RecFish Australia
- Research providers
- State/Territory Departments of Fisheries/Natural Resources/Agriculture

It is acknowledged that the list of beneficiaries is much longer, including e.g. the post-harvest industry, the ornamental fish industry, conservation interests, indigenous groups, pharmaceutical companies, research investors, extension services, consumers of seafood, and the public at large.

5 Budget

The Subprogram was established with an indicative budget of $1.5 million over a three-year period.

6 Methods

The Subprogram fulfils its role by:

- Being accountable for actions outlined in this strategic R&D plan;
- Adopting a proactive approach to aquatic animal health;
- Adopting a holistic approach to aquatic animal health;
- Adopting clear directions and processes;
- Providing a focal point for research;
- Promoting a collaborative/cooperative R&D environment;
- Advocating the importance of aquatic animal health; and

[9] CSIRO Livestock Industries
[10] Department of Fisheries, Government of Western Australia
[11] Aquafin CRC, University of Tasmania

- Communicating with Fisheries Research Advisory Bodies (FRABs) and other FRDC subprograms on:
  - ⇒ Research pre-proposals and full project applications received by the Subprogram – informing and seeking comment by FRABs/subprograms;
  - ⇒ Subprogram assessment of research pre-proposals and full applications; and
  - ⇒ Advice sought on health related pre-proposals and full applications submitted to FRABs or other subprograms.

The STC and SAC assist the Subprogram in fulfilling its role and managing its projects.

6.1 FRDC R&D projects

The Subprogram follows the FRDC's standard operating procedures for project approval and management, especially regarding communication with other subprograms and FRABs.

6.2 Meeting Objectives

The Subprogram achieves its three key objectives through the following methods:

Objective 1: Provide leadership, coordination, management and planning for aquatic animal health R&D

A) Planning

- Establishment and annual review of strategic R&D plan (update; identify gaps)

B) Development of applications

- Commissioned, unsolicited or forwarded (by FRDC, FRABs or other subprograms)

C) Assessment of applications

- Determine whether application fits criteria[12] (if not, provide advice/expertise/leadership)
- Evaluate need
- Evaluate feasibility
- Determine overall priority (against other applications)

D) Application funding

- Identify appropriate funding body/ies

E) Project management facilitation

- Assessment and execution of projects
- Communication/extension of results
- Encourage/facilitate adoption of results

F) Governance

- Reporting/accountability (FRDC)
- Structure (STC; SAC – expertise based)

H) Linkages

- Establish strategic alliances

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[12] See 7.1 below

Objective 2: Set and review national priorities of aquatic animal health research

- Establish R&D priorities in consultation with stakeholders, e.g. through the annual workshops of NAAH-TWG
- Annual update of strategic R&D plan
- Full review of strategic R&D plan every 5 years

Objective 3: Oversee the communication, extension and adoption of results of aquatic animal health research projects

Develop a communication strategy that includes:

- *Health Highlights* (Subprogram newsletter)
- Scientific workshops
- Website
- Provide scientific advice and communication to other subprograms and FRABs regarding aquatic animal health research pre-proposals, applications, projects and results
- Databases e.g. the Australian Aquatic Animal Health Information System (AAAHIS)

7 Research and Development

This section outlines the criteria used to determine whether a project falls under the Subprogram. Key research areas for the Subprogram are listed as a guide for applicants in developing projects for funding under the Subprogram.

7.1 Criteria

The following criteria are used to define a project under the Subprogram:

- Exotic or endemic aquatic animal disease of potential infectious aetiology, with potential or existing significant impact on Australian fisheries and aquaculture (includes also capture fisheries, recreational fisheries, indigenous fisheries and/or aquatic ecosystems);
- Emergency disease of national significance (e.g. based on Australia's *National List of Reportable Diseases of Aquatic Animals*);
- Addresses gaps in existing aquatic animal health research and contributes to the future understanding of aquatic animal diseases and their control (including diseases of new or potential species for aquaculture);
- Facilitates collaborative research to avoid duplication or gaps;
- Facilitates capacity development within Australia;
- Identified as a stakeholder priority (including industry, government and research stakeholders).

7.2 Key research areas

When developing project applications for funding through the Subprogram, the outcomes of the project should address at least one of these key research areas. Discrete RD priorities for the next years are listed under the pertinent areas.

7.2.1 Nature of disease and host-pathogen interaction

- Improved knowledge of the biology of disease agents (including epizootiology, taxonomy of pathogens, morphology, pathophysiology, histology, toxicology, etc)
- Improved knowledge on the host response to disease agents (aquatic animal immunology and immunomodulators)
- R&D to underpin knowledge about new and emerging diseases of significance
- R&D to underpin knowledge about disease risk associated with ornamental fish and recreational fishing.

PRIORITIES

- Midcrop mortality syndrome / Mourilyan virus / gill-associated virus / spawner-isolated mortality virus
- Herpesvirus and iridovirus infections in ornamental fish
- *Perkinsus* infections
- Parasitic protozoans
- Immunology in aquatic vertebrates
- Immunology of aquatic invertebrates
- Evaluate host-pathogen interactions for intractable diseases and identify risk factors to develop disease minimisation strategies

7.2.2 Aquatic animal health management

- R&D to underpin risk analyses (including disease risk minimisation procedures for exported and imported aquatic animals and products)
- R&D to facilitate inter-jurisdictional harmonisation of domestic and international approaches (common tests, common protocols [e.g. translocation], common certification)
- Development of protocols, methods and operational instruments to manage emergency aquatic animal disease outbreaks in Australia
- Methods of aquatic animal product treatments to prevent spread of disease (sterilisation, disinfection and decontamination)

PRIORITIES

- Risk assessment on the escape of live pathogens from abalone farms
- Risk assessment on the escape of live pathogens from ornamental fish to farmed fish
- Impact of micro-and macro-nutrition on immune health and expression of disease
- Immunomodulators to enhance vaccine efficacy

- Development of standard protocols to evaluate vaccine efficacy under farm conditions
- Development of probiotics for the control of disease or improved health of hatchery and farmed aquatic animals
- Identify and assess stress factors in molluscs to develop health management strategies for on-farm and post harvest stock

7.2.3 Endemic and exotic aquatic animal disease diagnostics

- Review and assessment of existing screening and diagnostic tests, and those under development
- Development of case definitions and diagnostic criteria
- Development and validation of screening tests and diagnostic tests
- Facilitate transfer of knowledge and technology in aquatic animal diagnostics

PRIORITIES

- Initiation of an aquatic animal component of the National Registry of Domestic Animal Pathology (or equivalent)
- Diagnostics for endemic iridovirus (tropivirus group – ornamental and farmed fish) (epizootic haematopoietic necrosis reference laboratory)
- Development of diagnostic tests for economically important diseases of ornamental fish, both enzootic and exotic
- Evaluation of flavobacteria as pathogens of aquatic animals and development of a practical diagnostic system for their identification

7.2.4 Surveillance and monitoring

- Support projects to enhance existing surveillance and monitoring programs and those under development
- Research into aquatic animal disease surveillance methodology
- R&D to underpin disease control programs, translocation, zoning, surveillance and monitoring, and risk analyses in relation to disease organisms

PRIORITIES

- Distribution and impact of iridovirus (epizootic haematopoietic necrosis virus) in wild stocks of native fish and introduced redfin perch
- Distribution of iridovirus (epizootic haematopoietic necrosis virus) in farmed salmonids in New South Wales, Victoria and Western Australia
- Random/structured surveillance of ornamental fish on release from quarantine
- Development of base line data of blood/haemolymph parameters and common diseases for all endemic Australian species under culture
- Development of tools for immune status monitoring as a means of implementing health management strategies
- Evaluation of causes leading to apparent increased virulence of disease agents

- Development of a national guidelines/strategy framework to ensure effective passive surveillance of aquatic animals, especially those under culture.
- Application of diagnostic tools for disease forecasting for improved health management strategies.

7.2.5 Best practice/national and international quality assurance

- Quality assurance (QA) and proficiency testing (e.g. white spot disease, epizootic haematopoietic necrosis, histology slides and nodavirus)
- R&D to underpin development of QA standards
- Facilitate the establishment of laboratory proficiency testing in detecting infectious diseases

PRIORITIES

- Continue ring testing for white spot virus
- Ring testing for viral encephalopathy and retinopathy, epizootic haematopoietic necrosis, and crayfish plague
- Inclusion of aquatic specimens into the 'slide of the month' series for histopathologists
- Identification of key factors for the development of Regional Codes of Best Practice for Health e.g. biosecurity for feed boats, dive teams, disposal of blood water etc.
- Development of interpretation guidelines for antibiotic sensitivity testing for bacterial pathogens of aquatic animals in Australia
- Evaluation of chemotherapeutic treatments for specific aquatic animal use and development of treatment best practices

7.2.6 Training and capacity building

- Human capital development – including training and capacity building for aquatic animal health specialists/veterinarians.
- Facilitate the development of training and extension tools
- Sustain and further develop technical skill base in aquatic animal health
- Facilitate R&D knowledge transfer in aquatic animal health

PRIORITIES

- Assessment of national capacity in aquatic animal health
- Development of resources for undergraduate education in aquatic animal health at Australian veterinary schools
- A continued focus on organised, continuing education with particular reference to aquatic animal health and aquatic animal pathology for veterinarians at undergraduate, post graduate and specialist levels
- Specialist training programs for microbiologists providing diagnostic services for aquatic animal health

- Consolidation of knowledge and capability for parasitology of aquatic animals
- Development and maintenance of databases and related resources for diseases and pathology of aquatic animals in Australia

8 Further information

- **Aquatic Animal Health Subprogram website:**

Go to the FRDC website www.frdc.com.au and follow the links:

Research and Development -> Subprograms -> Aquatic Animal Health Subprogram

- **Agriculture, Fisheries and Forestry – Australia website: www.affa.gov.au**
- **Contacts Aquatic Animal Health Subprogram:**

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FRDC Aquatic Animal Health Subprogram
Summary of Active Projects

Summary of Active Projects

within the Aquatic Animal Health Subprogram

Federal Budget Initiative: *Diagnostics Program*		
Project #	**Project**	**Principal Investigator**
2001/620	Aquatic Animal Health Subprogram: development of improved procedures for the identification of aquatic birnaviruses (November 2001 – December 2003) *Associated species:* salmonids	Ken McColl CSIRO Livestock Industries AAHL Fish Diseases Laboratory
2001/621	Aquatic Animal Health Subprogram: molecular diagnostic tests to detect epizootic ulcerative syndrome (*Aphanomyces invadans*), and crayfish plague (*Aphanomyces astaci*) (April 2002 – March 2004) *Associated species:* finfish and freshwater crayfish	Nicky Buller Department of Agriculture, Government of WA
2001/624	Aquatic Animal Health Subprogram: development of diagnostic procedures for the detection and identification of *Piscirickettsia salmonis* (November 2001 – December 2003) *Associated species:* salmonids	Mark Crane CSIRO Livestock Industries AAHL Fish Diseases Laboratory
2001/625	Aquatic Animal Health Subprogram: development of diagnostic capability for priority aquatic animal diseases of national significance: spawner-isolated mortality virus (March 2002 – March 2004) *Associated species:* crustaceans	Leigh Owens School of Biomedical Sciences James Cook University
2001/626	Aquatic Animal Health Subprogram: development of diagnostic tests for the detection of nodavirus (April 2002 – June 2004) *Associated species:* marine finfish	Nick Moody Oonoonba Veterinary Laboratory Queensland Department of Primary Industries
2001/628	Aquatic Animal Health Subprogram: vibrios of aquatic animals: development of a national standard diagnostic technology (October 2001 – June 2004) *Associated species:* finfish, shellfish and crustaceans	Jeremy Carson TAFI – Fish Health Unit Tasmanian Department of Primary Industries, Water & Environment
2001/630	Aquatic Animal Health Subprogram: validation of DNA-based (PCR) diagnostic tests suitable for use in surveillance programs for marteiliosis of rock oysters (*Saccostrea glomerata*) in Australia (April 2002 – July 2003) *Associated species:* rock oysters	Robert Adlard Queensland Museum
2003/620	Aquatic Animal Health Subprogram: establishment of diagnostic expertise for detection and identification of red sea bream iridovirus (RSIV) (May 2003 – April 2006) *Associated species:* finfish	Mark Crane CSIRO Livestock Industries
2003/621	Aquatic Animal Health Subprogram: development of diagnostic and reference reagents for epizootic haematopoietic necrosis virus of finfish (March 2003 – August 2004) *Associated species:* finfish	Richard Whittington University of Sydney
2003/622	Aquatic Animal Health Subprogram: development of molecular diagnostic expertise for the mollusc pathogen *Bonamia* sp. (October 2003 – June 2004) *Associated species:* Molluscs	Serge Corbeil CSIRO Livestock Industries AAHL Fish Diseases Laboratory
Federal Budget Initiative: *Manuals and Planning Program*		
2002/640	Aquatic Animal Health Subprogram: production of AQUAVETPLAN disease strategy manual for viral haemorrhagic septicaemia (September 2003 – March 2004) *Associated species:* finfish	Paul Hardy-Smith

2002/641	Aquatic Animal Health Subprogram: crayfish plague disease strategy manual (July 2002 – June 2004) *Associated species:* Crayfish	Fran Stephens Aquatilia Healthcare
2002/643	Aquatic Animal Health Subprogram: viral encephalopathy and retinopathy a disease strategy manual (July 2002 – December 2003) *Associated species:* finfish	Richard Miller School of Human Life Sciences University of Tasmania
2002/645	Aquatic Animal Health Subprogram: exotic disease training manual (July 2002 – June 2004) *Associated species:* finfish, crustaceans and molluscs	Shane Raidal Division of Veterinary and Biomedical Sciences Murdoch University
2002/647	Aquatic Animal Health Subprogram: production of an AQUAVETPLAN disease strategy manual for white spot disease of all WSV-susceptible crustaceans (August 2002 – September 2003) *Associated species:* crustaceans	Chris Baldock AusVet Animal Health Services Pty Ltd
2002/651	Aquatic Animal Health Subprogram: whirling disease – disease strategy manual (December 2002 – March 2004) *Associated Species:* finfish	Paul Hardy Smith
2002/652	Aquatic Animal Health Subprogram: enhancement of the emergency disease management capability in Victoria - developing a Victorian Control Centre Management Manual (October 2002 – August 2003) *Associated species:* finfish, crustaceans and molluscs	Anthony Forster Fisheries Victoria
2002/653	Aquatic Animal Health Subprogram: AQUAVETPLAN aquatic disease disinfection manual (October 2002 – January 2004) *Associated species:* finfish, crustaceans and molluscs	Kevin Ellard Livestock & Aquaculture Veterinary Consulting Services
2002/654	Aquatic Animal Health Subprogram: development of a training course on exotic diseases of aquatic animals (December 2002 – April 2004) *Associated species:* multiple species	Ken McColl CSIRO Livestock Industries AAHL Fish Diseases Laboratory
2002/655	Aquatic Animal Health Subprogram: design and organisation of a multi-state disease emergency simulation exercise (November 2002 – November 2003) *Associated species:* finfish, crustaceans and molluscs	Iain East Australian Government Department of Agriculture, Fisheries and Forestry
2003/640	Aquatic Animal Health Subprogram: subprogram conference ‘emergency disease planning and management (March 2003 - May 2004) *Associated Species:* multiple species	Mark Crane CSIRO Livestock Industries AAHL Fish Diseases Laboratory
2003/641	Aquatic Animal Health Subprogram: development of the control centre manual for managing aquatic disease emergencies in Queensland (March 2003 - March 2004) *Associated Species:* oysters, prawns	Tiina Hawkesford Department of Primary Industries, Queensland
2003/642	Aquatic Animal Health Subprogram: revision and expansion of the Australian aquatic animal disease identification field guide for publishing to cd rom (March 2003 - December 2003) *Associated Species*: finfish	Alistair Herfort Australian Government Department of Agriculture, Fisheries and Forestry
2003/644	Aquatic Animal Health Subprogram: NSW control centres manual (ccm) aquatic emergencies (April 2003 – November 2003) *Associated Species*: finfish, crustaceans; molluscs	Damian Ogburn NSW Fisheries
2003/645	Aquatic Animal Health Subprogram: aquatic animal disease emergencies – video and training kit (July 2003 – December 2004) *Associated Species*: finfish, crustaceans; molluscs	Wayne Tindall Big Time Media
2003/646	Aquatic Animal Health Subprogram: Australian aquatic animals diseases and pathogens database (July 2003 – December 2004) *Associated Species*: multiple species	Gus Boman F1 Solutions

2003/647	Aquatic Animal Health Subprogram: development of a database for Australian diagnostic laboratory expertise for diseases of aquatic organisms (July 2003 – October 2003) *Associated Species*: multiple species	Iain East Australian Government Department of Agriculture, Fisheries and Forestry
2003/648	Aquatic Animal Health Subprogram: the revision of the Tasmanian fish health plan and incorporation into the Tasmanian control centre manual (July 2003 – October 2003) *Associated Species*: finfish, crustaceans; molluscs	Mary Lou Conway Department of Primary Industries, Water and Environment, Tasmania
2003/649	Aquatic Animal Health Subprogram: industry's emergency preparedness and response to mass mortality of yellowtail kingfish *Seriola lalandi*: development of plans and protocols *Associated Species*: finfish	Martin Hernen South Australia Marine Finfish Association Inc (SAMFFA)
2003/650	Aquatic Animal Health Subprogram: update of the AQUAVETPLAN enterprise manual (semi-open systems) (July 2003 – October 2003) *Associated Species*: finfish, crustaceans; molluscs	Jo Sadler
	Federal Budget Initiative: *Training Program*	
2002/660	Aquatic Animal Health Subprogram: enhancement of emergency disease management through the education and training of the CCEAD participants on the CCEAD process (July 2002 – December 2003) *Associated species:* finfish, crustaceans and molluscs	Linda Walker Australian Government Department of Agriculture, Fisheries and Forestry
2002/661	Aquatic Animal Health Subprogram: enhancing the emergency disease response capability of NSW and Qld government agencies and industry bodies associated with oyster culture (October 2002 – October 2003) *Associated species:* molluscs	Matt Landos NSW Fisheries
2002/664	Aquatic Animal Health Subprogram: aquatic animal health emergency management training and incident simulation (October 2002 – February 2003) *Associated species:* finfish, crustaceans and molluscs	Melanie Ryan Seafood Training (SA)
2002/665	Aquatic Animal Health Subprogram: enhancement of the emergency disease management capability in Victoria - adapting the AQUAVETPLAN control centre management manual (October 2002 – June 2004) *Associated species:* abalone and trout	Anthony Forster Fisheries Victoria
2002/666	Aquatic Animal Health Subprogram: training course on exotic diseases of aquatic animals (July 2002 – June 2004) *Associated species:* finfish, crustaceans and molluscs	Mark Crane CSIRO Livestock Industries AAHL Fish Diseases Laboratory
2002/668	Aquatic Animal Health Subprogram: enhancing the emergency disease response capability of Department of Fisheries and industry bodies associated with non-*Pinctada maxima* oyster culture (December 2002 – September 2003) *Associated species:* molluscs	Brian Jones Department of Fisheries Government of Western Australia
2003/669	Aquatic Animal Health Subprogram: conduct of a multi-jurisdiction simulation exercise focused on health management in Australian aquaculture (September 2003 – March 2004) *Associated species:* finfish	Iain East Australian Government Department of Agriculture, Fisheries and Forestry
2003/670	Aquatic Animal Health Subprogram: emergency response by board microalgal identification for the finfish aquaculture industry (September 2003 – March 2004) *Associated species:* finfish	Judith-Anne Marshall University of Tasmania, School of Plant Science

2003/671	Aquatic Animal Health Subprogram: enhancing the emergency disease response capability of WA department of fisheries and industry bodies associated with freshwater crayfish culture (September 2003 – July 2004) *Associated species:* crustaceans	Fran Stephens Department of Fisheries Government of Western Australia

	Establishment of a joint industry/ government body for aquatic animal health management	
2002/600	Aquatic Animal Health Subprogram: facilitating the establishment of the Aquatic Animal Health Consultative Committee (AAHCC) as the primary industry-government interface for aquatic animal health (May 2002 – May 2004)	Eva-Maria Bernoth Australian Government Department of Agriculture, Fisheries and Forestry
2003/600	Aquatic Animal Health Subprogram: development of strategies for improved stock loss insurance and for development of a cost-sharing arrangement for emergency disease management in aquaculture (February 2003 - September 2003) *Associated Species*: multiple species	Iain East Australian Government Department of Agriculture, Fisheries and Forestry
	Aquatic Animal Health Subprogram R&D Projects	
1999/226	Aquatic Animal Health Subprogram: generation of diagnostic reagents for pilchard herpes virus (December 1999 – December 2002) - COMPLETED *Associated species:* Pilchards	Bryan Eaton CSIRO Livestock Industries
2001/093	Aquatic Animal Health Subprogram: strategic planning, project management and adoption (May 2001 – June 2004) *Associated species:* Finfish, crustaceans and molluscs	Eva-Maria Bernoth Australian Government Department of Agriculture, Fisheries and Forestry
2001/214	Aquatic Animal Health Subprogram: development of a disease zoning policy for marteiliosis to support sustainable production, health certification and trade in Sydney rock oyster (December 2001 – February 2005) *Associated species:* Sydney rock oysters	Robert Adlard Queensland Museum
2002/043	Aquatic Animal Health Subprogram: the production of nodavirus-free fish fry and the nodaviruses' natural distribution (December 2002 – June 2005) *Associated species:* Barramundi and other marine finfish species	Ian Anderson Department of Primary Industries, Queensland
2002/044	Aquatic Animal Health Subprogram: pilchard herpes virus infection in wild pilchards (January 2003 – December 2005) *Associated species:* Pilchards	Brian Jones Department of Fisheries Government of Western Australia
2003/216	Aquatic Animal Health Subprogram: detection and management of kingfish health issues in the yellowtail kingfish (*seriola ialandi*) industry - the foundation for a health programme for Australian finfish aquaculture (July 2003 – August 2004) *Associated species: Finfish*	Martin Hernen South Australian Marine Finfish Farmers Association Inc

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FRDC Aquatic Animal Health Subprogram Scientific Conference
“Emergency Disease Response Planning and Management”

Conference Programme

FRDC Aquatic Animal Health Subprogram Scientific Conference
"Emergency Disease Response Planning and Management"

Conference Programme

Wednesday 8 October 2003

8.00 am Registration

8.30 am Welcome and Introduction. Patrick Hone, FRDC

8.40 am FRDC AAH Subprogram: Summary. Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

9.00 am **Keynote Presentation:** Infectious Salmon Anaemia – An emerging global disease of salmon aquaculture. Ronald M. Stagg, Fisheries Research Services, Aberdeen, UK

10.00 am Coffee Break

PARASITES (Chair: Dr S Corbeil)

10.30 am Amoebic Gill Disease research progress. Barbara F. Nowak, University of Tasmania

11.00 am QX disease (*Marteilia sydneyi*) of commercial rock oysters (*Saccostrea glomerata*): research towards a defensible management strategy. Robert D. Adlard, Queensland Museum.

11.30 am Current status of perkinsosis among blacklip abalone in Australia, with a fresh look at transmission of the disease. Craig J Hayward and Robert JG Lester, University of Queensland

12 noon Parasite survey in Southern Bluefin Tuna in 2003 - preliminary results. Marty Deveney, Tom Bayly and Barbara Nowak, Aquafin CRC, PIRSA Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania

12.30 pm Lunch

FINFISH HEALTH (Chair: Dr K McColl)

1.15 pm Development of improved procedures for the identification of aquatic birnaviruses. Ken McColl, Kelly Steeper, Tam Chamberlain and Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

1.45 pm Development of cell culture, nested RT-PCR and immunodiagnostic tests for the detection of nodavirus in finfish. Nick Moody, QDPI, Oonoonba Veterinary Laboratory, Townsville, QLD

2.15 pm Characterisation of Tasmanian aquareovirus (TAV) in Atlantic salmon. Gemma Clark, Ken McColl and Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

2.45 pm	Molecular diagnostic tests to detect Epizootic Ulcerative Syndrome (*Aphanomyces invadans*) and Crayfish Plague (*Aphanomyces astaci*). Nicky Buller, Heather McLetchie, Brian Jones, Phil Nichols, Stan Fenwick, David Alderman, Dept Agriculture WA, Dept Fisheries WA, Murdoch University WA, Centre for Environment, Fisheries and Aquaculture Science, UK.
3.15 pm	Tea

CRUSTACEAN HEALTH (Chair: Dr P Walker)

3.30 pm	Development of diagnostic capability for priority aquatic diseases of national significance: spawner-isolated mortality virus. Leigh Owens and Brad R Cullen, James Cook University, Queensland
4.00 pm	Determination of the disease status of Western Australian commercial prawn stocks. Brian Jones, Department of Fisheries WA
4.30 pm	A monoclonal antibody specific for white spot virus of crustaceans. Mark StJ Crane, Joanne Slater, John White, Alexander D Hyatt and Sandra G Hengstberger, CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong
5.00 pm	AQUAVETPLAN disease strategy manual for white spot disease of prawns. Chris Baldock, Iain East and Dick Callinan, AusVet Animal Health Services Pty Ltd
5.30 pm	High throughput test development and health screening of Penaeus monodon used in the FRDC domestication project. Jeff A Cowley, Lee C Cadogan, KV Rajendran, Russell J McCulloch and Peter J Walker, CSIRO Livestock Industries, Brisbane
6.00 pm	Close

Thursday 9 October 2003

9.00 am	Keynote Presentation: Management of an exotic disease outbreak - Infectious Salmon Anaemia in Scotland. Ronald M Stagg, Fisheries Research Services, Aberdeen, UK
10.00 am	Aquafin CRC Health Program. Barbara Nowak, Tasmanian Aquaculture and Fisheries Institute
10.30 am	Coffee Break

AQUATIC ANIMAL DISEASE TRAINING AND TOOLS (Chair: Dr B Nowak)

11.00 am	Training video. Wayne Tindall, Big Time Media Pty Ltd, South Yarra
11.20 am	National database of Australian aquatic animal diseases and pathogens. Gus Boman and Steven Jones, F1 Solutions, Turner ACT
11.40 am	Revision and expansion of the Australian aquatic animal disease identification field guide. Alistair Herfort, Department of Agriculture, Fisheries and Forestry – Australia

12 noon	Aquatic animal diseases exotic to Australia: A training module for use in tertiary institutions. Shane Raidal, Frances Stephens, Barbara Nowak, Garry Cross, Kevin Ellard, Stan Fenwick, Phillip Nicholls, Murdoch University, WA
12.30 pm	Part 1: Development of an integrated approach to fish health emergencies within Tasmania. Mary Lou Conway and Kevin Ellard, Department of Primary Industry, Water and Environment, Tasmania Part 2: Development of a practically orientated Decontamination Manual for AQUAVETPLAN. Kevin Ellard, Livestock & Aquaculture Veterinary Consulting Services, Tasmania
1.00 pm	Lunch
1.30 pm	**FRDC AAH Subprogram Workshop (Chair: Dr P Hone)**
1.30 pm	Introduction: Progress report. Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong
1.50 pm	Group breakout and discussion
2.15 pm	Feedback
2.30 pm	**NAAHTWG Workshop (Chair: Dr B Jones)**
2.30 pm	Introduction: Progress report. Brian Jones, Dept Fisheries, WA
3.00 pm	Tea
3.30 pm	Group breakout and discussion
4.00 pm	Feedback
4.15 pm	ARC Networks. Shane Raidal, Murdoch University, WA
4.30 pm	Feedback
5.00 pm	Feedback on disease database/video, other training tools
5.30 pm	Close
7.00 pm	Conference Dinner, Four Points Sheraton, Geelong

Friday 10 October 2003

9.00 am	Keynote Presentation: Eradication of VHS and ISA – Scottish experience in an international context. Ronald M Stagg, Fisheries Research Services, Aberdeen, UK
10.00 am	AAHL's diagnostic capability. Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong
10.30 am	Coffee Break

PILCHARDS (Chair: Dr M Crane)

11.00 am — Pilchard herpesvirus infection in wild pilchards. Melanie Crockford1, Brian Jones1, Mark StJ Crane2, Kenneth McColl2, Richard Whittington3, 1Dept Fisheries WA, 2CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong, 3University of Sydney, NSW

11.30 am — Development and preliminary characterisation of pilchard (Sardinops sagax neopilchardus) cell lines derived from liver and heart tissues. Lynette M Williams, Mark StJ Crane & Nicholas Gudkovs, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

12 noon — Isolation of a new virus from wild-caught pilchards (Sardinops sagax neopilchardus) in Australia. Mark StJ Crane, John Young, Alexander D Hyatt, and Lynette M Williams, CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong

12.30 pm — Lunch

BACTERIOLOGY (Chair: Mr N Gudkovs)

1.30 pm — Vibrios of aquatic animals: Towards a national standard diagnostic technology. Jeremy Carson[1], Teresa Wilson[1], Nicholas Gudkovs[2], Melissa Higgins[1] and Trevor Bryant[3], [1]Fish Health Unit, Tasmanian Aquaculture & Fisheries Institute, University of Tasmania, [2]CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong and [3]Department of Medical Statistics and Computing, University of Southampton, UK

2.00 pm — Development of diagnostic procedures for the detection and identification of *Piscirickettsia salmonis*. Serge Corbeil and Mark StJ Crane, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

FINFISH IMMUNOLOGY (Chair: Dr B Nowak)

2.30 pm — Finfish immunology – a review. Ken McColl, CSIRO Livestock Industries, AAHL Fish Diseases Laboratory, Geelong

3.00 pm — Vaccination versus non-specific immunostimulation in fish - Lessons learned from the teleost antibody response. Richard N. Morrison[1], A. Bruce Lyons[2], Barbara F. Nowak[1], Mathew Cook[3], John D. Hayball[2], [1]School of Aquaculture and CRC for Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Launceston, [2]Hanson Institute, IMVS, Adelaide, [3]CSIRO Marine Research, Hobart.

3.30 pm — Tea

4.00 pm — Close

Conference Participants

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Keynote Presentations: Summaries

Infectious Salmon Anaemia – An emerging global disease of salmon aquaculture

Ronald M Stagg **Click here for PowerPoint presentation**

Fisheries Research Services, PO Box 101, Victoria Road, Aberdeen UK

Introduction

Infectious salmon anemia (ISA) is a contagious and significant disease of farmed Atlantic salmon which first appeared in Norway in 1984 (Thorud & Djupvik, 1988). ISA has since emerged in all of the major Atlantic salmon producing countries in the world (Table 1) although there are still questions about the aetiology of the disease in Chile where virus has been isolated only in Coho salmon with clinical signs atypical of ISA. In Ireland the virus has recently been isolated from a marine rainbow trout farm but no signs of clinical disease were evident.

Table 1 First reports of ISA in Atlantic salmon producing countries

Country	Date	Species	Comments
Norway	1984	Atlantic salmon	Demonstrated to be infectious in 1987
Canada	1996	Atlantic salmon	Initially identified as HKS*
Scotland	1998	Atlantic salmon	
Faeroe Islands	2000	Atlantic salmon	
Chile	2001	Coho salmon	Complex aetiology
United States of America	2001	Atlantic salmon	
Ireland	2002	Rainbow trout	Virus isolation

* HKS Haemorrhagic Kidney Syndrome

Aetiology

The causative agent (ISAV) is an enveloped, single-stranded, negative-sense ribonucleic acid (RNA) virus approximately 45-100nm in diameter. The entire genome of ISAV and the identity of many of the gene products have now been discovered. The genome is typical of the Orthomyxoviridae family of viruses being organised into 8 segments that appear to encode polymerase, nucleoprotein, haemagglutinin, esterase, matrix and non-structural proteins. However the virus is sufficiently distinct to merit a separate genus (Krossoy et al. 1999) and the international committee on the taxonomy of viruses has suggested the name Isavirus. A most notable feature of the haemagglutinin gene is the presence of a highly polymorphic region and the suggestion that variants might arise as a consequence of deletion mutations from an ancestral wild type sequence (Cunningham and Snow 2003). This has considerable epidemiological and management implications in terms of the effectiveness of control regimes relying on vaccination and the emergence of new, possibly more pathogenic strains in aquaculture.

Epizootiology

The temperature sensitivity of ISAV (Falk et al., 1977) will restrict the host range to poikilothermic species and observations indicate that ISA is primarily a disease of cultured marine Atlantic salmon (Salmo salar). Experiments indicate that salmon can be asymptomatic carriers since survivors of experimental challenge can cause mortality in naïve fish several months after the original challenge (Totland et al., 1996) and can remain infectious for up to 18 months (Griffiths and Melville, 2000). Most salmonids including brown trout and sea trout (Salmo trutta), rainbow trout (Oncorhynchus mykiss) and Arctic char (Salvelinus alpinus) have the potential to act as carriers and to transmit ISAV (Nylund & Jakobsen, 1995; Nylund et al., 1995, Snow et al. 2001a). In Ireland a non-clinical outbreak (virus isolation only) in a rainbow trout farm has been

reported. While there is good evidence for salmonid fish as potential carriers of ISAV there is little evidence for, or against, non-salmonid fish species acting as potential vectors. Hjeltnes (quoted by Nylund & Jakobsen, 1995) tested the susceptibility of goldsinny wrasse (*Ctenolabrus rupestris*) and turbot (*Scopthalmus maximus*) by experimental challenge with ISAV but failed to demonstrate evidence of infection or carrier status. There are unconfirmed reports (Nylund personal communication cited in Krossøy *et al.*, 1999; Mullins *et al.*, 1999) that herring (*Clupea harengus*) may act as carriers of the viruses. A comprehensive survey of over 2000 fish failed to detect ISAV in those sampled from the natural environment off the Maine coast (MacLean *et al*. 2003) suggesting a marine reservoir in non-salmonid species is unlikely.

Epidemics of ISA have occurred in all of the major salmon producing regions and primarily occur in Atlantic salmon cultured in seawater. Although there is an increasing body of evidence that wild fish may be infected, possibly with strains of low pathogenicity, transmission from farm to farm primarily occurs through transfer of infected fish or by clinical material. Poor hygiene therefore facilitates transmission particularly via equipment such as harvesting and grading equipment, wellboats, and mortalities for disposal. Proximity to infected farms and to discharges from processing or harvesting effluent increases the probability of a farm becoming infected (Jarp & Karlsen, 1997). Hubs and re-cycling of infective material from processing plant or harvesting stations to farms via wellboats are particularly important routes of contagion. Although horizontal transmission is the primary means of spread there has been some debate about the role of vertical transmission especially in the context of the international trade in salmonid eggs. However vertical transmission has not been demonstrated experimentally (e.g. Melville & Griffiths, 1999) and the OIE have determined that vertical transmission is not a risk for international trade providing there is adequate egg disinfection.

Control

Since ISA is a notifiable disease in the major salmon producing countries measures to minimise spread are managed by control of trade in live and dead fish, movement of transporters (ships and trucks), personnel and other equipment entering and leaving infected sites. Particular attention is paid to harvesting and processing operations and the disinfection of all effluents from these operations including the vehicles or vessels used in transport. Disinfection and containment are particularly effective in reducing the risk of transmission and ISAV has been shown to be sensitive to a range of convenient disinfectants (Smail et al., 2001). Effective control is also associated with strict zoning of the coastal area around farms with outbreaks and extension of the biosecurity controls to un-infected farms in these zones. The basis for establishing zones can vary between countries, for example, contiguous hydrographic zones using a tidal excursion model are used in Scotland (Stagg, 2003) and 5 km zones in Norway on the basis of the risk factor study carried out by Jarp & Karlsen (1997).

The acute nature of the disease, the inability to control mortality and the risk of spread of disease from infected stocks has meant that, to greater or lesser degrees, eradication measures are also used to manage the disease. This requires the removal of infected pens or entire farms and the temporary closure and fallowing of the farm once all stocks have been harvested or destroyed. In Norway the peak incidence (80 cases per annum) of ISA in Norway occurred in 1990 (Thorud and Håstein, 2003). At this time, it was realised that the disease could not be eliminated unless the farm was emptied of fish and fallowed and in 1996 it was determined that a pen had to be emptied of fish if the mortality rate exceeded 0.05% per day. The speed of de-population of affected farms would also appear to be critical and varies from country to country. In Scotland where de-population of whole farms was achieved between 10 and 50 days from diagnosis the disease appears to have been eliminated whereas in

countries like the Faeroe Islands and New Brunswick, Canada with more prolonged depopulation strategies outbreaks of ISA are persistent. It is notable that the revised Norwegian contingency plan there are proposals to limit the length of time fish may remain on infected farms to 80 days in an attempt to further reduce the number of outbreaks occurring.

Vaccination is an option for future control since it is clear that fish mount a strong immune response when challenged with ISAV and several workers have demonstrated a protective effect in vaccination experiments (Brown et al. 2000; Jones et al. 1999). However, the occurrence of different ISA strains (Cook et al, 2003), the possibility that new haemagglutinin sequences arise by deletion mutations (Cunningham & Snow, 2003) and the parallels with influenza (Kibenge et al., 2003) mean that much more effort will be required before effective field vaccines are available. It is also important that the criteria for assessing vaccine efficacy should eliminate virus replication and shedding as well as reducing the impact of clinical disease.

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Management of an exotic disease outbreak - Infectious Salmon Anaemia in Scotland

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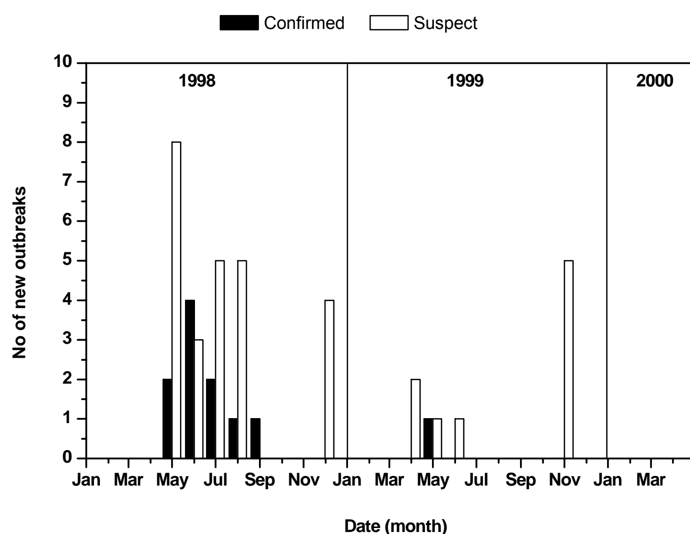
Introduction

In 1998, an epidemic of Infectious Salmon Anaemia (ISA) occurred in marine salmon farms in Scotland. A total of 11 farms were confirmed and a further 25 farms were suspected of containing infected fish. The last suspected case was in November 1999 and the epidemic was officially over on 16 January 2002 when the last farm that shared coastal waters with a suspect case had completed the requisite fallow period (Fig. 1).

Fig. 1. Incidence of ISA cases (farms) in Scotland January 1998 - May 2000



Legislative framework

In the European Union, ISA is a List I disease and a strong legislative framework had already been established in 1993 to enable its control and eradication (Directive 93/53/EC). This required that the management of ISA in Scotland be based upon:

- the disease being notifiable;
- regulations to prevent the import of the disease from infected zones;
- surveillance to demonstrate continued freedom or to delimit an outbreak;
- measures to deal with outbreaks including biosecurity measure, eradication and disposal of dead or culled stock; and
- mechanisms to notify the international community of the presence of the disease and to introduce protective measures as regards trade.

Notification and surveillance

ISA has been notifiable in the United Kingdom since 1991 and is supported by an official inspection of all fish farms at least once a year by the Official Service. There are powers of entry and powers to take any samples required to diagnose suspected disease. Although surveillance provides confirmation of absence of an infectious agent, it is noticeable that the outbreak of ISA was first identified through notification of the disease (Rodger et al. 1998). It follows from this that an important aspect of early

warning of emergence of new diseases is that notification should be mandatory and that there should be good relations between the official service and industry to promote reporting.

Measures on suspicion or confirmation of cases

The existing United Kingdom regulations allowed a range of measures to be put in place immediately ISA was notified with the prime objective of containing and eradicating the disease. On suspect farms and farms in the same coastal zone as confirmed and suspect farms measures were put in place to:

- control the movements of live and dead fish; people and materials liable to transmit the disease;
- establish surveillance and control zones;
- prevent restocking of farms until they had been harvested, fallowed and disinfected to minimise the risk of re-emergence; and
- conduct an epizootic investigation to confirm the presence of the diseases and to establish the source and spread of the disease.

In addition complete and immediate de-population of all fish from confirmed farms was required. Such fish could be harvested for sale, or if unsuitable for harvest, or if the fish showed clinical signs of disease they were destroyed.

Epizootic investigation

A requirement of the legislation is that an epizootic investigation is carried out to determine the source of the infection responsible for the outbreak and the spread to other farms. Detailed investigations were made of the sources of fish (smolt suppliers) at the primary outbreak and the possible connections to other sources of infection (primarily Norway at the time of the outbreak). These investigations identified two likely sources of infection – either importation accidentally in a well boat that had been operating previously in Norway or emergence from a wild source due to conditions favouring transmission and emergence on the primary infected farm.

Gaps in knowledge

Despite this legislative framework and the existence of contingency plans, further development of the decision making process and underpinning science was required to manage the outbreak.

Although the legislation required the establishment of zones the basis for this was not described. This required a rapid review of current practice, the establishment of a multidisciplinary tem of coastal oceanographers and epidemiologists and the development of the tidal excursion model as a practical tool for zone delimitation (Stagg, 1993). At that time there was some information on the environmental survival of ISAV but our understanding of the passive dispersal of ISA from fish farms and the ability of contaminated waters to infect naïve populations of fish was poor. In addition hydrographic data on individual sites was not easily available and nor was there sufficient time to survey sites or model dispersal patterns around individual farms. These constraints and a complex coastline meant that a simple and robust method was needed to identify adjacent farms at risk using existing site locations and available generic hydrographic information. The hydrographic information used for such purposes was the maximum spring tide current obtained from a tidal atlas. This data was translated into tidal excursions, in kilometres, around each fish farm. Most salmon farms are located in relatively sheltered locations and, therefore, the maximum tidal amplitudes were restricted to 0.51 ms-1 (1 knot) in mainland Scotland and 0.255 ms-1

(0.5 knot) in Shetland giving tidal excursions of 7.3 and 3.6 km respectively. An indication of the validity of this approach can be obtained from the following:

- The output of the model compares favourably with the results of a Norwegian case control study (Jarp and Karlsen, 1997) on the epidemiology of ISA. This study established an inflection in regressions of probability of infection against distance at 5km from an infected farm.
- Analysis of all RT-PCR data on ISAV infection in salmon farms following the Scottish outbreak (Stagg *et al*., 2001) also showed that the majority of RT-PCR signals came from within the surveillance zones of the tidal excursion model established to contain the disease.
- The successful eradication of ISA is an indication of the validity of the model. However, since smaller sized zones were not tested it is possible that this was too precautionary and the disease may have been eradicated with smaller zones.

The conclusion from this evidence is that in the case of ISA the use of the tidal excursion model provided a useful management tool that compares favourably with the epidemiological information and resulted in control of the disease.

Although the principles of fallowing, to prevent re-infection of cage farms, are widely accepted by regulators and industry alike the scientific evidence for such benefits are poorly established (Stewart, 1998). In particular determination of the length of fallow period needed to ensure the probability of re-emergence is negligible. It may be assumed that there will be a relationship between risk of re-emergence and the level of infection on a site and the time since that infection was detected when the site is under continued surveillance. Using these principles a risk assessment scheme was developed to determine the minimum fallow period to be applied to sites affected by the Scottish outbreak (Stagg, 1993).

Disinfection protocols and biosecurity arrangements are one of the principle controls applied on infected sites. Fortunately, much work had been done previously (Torgersen & Håstein, 1995) on the sensitivity of pathogens to various disinfectants including ISA. However, the most practical disinfectant for on farm use was hypochlorite. This caused some considerable difficulty in terms of its caustic properties giving rise to health and safety concerns and effects on equipment such as diving suits and nets and also environmental concerns due to its potential to react with organic material to form persistent organochlorines. Research was put in place to develop testing methods and evaluate the efficacy of a wide range of disinfectants such as chloramine-T, chlorine dioxide, iodophors and peracetic acid mixtures against ISAV (Smail et al. 2002).

Unexpected issues

In the management of an outbreak a huge range of public interest issues arise. Political, public and media interest can be very intense and place strong pressures on scientists and policy makers dealing with an outbreak. Apart from the obvious communication and management issues that arise new scientific questions are asked for which there is little scientific information on which to base policy or for which there are high levels of uncertainty. In the case of the ISA crisis in Scotland three issues of particular had to be dealt with.

- Supermarkets, presumably in response to public and media speculation, raised question about the human health implications of consuming fish infected with ISA. Regulations allowed fish from infected farms to be sold for human consumption as long as the fish did not show clinical signs of the disease and checks by veterinarians and fish health inspectors were established in processing plants to

safeguard these conditions. Nevertheless an independent food safety committee instigated a review of the human health implications and some supermarkets boycotted the purchase of fish from ISA suspect or confirmed farms.
- It was discovered that oysters were being moved from a zone affected by ISA for relaying in an unaffected zone. Interim measures requiring depuration prior to relaying were instigated to reduce the risk of transmission. Experiments were conducted which did not demonstrate a significant risk and the measures rescinded however there is still considerable work to do in this area.
- Wild fisheries interests were hugely concerned at the risk to stocks for ISA infected farms and at the same time fish farmers were concerned at the potential for transmission from wild to farmed fish and the possibility that ISA might emerge at any time from a wild source. This stimulated a sizeable scientific program to evaluate the prevalence ad extent of ISA in wild fish (Raynard *et al*., 2001) which showed the presence of the virus by RT-PCR in wild salmonid populations.

Stakeholder involvement

A principle need in managing an outbreak like ISA is the communication needed with stakeholders. Starting in an ad hoc way industry government liaison rapidly evolved with the establishment of Joint Industry/Government Working Group on ISA in October 1998. At one stage this group was meeting every two weeks. Its terms of reference were to review current industry practices and to identify the risk factors that might increase the likelihood of transmission of ISA and agree suitable risk reduction measures. The group also served, as an extremely useful communication forum, where government could explain the progress of the epidemic and policy developments and industry could articulate concerns and impacts. The ISA JWG produced a final report in January 2000 (Anon, 2000) which was translated into a code of practice for the industry. Another output of this group was changes to the regulatory regime and modifications to the EU directive to enable a more flexible response to the disease and to allow the use of vaccination in the context of the eradication regime.

Conclusion

Following the outbreak the Royal Society of Edinburgh which is Scotland's national academy of science established a working party to investigate the scientific issues surrounding the disease and its control. The EU also investigated whether the UK had properly implemented the legislation governing the control of the disease. The conclusions from both of these reports (RSE, 2002; EC, 2000) are of general applicability and indicate that:

- The UK had fully implemented the required legislation;
- Clinical outbreaks of ISA in Scotland have been eliminated; and
- The eradication policy through de-population should continue.

There are still some outstanding questions that need resolution and the most important of these relates to the significance of the observations of ISA virus in wild fish and the implications of this for the status of the of the disease and the risk to trade.

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Eradication of VHS and ISA – Scottish experience in an international context

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Introduction

In the context of diseases of aquatic organisms eradication usually refers to the elimination of infection or disease from a defined geographical area (zone or country) such that the incidence of the disease or infection is reduced to zero. Eradication usually implies the removal of infected stocks and the introduction of measures to prevent the re-establishment of transmission. The adoption of an eradication strategy implies that the pathogen in question is exotic (established by passive, active and/or targeted surveillance) and that the removal of infected stocks following an outbreak is part of a process to re-establish disease free zones. This is important in terms of the trade rules governing the movements of aquatic animals in the context of the SPS agreement and the European Union (EU) common market. In Scotland research undertaken following the emergence of viral haemorrhagic septicaemia (VHS) in farmed turbot in 1994 and infectious salmon anaemia (ISA) in farmed salmon in 1998 have revealed the existence of wild reservoirs of the agents of these diseases. These wild reservoirs clearly pose some risk for disease re-emergence but this is much lower than the risk posed by allowing trade in live fish from zones or countries where disease outbreaks occur on farms. This paper provides a summary of the work that took place in Scotland after the occurrence of these two disease outbreaks and discusses the implications for the international framework currently established to regulate trade in live fish.

VHS – disease emergence from a marine reservoir

VHS is one of the most important diseases of farmed rainbow trout (*Oncorhynchus mykiss*) in Europe causing losses of the order of 70 mECU per year (Lorenzen & Olesen, 1997). VHS is caused by the Egtved virus (VHSV) and is a member of the rhabdovirus family. In the European Union VHS is classified as a List II disease which means that zones may be established which are free of the pathogen. To prevent introduction of the pathogen restrictions on trade may be applied in such zones to ensure fish entering are of an equal or greater health status and a program of inspection and surveillance must be carried out to demonstrate continued freedom from the pathogen. Some Member States have sought to extend the size of these approved zones by establishing a stamping out programme. Such programmes require the systematic clearing of infected stocks, comprehensive disinfection and fallowing, re-socking with certified virus free fish, imposition of strict biosecurity and enhanced surveillance to detect re-emergence at the earliest opportunity. In Denmark a very successful programme has been established using this approach which has seen the number of infected farms fall from 400 in 1965 to about 26 in 1997 (Olesen, 1998).

Initially, VHS was considered a disease affecting freshwater salmonids and even outbreaks occurring in marine rainbow trout farms in the 1980's were considered to originate in freshwater (Jorgensen, 1992). However, in 1989 VHSV was isolated in chinook (Hooper, 1989) and coho salmon (Brunson *et al.*, 1989) in the United States. This finding precipitated a series of studies that have established a marine reservoir of VHSV in the North Sea (e.g. Smail 1995) and the west-coast of the USA (Meyers and Winton, 1995). It also led to a re-appraisal of the marine origins of the outbreak of VHS in turbot in Germany (Schlotdfelt, 1991). In 1994 an outbreak of VHS occurred in farmed turbot in Scotland. The outbreak occurred in the Great Britain approved zone where VHS had never been observed in farmed stock despite an annual programme of

inspection and testing. The outbreak resulted in relatively low mortality and never exceeded 6% of the total stock but the epizootic investigation indicated the most likely source of the infection was from a marine reservoir possibly through the feeding of marine fish.

One of the consequences of this conclusion was a detailed European Union program to clarify the threat of VHS from marine sources. A widespread survey of the European continental shelf provided detailed information on the prevalence of VHSV in marine stocks and also material to allow comparative pathogenicity studies and genotyping. The results show the existence of four genotypes based on the sequence analysis of the nucleoprotein gene (Snow *et al.* 1999) and that these genotypes have a range of pathogenicity that varies between the species examined (cod, halibut, trout, and salmon). Such findings have implications for the management of zoning and this is complicated by the diversification of aquaculture and the moves to develop cod, haddock and marine species farming alongside conventional salmonid aquaculture. These findings point to the need to consider the different VHSV strains separately and an understanding of the pathogenicity of a strain in terms of the management of outbreaks and the rules governing trade between zones.

ISA – an exotic disease?

Infectious salmon anaemia first emerged in Norway in 1984 and incidence of cases rose rapidly peaking in 1990. Based on a combination of experience and epidemiological studies a series of measures to improve biosecurity and to remove the foci of infection were put in place (Thorud & Håstein, 2003). These measures were introduced progressively in a time line from 1988 to the present and included: making ISA notifiable; requiring health certification and hygienic measures in hatcheries; banning the use of seawater in hatcheries; preventing movements of fish already in the sea; applying hygienic measures and regulations on transport; disinfecting waste water from slaughterhouses and processing plants; introducing combat zones; slaughtering pens where mortality exceeded 0.05% per day and finally introducing a time limit by which farms have to be de-populated. These measures gradually reduced the incidence of the disease and their success illustrates the importance of biosecurity and measures to prevent the farm to farm spread of the disease. In Scotland where a regulatory regime was already in place to combat the disease the initial outbreak was contained. Genetic analyses of the virus isolated from outbreaks in key geographical locations and contact tracing information showed the outbreak to originate at a primary location and to have been spread to the other locations by anthropogenic means. A comparison of the approach taken in Norway, Canada, Scotland, the Faeroe Islands and the United States shows that the most successful control programs, in terms of eliminating or reducing the incidence of disease, have been associated with the application of strict biosecurity and rapid de-population strategies.

Despite the clear evidence for spread from farm to farm in the Scottish outbreak reports that sea trout and herring were potential carriers of ISA stimulated a debate about the possible origins of ISA in Scotland. Examination of the evidence indicated that either importation from Norway or a wild origin might have been the source of the primary outbreak (Stagg *et al.* 2001). The epizootic investigation revealed the presence of ISAV (mainly by RT-PCR) in wild salmonids over a considerable geographic range, in both seawater and freshwater and remote from outbreaks. Prevalence was low but appeared to decline in parallel with the elimination of outbreaks on farms but whether there was a causal link (such as spillover from farm to wild or elevated prevalence in the wild driving the epidemic) could not be determined from the data. The finding of a putative ancestral variant of the haemagglutinin gene in wild migrating salmon (Cunningham *et al.* 2002) raises the possibility that farm variants, possibly with higher pathogenicity and potential for farm-to-farm spread, have arisen by deletion mutations

from a wild type gene. Such a hypothesis has implications for control strategies since it provides an indication of the relative significance of observations of the virus in wild fish and the need to control farm-to-farm or zone to zone spread of the virus.

Implications for international trade and disease management

A central tenet of international control strategies both in the EU and the OIE is that trade should not jeopardise the health status of receiving zone or country. In order to protect the notions of free trade much debate and effort has gone into developing criteria for determining whether diseases should be notifiable and the surveillance requirements to justify establishing freedom. The OIE for example has recently published revised guidelines based upon determination of consequence, spread and the ability to diagnose the presence of the agent. The work carried out following the outbreaks of VHS and ISA in Scotland indicates that there is also a new dimension to consider and that is the range of virus diversity and host susceptibility. The existence of wild reservoirs of VHSV and ISAV that do not necessarily predict the occurrence of disease also needs to be considered. Clearly farms located in these zones are at much greater risk from trade with zones were clinical outbreaks occur than from the local wild reservoir. There is a need to re-evaluate the criteria for freedom of disease to take into account the diversity of these pathogens and to properly address the trade risks in such circumstances.

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Conference Abstracts

QX disease (*Marteilia sydneyi*) of commercial rock oysters (*Saccostrea glomerata*): research towards a defensible management strategy

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Funding sources: FRDC Project: 2001/630, FRRF

Introduction

The establishment of scientifically defensible zoning and translocation policies is critical to the future development of Australian aquaculture and the maintenance of international market access by supporting export health certification, particularly for diseases classified as either reportable or notifiable.

Australia's edible oyster industry has for decades been subjected to periodic epizootics of disease induced by a range of protozoan parasites that produce mortality and morbidity of commercial oyster stocks. The most significant of these agents is the notifiable pathogen *Marteilia sydneyi*, the aetiological agent of QX disease, which affects the Sydney Rock Oyster, *Saccostrea glomerata*.

Growth of rock oysters often involves the translocation of live oysters between various rivers on the NSW and Queensland coasts at various stages of their life cycles. This regular translocation presents a potential threat to the industry because previous data on QX disease indicates that it had a limited geographic distribution.

It was recognised that management of this pathogen would be predicated on zoning policy with areas of disease freedom established from which trade can continue unaffected by the presence of disease elsewhere. As such, 2 projects funded by the FRDC, FRRF and through the Federal Government's Building a National Approach to Animal and Plant Health initiative are being undertaken to establish baseline data for QX disease. The major objective of these projects is to field test the zoning policy framework in a practical context in Australia. Subsidiary objectives include validation of molecular diagnostic procedures to provide best cost/benefit for ongoing monitoring and surveillance, while maintaining appropriate standards for detection specificity and sensitivity.

Materials and Methods

There is a major field-sampling component to this project, with oyster samples collected from a minimum of 22 estuaries from commercial growing areas in NSW (2001-2003) and Queensland (2002-2003). Initially, 250 oysters per year for 3 years were sampled at random from within the commercial lease areas of each estuary. Subsequently, sample sizes were reduced to 150 oysters per estuary once the sensitivity of available diagnostic tests for *M. sydneyi* was assessed. At all times, sampling and diagnostic procedures were aimed at delivering a minimum detectable level for *M. sydneyi* at 2% (internationally recommended level of detection for surveillance).

Diagnosis of the presence of *M. sydneyi* was first undertaken using stained tissue imprints (cytology) of the digestive gland of each oyster, given that this technique is rapid and relatively inexpensive. This, and other diagnostic techniques, require sub-sampling of target tissue, thus an investigation of homogeneity of infection throughout the digestive gland was required.

A standard diagnostic procedure (ANZSDP) was developed based on PCR amplification of a short (ca. 200bp) DNA motif within the ITS1 region of rDNA of *M. sydneyi*, and validated against field samples (Adlard & Worthington Wilmer, 2003). This test is now being applied to routine diagnostic assessment of samples collected in 2003 to determine the distribution of the disease agent.

Results and Discussion

Examination of serial sections cut through the length of the digestive glands of 50 oysters collected during this project and previously found to be infected (by cytology), demonstrated that in all cases, infections were homogenous throughout the target tissue with variation between individual hosts being related to the percentage of digestive tubules infected by the pathogen. It is considered that this study validates the process of sub-sampling of digestive gland tissue for diagnostic purposes. However homogeneity throughout the digestive gland of oysters occurs only when disease development has progressed either to pre-sporulation stages (proliferation via nurse cells in the epithelium of digestive gland tubules) or to sporulation.

In 2002, using cytological examination of tissue imprints, samples taken from 4 estuaries (Southern Moreton Bay, Richmond, Clarence, Georges) were diagnosed as positive for *Marteilia sydneyi*.

A total of 1,837 of these oysters were then screened for the presence of *Marteilia sydneyi* using PCR, while validating the ANZSDP. Of these, 142 unexpected positives for *M. sydneyi* were found in oysters scored as negative by cytological examination. Of these, 74 were identified in oysters sampled from estuaries with no prior record of *M. sydneyi*. These represent oysters from NSW: Hastings R, Wallis Lake, Port Stephens, Bateman's Bay, Tuross Heads, Narooma, Merimbula; and QLD: Northern Moreton Bay and Central Moreton Bay. Note that Crookhaven/Shoalhaven and Hawkesbury R samples were the only estuaries tested by PCR techniques which returned negative PCR results for 100 oysters screened per estuary.

Examination of archived tissues from these estuaries using histological and DNA probe *in situ* hybridisation techniques confirmed the presence of the pathogen in an atypical, (with respect to seasonality) early stage of development. No proliferation through to sporulation (development of spores) was identified in the samples examined to date. As a result diagnosis for samples collected in 2003 is currently being undertaken using PCR.

It has become apparent that the pathogen *M. sydneyi* is more widespread than was previously thought. Certainly, the sensitivity of diagnosis provided by DNA tests has considerably enhanced our ability to detect the agent. There are now at least two obvious scenarios: the agent has recently been introduced into estuaries from which it has been previously unreported and there is an immediate potential for epizootic outbreaks; or it has been introduced (perhaps repeatedly) and does not progress to disease outbreak perhaps due to effective immune defence. Regardless, of these scenarios, domestic management of this pathogen by exclusion from areas deemed disease free will require reassessment.

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AQUAVETPLAN disease strategy manual for white spot disease of prawns

Chris Baldock, Iain East and Dick Callinan ***Click here for PowerPoint presentation***

AusVet Animal Health Services Pty Ltd, Dept Agriculture, Fisheries & Forestry – Australia, NSW Fisheries

FRDC Project: 2002/647

Introduction

White spot disease (WSD) is caused by white spot virus (WSV), also known as white spot syndrome virus (OIE 2003a). It is a highly contagious disease of penaeid prawns characterised by the rapid onset of high levels of mortality in farmed prawn populations. Outbreaks are preceded by cessation of shrimp feeding followed within a few days by the appearance of moribund shrimp at the edge of ponds and then mass mortality. Since first being reported in the early 1990s, WSD has exhibited pandemic behaviour in Asia and the Americas. It is arguably the most serious disease affecting prawn cultivation and is one of the crustacean diseases listed by the World Organisation for Animal Health (OIE 2003b). WSV also infects a wide range of other crustaceans, often without causing any clinical signs. Although WSV was detected in and around two aquaculture facilities in Darwin in November 2000, it did not result in a disease outbreak and was promptly eliminated. The source of infection was attributed to the use of imported green prawns as feed for cultivated crustaceans. Subsequently, an Australia-wide survey has demonstrated freedom (Animal Health Australia 2002). Because of its potential devastating impact on Australia's farmed prawn industry, WSD is a high priority for Australia's emergency disease preparedness planning and a disease strategy manual is being prepared as part of AQUAVETPLAN.

Materials and Methods

The document follows the standard format for disease strategy manuals with three main sections: Nature of the disease; Principles of control and eradication; Preferred control strategies in Australia. The first section is a review of what is known about the disease and its causative agent, the second explores the options for control and eradication while the third offers the preferred option for Australia. The authors prepared a first draft based on a literature review and consultation with internationally recognised experts. This draft was peer reviewed by two international experts on WSD in May 2003 and comments incorporated. The next draft was circulated for comment in June to 32 technical experts and policy makers throughout Australia plus the Australian Prawn Farmers' Association and Queensland Crayfish Farmers' Association. These comments were incorporated into a final draft completed in September.

Results and Discussion

There are three possible response options for WSD in Australia:

- Option 1 - *eradication* with the view to having Australia return to being free from WSV;
- Option 2 - *containment, control and zoning* of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas; and
- Option 3 - *control and mitigation of disease* where it is accepted that the virus will remain endemic in Australia.

The choice of response option will be decided by the Director of Fisheries and/or the CVO of the State/Territory in which the outbreak occurs, following initial epidemiological investigations.

All of these response options involve the use of a combination of strategies, which may include:

- *quarantine and movement controls* on crustaceans, their products and things in declared areas to prevent spread of infection;
- *destruction* of all clinically diseased or dead prawns as soon as possible, to prevent further virus shedding;
- *decontamination* of facilities, products and things to eliminate the virus from infected premises and to prevent spread of infection;
- *surveillance* to determine the source and extent of infection and to provide proof of freedom from infection;
- *zoning* to define and maintain zones of different disease status; and
- *hygiene and biosecurity measures* aimed at mitigating the on-farm effects of WSD.

Eradication may not be feasible if epidemiological investigations determine that WSV infection is widespread across most or all Australian prawn producing zones, has no controllable point source or is otherwise unable to be contained. Similarly, the feasibility of zoning and containment will depend on farm management practices, the extent to which infection has already spread and the location, distribution and migratory behaviour of infected species. If infection is widespread, and there is evidence of widespread infection in available wild broodstock populations control and mitigation of the disease is likely to be the most appropriate option.

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Australian Aquatic Animal Diseases and Pathogens National Database

Gus Boman and Steven Jones ***Click here for PowerPoint presentation***

F1 Solutions, Level 3, 97 Northbourne Ave, Turner ACT 2612

FRDC Project: 2003/646

The creation of a national database of Australian aquatic animal diseases and pathogens aims to deliver on one of the key recommendations of the National Taskforce on Imported Fish and Fish Products. F1 Solutions, an information technology company specialising in database development, has commenced work to construct this database.

F1 Solutions has completed gathering the requirements from key users and stakeholders. These requirements have been categorised into technical, management (business rules/policy) and operational (e.g. data entry and database maintenance) requirements. Critical success factors based on the information provided have been identified.

Conference attendees are encouraged to attend this session to consider the issues, the options and provide input into shaping what might become an invaluable tool on a national scale.

This presentation is designed to:

1. Inform the audience of the **key requirements** specified by the users and
2. Engage the audience to **obtain** their **feedback** on important and critical aspects of the database system, such as,
   a. Database content.
      - Should the database be a repository or a facilitator of access to externally held information sources on Australian aquatic animal diseases and pathogens or a hybrid?
      - Would a staged approach be appropriate, whereby the database is initially developed as a more modest “facilitator”, and then is expanded to a fuller repository?

   b. Database maintenance.
      - Should all information submitted be automatically accepted, or only that information that includes “evaluation information” (eg. the name of submitter)?
      - Alternatively should all the information submitted be validated, and if so how and by whom? Could the responsibility for aspects of this function be distributed amongst a number of experts and interested persons?

   c. Database value.
      - How can the database quickly become a useful tool?
      - How will an initial “critical mass” of quality information be entered into the database?
      - What will encourage people to continue to input information?

   d. Competitiveness.
      - What should be the rules governing viewing of sensitive information by overseas organisations?
      - How will the information contained in the database impact trade negotiations?

Molecular Diagnostic Tests to Detect Epizootic Ulcerative Syndrome (*Aphanomyces invadans*) and Crayfish Plague (*Aphanomyces astaci*)

Nicky Buller[1], Heather McLetchie[1], Brian Jones[2], Phil Nichols[3], Stan Fenwick[3], David Alderman[4] ***Click here for PowerPoint presentation***

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FRDC Project: 2001/621

Introduction

Aphanomyces species belong to the water molds (Oomycetes) from the family *Saprolegniaceae* and comprise several pathogens of fish, crustaceans and plants. *Aphanomyces invadans* appears to be the primary agent (often following an initial insult such as acid sulphate run-off) of Australian red spot disease: synonymous with both Epizootic Ulcerative Syndrome (EUS) in Southeast Asia and mycotic granulomatosis from Japan. EUS is found throughout South East Asia, India, Pakistan and has been implicated in mortalities of menhaden in the USA, (Blazer *et al.* 2000). In Australia it is one of the major diseases of silver perch, and affects ornamental fish, barramundi and other freshwater and estuarine fish. The disease causes high mortality, and up to 80% of the fish may be affected.

Aphanomyces astaci is the causative agent of Crayfish Plague, a disease exotic to Australia but endemic in Europe. North American crayfish are generally immune to the disease, but become carriers. Crayfish from Australia are very susceptible and the fungus is considered to have serious pathogenic potential in native crayfish fauna (Unestam, 1976). In countries where the disease occurs, outbreaks may be associated with 100% mortality. In Europe, attempts to protect native stocks from Crayfish Plague, by banning the importation of crayfish, have been unsuccessful. Both fungal diseases once established are almost impossible to eradicate.

Currently, detection of both diseases relies on clinical diagnosis, histological examination and culture of the fungus. With histological diagnosis, fungal elements may not be seen until the lesion is well advanced. These methods are time-consuming because of the prolonged culture period of up to 15 days and do not provide definitive diagnosis from other *Aphanomyces* species. Additionally, there are difficulties in growing *Aphanomyces* species, and unreliability of the fungus sporulating in artificial media - a requirement for distinguishing morphological features crucial for identification.

Materials and Methods

Fungal strains

A. invadans cultures have been obtained from WA, NSW, USA and Thailand. Formalin-fixed crayfish infected with *A. astaci* have been obtained from UK. Strains of *Saprolegnia*, *Aspergillus*, *Penicillin*, and *Trichoderma* are being used in specificity studies.

Methods

Storage of Cultures: Assess different methods for the short-term and long-term storage of Oomycetes fungi.

DNA Extraction Methods: Test different DNA extraction methods for use against a variety of infected material. Test Puragene, Instagene, phenol/chloroform, Qiagen DNAeasy mini plant tissue kit, DNAzol, Enza fungal extraction kit and simple extraction methods such as boiling, and use of SDS. Addition of a proteinase K step and grinding in liquid nitrogen were assessed to determine if extraction of DNA could be improved.

PCR and FISH

Use Polymerase Chain Reaction (PCR) and Fluorescent In situ Hybridization (FISH) for the detection of both fungi from culture material, paraffin wax blocks, formalin-fixed material and fresh tissue. *A. astaci*: assess published primers and compare to primers designed from the internal transcribed spacer regions (ITS). *A. astaci:* Design primers from the trypsin protease gene precursor, and to the chitinase genes.

Results and Discussion

Culture Storage

Oomycetes fungi are difficult to keep viable and are not amenable to storage at –80°C or freeze-drying. Cultures are being maintained in sterile distilled water, GP broth, GP slopes with and without a paraffin oil overlay. The recommendations are that Oomycetes fungi are stored on slopes with or without paraffin oil overlay and that they are subcultured every 6 months to maintain viability. Distilled water is suitable for short-term storage but for longer storage broth and slopes appear to be more suitable. Assessment is continuing.

DNA Extraction Methods

Six different DNA extraction methods plus the use of additional reagents have been assessed for use on a variety of material such as plate cultures, broth cultures and wax-embedded blocks fish tissue containing *A. invadans*, and wax-embedded blocks and formalin-fixed tissue for *A. astaci*. Only two methods have proved suitable for the extraction of fungal DNA from broth cultures and formalin-fixed tissue and these were the Qiagen DNAeasy mini plant DNA extraction kit and the DNAzol reagent from Invitrogen. None of the other methods produced DNA from the material tested. Almost all methods for the extraction of DNA from fungi recommended grinding the hyphae in liquid nitrogen, however, in our hands this hindered rather than improved the quantity of DNA extracted. Both the Qiagen kit and the DNAzol reagent were used as recommended by the manufacturers but without the recommended liquid nitrogen step.

A. invadans PCR

The published primers (AIFP1, AIFP2) generate non-specific product at a molecular weight similar to the 98 bp product from *A. invadans*. The product sits at about the same position as excess primer material (high concentrations of primer are required) therefore the results are not easy to read on the gel. These primers were tested with the real time PCR methodology and would be suitable for this method, although further work on specificity needs to be done.
To improve the amplification of *A invadans*, 5 primers were designed from the internally transcribed spacer regions, which are non-conserved, non-coding regions and are used for species differentiation. The 18S and the 28S rRNA are highly conserved throughout the Oomycetes fungi and are therefore not suitable for primer design.

A. astaci PCR

Twenty primers have been designed and tested for *A. astaci* and so far amplification has not been successful from formalin-fixed tissue. However, universal primers to the

internal transcribed spacer regions are being used to amplify template that can be used to obtain sequence information that will allow the design of specific primers.

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Vibrios of Aquatic Animals: Towards a National Standard Diagnostic Technology

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FRDC Project: 2001/628

Introduction

Vibriosis is one of the most prevalent causes of marine aquatic animal disease and affects a wide range of farmed species including finfish, crustacea and shellfish. In Australia, catastrophic losses are regularly observed in larvae and juveniles in hatcheries as well as in adults. Vibriosis is a national problem that affects aquaculture from the temperate regions of the south through to tropical northern Australia. The purpose of this project is to develop practical and reliable laboratory based test systems for the identification of Australian *Vibrio* species. No single species of *Vibrio* is associated with the condition Vibriosis. The taxon is large and currently comprises 74 species in the genera *Vibrio, Photobacterium, Grimontia, Enterovibrio* and *Moritella* of which 27 cause disease in aquatic animals and a further 10 are known human pathogens. In Australia, significant disease outbreaks due to Vibriosis occur in salmonids (*V. anguillarum* and *Ph. damselae* ssp. *damselae*), barramundi (*V. harveyi*), snapper (*V. splendidus*) and striped trumpeter larvae (*V. anguillarum*). Overseas, serious epizootics have been associated with *V. salmonicida*, *M. viscosa* and *Ph. damselae* ssp. *piscicida*; these species are currently thought to be exotic to Australia. While some specific species have been associated with acute disease, significant cumulative production losses also occur on farms and in hatcheries as a result of opportunistic infection. Reported simply as non-specific vibriosis, most of the bacteria isolated from this common condition are not identified. Typically, diagnostic laboratories can only identify 40% of these isolates. Set against a rapidly expanding and diversifying aquaculture industry, diagnostic laboratories in Australia are struggling to provide meaningful identification of many of the *Vibrio* species they isolate. In the absence of a reliable framework for identification, it is unlikely that new or emerging diseases will be recognised against the background of non-specific infection encountered day to day.

Identification methods for *Vibrio* species vary enormously in their complexity, utility and accuracy. Currently there are no national or international standard methods, a deficiency that underlies the inadequacy and inefficiency that characterises existing procedures. Commonly used diagnostic kits developed for bacteria of medical importance are unreliable and of limited use. While computerised identification methods for *Vibrio* species have proved useful, at present they remain unwieldy, due to the large number of tests required to achieve a reliable identification. Nevertheless with refinement, computer assisted identification using miniaturized biochemical tests represents the most promising means of identifying presumptive isolates. This is a proven technology that serves as the basis for many existing commercial identification systems.

Materials and Methods

A comprehensive library of 805 isolates of Vibrionaceae was obtained from farmed aquatic animals from temperate and tropical regions of Australia. In addition to these wild type strains, reference and type strains representing 53 species were obtained; the

remaining 22 species not included comprised 13 species newly described or from niche environments or considered outside the scope of the survey. Isolates were characterised using a panel of 107 tests formatted as miniaturised tests developed to emulate conventional macro-format tests used for the identification of Vibrionaceae. Following profiling of the library, a random selection of 12% of the library was retested to determine test reproducibility. Phylogenetic analysis of the complete library has been undertaken by PCR amplification of the 16S rRNA gene and subsequently sequencing the first 684 base pairs. An evaluation has been made of the specificity and utility of PCR primer sets that have been described for the fish pathogens *V. anguillarum, V. ordalii, V harveyi, V. vulnificus, Ph. damselae* ssp. *damselae* and *Ph. damselae* ssp. *piscicida*. Primer sets targeting a range of constructs are being tested against the library of Vibrionaceae and PCR conditions optimised. Screening is undertaken with template concentrations from 50fg-500ng. Specific negative controls comprising a mix of DNA from 32 non-target species are included. Performance of the primer sets was also evaluated in the presence of Atlantic salmon kidney spiked with 10^1 to 10^6 cfu/g tissue of the target organism. Identification by a predictive panel of phenotypic tests will be undertaken by computer-assisted identification. The software for this has been developed and implemented.

Results

The library is based on 62 species of aquatic animal representing finfish, shellfish and crustacea. Of the 805 isolates, 94 are type or reference strains, 440 isolates were submitted as named species with the balance of 271 isolates as un-named species. A major focus has been on test development and implementation. Marked improvements in test reliability and performance were achieved for decarboxylase tests, fermentation tests and sole carbon source utilisation tests. New tests were introduced including tolerance to bile, crystal violet and cobalt chloride and the release of free amino acids from peptone media. A good balance of test performance was achieved with an almost linear range of test activity between 99% test positive for growth at 1% NaCl through to 0.2% strains positive for utilisation of butyramide as a sole carbon source. Test redundancy was low: only Gram reaction, deaminiation of threonine and serine were all negative; no tests were all positive. Test reproducibility, determined during phenotyping of the library, was high; using all test data, the global test error p was 4.69%, below the recommended test error limit of 5%. Tests with low levels of reproducibility included utilisation of oxaloacetate, tolerance to potassium tellurite and crystal violet and alginase production. Excluding these tests reduced the error to 4.0%. Purified DNA has been prepared and 16S rRNA amplified for all 805 isolates. Sequencing has largely been completed and has now to be analysed phylogenetically. For the six nominated pathogens, a total of 13 primer sets are being tested. The target sites range from 16S rRNA through to haemolysin and urease genes. The utility of these constructs will be assessed on intra- and inter-species specificity and their apparent sensitivity. A production version of the software package, PIBWin has been released. The software is used for matching the characterisation profile of an unknown with a database of reactions that is definitive of the different species of *Vibrio, Photobacterium* and *Moritella*. The best match is based on a probabilistic assessment measured as the Willcox identification score and the quality of the match is measured by the modal likelihood value. The Windows version of the program allows full archiving and exporting of data. The program is freely available from http://www.som.soton.ac.uk/staff/tnb/pib.htm

Discussion

The goal of this project is to establish a robust and practical identification system for the identification of this large and ubiquitous group of bacteria that are the cause of both acute and chronic disease in a wide variety of farmed aquatic animals. As the

phenotypic tests are in miniaturised format, the technology is suitable for routine high or low volume use in diagnostic laboratories. Provision of PCR primers for major pathogens will extend test capability and enable an alternative means of confirmatory testing and provide a basis for screening samples to detect specific pathogens. The progress described relates primarily to the generation of data, both phenetic and genetic, which will be analysed by numerical taxonomic and phylogenetic methodologies. The numerical taxonomic analysis will establish the phenetic relationships of the isolates. Boundaries of the clusters identified by numerical taxonomy will be defined using the phylogenetic data derived from 16S rRNA sequences. The phenotypes of the species will be defined using the characterisation data generated in this study and will be used to create a probability identification matrix. It is anticipated that identification of known species can be achieved using between 24-36 tests in a microtitre tray and when used in conjunction with PIBWin, diagnostic laboratories will have a realistic basis on which to identify members of the Vibrionaceae.

Characterisation of Tasmanian Aquareovirus (TAV) in Atlantic salmon

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CSIRO Livestock Industries

Introduction

Aquareoviruses are double-stranded (ds) RNA viruses belonging to the Family *Reoviridae*. They are non-enveloped viruses with a multi-layered capsid that contains 11 segments of dsRNA. These encode seven structural and five non-structural proteins (Rangel et al, 1999; Subramanian et al, 1994). The viruses grow in a variety of fish cell lines and induce a characteristic cytopathic effect that includes formation of syncytia.

Over 40 reported aquareovirus isolates, belonging to 6 distinct genogroups, have been isolated from fish, shellfish and crustaceans around the world. The role of aquareoviruses as disease-causing agents is varied with a majority of isolates reportedly taken from 'healthy' fish. Only a few members of the group cause overt disease, including haemorrhagic disease, hepatitis and pancreatitis (Lupiani, et al 1995). Pathogenesis studies of other aquareoviruses also report subclinical infections, and these may be associated with a viral carrier state (Lupiani, et al 1995).

In 1989 an aquareovirus was isolated from farmed Atlantic salmon in southeast Tasmania, and since then it has been consistently isolated from samples submitted as part of the Tasmanian Fish Health Surveillance Program from the southeast region. No clinical or pathological signs have been associated with infection in the field. Infection trials undertaken at CSIRO-AAHL in the early 1990s have shown a subclinical infection with necrotic lesions present in a variety of tissues (unpublished data).

Materials and Methods

Neither the pathogenesis of TAV nor the significance of TAV as a direct or indirect pathogen of Atlantic salmon is well understood. The aim of this project is to characterise TAV by molecular techniques and conduct pathogenesis studies. Molecular characterisation of the virus will involve sequencing part or all of the genome to facilitate virus classification. This will also allow the development of generic, and specific, diagnostic PCRs.

Pathogenesis studies will include infection trials, to identify tissue tropism of the virus and to examine aspects of the immune response. Expression of a number of cytokines and other important immune regulatory factors will be measured during infection by means of quantitative PCRs. The cytokine profile initiated during infection influences the production and differentiation of T-cell subsets, which are important in the activation of specific and innate immune responses (Roitt et al, 2001). Characterisation of the cytokine profiles induced during TAV infection will be used to gain a better understanding of virus-host interactions and of a potential underlying role for TAV with infection by other important pathogens in southeast Tasmania.

Results and Discussion

Results to date and future plans will be presented and discussed.

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Development of diagnostic procedures for the detection and identification of *Piscirickettsia salmonis*

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FRDC Project: 2001/624

Introduction

Piscirickettsia salmonis, an obligate intracellular bacterial pathogen of salmonids, is the aetiological agent of the disease piscirickettsiosis (Fryer and Mauel, 1997). *P. salmonis* is classified as a member of the Proteobacteria gamma subdivision based on 16S rRNA sequence. Several salmonid species including coho salmon (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*) are susceptible to infection. Outbreaks of the disease in farmed fish can cause substantial mortalities leading to significant financial loss. To date, piscirickettsiosis has been identified in Chile, Ireland, Norway, Scotland, and both the east and west coasts of Canada.

The recent detection of a rickettsia-like organism (RLO) in Tasmanian farmed Atlantic salmon has highlighted the need to develop diagnostic assays that can detect and differentiate, at the antigenic and molecular level, the Tasmanian RLO from exotic isolates of *P. salmonis*.

Materials and Methods

Rickettsia DNA extraction

Formalin-fixed and fresh salmon tissues (liver, kidney, brain) were rinsed in PBS and DNA extracted using a QiaAmp DNA minikit (Qiagen) according to the manufacturer's instructions.

PCR amplification of the Tasmanian RLO rDNA genes

Regions of the 16S and the internal transcribed spacer (ITS) of the rDNA operon of the Tasmanian RLO were amplified following methods described by Mauel *et al.* (1996) using the PS2S and PS2AS primer set and Marshall *et al.* (1998) using the ITS1 and RTS4 primer set respectively.

Sequence determination

The ITS sequence was determined by sequencing PCR products inserted into a cloning vector using universal primers T7 and SP6. The 16S rDNA partial gene sequence was determined by direct sequencing of the PCR product.

Phylogenetic analysis

The Tasmanian RLO and *P. salmonis* sequences were aligned, and phylogenetic trees were constructed from the sequence data using the DNA Distance + Neighbor programs in PHYLIP Phylogeny Inference Package Version 3.2 (Felsenstein, 1989; Biomanager, Australian National Genomic Information Service). For comparisons between the Tasmanian RLO and the *P. salmonis* isolates using the partial 16S rDNA gene and ITS region, 445 and 265 bases were utilized respectively, bootstrapped 100 times.

Immunoperoxidase/Immunoelectron microscopy assays

These assays were performed following standard procedures (using a polyclonal sheep anti-*P. salmonis* (LF-89) serum (Microtek) and a normal sheep serum) (c.f. AAHL Quality assurance manual and Hyatt, 1989; 1991).

Multiplex TaqMan Assay and instrumentation

c.f. Corbeil et al., 2003.

Results and Discussion

The immunoperoxidase assay performed on RLO infected fish tissues revealed weak staining in comparison to the bright staining observed on *P. salmonis* (LF-89) infected tissues. Uninfected tissues did not show significant staining. These results suggest the presence of antigenic similarity between the Tasmanian RLO and the *P. salmonis* isolate LF-89. Immuno-electron microscopy allowed observation of the Tasmanian RLO in infected fish tissues. Specific immunogold staining was also observed in infected fish tissues. These observations support the immunoperoxidase staining result; *P. salmonis* antiserum (directed against the LF-89 isolate) cross-reacts with the Tasmanian RLO, hence suggesting the presence of closely related antigenic determinants between the RLO and *P. salmonis*.

Sequencing and phylogenetic analysis of the 16S partial sequences of the Tasmanian RLO and 5 *P. salmonis* isolates revealed high levels of similarity with values ranging from 93.4 to 95.7 percent. In addition, the percent similarity for the ITS sequences were slightly lower with values ranging from 86.8 to 89.0 percent.

Results obtained from the analysis performed on the partial gene sequence of the 16S rDNA indicate that the Tasmanian RLO is closely related to *P. salmonis*, particularly to the Chilean isolate EM-90. In addition, we chose to amplify and sequence the RTS-1/RTS-4 amplicon of the ITS region as it has been suggested as a promising region for monitoring genetic variation in *P. salmonis* (Marshall *et al*., 1998). This DNA region allowed us to further evaluate the genetic relatedness of the Tasmanian RLO with strains originating from other geographical regions. We found the ITS sequence of the Tasmanian RLO slightly less conserved than the 16S sequence when compared with the five exotic *P. salmonis* isolates. The phylogenetic analysis also indicates that the Tasmanian RLO contains a 19 base pair deletion at the 3-prime end of the ITS region of the rDNA operon, indicating a significant genetic divergence from *P. salmonis* isolates, exotic to Australia.

The real-time PCR TaqMan assay developed as a diagnostic assay was specific to *P. salmonis* and to the Tasmanian RLO. In addition, the assay offers a level of sensitivity comparable to the conventional nested PCR recommended by the Office International des Epizooties.

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High throughput test development and health screening of *Penaeus monodon* used in the FRDC domestication project

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This work forms part of a multi-organisational (CSIRO/AIMS/QDPI) and industry (Gold Coast Marine Aquaculture/Seafarm) research project on 'Understanding and removing the barriers to *Penaeus monodon* domestication'. The project is led by the Australian Prawn Farmers Association (APFA) and supported by the FRDC.

Introduction

Attempts to selectively breed *P. monodon* derived from the Pacific Coast of Australia have been hampered by viral disease and mortalities. Testing of diseased, captive-reared stocks has indicated that gill-associated virus (GAV) and Mourilyan virus (MoV) have contributed to this problem. RT-nested PCR screening over several years has shown that GAV, and possibly MoV, are highly prevalent and can exist as unapparent/chronic infections of wild and farmed *P. monodon* from this region. At least for GAV, there is experimental data to suggest this high infection prevalence is perpetuated by vertical transmission of the virus. There is also circumstantial evidence that certain stressors can induce acute GAV infection states in chronically infected carriers.

The use of virus-free wild broodstock appears to be a crucial prerequisite for a successful domestication program. PCR screening of wild *P. monodon* from Western Australia and the Gulf of Carpentaria (GoC) has identified prawn populations that appear to be free of GAV, yellow head virus (YHV) and white spot syndrome virus (WSSV). As the Queensland Government presently prohibits interstate translocations, founder (G0) *P. monodon* broodstock were collected near Weipa, GoC, and transferred to biosecure facilities at the Gold Coast Marine Aquaculture hatchery and at CSIRO Marine Research in southeast Queensland. Progeny GoC-G1 families have been produced from the GoC-G0 founder stocks and are being reared to maturity at these facilities. The GoC-G1 stocks are now approaching a size/age to initiate spawning, allowing assessment of their reproductive performance and generation of GoC-G2 families. We report here health screening of these stocks and the development of high throughput real-time TaqMan PCR tests to assist in this process and for use in the certification of the stocks as specific pathogen free (SPF). Other health-related aspects of the project are aimed at determining whether GAV and MoV infection levels impact on spawning efficiency, the mechanism by which these viruses are transmitted vertically and whether egg disinfection can generate virus-free progeny from infected broodstock.

Materials and methods

Standard histological staining techniques have been employed to examine prawn tissues for histopathology indicative of viral and bacterial infections, and to identify epicommensal gill fouling organisms. *In situ* hybridisation (ISH) using DNA probes to GAV and MoV has also been conducted to identify chronic or acute states of infection.

Conventional PCR and RT-PCR tests have been established for most known viral pathogens of *Penaeus monodon*. However, as each PCR method employs different test conditions, the application of multiple tests is technically problematic. Using an ABI PRISM® 7900 HT, 384-well Sequence Detection System, real-time TaqMan-probe

based PCR tests are being developed for exotic viruses including WSSV, YHV, Taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), *P. monodon* hepatopancreatic parvovirus (HPV) and the indigenous viruses GAV, MoV, spawner-isolated mortality virus (SMV) and monodon baculovirus (MBV). The design and development stage of the TaqMan PCR tests will be reported.

Results and Discussion

Gill nucleic acid from thirty founder GoC-G0 *P. monodon* broodstock (5 pools of gill from 6 prawns) tested negative by conventional PCR for WSSV, YHV and GAV, but MoV was detected at very low levels in 3 of the 5 gill pools. Gill, lymphoid organ (LO), haemocyte and hepatopancreas were collected from another ten GoC-G0 *P. monodon*. Gill DNA was tested for WSSV, LO RNA for GAV and YHV and both LO and haemocyte RNA for MoV. Very low levels of MoV were again detected in 9/10 haemocyte and 1/10 lymphoid organ samples. The data suggests the GoC *P. monodon* harboured very low-level MoV infections. As the stocks were tested within days of transfer to southeast Queensland, the infections did not appear to be acquired after translocation. Histological examination of some of the GoC-G0 stocks also identified a low-grade bacterial infection in a single prawn but there was no evidence of any pathology indicative of viral infection. However, the MoV infection levels were too low to be detected by histology or ISH.

Two G1 families were generated soon after the arrival of the GoC-G0 prawns. However, the majority were maintained in biosecure facilities for 9 months during 2002 to reach a size more suitable to spawning. Two biosecure facilities were employed at the GCMA hatchery and CSIRO-MR, Cleveland. PCR testing during this period indicated that both the G0 and G1 stocks held at CSIRO-MR acquired relatively high levels of GAV infection (1-step RT-PCR-positive). The lymphoid organs of these prawns also displayed extensive spheroid formations that were positive by ISH for GAV but not MoV. This prompted the implementation of new operational practices and upgrading of the CSIRO-MR biosecurity facilities. In November 2002, two new GoC-G1 families were generated from the G0 stocks held at GCMA and reared in the biosecure facilities at both GCMA and CSIRO-MR. In March-April 2003, low-level MoV infections were detected by PCR in 5/10 prawns and low-level GAV infections in 2/10 prawns sampled from GCMA. However, the infections could not be confirmed by ISH. In June 2003, MoV was detected in 55% to 80% of the prawns reared at the 2 locations and infection levels appeared to be slightly higher than detected earlier. Low-level GAV infections (nested RT-PCR-positive) were also detected in 2/29 prawns from GCMA and in 7/10 prawns from CSIRO-MR. Although some haemocytic nodules were detected within the lymphoid organs during histological examination, neither MoV nor GAV were detected by ISH. This suggested that the infections levels were below the sensitivity limit of ISH and that the nodules were due to another agent. Preliminary sequence analysis of MoV and GAV amplicons obtained from GoC stocks has not yet identified the geographical origin of the infections. Additional samples and amplicons derived from other genome regions will be sequenced to examine the molecular epidemiology in more detail.

Although commonly infected with GAV, *P. monodon* stocks from the Pacific Coast (PC) are readily accessible and so are also being bred as part of the domestication program. Egg numbers and nauplii hatching rates were recorded for all PC *P. monodon* spawned at GCMA in the 2002 hatchery run. Comparable data were collected for 40 captive-reared PC *P. monodon* spawned at AIMS. RNA was prepared from gill biopsies of the 40 AIMS females and 20 each of the best, middle and poorest performing females (60 in total) from GCMA. Conventional RT-nested PCR testing of the 60 GCMA samples for MoV and GAV suggested that viral loads have little if any influence on spawning efficiency. Real-time GAV PCR testing at AIMS has supported this data for GAV. A

real-time PCR test for MoV under development at CSIRO-LI will also be used to assess more accurately the effect of MoV infection on spawning performance.

CSIRO-LI is also collaborating with AIMS to determine experimentally whether MoV (like GAV) is transmitted vertically during spawning. More comprehensive experiments will also be conducted to determine the exact mechanism of vertical transmission of each virus. Data currently available indicates that GAV is present in or on the egg membrane as most of the virus associated with fertilized eggs is lost upon hatching of nauplii. Experiments will also determine if egg washing with disinfection agents can produce virus-free progeny. Data on the vertical transmission mechanisms will assist the formulation and implementation of egg disinfection practices.

AAHL Fish Diseases Laboratory – Diagnostic Capability

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Introduction

In 1981 a national fish health reference laboratory was fully established for the first time under a joint agreement between the Victorian Dept of Agriculture, the Commonwealth Dept of Health (Quarantine) and the Commonwealth Bureau of Animal Health at the RVL Benalla, Vic. At that time the laboratory was responsible for addressing national issues related to fish health – diagnosis of endemic and exotic diseases and responding to quarantine issues. Over the years, as aquaculture activities expanded in Australia, it was decided that facilities at Benalla were inadequate to support a national fish health laboratory. Thus, in 1989, the Australian Fish Health Reference Laboratory was relocated to AAHL. In 1993 an external review of AFHRL was conducted and since that time AFDL has been addressing the major issues emphasised during that review as outlined below.

Mission Statement

To provide specialist advice and laboratory services to AQIS (now Biosecurity Australia) and other Commonwealth and State government agencies on infectious diseases of finfish and aquatic invertebrates.

To achieve this mission, AFDL is addressing the following responsibilities:

Diagnosis

- Primary responsibility for diagnosis exotic diseases, in testing for suspected outbreaks of exotic disease, and in developing improved diagnostic procedures
- Export and import testing and certification where there is a special requirement
- Referral laboratory where testing requires use of exotic pathogens and in relation to emerging diseases
- Providing expertise in the conduct of testing for exotic diseases
- Provision of special diagnostic reagents

Research and development of diagnostic tests for exotic diseases

- Generic technology for improved diagnosis of endemic and exotic diseases
- Identification of latent infections and disease carriers
- Collaboration in other areas of research, on aquatic animal diseases deemed important to industry, with other research providers and industry

Training and technology transfer to other fish health laboratories

- Provision of laboratory training for infectious disease diagnosis and transfer of procedures developed at AAHL to other aquatic animal health laboratories

Recent and Current Activities

- Member of the CCEAD joint pilchard scientific working group (provision of advice)

- Member of FRDC AAH Subprogram scientific advisory committee (provision of advice)
- Member of NAAHTWG (provision of advice)
- Provision of virology services for the Tasmanian fish health surveillance program (on-going)
- Provision of export certification services for DPIWETas/AQIS re: salmonid ova exports (on-going)
- Import a representative sample of a broad range of exotic pathogens of aquatic animals (on-going)
- Provision of diagnostic services for aquatic animal diseases, especially exotic diseases (on-going)
- ACIAR-funded project: Development of diagnostic antibodies for white spot disease. Collaborative partners include CSIRO LI (Brisbane) and Univ. Mahidol, Bangkok (2002-3)
- With DPIWE Tasmania, FRDC-funded project to undertake development and provision of an aquatic animal bacteriology training workshop for state diagnosticians (2000-2001)
- AFFA-funded project on establishment of diagnostic procedures for the detection and identification of infectious salmon anaemia virus, ISAV (2000-2001)
- FRDC-funded project on the development of diagnostic procedures for the detection and identification of pilchard herpesvirus (1999-2002; and in collaboration with WA Fisheries and USyd 2002-2005)
- FRDC-funded project on barramundi fry nodavirus (NNV) in collaboration with QDPI (1999-2001; 2002-2004)
- Aquafin CRC/FRDC-funded project on development of tuna cell lines (2002-2006)
- FRDC-funded project on development of SDT for aquatic birnaviruses (2001-2003)
- FRDC-funded project on development of SDT fro Piscirickettsia-like organisms (2001-2003)
- FRDC-funded project with DPIWE, Tas. Establish national reference laboratory for V*ibrio* spp. of aquatic animals (2001-2004)
- Provision of export certification services for DNREVic/PIRSA/BA re: export of live carp to UK/Europe (on-going)
- FRDC-funded project on the establishment of diagnostic expertise for detection and identification of red sea bream iridovirus (2003-2006)
- FRDC-funded collaborative project, with OIE Reference Laboratory for mollusc diseases, on development of molecular diagnostic expertise for the mollusc pathogen *Bonamia* sp. (2003-2004)
- AFFA-funded project on development of inter-laboratory proficiency test for diagnostic laboratories – Detection and identification of white spot syndrome virus in crustaceans (2002)
- Development of improved procedures for the detection and identification of important aquatic animal pathogens: *Aeromonas salmonicida*, *Myxobolus cerebralis*, white spot syndrome virus, *Oncorhynchus masou* virus (on-going)
- FRDC-funded project to develop and conduct a training course on exotic diseases of aquatic animals (2002-2004)

A monoclonal antibody specific for white spot virus of crustaceans

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Introduction

White spot syndrome, first reported in Taiwan and subsequently reported in Japan in 1993, Thailand (1994) and other Asian countries, USA (1995), S America (1999), C America (1999) and the Philippines (1999), has been responsible for huge economic losses globally. Despite this rapid spread through all prawn producing regions of the world, white spot syndrome virus (WSSV) remains exotic to Australia (East et al 2002). It is known that viable viral pathogens are carried by commodity prawns traded internationally (Lightner et al 1997; Nunan et al 1998; McColl et al 2003), that WSSV is resistant to freezing (McColl et al 2003) and together with a broad host range (see Hill 2002), these properties identify WSSV as a significant threat to the prawn farming industry in Australia.

To assist with the rapid, sensitive and specific detection and identification of WSSV a number of diagnostic reagents have been developed. In this report a monoclonal antibody is described which can be applied to a broad range of immunodiagnostic assays and should be useful for the rapid detection and identification of WSSV as well as further study of the pathogenesis of the virus and its interaction with host species.

Materials and Methods

Using standard procedures, WSSV was harvested from experimentally infected prawns (*Penaeus monodon*), purified, and inoculated (i.p.) in to six-week-old, female Balb/C mice (10 ug protein/mouse) using Montanide ISA50V adjuvant (Seppic, Paris). Booster inoculations (10 ug viral protein/mouse), including adjuvant, were given at 2 and 4 weeks following the primary inoculation. Mice were tail bled at least 2 weeks later, and the magnitude and specificity of the resultant virus specific antibodies were measured by ELISA and western blotting respectively.

Using 50% polyethylene glycol (MW 1500) as previously described splenic lymphocytes were fused with sp2/0 murine myeloma cells. After cultivation in selective (HAT) medium for 10 days, hybridoma supernatants were screened for production of WSSV-specific antibodies in an indirect ELISA.

Hybridoma cell lines which yielded positive results in the primary screen were expanded and cryo-preserved. Hybridomas producing Mabs with strong reactivity and specificity for WSSV were cloned twice by limiting dilution and cryopreserved.

One particular hybridoma cell line was selected for further characterisation using enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC) on prawn tissues, immunodot blot and negative contrast immuno-electron microscopy (NCIEM).

Results and Discussion

Ten out of the 800 hybridomas produced Mabs with a strong positive reaction, by ELISA, to WSSV-infected haemolymph (absorbance >1.0) and low background reaction to non-infected haemolymph (absorbance <0.05). These 10 cell lines were expanded and the supernatants further screened by dot blot and IHC to ensure specificity.

While primarily developed for undertaking screening of supernatants, a simple and specific immunodot blot assay was developed for the rapid detection of WSSV infection in prawn haemolymph.

By IHC, inclusion bodies of infected cells in WSSV-infected prawn tissue were stained with the substrate. There was no reaction with tissue from non-infected prawns. All of the 10 supernatants were able to detect infected cells, each to varying staining intensities. Confirmation of specificity was achieved when colloidal gold was used in TEM to visualise that the Mabs bound to the envelope of the WSSV virion.

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Isolation of a new virus from wild-caught pilchards (*Sardinops sagax neopilchardus*) in Australia

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Introduction

The 1995 mass pilchard mortality event which occurred around the southern coast of Australia (Hyatt et al. 1997) was followed by a similar event in 1998/9. In both incidences, a herpesvirus was observed in gill epithelium, associated with severe tissue lesions. It was concluded that the fish died from anoxia due to impaired gill function resulting from this infection. During the disease investigations numerous samples of tissues from affected pilchards were processed for virus isolation on several fish cell lines. The pilchard herpesvirus could not be grown in any of the cell lines used. However, from one submission of pilchard gill tissue, during the 1998/9 investigation only, a further virus was isolated, initially on CHSE-214 cell cultures. This report describes the isolation and initial characterisation of this virus, the occurrence of which is assumed to be purely incidental and not associated with the mass pilchard mortalities of 1995 and 1998/9.

Materials and Methods

During the 1998/9 mass pilchard mortality event, submissions of fresh pilchard tissue were processed for virus isolation on fish cell lines, using standard protocols (OIE, 2000; Crane et al., 2000). Briefly, duplicate cell cultures of CHSE-214, RTG-2 and EPC cell lines, established in 24-well culture plates, were inoculated with aliquots of tissue homogenates and incubated at 15°C. At seven days post-inoculation (p.i.), material from cultures displaying cytopathic effect (CPE) was passaged on to fresh cell cultures which were incubated as described above. When CPE was evident, tissue culture supernatants from these cultures were passed onto 24-well plates containing Thermanox™ coverslips with cell monolayers attached. When CPE developed, the coverslip cultures were fixed and processed for electron microscopy. Cell cultures from this passage were also used for immunoperoxidase tests using polyclonal antibodies specific for either VHSV, IPNV or IHNV (Crane et al., 2000).

Results and Discussion

At 2 days p.i., viral CPE was evident in the primary cultures of CHSE-214 cells inoculated with one of the pools of gill tissue submitted. CPE continued to develop until there was total destruction of the cell monolayer. On sub-culture of these supernatants and re-culture of original material only some cultures developed CPE. In cultures that did develop CPE, the CPE commenced with the formation of discrete plaques, areas of cell rounding and lysis, which developed further until the complete cell monolayer was destroyed. Cell culture supernatant with the virus isolated from the index case was aliquoted and stored at -80°C.

Infected cultures were processed for immunoperoxidase tests for VHSV, IHNV and IPNV. All cultures were negative (results not shown). Similarly, PCR analysis for detection and identification of VHSV, IHNV, IPNV, were negative.

The virus survived freezing at -80°C and, when inoculated onto a range of fish cell lines including CHSE-214, RTG-2, EPC, FHM, BF-2 and SHK-1, it readily infected CHSE-214 and SHK-1 cell cultures and demonstrated typical viral CPE. The cell lines RTG-2, EPC, FHM and BF-2 appeared to be refractory to infection.

Ultrastructurally, the virus exhibited novel features which were similar to, but different from, the orthomyxo-like virus that causes infectious salmon anaemia in Atlantic salmon (Dannevig et al., 1995). Immunodiagnostic tests using ISAV-specific antibodies and molecular assays using ISAV-specific PCR primers demonstrated unequivocally that the virus derived from pilchards was not ISAV (Dannevig pers. Comm.).

The virus was isolated from only one pool of pilchard tissues collected during the 1998/9 mass pilchard mortality event and is not implicated as the causative agent of this disease. Indeed, it is highly unlikely that this virus performed any role in the epizootics of either 1995 or 1998/9. There is little correlation between the occurrence of this virus and the two pilchard epizootics. In contrast, there was a high correlation of the incidence of disease with the presence of a herpesvirus in the gills of affected fish (Hyatt et al., 1997).

Ultrastructural analysis indicates that the virus belongs to the Orthomyxoviridae and it is interesting to note that the virus grows well in SHK-1 cells, similar to some, but not all, strains of ISAV (Bouchard et al., 2001). Moreover, it is interesting to note that investigations aimed at determining the natural reservoirs for ISAV have shown that herring (Clupea harengus) is susceptible to infection and may be an asymptomatic carrier of ISAV (Nylund et al., 2002). The relationship of the pilchard orthomyxo-like virus to other similar viruses is under current investigation. Further characterisation of biochemical and molecular properties will allow correct classification of this virus.

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Pilchard herpesvirus infection in wild pilchards

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FRDC Project: 2002/044

Introduction

Between March and September in 1995, high mortalities of pilchards *(Sardinops sagax neopilchardus)* were seen for over 5000 kilometres (km) of the Australian coastline – their entire geographical range – and also in over 500 km of the New Zealand coastline. The Australian epizootic moved progressively, in a "bush-fire-like" manner against the prevailing Leeuwin and East Australian currents, at a rate of approximately 21 km per day to the west and 40 km per day to the east. The estimated loss to the pilchard fishery after the 1995 epizootic was approximately $12 million.
Following investigation, it was concluded that a herpesvirus was the most likely cause of the mortalities, although the source of the virus remains unknown. Several models for introduction or emergence and transmission have been proposed but are still inconclusive.

A second mortality event began in October in 1998 and spread throughout the Australian pilchard range by mid 1999, and again, a herpesvirus was present in the gills of diseased fish. Although the spread of the mortalities was much slower in 1998/9, higher mortality rates were observed, particularly in Western Australia, where an estimated 70% of the pilchard population on the south coast died, resulting in a severe depression of Australian pilchard stocks.

To date, Polymerase Chain Reaction (PCR) techniques have been developed by AAHL and the Department of Fisheries WA, and an *in situ* Hybridisation (ISH) technique has been developed by the Department of Fisheries WA, for the detection of *Pilchard herpesvirus* (PHV). Limited sequence data has been obtained, and it is in this project that these molecular tools will be used to take the research to the next level.

Though we now have some basic molecular tools we still do not know if the virus is present in the pilchard population as a latent virus, or whether or not it is coming in to the country through imported pilchard bait. There is a risk that another epizootic, similar to the 1995 and 1998/9 events, may occur.

The aims of this project are to:

- improve the molecular techniques available, which are currently based on limited sequence data
- investigate basic aspects of the virus and the disease, such as the tissue distribution of virus in infected fish, and the correlation between disease in fish and the presence of virus
- survey wild pilchard populations to determine whether the virus is still currently detectable and capable of causing disease
- compare the herpesvirus strains from 1995 and 1998/9, and to compare, at the molecular level, this herpesvirus and this disease with other similar herpesvirus fish diseases which have been reported elsewhere in the world.

Materials and Methods

One method that will be explored involves the comparison of related herpesvirus sequences to design primers that relate most closely with the sequences being compared, with some degree of degeneracy, and use these primers to amplify PHV DNA of unknown sequence that can then be characterised. This was the method used to obtain the first sequence data for PHV, using a comparison of *Oncorhynchus masou herpesvirus* (OMV) and *Channel catfish herpesvirus* (CCV).

Another method that will be tried makes use of a DNA library that has been prepared by digesting purified virus with restriction enzymes and ligating the resulting various fragments into a suitable vector. Clones can be grown on LB agar in petri dishes, purified, and then used in PCR and/or sequencing to characterise the PHV DNA that is inserted into that clone.

The larger sequence data obtained from either of these methods can then be used to further develop the currently available tests in terms of sensitivity and specificity, and to compare more extensively the similarity between the 1995 and 1998 viruses, and compare these virus(es) to other related herpesviruses.

Results and Discussion

The project commenced in 2003, so results at this stage are limited. Molecular tools have been exchanged between the Department of Fisheries WA, AAHL and University of Sydney for validation of currently available techniques. Research aimed at obtaining more sequence data is underway.

Parasite survey in Southern Bluefin Tuna in 2003 - preliminary results

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Click here for PowerPoint presentation

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Aquafin CRC

Introduction

Southern bluefin tuna aquaculture started in 1991 and has grown into the largest finfish aquaculture in Australia with an export value of $290 million. It is based on fattening of wild caught fish in sea-cages over a period of 3-6 months. Continuous husbandry improvements have ensured very low mortality. A tuna health risk assessment, completed early this year, recommended a health surveillance and monitoring program (Nowak et al 2003). This parasite survey is the first part of this program, funded by Aquafin CRC as FRDC project 2003/225. The aim of this survey was to investigate the presence of parasites in harvested tuna, to provide background information for health surveillance and other tuna health projects.

Materials and Methods

Seven companies provided samples from harvest. Seventy-four fish were sampled during the first two months of the project. Gills and internal organs (excluding kidneys) were collected at harvest and put on ice. Within a few hours of sampling these organs were examined for the presence of parasites and any abnormalities. Forty three hearts were flushed with PBS to determine the presence of *Cardicola forsteri*. One of the farms reported swimmer syndrome and fish were sampled for the presence of *Uronema nigricans*. At this farm, sixty two healthy fish were sampled at harvest, and 31 morts and 2 fish showing swimmer syndrome were examined. Samples were examined by light microscopy.

Results and Discussion

A wide range of parasites was found in the sampled fish. *Cardicola forsteri* was found in hearts of 13 fish out of 43 fish screened for this parasite at harvest. The intensity of infection ranged from 1-7 flukes per heart. None of the harvest fish screened for the presence of *Uronema nigricans* were positive, whereas both fish showing swimmer syndrome were positive and 18 out of the 31 morts were positive. The test used for *Uronema* lacks sensitivity, and therefore may result in false negative results. As it only detected the parasite in fish which were already showing the syndrome or which had died due to infection, it may have a limited practical value.

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Part 1: Development of an integrated approach to fish health emergencies within Tasmania

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The Tasmanian aquaculture industry is a significant export earner for the State and infectious disease, both endemic and exotic, is a major threat to the financial viability of the industry. In recognition of the specific roles and responsibilities of State authorities within animal disease control frameworks, the relevance of an accepted fish health plan developed for Tasmanian conditions has been a high priority for both industry and government. Although the Tasmanian Fish Health Emergency Management Plan was originally developed following a workshop on the prevention and response to disease emergencies in aquaculture in Tasmania held in July 1996, this plan was never ratified by government and never incorporated into a multi-agency approach to emergency animal disease response.

The incorporation of the Tasmanian Fish Health Emergency Management Plan into existing emergency animal disease response plans together with the development of a specific aquatic control centre manual has obvious resource management advantages and enables Tasmanian to contribute more effectively to national decision-making for emergency animal disease response plans.

Tasmania has undertaken two significant emergency disease responses to suspect exotic disease incursions during recent years. Although both events proved not to be caused by exotic pathogens, the response did highlight deficiencies in the existing Fish Health Emergency Management Plan. Identified problems centred on the lack of incorporation of this document into existing emergency plans and thus a lack of acceptance by other agencies required to provide support during such events.

Similarly, during 2002 the national foot & mouth disease exercise, 'Operation Minotaur', highlighted the need for a whole of government approach to animal disease emergency response. Following this exercise the Tasmanian Department of Primary Industry Water & Environment undertook major reviews of all response plans. These plans now give the State Emergency Service greater responsibility for coordination of responses to emergency animal disease events as well obtaining formal agreement from industry and other government agencies for the provision of services.

Key objectives of this project have been to:

- Undertake a major revision of the Tasmanian Fish Health Emergency Management Plan and incorporate this plan into the existing Tasmanian Animal Disease Emergency Management Plan,
- Produce a control centre manual outlining standing operational procedures for Tasmanian fish health emergencies, referred to as 'AquaTOM' (the Tasmanian Aquatic Operational Plans & Resources Manual), which is consistent with the existing manuals for terrestrial livestock emergencies and
- Audit the plan and manual according to 'National Animal Health System Performance Standards'.

The final result has highlighted the importance of fish health emergencies to staff involved in the animal health emergency response process whilst aquatic plans and procedures are now consistent with other operational plans resulting in a greater efficiency in the utilisation of resources.

Part 2: Development of a practically orientated Decontamination Manual for AQUAVETPLAN

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FRDC Project: 2002/653

Anyone investigating decontamination procedures for emergency animal disease response will note that there are currently a number of existing manuals relating to terrestrial animal disease but very few specifically addressing the area of aquatic disease. The proposed AQUAVETPLAN Decontamination Manual does not aim to reproduce the existing AUSVETPLAN Disinfection Manual but instead hopes to complement information contained within this and other manuals.

Disinfection of aquaculture facilities presents a unique set of issues and problems that must be addressed by those undertaking decontamination procedures. Issue such as the disinfection and disposal of large volumes of contaminated water, the cleaning of boats, divers or large marine equipment, the access to supplies of freshwater at marine sites and the corrosion of equipment by chemicals are all examples of issues that must be considered during such events.

The manual aims to provide practical information relating to cleaning and disinfection procedures for aquaculture and fish handling establishments. In an attempt to provide a concise and easy to follow document, wherever possible, summary tables and job sheets relating to diseases and production enterprises will be utilised rather than detailed descriptions.

In order to maintain continuity the manual will follow the same general profile of the AUSVETPLAN Decontamination Manual whilst incorporating principles and terminology used within the AQUAVETPLAN Enterprise Manual. Those diseases listed on Australia's National List of Reportable Diseases of Aquatic Animals will be used as a guide.

The Manual will be divided into three major sections together with an introduction.

Section 1: General Principles.
Description of basic principles relating to cleaning and disinfection procedures, the action of chemical disinfectants and the legislation relating to the use of such chemicals.

Section 2: Check lists for specific diseases and enterprises.
Summary tables listing the procedures and chemical agents effective against specific pathogens together with recommendations on cleaning and disinfection techniques for specific enterprises listed within the AQUAVETPLAN Enterprise Manual. Suggested standard operating procedures for cleaning and disinfection of specific equipment.

Section 3: Appendices
Lists of chemical suppliers within states and appropriate contact personnel.
Check lists for equipment and procedures.

Current status of perkinsosis among blacklip abalone in Australia, with a fresh look at transmission of the disease

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FRDC Project: 2000/151

Introduction

Populations of wild blacklip abalone (*Haliotis rubra*) have suffered severe dieback in the northern coast of New South Wales for over a decade. In South Australia, a significant proportion of wild blacklip continue to develop unsightly lesions that lead processors to reject them, or grade them as inferior – and this has occurred for more than 30 years. Both of these problems are associated with a dinoflagellate protist, *Perkinsus olseni*. We aimed to determine the nature and extent of a known *Perkinsus* 'hotspot' field site in South Australia, and to examine transmission rates in culture abalone in the laboratory, with a view to recommending appropriate management strategies.

Materials and Methods

Three batches of 200 uninfected abalone were tagged and taken from West Bay to a known disease 'hotspot', Taylor Island, in January of three consecutive years (2001-2003). After three months, local and translocated abalone were tested for *Perkinsus* using the standard Ray test (culturing in fluid thioglycollate for 7 d followed by flooding in iodine to reveal swollen hypnospores).

In addition, in a series of lab experiments, we exposed commercial abalone juveniles (<3 cm, obtained from government and commercial facilities), to zoospores of *Perkinsus*. These were maintained in recirculating seawater aquaria with unexposed control groups for varying periods of time, up to 8 months. Abalone that perished during experiments were tested for *Perkinsus* using the Ray test, as were all surviving animals at the time each experiment was terminated.

Results and Discussion

P. olseni was transmitted to translocated abalone at markedly different rates from year to year in the disease 'hotspot' (Figure 1). After 3 months in the hotspot, the percentage of abalone acquiring *Perkinsus* was similar in 2001 and 2003, but more rapid than those translocated in 2002. A similar overall pattern of infection was found in the resident abalone: prevalence was also higher in 2001 and 2003 compared to 2002 (Figure 1). We conclude that the rate of transmission is extremely high, though it varies from year to year.

Additional experiments in the laboratory with cultured *H. rubra* indicate *Perkinsus* does not kill abalone, even at the relatively high temperatures of 18°C nor even at 24°C. In all experiments, control abalone maintained in the same tanks as the exposed abalone remained uninfected, indicating that transmission of the protist may require death of the molluscan host. In the most recent experiment, two groups of abalone were exposed to a sudden 7C rise in temperature and held at this temp for 1 d or 2 d, before exposure to *Perkinsus*. After 2 months at 18°C, there were no significant differences in rate of infection between the heat-stressed and the untreated abalone (93% vs. 80%; $\chi^2_{1,1df}$ p=0.3635); none showed any signs of disease.
Evidence from several other different sources also indicate that *P. olseni* is not a virulent pathogen, but rather a symbiont that is probably widespread and that

proliferates in abalone (and other molluscs) only after these animals have been stressed by environmental variables. Stress was recently discovered to impair mollusc immunity by causing elevated levels of stress hormones, particularly noradrenaline, which in turn inhibit the major line of mollusc defence (phagocytosis by hemocytes) in a dose-dependent manner; these researchers also found that noradrenaline induces hemocytes to express an isoform of the *hsp70* stress protein, and decreases the release of reactive oxygen species by suppressing the binding of Interleukin-1-alpha to hemocyte receptors (1-5). A link between stress and immunity has also been shown in the European abalone (6).

Indications of our studies are that *P. olseni* is symbiont that proliferates after molluscs have been stressed. We now investigate sea surface temperature anomalies during El Niño episodes, and the phenomenon of cold, internal waves, that occasionally plunge particular rocky reefs and semi-enclosed bays under cold water each tide. We suspect that it is a combination of three environmental conditions – abnormally high sea surface temperature, internal waves, and local topography – that will ultimately explain why abalone disease hotspots are so strongly localised, even to distances of just tens of metres.

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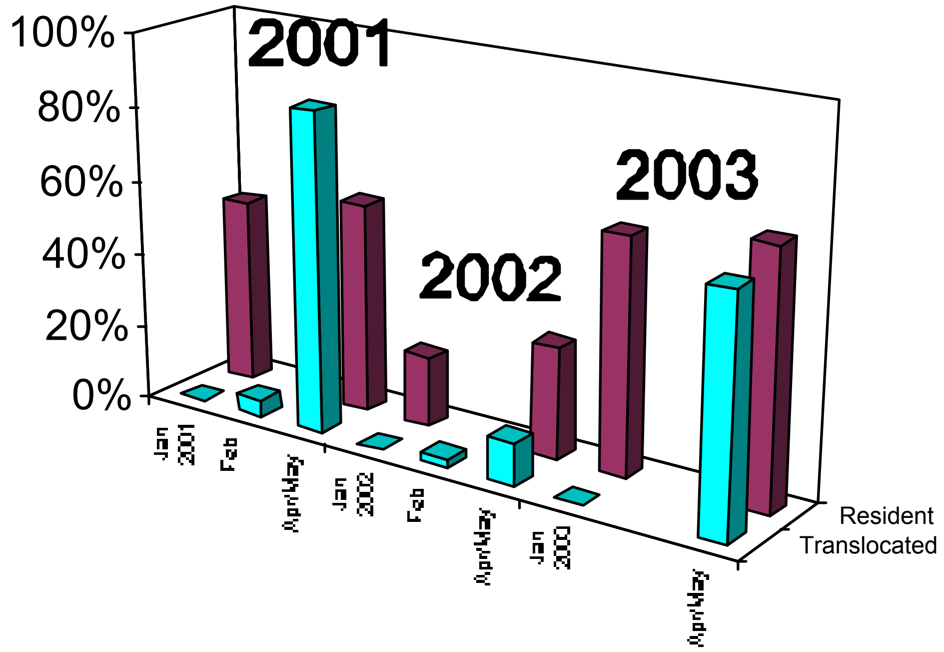

Prevalence of *Perkinsus* among blacklip abalone at Taylor Island over 3 summers
100%
80%
60%
40%
20%
0%
2001
2002
2003
Jan 2001
Feb
Apr/May
Jan 2002
Feb
Apr/May
Jan 2003
Apr/May
Resident
Translocated

Revision and Expansion of the Australian Aquatic Animal Disease Identification Field Guide

Alistair Herfort ***Click here for PowerPoint presentation***

Department of Agriculture, Fisheries and Forestry – Australia

FRDC Project: 2002/642

Introduction

During 1998/1999, Commonwealth, state and territory government agencies and key members of the private sector signed on to 'AQUAPLAN-Australia's national strategic plan for aquatic animal health (1998-2003)'.

A key priority under AQUAPLAN Program 5 - Awareness, was the preparation of an Australian Aquatic Animal Disease Identification Field Guide (Field Guide), which was officially released in March 2000.

The Field Guide deals with the major viruses, bacteria, fungi and parasites known to affect finfish, molluscs and crustacea overseas, as well as some enzootic (endemic) diseases of aquatic animals in Australia providing reference to 30 of the 32 diseases on Australia's then current, "National List of Reportable Diseases of Aquatic Animals".

With advances in knowledge and diagnostic capacity for the diseases currently covered by the existing Field Guide, the Aquaplan Business Group[13] and its Scientific Advisory Committee, recommended at its 9th meeting (ABG/SAC–09) 25th July 2002, that the Field Guide be revised to update information on existing entries. The Field Guide was also to be expanded to incorporate recent and on-going changes (additions and deletions) to the OIE and NACA[14] disease lists (as revised in the Australian National List of Reportable Disease of Aquatic Animals). ABG/SAC further recommended that the revised Field Guide be published on CD ROM, thereby reducing production and distribution costs allowing for wider dissemination of the product and periodic revision in line with on-going changes to National and regional disease lists.

ABG/SAC also recommended the production of Fish Disease Fact Sheets similar to those posted on the OIE website, and agreed that a revised Field Guide should also satisfy this requirement. It was further recommended that the Field Guide be revised with a greater focus on the technical audience such as State and Territory fisheries and aquaculture managers and staff, border protection officers, and those engaged in aquatic animal veterinary health, while remaining relevant to a non-technical audience.

A successful R&D funding application to the FRDC AAH Subprogram secured funding in February 2003 to carry out this project - Project 2002/642: revision and expansion of the Australian Aquatic Animal Disease Identification Field Guide for publishing to CD ROM.

Materials and Methods

The primary objectives of the FRDC AAH subprogram project 2002/642 are:

1. To update information in the existing Australian Aquatic Animal Disease Identification Field Guide, with advances in knowledge and diagnostic capacity

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[13] Prior to establishment of the FRDC AAH Subprogram, steerage of this program was undertaken by the Aquaplan Business Group (ABG).

[14] Office International des Epizooties and Network of Aquaculture Centres in Asia-Pacific

2. To be more relevant to managers of fisheries and aquaculture, providing a snapshot of diseases of concern, assisting informed decision making with regard to aquaculture development and sustainability
3. To produce the revised Field Guide in a cost-effective and easily distributable format

This project is largely a review of existing and emerging scientific literature pertaining to aquatic Animal health management of the diseases listed by the OIE and NACA, with additional diseases of particular socio-economic concern to Australia, comprising Australia's National List of Reportable Diseases of Aquatic Animals. Sources of information include:

- The OIE fish disease commission website (various sections)
- OIE Collaborating Centre for Information on Aquatic Animal Diseases, CEFAS
- Network of Aquaculture Centres in Asia-Pacific website (various sections)
- Journal of Fish Diseases
- Journal of the European Association of Fish pathologists
- AQUACULTURE
- Diseases of Aquatic Organisms
- Various sourced via Winspirs and other scientific literature search engines

Results and Discussion

Changes to the original version of the Field Guide include:

1. Production to CD ROM (with the provision for printing individual disease pages as fact sheets) and WWW access
2. The inclusion of all diseases on Australia's National List of Reportable Diseases of Aquatic Animals as endorsed by PISC[15] in September (from 30 to 45), with updated information on all those from the previous (original) edition
3. Addition of hyperlinks at the bottom of each disease page linking to latest available diagnostics
4. A strong statement warning of the limitations of relying on gross pathology in reaching a definitive diagnosis
5. An 1800 hotline numbers for each State and Territory
6. An improved differential diagnostic table and key

The first draft of the revised Field Guide was approved as the first milestone by the FRDC AAH Subprogram SC and SAC 1 May 2003, and was distributed to NAAH-TWG for comment at their June workshop in Perth. Comments from each group were incorporated into the second draft of the revised Field Guide, which was circulated 31 July, for peer review by all NAAH-TWG, FRDC AAH Subprogram SC and SAC members, international experts, state and territory fish pathologists, relevant CSIRO specialists and related project Principal Investigators (a total of 13 primary peer reviewers and 22 secondary reviewers). A first draft of the CD was also presented to the NAAH-TWG June meeting, with subsequent comments factored into a revised Terms of Reference for the consultancy building the CD.

A request for photographs of gross pathology and images of histology was circulated to all OIE reference labs for diseases on the OIE Disease List and relevant specialists of non-OIE listed diseases.

Pending are the reviewers' comments, and images to go into the 2nd working draft of the CD ROM, which will be distributed for further comment by NAAH-TWG and peer reviewers following its expected release at the end of September.

[15] Primary Industries Standing Committee

A database of tertiary aquatic animal health related courses, offered across Australia has been established to assist with dissemination of the final product, in an attempt to reach as many undergraduate students who already have, or may well develop, a professional interest in aquatic animal health management.

This project closely resembles a related FRDC AAH subprogram project, namely 2002/645, which is targeting the more technical audience, focusing on the detailed diagnostic aspects of disease management. The Field Guide is intended to compliment related aquatic animal disease management and operational resources.

Determination of the disease status of Western Australian commercial prawn stocks

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FRDC Project: 1998/212

Introduction

There is little published information on the disease status of the prawns on the north-west shelf, yet these prawns (*Fenneropenaeus merguiensis, Metapenaeus endeavouri, Penaeus esculentus* and *M. latisulcatus*) form the basis of a commercial fishery worth in excess of A$42 million in 2001-2002. There are also stocks of *P. monodon* on the shelf which form an important source of broodstock for the developing aquaculture industry in Western Australia, and potentially also for the Northern Territory and Queensland.

Unfortunately, prawns are infected with a variety of viral diseases, many of which have been translocated to new areas with movements of the host prawn – mainly for aquaculture but in some cases through frozen product destined for human consumption.

There is a zoogeographic barrier at the Torres Strait so there is no reason to expect that the prawns in Queensland and New South Wales will have the same diseases as those in Western Australia. This is particularly so since the northwest shelf has had little, if any, exposure to other areas through translocations. This presents a unique opportunity to study the viruses and other diseases that may have co-evolved in the area with the prawns. This isolation is already under threat, with, for example, the movement for aquaculture purposes of Gill Associated Virus (GAV) infected post-larvae from Queensland into the Northern Territory.

Thus, there are two disease risks for which this project provides background data. The first is the importation into Western Australia of prawns from other states and from the Northern Territory. To assess adequately the disease risk posed by the imports we need to understand the local disease status and this has been achieved. The second risk is that diseases endemic in Western Australia may pose a risk to aquaculture establishments in other States. This report provides a basis on which those states can assess the risk to their own industries.

Materials and Methods

During the five years of the project over 2500 prawns were examined for disease, mainly by histology, but also by PCR for White Spot Syndrome, Yellow Head Virus, and Gill Associated Virus. Most of the prawns were sourced from the wild fishery, but both *P. monodon* and *P. esculentus* are now being spawned and on-grown in Western Australia under pilot scale or commercial conditions. The disease investigations associated with these nursery or grow-out operations have also been used in compiling this report.

Results and Discussion

The two problems so far encountered in these prawns under aquaculture conditions are Monodon baculovirus-like virus (MBV-like virus) (in *P. esculentus* only) and bacterial problems (both species).

Overall, Western Australian prawns are exposed to MBV-like virus and Hepatopancreatic parvo-virus (HPV). Based on limited electron microscopy, an eosinophilic virus-like inclusion in epithelial cells particularly in the midgut, and similar to HPV but with different staining characteristics, may be a fixation artefact. There are a number of syndromes, including a new nerve syndrome in *P. monodon*, that may be associated with un-recognised viruses but could equally be due to autolysis and fixation artefacts. Further work is clearly required on these. There is also a rich fauna of metazoan parasites in most of the wild prawns. These are of little concern for aquaculture but probably deserve some taxonomic attention.

The lack of MBV-like virus and HPV in *P. monodon*, and the absence of GAV in any species are of particular note.

Finfish immunology – a review

Ken McColl ***Click here for PowerPoint presentation***

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Within the AAHL Fish Diseases Laboratory (AFDL), there is a developing interest in understanding the immune responses of finfish. This interest stems from (1) current projects on viral diseases of finfish that have inadvertently suggested that an investigation of the role of antiviral immune responses may be productive in developing control strategies for certain diseases, and (2) a developing interest in immunomodulation of fish with the attendant advantages that it might confer in terms of resistance to disease and in productivity gains.

This review paper aims to:

- Discuss the phylogenetic diversity of fish, and thereby emphasize the diversity of the immune response in fish.
- Summarize what is known about innate immune responses in fish with particular emphasis given to cytokines.
- Summarize aspects of the adaptive immune response in fish.
- Demonstrate how the innate and adaptive immune responses are integrated with each other, using a viral infection as a model.
- Hypothesize on the role of underlying viral infections during dual infections of fish.
- Summarize, in general terms, the reagents that are available for studies on the innate and adaptive immune responses of fish.

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Development of improved procedures for the identification of aquatic birnaviruses

Ken McColl, Kelly Steeper, Tam Chamberlain and Mark Crane

Click here for PowerPoint presentation

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FRDC Project: 2001/620

Introduction

Based on their geographical distribution and the diversity of hosts that are infected (finfish, molluscs, crustaceans), aquatic birnaviruses (aquabirnaviruses) of the Family Birnaviridae are generally regarded as the most widespread pathogen of aquatic animal species. The term aquabirnavirus includes both virulent and avirulent viruses, with those that are pathogenic for salmonids being known as Infectious Pancreatic Necrosis virus. IPN viruses may cause up to 100% mortality in fry and smolt, and survivors often become chronic shedders of virus.

Serological and molecular tools have been used in an attempt to classify the aquabirnaviruses, but their taxonomy remains complex and unclear. Currently, the aquabirnaviruses are divided into two serogroups, A and B (Hill and Way, 1995). Within the former, nine serotypes are recognized, whereas only one serotype is recognized in serogroup B. Blake et al (2001) used deduced amino acid sequences to study the phylogenetic relationships between serogroup A aquabirnaviruses. They proposed that there were six genogroups within serogroup A, and that, in general, these genogroups correlated with the geographical origin and serological classification of viruses. It is unclear where the marine aquabirnaviruses fit into these classification schemes.

The isolation of Tasmanian aquabirnavirus (TAB) from farmed salmonids and wild fish in Macquarie Harbour, Tasmania in 1998 (Crane et al, 2000) highlighted a need for improved procedures for the identification, and characterisation, of aquabirnaviruses. In particular, there was a need for (1) a generic polymerase chain reaction (PCR)-based test that would allow confirmation of any aquabirnavirus, virulent or avirulent, that had been identified in cell culture; (2) a PCR test, specific for TAB, that could also be used on tissue-culture supernatant fluid; and (3) a molecular-based test that allowed differentiation of virulent and avirulent aquabirnaviruses (this was considered a very challenging objective).

Materials and Methods

Twelve serogroup A aquabirnaviruses, representing all nine serotypes, were used in this study. They included 10 isolates of TAB, two isolates from New Zealand (Tisdall and Phipps, 1987), a Thai virus, DPL, of unknown serotype, and a number of imported IPN isolates (West Buxton, VR299 and Erwin [serotype A_1], Sp [A_2], Ab [A_3], He [A_4], Te [A_5], Canada 1 [A_6], Canada 2 [A_7], Canada 3 [A_8] and Jasper [A_9]). Two serogroup B viruses (TV-1 and an uncharacterised isolate) were also examined.

Indirect immunoperoxidase (IPX) tests were developed for the detection of aquabirnaviruses using either (1) a panel of 11 commercial anti-IPN monoclonal antibodies (DiagXotics, Wilton, CT, USA) to detect virus in tissue culture, or (2) a sheep anti-IPN polyclonal antibody (Microtek, Saanichton, Canada) as the primary antibody for detection of virus in tissue sections. After the original 1998 isolate of TAB (TAB 98) was fully sequenced, and the VP2 regions of TAB 02 and of two NZ isolates were also

determined, a generic birnavirus PCR, and a TAB-specific PCR were designed. Deduced amino acid (aa) sequences from the VP2 of a number of so-called "virulent" and "avirulent" isolates of IPN were aligned in an attempt to identify aa residues that might be a marker of virulence.

Results and Discussion

In cell culture, TAB reacted with the same range of mAb s as the DPL and NZ strains of IPN, and this group of viruses appeared to be much closer (in their reactivity profile) to the Sp serotype than to the Ab serotype (although both TAB and NZ were originally described as being Ab-like). Interestingly, a virus belonging to the Sp serotype is the only other IPN serotype recorded from Thailand. Using the IPX technology, aquabirnaviruses have also been demonstrated in histological sections of experimentally infected fish.

TAB 98 has been completely sequenced as has the VP2 gene of TAB 02. There is at least 99% similarity between the viruses in the VP2 region (at nt and aa levels). Two NZ isolates are also 94-97% similar to TAB 98 over the VP2 region, and phylogenetic analysis of VP2 sequences placed TAB 98 and the NZ isolates within the same genogroup, along with the DPL and Sp strains of IPN. Therefore, the results of serological and genetic studies on TAB and the NZ birnaviruses were consistent.

Based on these sequence data (and those generated by Blake et al, 2001), diagnostic PCRs were designed (Figure 1, Table 1). A generic aquabirnavirus PCR has been shown to amplify 12/12 serogroup A viruses, including TAB, the NZ isolates, and representatives from all nine serotypes. It does not detect either of the serogroup B viruses in our lab, and attempts to design truly generic PCR primers based on the VP1 gene or the VP5-VP2 overlap region, have not been successful. Nevertheless, given that most, if not all, virulent aquabirnaviruses seem to belong to serogroup A, our generic PCR is probably a very adequate diagnostic tool for the present.

A TAB-specific PCR was also designed, and it has successfully detected all 10 isolates of TAB that are held at AAHL. Importantly, this PCR failed to amplify products from any other serogroup A or B viruses, including the Sp, DPL and NZ strains.

When deduced amino acid (aa) sequences from the VP2 of a number of so-called "virulent" and "avirulent" isolates of IPN were aligned, aa 245 was identified as being a potential marker of virulence (R in "virulent" strains, K in "avirulent"). However, assessing the virulence of an IPN isolate is a very subjective process, and markers of virulence are more likely to be identified using a reverse genetics approach.

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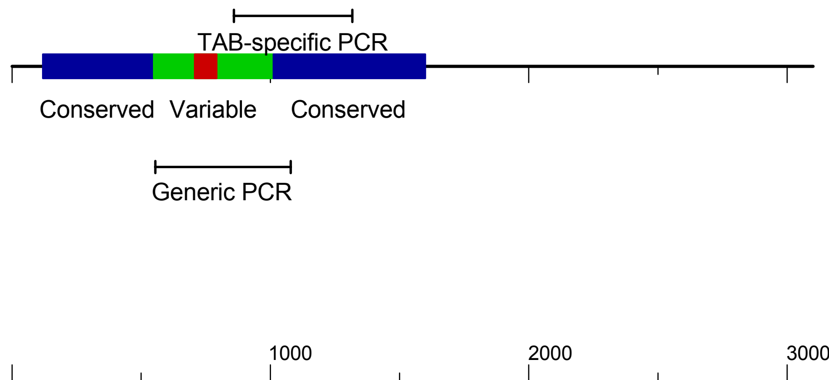

Figure 1. Diagrammatic representation of an aquabirnavirus segment A showing conserved and variable regions of the VP2 gene. The locations of the generic aquabirnavirus PCR, and the TAB-specific PCR are shown.

Table 1. Primers for diagnostic aquabirnavirus PCRs

PCR	Forward primer	Reverse primer
Generic[1]	5'-acgaaccctcaggacaa-3'	5'-gattgctgtctgctggttga-3'
TAB-specific[2]	5'-cggacaaccccccaacg-3'	5'-tggcgtagttcaggccctct-3'

[1]Forward primer: GAB F2; reverse primer: GAB R2

[2]Forward primer: A3F; reverse primer: A1R

Development of cell culture, Nested RT-PCR and immunodiagnostic tests for the detection of nodavirus in finfish

Nick Moody ***Click here for PowerPoint presentation***

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FRDC Project: 2001/626

Introduction

Viral Encephalopathy and Retinopathy (VER), or Viral Nervous Necrosis (VNN), is caused by a nodavirus (Nervous Necrosis Virus, NNV) and is a common disease of cultured marine finfish larvae. At least 23 different marine fish species have been recorded as having VNN (Munday et al, 2002). A range of diagnostic tests, including isolation in cell culture, immunohistochemistry (IHC), immunofluorescent antibody test (IFAT) and RT-PCR, has been published by the International Office of Epizootics for nodavirus diagnosis (OIE, 2000). The main objectives of this project are to develop, optimise, validate and standardise cell culture, IHC, IFAT and nested RT-PCR protocols for the detection of NNV and transfer these protocols to laboratories in Australia and New Zealand through and Australian and New Zealand Standard Diagnostic Protocol (ANZSDP).

Materials and Methods

Nested RT-PCR

The standard NNV Nested RT-PCR implemented at the Oonoonba Veterinary Laboratory uses the RT-PCR primers and cycling conditions described in Nishizawa et al (1994) and the nested primers and cycling conditions described in Thiery et al (1999). Template volume, $MgCl_2$, primer and enzyme concentration, and cycling parameters were optimised to improve the sensitivity of the test.

Four different RNA extraction protocols, reverse transcriptases and *Taq* polymerases were evaluated using this standard nested RT-PCR protocol to determine the variability of different reagents and kits. All testing was conducted on replicated, serially diluted samples, which were known to contain nodavirus. Material generated during previous infectivity trials was used to optimise sample preparation protocols and is currently being used to validate the nested RT-PCR. Clinical material has been tested from barramundi in Queensland and the Northern Territory and striped trumpeter from Tasmania to assess the ability of the nested RT-PCR to detect endemic nodavirus isolates from a range of tissue types. Two exotic nodavirus isolates have been tested at AFDL, to determine the ability of the test to detect exotic nodavirus isolates.

Immunodiagnostics

Polyclonal antibody production in sheep was initiated by intramuscular inoculation at two sites using recombinant coat protein (rCP), from either Sleepy Cod Nervous Necrosis Virus (SCNNV) or Barramundi Nervous Necrosis Virus (BNNV), and Freund's Complete Adjuvant. Subsequent inoculations used Freund's Incomplete Adjuvant. Prior to booster inoculation, jugular blood samples were collected into lithium heparin and untreated vacutainer tubes. For production blood was collected thirty days post-inoculation, clarified by centrifugation and stored in 0.9ml and 4.5ml aliquots at -80°C. Sensitivity and specificity were assessed by dot blot using rCP as the capture antigen, sheep α-NNV rCP as the primary antibody, mouse α-sheep HRP conjugate as the secondary antibody and AEC-DMF as the chromogen.

For immunohistochemistry (IHC) and Indirect Immunofluorescence Test (IFAT) test development, deparaffinised 3-5μm thick sections were trypsinised, washed and blocked with BSA. Sections were washed and incubated with different dilutions of Sheep α-NNV rCP followed by washing and incubation with different dilutions of either mouse α-sheep HRP conjugate (IHC) or mouse α-sheep Cy2™ conjugate (IFAT).

Cell line initiation and screening

Cell lines were initiated by aseptically producing a single cell suspension of brain tissue from barramundi. Cells were cultured in Ae MEM (MEM containing 1000IU/ml benzylpenicillin and 1mg/ml streptomycin sulphate) and 20% FBS in 25cm² tissue culture at 25°C. Several cell lines were cloned and the clones and stock cell lines assessed for susceptibility to NNV when consistent growth rates were obtained. To assess the susceptibility of the cell lines, 80% confluent monolayers, cultured in 25cm² flasks, were exposed to 1ml of a clarified nodavirus-infected barramundi homogenate for 60 minutes, incubated at 25°C and monitored daily for the appearance of CPE. Different concentrations of polybrene and DEAE-dextran have been trialled in an effort to increase the susceptibility of the cell lines and pre-treating of the flasks with 2.5% gelatin had been used in an attempt to increase the plating efficiency of the cells.

More recently the optic tectum and cerebellum have been used to initiate cell lines as these areas of the brain are more susceptible to nodavirus infection than other parts of the brain.

Results and Discussion

The different stages of the Nested RT-PCR have been optimised and the test will detect nodavirus in cell culture supernatant, homogenised fish and broodstock spawning fluid and blood samples. The test will detect endemic nodavirus isolates and at least two exotic nodavirus isolates. Optimisation of broodstock spawning material requirements and tissue disruption protocols are ongoing.

Although over 15 new brain cell lines have been produced, none have been identified as appropriate for culture of NNV. Development of a susceptible cell line is ongoing. Addition of Polybrene and DEAE-dextran did not result in an increase in the appearance of any CPE. Overnight pre-treatment of flasks with 2.5% gelatin increase the plating efficiency 13-fold.

The polyclonal antibodies are highly specific and there is excellent cross-reactivity between the two isolates used to produce them. Used in the IHC and IFAT at a 1/1000 dilution, the polyclonal antibodies can detect BNNV in clinically and subclinically-infected barramundi, sleepy cod, barcoo grunter, silver perch and golden perch and in sections of NNV-infected finfish from five overseas countries. The IHC is more sensitive than the IFAT and has detected nodavirus in experimentally infected barramundi from 4 days post infection. While positive staining has been seen in the spinal cord, brain and retina, no staining has been seen in any other tissue.

The IFAT and IHC are still undergoing optimisation, validation and comparative testing with the Nested RT-PCR. Approximately 1000 fresh and fixed samples, resulting from infectivity trials conducted during FRDC 1999-205 are being used for validation and determination of the comparative sensitivity of the different tests.

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Vaccination versus non-specific immunostimulation in fish - Lessons learned from the teleost antibody response

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Fish are the lowest phylogenetic group to generate an “acquired” immune response. As per higher vertebrates, recombinatorial mechanisms are fundamental to this process. Humoral adaptive (antibody-mediated) immunity has long been demonstrated and provides protective immunity against bacterial pathogens such as *Vibrio anguillarum* in Atlantic salmon (*Salmo salar*). Despite this, the fish antibody response is considered relatively simple, particularly against the backdrop of the mammalian antibody paradigm. For example, in fish there is little evidence of an anamnestic antibody response (magnitude, speed & affinity) to secondary immunisation. Affinity maturation does not appear to be limited by a lack of somatic mutations in fish immunoglobulin (Ig) variable region genes. Rather, fish lack the structural organisation of mammals (eg. germinal centres) within relevant organs to select B cell mutants with greater affinity (Figure 1). In addition, the polymeric immunoglobulin M (IgM) is the predominant class of immunoglobulin produced in response to primary and secondary immunisation suggesting that class switching of immunoglobulin isotypes does not occur.

Within germinal centres of mammals, B cells selected to enter the peripheral B cell pool are rescued from programmed cell death by activation via contact with activated T cells and soluble growth factors (Figure 2). Using snapper (*Pagrus auratus*) as a model, we have shown in preliminary experiments that fish may also use T cell-dependent B cell activation. This implies that T cells may indeed activate B cells in fish but given the lack of germinal centres, this T cell-derived activation may simply expand the peripheral B cell pool. Therefore the fish host defence will be limited by this relatively simplistic antibody response model. We argue that fish place a greater reliance on innate immunity for disease protection and this provides further impetus for the use of non-specific immunostimulation in aquaculture.

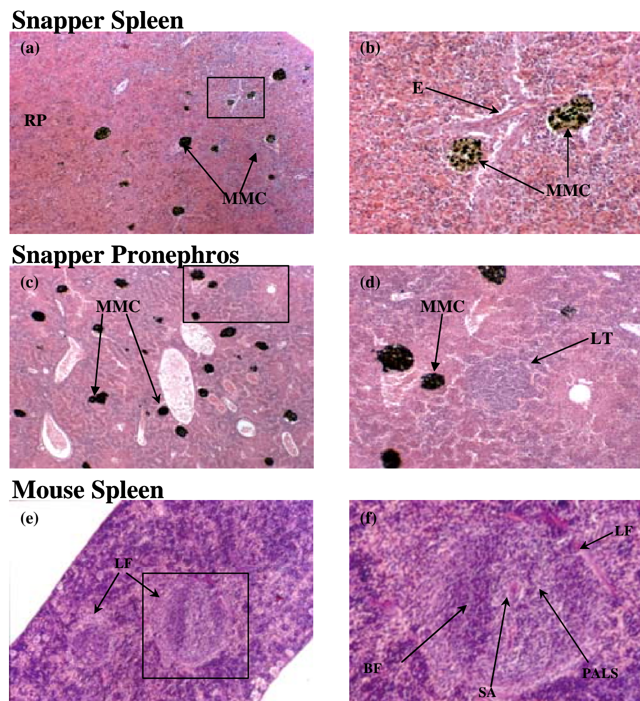

Figure 1. The snapper spleen and pronephros lacks the clear cellular demarcation of the murine splenic lymphoid follicles which may affect snapper B cell selection and thus affinity maturation. (a) snapper spleen showing red pulp (RP) and diffuse white pulp. (b) Inset of (a) showing an ellipsoid (E) and melanomacrophage centres (MMC). (c) Snapper head kidney with prominent putative lymphohemopoietic tissue (LT) and MMC. (d) higher magnification of the putative LT in (c). (e) low magnification of a mouse spleen showing the demarcation of cells forming primary lymphoid follicles. Germinal centres form in the centre of the LF after administration of antigen. (f) high magnification of the LF in (e). The splenic arteriole (SA) is central in the LF, while the periarteriolar lymphoid sheath (PALS) and B cell follicular zone surround the SA. Snapper spleen and pronephros stained with H and E and the mouse spleen stained using Voerhoff's/Masson's trichrome stain.

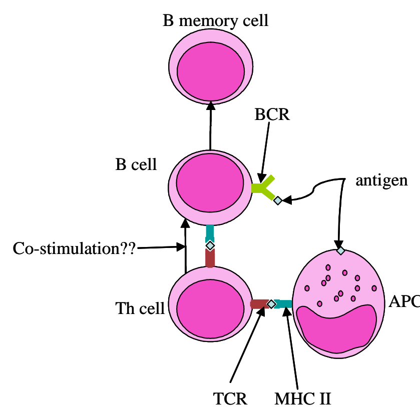

Figure 2. Are co-stimulatory signals by T cells required to initiate expansion of the memory B cell pool in fish? In mice, antigen is recognised by both APC and B cells and is presented in peptide form using MHC II molecules to T helper (Th) cells. Th cells provide B cells with a co-stimulatory signal via CD40L to rescue B cells from apoptosis and initiate clonal expansion. Th cell = T helper cell, APC = antigen presenting cell, TCR = T cell receptor, MHC II = major histocompatibility complex class II molecule, BCR = B cell receptor.

Amoebic Gill Disease research progress

Barbara F. Nowak **Click here for PowerPoint presentation**

School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania and Aquafin CRC

Introduction

Amoebic Gill Disease (AGD) is the main health problem in Atlantic salmon culture in Tasmania (Nowak et al 2002). The disease is usually diagnosed by histopathology showing presence of paramoebae and associated characteristic AGD lesions (Nowak et al 2002, Adams and Nowak 2003). While *Neoparamoeba pemaquidensis* is considered to be the primary pathogen in this disease (Nowak et al 2002), Koch's postulates have not been fulfilled. Salmon industry in Tasmania uses freshwater bathing to treat AGD infected fish. This bathing has to be repeated a number of times and can significantly increase cost of salmon production. Additionally, the quantity and quality of fresh water available on a salmon farm may be another limiting factor. There is an urgent need for effective control and treatment to reduce the effects of AGD on salmon industry in Tasmania. Aquafin CRC funds a comprehensive research program focussed on AGD.

Materials and Methods

In laboratory experiments fish were infected using gill isolated amoebae from salmon from AGD infection tank (Bridle et al 2003). AGD outbreaks were confirmed using histopathology (Adams and Nowak 2003, Bridle et al 2003) and immunocytochemistry (Bridle et al 2003). *Neoparamoeba pemaquidensis* was detected in environmental samples using culture on marine agar, followed by PCR, DAPI staining and IFAT (Crosbie et al 2003).

Results and Discussion

We have shown that *Neoparamoeba pemaquidensis* is widely distributed in marine and estaurine sediments around Tasmania (Crosbie et al 2003). While the amoebae were viable as determined by culture, it is not known if they were infectious. This would be difficult to test experimentally as amoebae loose their infectivity during culture (Morrison, unpublished). AGD outbreaks were detected on salmon farms 13 weeks posttransfer and coincided with the cessation of halocline and increased water temperature (Adams & Nowak 2003). Lesion pathology suggested that *Neoparamoeba pemaquidensis* is a primary pathogen and does not require pre-existing or co-existing gill damage for attachment. Current research increased our understanding of pathophysiology of AGD and role of mucus in this disease (Roberts and Powell, 2003). These results are already applied in research on treatment of AGD (Roberts and Powell, unpublished). Positive effects of CpGs injection 6 days before infection (Bridle et al 2003), suggest that if an effective dose of immunostimulant is applied at an appropriate time before infection, the mortalities due to AGD outbreak may be delayed and reduced. Currently we are investigating effects of commercially available oral immunostimulants.

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Aquafin CRC Health Program

Barbara F. Nowak ***Click here for PowerPoint presentation***

Aquafin CRC and School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania

Aquafin CRC

Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC) was established in 2001. Aquafin CRC contributes to the growth and competitiveness of aquaculture industry, develops effective research collaborations, provides education and training. It also allows for harmonisation between industry sectors. Aquafin CRC focuses on southern bluefin tuna (62%) and Atlantic salmon (32%), additionally snapper and striped trumpeter are investigated as future commercial aquaculture species. The main objectives of Health Program are to reduce economic impact of disease in farmed finfish, improve industry and government responsiveness to disease outbreaks and to develop environmentally friendly approaches to disease management. The Program consists of two subprograms - Diagnostics and Risk Assessment and Management and Control of Amoebic Gill Disease in Atlantic salmon. Two projects within Diagnostics and Risk Assessment have already been completed. These included Tuna health risk assessment and Development of diagnostics for bacterial pathogens. The continuing project is on development of tuna cell lines. A new project on the effects of farming practices on tuna health commenced this July. The subprogram on Control of Amoebic Gill Disease includes four projects: Treatment and control of AGD, Host-pathogen interaction in AGD, Epidemiology of AGD and AGD vaccine development. These projects will be completed late next year. Both subprograms will include new research projects from 2004/05. Aquaculture industry needs in the area of training and human capital development are recognised and addressed within Health Program.

Development of diagnostic capability for priority aquatic diseases of national significance: spawner-isolated mortality virus

Leigh Owens and Brad R. Cullen ***Click here for PowerPoint presentation***

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FRDC Project: 2001/625

Abstract

Spawner-isolated mortality virus (SMV) was first identified as being associated with epizootic mortalities in *Penaeus monodon* spawners held in a research facility. It has also been one of the pathogens associated with mid-crop mortality syndrome (MCMS). This syndrome first manifested as mortalities in Australian prawn farms during the 1994 season. Epidemiological evidence suggested an association between the SMV status of broodstock and subsequent survival of progeny. A polymerase chain reaction (PCR) was developed to detect the presence of SMV, with positives confirmed by internal dot blot. This was used to detect the virus in prawn faeces. Through testing 909 broodstock in 9 different groups, prevalence of SMV ranged from 0% to 24% among groups, being higher in *P. monodon* than *P. merguiensis*. Overall prevalence in spawners was 8.5%. Survival in hatchery tanks of progeny from SMV-positive spawners was lower than those from SMV negative spawners with reductions of 23%, 7.3% and 18.9% in 3 studies. Survival was negatively related to the proportion of postlarvae from SMV-positive spawners, with a decrease in survival of 5.6% for each 10% increase in the proportion of postlarvae coming from SMV positive spawners.

The current project has been primarily concerned with producing a PCR/ELISA to enhance the diagnostic capability of SMV. The development of the assay has been completed, with assay validation and testing of clinical samples well underway. A non-infectious positive control for the assay has been produced, and is being distributed to laboratories interested in testing for SMV. Advantages of the test have included increased sensitivity (able to detect down to 5 copies of positive control plasmid), test results are easy to read and objective, it allows for discrimination between specific PCR amplification and spurious bands, it is easier to run than the previous system of gels and dot blots, the method could be adapted for high sample throughput due to its 96-well format and it would be possible to package into a kit form. This would potentially allow spawners and postlarvae to be screened for the presence of SMV across the Australian prawn industry. Appropriate strategies could then be put in place to optimise productivity.

So far the best tissues to target have included the intestine followed by soft tissues in the cephalothorax. Gill and pleopod do not appear to be effective target tissues, so for non-lethal testing of spawners, detection in faeces is recommended. DNA quality from samples is continually assessed. Findings and recommendations for appropriate collection and transport of prawn samples for testing shall be presented. The assay is suitable for use on postlarvae, with spin-column methods of DNA extraction (Roche) showing no observable difference in using postlarvae with or without prior removal of eyestalks. Use and validation of the assay, as well as relevant clinical data from prawn sampling shall be discussed.

Aquatic Animal Diseases Exotic to Australia: A Training Module for Use in Tertiary Institutions

Shane Raidal, Frances Stephens, Barbara F. Nowak, Garry Cross, Kevin Ellard, Stan Fenwick, Phillip Nicholls ***Click here for PowerPoint presentation***

Murdoch University, WA

FRDC Project: 2002/645

Introduction

Improving the awareness of veterinary, aquatic science and aquaculture students of the risk to Australia following incursions of exotic diseases of aquatic animals was identified as a key feature of the AQUAVETPLAN. In many instances students are either unfamiliar with the diseases of aquatic animals or are familiar with some endemic diseases but have little or no understanding of exotic diseases. This project aimed to address this problem.

Materials and Methods

Initially, a current list of reportable diseases that are also exotic to Australia was compiled. A literature review of the diseases was then undertaken. The main aim of the literature review was to identify key species (host range) and geographical range for the disease, clinical signs and histological features of diseased animals and epidemiological features of the disease.

Photographs were obtained from collections within Australia and from overseas.

Results and Discussion

Since the commencement of this project several diseases have been added to the list but none have been removed. Nevertheless the latest information and policies have been incorporated as best possible. A draft copy of the training manual has been completed and is available for peer review. The draft manual can be viewed on the Murdoch University fish health website (http://wwwvet.murdoch.edu.au/fish/exotic/Exotic-disease-manual-DRAFT.pdf). Aquatic animal health professionals who work, or have worked with the diseases, and representatives of tertiary institutions in Australia will be asked to review and comment on the document. Information was obtained from OIE publications and other reviewed literature such as journal articles and books. Photographs have been obtained from overseas or from experimental infections at the Australian Animal Health Laboratory. The information has been provided in brief, point format and a list of key references such as review articles has been provided. More detailed information is available in the cited references, if required. Diagnostic techniques have been listed but not described in detail as the emphasis of the training manual is on describing the clinical signs of disease, basic diagnostic techniques such as histology, and the epidemiology of the diseases. This is considered to be of more importance in a training manual targeting veterinary and aquaculture students. As research into many of the diseases is still progressing, knowledge of many of the diseases that are included in this training manual is being frequently refined. This has made the compilation of information more difficult than would have been the case if all of the diseases and their epidemiology were well understood. It also demonstrates that information in the training manual will require frequent updating before and after completion of the project.

Aquatic Animal Health Subprogram: Aquatic Animal Disease Emergencies – Video and Training Kit

Wayne Tindall

Big Time Media

FRDC Project: 2003/645

Summary

The purpose of this presentation is:

To provide an introduction to Big Time Media

To play an Intro Video Clip

To provide an introduction to the Interactive CD ROM outline and scope that BTM is developing

To provide an outline of proposed filming schedule (late October)

To provide an introduction to the discussion forum for feedback on the project as it develops

To provide an introduction to the concept of a dynamic Web Site for future updates

Invite the audience for discussion and interviews during the conference.

To make a request for any video footage that may assist in the development of video clips for the CD ROM.

Development and Preliminary Characterisation of Pilchard (*Sardinops sagax neopilchardus*) Cell Lines Derived from Liver and Heart Tissues

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FRDC Project: 1999/226

Introduction

In 1995 and again in 1998-99, mass mortalities of pilchards *(Sardinops sagax neopilchardus)* occurred around the southern coastline of Australia. In the 1995 mortality, a herpesvirus was observed in the gills of all affected fish examined [Hyatt et al. 1997, Whittington et al. 1997]. Numerous attempts to isolate this pilchard herpesvirus on various poikilothermic cell lines have not been successful [Hyatt et al. 1997]. During the 1998-99 mortality, a herpesvirus was again observed in affected fish but in lower numbers (Hyatt, pers. comm.). The inability to isolate and grow this virus in fish cell lines such as CHSE-214, RTG-2, BF-2, FHM or EPC has severely limited the progress of research on this virus. During the disease investigations, it became evident that a pilchard cell line, susceptible to infection by the pilchard herpesvirus, would facilitate further studies with this virus. This report describes the establishment of two pilchard cell lines which should provide diagnostic and research tools for pilchard biology in general, and the study of various marine viruses in particular.

Materials and Methods

During the 1998-99 pilchard mortality event, pilchards were collected in collaboration with staff from the Marine and Freshwater Resources Institute, Queenscliff, Victoria and a commercial fisher from Lakes Entrance, Victoria. In an attempt to obtain tissues from normal uninfected fish, juvenile pilchards approximately 10 months of age were netted from a fishing trawler off the coast of Lakes Entrance in Victoria, Australia in November 1998. Tissues were derived from these pilchards which were netted ahead of the mortality front.

Standard procedures (explant and trypsinisation) were used to establish primary cell cultures from all major internal organs [Freshney, 1994; Nicholson 1985]. Several culture media (EMEM, M199, L-15) were used supplemented with 2mM glutamine, 20% (v/v) foetal bovine serum (FBS), 50µg/ml gentamycin sulphate and 5µg/ml amphotericin B. All cultures were incubated at 22°C.

After several weeks of culture and maintenance, confluent cell monolayers of liver and heart cultures were obtained and these have been maintained in single strength EMEM and sub-cultured (split ration of 3:1) using standard techniques (treatment with 0.25% trypsin/versene solution to obtain a single cell suspension).

Parent cell lines and clones thereof have been cryopreserved using standard techniques. Viable cultures can be recovered from liquid nitrogen storage. In addition, the temperature range permissive for cell growth and susceptibility to a range of viruses was determined.

Confirmation that the cell lines were derived from pilchards was sought using a polymerase chain reaction (PCR) specific for *Sardinops spp.*, in combination with sequence analysis of the PCR product.

Results and Discussion

Confluent monolayers of cells derived from liver and heart tissue were obtained after approximately 5 weeks incubation. After initial sub-culturing and incubation for various time periods, monolayer cultures could be sub-cultured on a weekly basis, using trypsin-versene solution to obtain a single cell suspension. A split ratio of 3:1 was used to establish new cultures which grew to confluency in 5 to 7 days at an incubation temperature of 22°C.

The pilchard liver cell line has been sub-cultured in excess of 80 passages over a 4-year period, while the pilchard heart cell line has been sub-cultured in excess of 40 passages over the same 4-year period. Cultures have been grown in a standard cell culture medium (EMEM) supplemented with 10% FBS, 2mM glutamine and 10 mM hepes.

Pilchard liver cell cultures were susceptible to infection by IPNV, IHNV, VHSV, EHNV, pilchard orthomyxo-like virus, SVCV and Atlantic salmon reovirus. Cultures inoculated with these viruses demonstrated viral CPE. Growth of IHNV appeared to be temperature dependent. At 15°C the virus appeared to grow well but IHNV did not appear to grow in cultures incubated at 19°C. The pilchard liver cell line was refractory to infection with OMV and ISAV.

The high degree of DNA homology between sequences obtained from PCR analysis of the cultured cells, and pilchard spleen, and *Sardinops spp.* sequences in Genbank confirms the pilchard origin of both the liver and heart cell lines. The data also establish the likely origin of the cell lines to be either *Sardinops neopilchardus* or *S. ocellatus*. Since *S. ocellatus* is not known in the waters of south-eastern Australia, where the original samples for cell culture were obtained, it is reasonable to assume that the cells were derived from *S. neopilchardus.*

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